Electrokinetic biased Deterministic Lateral Displacement: Scaling Analysis and Simulations

Victor Calero [a], Pablo Garcia-Sanchez [b], Antonio Ramos [b] and Hywel Morgan *[a]

[a] School of Electronics and Computer Science, University of Southampton, United Kingdom.

[b] Departamento de Electrónica y Electromagnetismo, Facultad de Física, Universidad de Sevilla, Spain.

* Email: hm@ecs.soton.ac.uk - Tel: +44 2380593330

Abstract

Deterministic Lateral Displacement (DLD) is a microfluidic technique where arrays of micropillars within a microchannel deflect particles leading to size-based segregation. We recently demonstrated that applying AC electric fields orthogonal to the fluid flow increases the separation capabilities of these devices with a deflection angle that depends on the electric field magnitude and frequency. Particle deviation occurs in two distinct regimes depending on frequency. At high frequencies particles deviate due to negative dielectrophoresis (DEP), and at low frequencies (below 1 kHz) particles oscillate perpendicular to the flow direction due to electrophoresis. Particles were deflected at significantly lower voltages. In order to characterize the enhanced separation at low frequencies, the induced deviation was compared between the two frequency ranges. For high frequencies, we develop both theoretically and experimentally the scaling laws for the dependence of particle deviation on several parameters, namely the amplitude of the applied voltage, particle size and liquid velocity, where DEP forces compete with viscous drag. A novel theoretical framework is presented that enables simulation of particle trajectories subjected to DEP forces in DLD devices. Deviation angles predicted by simulations are in very good agreement with experimental data. At low frequencies (below 1 kHz), particles follow the same scaling law, but with much lower voltages. This indicates that electrokinetic phenomena other than DEP play an important role in driving particle behaviour. Experiments show that at low frequencies, particle motion is affected by quadrupolar electrohydrodynamic flows around the insulating pillars of the DLD array. We quantify the difference between the two frequency regimes and show that an electrokinetic model based only on DEP forces is limited to frequencies of 1 kHz and above.

Keywords: Electrokinetics; Electric fields; Microparticles; Dielectrophoresis; Electrophoresis; Microfluidics

1. Introduction

Deterministic Lateral Displacement (or DLD) is a continuous-flow technique that passively separates particles based on size using arrays of microposts within a microchannel [1-3]. The technique has been widely used in numerous applications [4-6]. The post array has a special geometry that induces particle separation (see Figure 1a). Inside the DLD channel, rows of posts are tilted at a small angle (typically <6°) so that particles bigger than a given size threshold called the critical diameter D_c, bump onto the posts displacing at a tilt angle θ (also called deviation angle). Particles smaller than D_c continue to flow straight through the device without deviation, zigzagging around the posts. Thus, larger particles reach the end of the channel having been displaced laterally and separated from the smaller particles [7].

The DLD mechanism is explained diagrammatically in Figure 1b where separation relies on a separatrix streamline (black line in Figure 1b) [8]. This streamline separates the flow that passes above and below a given post (dark post in Figure 1b). Separation occurs when particles interact with the previous post (upstream). If the particle is bigger than D_c (red), it hits the post, is pushed across the separatrix and moves into the portion of fluid that passes above the black post. On the other hand, if the particle diameter is smaller (green), it stays within the same fluid lamina and passes below the black post. This process is repeated every time a particle encounters a post and is magnified throughout the DLD channel.

The value of D_c only depends on the geometry of the post array. In 2009, Davis *et al*. derived an empirical estimate of D_c for arrays of circular posts from geometrical parameters [10]:

$$D_c = 1.4 G N^{-0.48} \quad (1)$$

where G is the gap between the posts and N is the periodicity of the array ($N = \lambda/\Delta\lambda = 1/\tan(\theta)$). Although DLD particle separation has in general high precision and resolution, it also has some drawbacks when compared to other microfluidic based separation techniques. First, D_c depends only on the geometry of the array and cannot be easily modified, limiting the range of potential applications, i.e. a given DLD device can only be useful to process the specific sample it was designed for. Also since DLD performs binary separation, its utility for processing complex mixtures with more than two components is very restricted. In addition, DLD is a size-based separation technique where other physical properties of the particles, such as electrical or mechanical properties have no influence on separation. Finally, due to the very small dimensions of the DLD channels, the throughput of DLD devices is very low compared to other microfluidic sorting techniques.

Several approaches to overcome these drawbacks have been reported. A modified geometry, such as non-circular post shapes [11-13] or different lateral and horizontal gaps [14], can lead to improved separation of non-spherical particles with higher efficiency and throughput. Deformability and shape-based DLD separation has also been reported [15,16]. Several DLD devices can be combined for enhanced sorting. A staggered DLD with different array geometries within the same device can be used for fractionation of samples composed of more than two components [4,17] and numerous DLD devices can be fabricated on the same chip and run in parallel to compensate for the low throughput of this technique, which is a big drawback particularly for nanoparticle DLD separation [18].

The tunability of the DLD can be significantly improved by combining it with other forces that modify the paths of particles through the channel. Devendra *et al.* used gravity to drive the flow and control the separation [19]. Beech *et al.* [20] showed how the elastomeric properties of a PDMS device can be used to actively modify the dimensions of the geometry and modify the D_c value. Zeming *et al.* [21] reported successful control of the effective diameter of the particles by changing medium conductivity and hence the electrostatic interaction with the posts. Li *et al.* [22] used viscoelastic flows to add a lift force from the walls and alter the particle trajectories.

The electrical manipulation of microparticles suspended in electrolytes has grown exponentially in the last decades and has been demonstrated as suitable for particle trapping [23-25], isolation [26,28] and separation [29-31]. Combining DLD with electric fields leads to a tuneable particle separation system [9,32-35]. Similar to the insulator based dielectrophoresis technique (iDEP) [36-40], application of an electric field to the DLD channel creates high and low electric field regions around insulating posts that modify the electric field lines giving rise to field gradients and regions of dielectrophoresis (DEP). This force acts on particles and alters their trajectories. This mechanism was first reported by Beech *et al.* in 2009 [33]. They applied a low frequency AC electric field along a DLD channel, parallel to the fluid flow, placing electrodes at the inlet and the outlet of the device demonstrating tuneable deflection of particles smaller than the critical diameter.

In recent work [34], we designed a device where the AC electric fields were perpendicular to the fluid flow and described the full frequency-dependent behaviour of the electric field induced deviation of particles smaller than the critical diameter. Experiments showed that the particle behaviour is much richer than previously reported and strongly dependent on the frequency of the AC signal and applied field magnitude. A transition between two different regimes was observed at around 1 kHz. At low frequencies, forces such as electrophoretic-induced particle oscillation dominate, whilst above this frequency, these low frequency effects become negligible and DEP dominates. This effect is outlined in Figure 2.

At high AC frequencies the particles are deflected by the action of negative DEP which repels particles from high electric field regions around the posts (Figure 2b and 2d). Figure 2d shows a comparison between the simulated trajectories of a 1 μm particle in a 6.3 μm D_c DLD when a nDEP force acts on the particle (white line) and in the absence of such force (transparent line). Particles experiencing either weak nDEP or pDEP (particles attracted towards high electric field regions) do not bump along the posts of the DLD. At low frequencies (below 1 kHz) particles oscillate perpendicular to the flow direction along the electric field lines due to electrophoresis (see Figure 2c and 2e). In this low frequency range, particles experiencing weak nDEP or pDEP are also deflected. The critical electric field magnitude that governs particle deviation is much lower at frequencies below 1kHz. These results indicate that at low frequencies, DEP is not the only force acting on the particles; other electrokinetic phenomena must be involved in driving the electrically induced deviation.

In this paper we provide further experimental characterisation of the AC electrokinetic behaviour of particles in a tuneable DLD, along with a theoretical analysis. We describe the scaling laws that govern particle behaviour at high and low frequencies. We also present a numerical model to explain the high frequency deviation and compare predictions with experimental measurements. It is shown that the low frequency induced deviation is not controlled by the amplitude of the particle electrophoretic oscillations along the electric field lines but by a combination of several phenomena. At low frequencies electrohydrodynamic flows were observed around the insulating (PDMS) pillars of the DLD array that probably play an important role in controlling the particle trajectory; these flows could be related to those reported by Zehavi *et al* [41] for AC fields.

2. Theoretical analysis

2.1 High frequency: DEP deviation

A particle moving within a fluid under the action of a force $\boldsymbol{F}$ reaches a stationary state given by:

$$\boldsymbol{F} = 6\pi a\eta(\boldsymbol{u} - \boldsymbol{v}_f) \quad (2)$$

where a is the particle radius, η the dynamic viscosity, $\boldsymbol{u}$ the particle velocity and $\boldsymbol{v}_f$ the velocity of the fluid. If the size of the particle is very small, inertial effects can be neglected so that the stationary state is reached instantaneously after the force is applied.

When an electric field is applied to a DLD device, the presence of insulating posts distorts the field, creating gradients of electric field magnitude, as shown in Figure 2d. This non-uniform electric field gives rise to a DEP force that acts on the particles as they flow along the DLD channel. At high frequencies, it is assumed that DEP is the only electrical force acting on the particles. The time average DEP force acting on a particle subjected to an electric field $\boldsymbol{E} = \mathrm{Re}[\boldsymbol{E}_0(\boldsymbol{r})e^{i\omega t}]$ is given by the formula:

$$\boldsymbol{F}_{DEP} = \pi a^3 \varepsilon \mathrm{Re}[f_{CM}]\boldsymbol{\nabla}|\boldsymbol{E}_0|^2 \quad (3)$$

where $\boldsymbol{E}_0$ is the electric field phasor, ε is the dielectric constant of the suspending medium and f_{CM} the Clausius-Mossotti factor that relates the polarisabilities of the medium and the particle. If $\mathrm{Re}[f_{\mathrm{CM}}] > 0$ (i.e. the particle is more polarisable than the medium), the particle is attracted towards the high electric field gradient regions and the force is called positive DEP (pDEP). If $\mathrm{Re}[f_{\mathrm{CM}}] < 0$ (i.e. the particle is less polarisable than the medium), the particle is pushed away from the high electric field gradient regions and the force is termed negative DEP (nDEP).

Combining equations 2 and 3 gives the following expression for the particle velocity:

$$\boldsymbol{u} = \boldsymbol{v}_f + \frac{a^2 \varepsilon \mathrm{Re}[f_{\mathrm{CM}}]}{6\eta}\boldsymbol{\nabla}|\boldsymbol{E}_0|^2 \quad (4)$$

A dimensionless form of this expression can be derived using the DLD posts radius (R), a typical fluid flow velocity (U) and a typical electric field magnitude (E_0) as references:

$$\tilde{\boldsymbol{u}} = \tilde{\boldsymbol{v}}_f + \mathrm{sgn}(\mathrm{Re}[f_{\mathrm{CM}}])N\tilde{\boldsymbol{\nabla}}\left|\tilde{\boldsymbol{E}}_0\right|^2 \quad (5)$$

where sgn(x) is the sign function and *N* is a dimensionless parameter given by:

$$N = \frac{\varepsilon E_0^2 a^2}{6\eta R U}|\mathrm{Re}[f_{\mathrm{CM}}]| \quad (6)$$

Here the tilde indicates dimensionless magnitudes. As the particles flow through the DLD array, the DEP force modifies their paths. As depicted in Figure 2d, particles that experience nDEP are pushed away from the regions between the insulating posts in the same row (where the high electric field gradient regions are created). If the force is strong enough, it overcomes the fluid flow and leads to deviation of particles smaller than the critical diameter, i.e. the nDEP prevents the particles from passing between the posts and forces them to bump, switching from zig-zag to displacement mode.

2.1 Low frequency

At low electric field frequencies (<1 kHz), an electrophoretic force must be considered in addition to a DEP force, so that the particle velocity becomes:

$$\boldsymbol{u} = \boldsymbol{v}_f + \boldsymbol{v}_{\mathrm{DEP}} + \boldsymbol{v}_{\mathrm{EP}} \quad (7)$$

where $\boldsymbol{v}_{\mathrm{EP}} = \mu_{\mathrm{EP}}\boldsymbol{E}$, is the electrophoretic velocity and $\boldsymbol{v}_{DEP}$is the dielectrophoretic velocity. The fact that an alternating electric field is applied means that $\boldsymbol{v}_{EP}$ oscillates with frequency ω. In addition, the dielectrophoretic force has an oscillatory component on top of the time average given by equation 3 with frequency 2ω. The electrophoretic component drives particles to oscillate along the electric field lines (see Figure 2e) with an amplitude proportional to $|\boldsymbol{E}_0|/\omega$. The amplitude of the electrophoretic plus DEP oscillations vanishes as the frequency increases. Therefore, the time-averaged $\boldsymbol{v}_{\mathrm{DEP}}$ dominates and for high frequencies the particle velocity is given by equation 4. Note that the influence of electroosmosis is not considered since all the microfluidic devices were pre-treated with surfactant (Pluronics F-127) to avoid particle adhesion and minimise the electroosmotic flow [42].

3 Materials and methods

3.1 DLD devices and experimental setup

A diagram of the DLD devices used in this work is shown in Figure 3a. The device consists of a straight microchannel 31.2 mm long and 2.6 mm wide, with an average height of 8 μm and an array of tilted circular microposts (geometry shown in Figure 1a). The parameters of the DLD array were: $D_p = 18$ μm, $G = D_p$, $\lambda = 36$ μm, $\Delta\lambda = 2$ μm, $N = 18$ and $\theta = 3.18°$.

The DLD device has three inlets. The top and bottom inlets (Figure 3) were used for sheath flow, with the central inlet for the sample. The fluid flow was controlled with a three-channel pressure controller (Elveflow OB1 MK3). This focused the particles into a stream of around the same width as the sample inlet. The two outlets at the end of the channel are used to collect the deflected particles (red arrow – top outlet) or the particles flowing directly through (green arrow – bottom outlet).

The device was fabricated using photolithography, with an SU8 master made onto a Silicon substrate. This master was used to make the Polydimethylsiloxane (PDMS) devices. These were bonded using plasma activation of the PDMS to a glass slide with a pair of planar platinum electrodes. The electrodes are 31.2 mm long with a 2.2 mm gap set along the channel edges. These electrodes create a uniform electric field along the channel width, perpendicular to the fluid flow. The electrodes were connected to a signal generator (TTi Inc TGA12104) and a 50x voltage amplifier (Falco Systems High Voltage Amplifier WMA-300) that delivered up to 320 Vpp. To filter out any DC component from the amplifier, a 10 μF capacitor was connected in series between the amplifier and the electrodes

3.2 Samples

Fluorescent carboxylate polystyrene particles of 1, 2 and 3 μm diameter were used for experiments. The suspending electrolyte was KCl with conductivities of 2.8 mS/m, 3.1 mS/m, 6.1 mS/m, 15.7 mS/m and 50 mS/m. Prior to use, the PDMS DLD devices were primed with 0.1% (w/v) Pluronics F-127 for at least 1 hour to prevent particles from sticking to the channel walls and clogging the devices. The Pluronics also significantly reduced any electroosmotic flow. Particles were observed with an inverted microscope (Zeiss Axiovert 200) and a camera (Hamamatsu ORCA-ER C4742-95).

3.3 Measurements of the deviation angle

To quantify the deviation angle in the presence of an electric signal, videos of fluorescent particles were recorded for at least 2 minutes. The images were recorded at the end of the channel after the stationary state was reached, i.e. where the deflection angle did not change with time. Particle deflection was quantified from the maximum in the light emission (proportional to the number of particles) as a function of lateral position.

The deviation angle was derived by comparing the initial lateral position of particles at the inlet with the final lateral position at the outlet. Figure 3b shows a diagram of the measured angle compared to particle trajectories in bump mode and the tilt angle of the array. The measured experimental angle θ_{exp} was determined from the total lateral displacement throughout the channel (black line in Figure 3b); note that the maximum does not correspond with the tilt angle of the posts θ_{geom} (red line in Figure 3b). Particles in full deflection mode reach the top of the channel before the outlet and concentrate in a region of the device where there are straight posts (concentration region in Figure 3b) flowing with no deflection. Thus, the measured deviation angle is always smaller than the angle defined by the array geometry. This implies that there is a small range of angles that cannot be discerned, range that goes from the maximum θ_{exp} (corresponding to the maximum lateral deflection) to θ_{geom}.

3.4 Simulations

Numerical simulations were performed using the commercial finite element solver COMSOL Multiphysics v5.3a (COMSOL AB, Stockholm, Sweden). The following COMSOL packages were used: Single Phase Flow to solve the

Stokes equation (creeping flow) and calculate the fluid velocity field, Electric Currents to calculate the electric field distribution and Mathematical Particle Tracing to compute the particle trajectories. The mesh refinement level was improved, and the time step was reduced until the simulation results converged. Data from the numerical calculations were analysed using MATLAB R2017a (The MathWorks Inc, Natick, Massachusetts, USA).

4 Experimental Results

4.1 High frequency induced deviation

The induced deviation of particles at high frequencies was measured using particles of three different sizes (1, 2 and 3 µm), all smaller than the critical diameter of the devices. The frequency of the applied field was 50 kHz and the conductivity was chosen so that the value of $\mathrm{Re}[f_{CM}]$ was approximately -0.5 in all cases, i.e. the particles always experiences the maximum value of nDEP (equations used for this calculations are found in section 1 of the Supplementary Material). For high enough voltages, the particles switch from zig-zag to bump mode and start to deviate at the tilt angle of the posts. The voltage threshold depends on both particle radius and flow velocity. Another important observation is that the larger particles (3 and 2 µm) speed up significantly when the electric field is applied whilst the 1 µm particles flow at a constant velocity. This effect can be explained by repulsion of particles from the top and bottom walls of the narrow DLD channel (8 µm height), an effect that is strongly dependent on particle size. Particles are pushed away from the top and bottom walls and focused into the central plane of the parabolic flow profile where the fluid speed is 1.5 times higher than the mean. The consequences of this effect are discussed below and detailed calculations are provided in the Supplementary Material.

The average electric field magnitude E_0 is equal to the voltage amplitude divided by the distance between the electrodes (2.2 mm). The fluid velocity U was calculated from the pressure difference between inlets and outlets. For this purpose, the fluidic resistance R_h of the DLD devices was determined from the volume of liquid flowing out of the device over time at a fixed pressure. This was $R_h = 170.6$ mbar min/µL. U corresponds to the average fluid velocity in the channel cross-section in the middle of the gap between two columns of posts. Thus, U can be calculated from the measured value of R_h, the applied pressure difference Δp and the channel cross-sectional area $h \times w$ (height h and width w) according to:

$$U = \frac{\Delta p}{w h R_h} \quad (8)$$

Figure 4 summarises experimental results for the high frequency induced deviation of particles, plotted as a function of the magnitude of the electric field for two different particle sizes (3 µm and 2 µm), both smaller than the critical diameter (6.3 µm), and for different flow rates. The conductivity of the suspending medium was 15.7 mS/m so that $\mathrm{Re}[f_{CM}]$ ~ -0.5. The error in the measured angle was estimated from the width of the particle stream which was observed to be approximately the same for all measurements. Error bars are showing in Figure 4a, although all the experimental points in the plots have the same experimental error.

Figures 4a and 4c show the dependence of deviation angle with applied voltage for three different values of fluid velocity. The plot shows that the induced deflection reduces abruptly below a given voltage indicating that the nDEP force is too weak to overcome the Stokes force.

As expected, for higher flow velocities the applied voltage necessary to force the particles to switch from the zig-zag to displacement mode increases. Figures 4b and 4d show the deviation angle plotted as a function of $E_0 a/\sqrt{U}$, a parameter proportional to $\sqrt{N}$ (see eq. 5 and 6). These plots show that the data for different fluid velocities (U) collapses into a single line in accordance with scaling laws that predict that nDEP is responsible for the deviation. When a high frequency electric field is applied, a competition exists between the fluid flow that pushes the particles to zig-zag and the nDEP force that repels the particles from the gap between the posts.

To establish how the scaling varies with particle size, the deviation angle for different particle sizes was measured. Figure 5 shows the deviation angle as a function of the parameter $E_0 a/\sqrt{U}$ for three different sized particles (3 µm, 2 µm and 1 µm) with a 50 kHz AC field. Note that the 1 µm particles were suspended in a higher conductivity medium (50 mS/m) to ensure that $\mathrm{Re}[f_{CM}]$ is the same as for the other two particle types (i.e. -0.5). The curves for the 3 µm and 2 µm particles overlap and follow the expected dependence on particle radius as predicted by theory. However, the 1 µm particles have a different response. As mentioned above, when the electric field was applied, the average velocity of the 3 and 2 µm particles increased, while the average velocity for the 1 µm particles was unchanged. This effect could explain the anomaly shown in Figure 5. Larger particles require a stronger nDEP force to overcome the increased Stokes drag so that a higher electric field is required to induce deflection. An explanation for this observation is given in the Simulation section.

4.2 Low frequency induced deviation

At low frequencies, the deviation mechanism changes drastically. As described in our previous publication[32], when a low frequency AC electric field is applied orthogonal to the fluid flow, particles oscillate at the frequency of the applied field along the field lines due to electrophoresis. Driven by this oscillation, particles smaller than the critical diameter are forced to bump on the posts and follow the tilt angle. This occurs above a threshold value of electric field which depends on the frequency of the AC signal, the medium conductivity, the particle size and the flow velocity.

This mechanism differs from nDEP induced particle deviation. For particles that experience nDEP, the applied voltage necessary to achieve full deflection at low frequencies (where the EP oscillation is significant), is lower than at higher frequencies (no EP oscillation). This difference in voltage cannot be attributed to changes in the DEP behaviour since the DEP force remains constant as the frequency is reduced. Secondly, particles that experience weak nDEP or even pDEP are also forced to bump by the low frequency AC field (as shown in our previous publication [34]), even though a pDEP force points in the opposite direction, attracting particles to the region between posts of the same row. These observations demonstrate that DEP is not responsible for the observed deflection at low frequencies. At higher frequencies, and for particles experiencing pDEP no particle deflection occurs, only trapping in the high electric field regions (as expected).

It might be assumed that the low frequency deflection depends on the amplitude of the EP oscillation, but experimental observations suggest this is not the case. Figure 6 show the superposition of several images recorded with a high-speed camera. It shows the trajectories of 1 µm particles moving around the pillars where they oscillate due to the low frequency applied electric field as they flow along the channel (see Figure 2e). Although the oscillation amplitude is the same for both cases (100 Hz/400 Vpp in Figure 6a and 50 Hz/200 Vpp in Figure 6b), the particles behave differently. In Figure 6a the particles bump on the posts while in Figure 6b they zig-zag around them because the electric field is lower.

Particles oscillate along the electric field lines (distorted around the posts) at the frequency of the applied field. The trajectories followed by the particles indicate that the oscillation is caused solely by electrophoresis and not by electroosmotic flow which is supressed by pre-treatment of devices with Pluronics F-127. According to Viefhues *et al.* [43] the electroosmotic mobility of Pluronics pre-treated PDMS is approximately 50 times lower than the measured electrophoretic mobility of the particles. In addition, the direction of a significant EO flow would be directed towards the walls near the electrodes, resulting in flow recirculation that has not been observed experimentally. Typical measured particle oscillation velocities were 6 mm/s (Figure 6a) and 3 mm/s (Figure 6b), This is in line with simple calculations using the Helmholtz-Smoluchowski equation, as the electric field in Figure 6a is double that in Figure 6b. The electrophoretic measured velocities correlate to a particle zeta potential of around of -100 mV, slightly higher than measured (-71 ± 4 mV).

To further investigate the deflection mechanism, particle deviation was measured as a function of the electric field magnitude for different particle sizes, flow rates and conductivities, but at a fixed AC frequency (50 Hz). The data is summarised in Figures 7, 8 and 9. Figure 7a and 7c show the deviation angle for 3 and 1 µm diameter microspheres as a function of voltage for different flow rates, with an electrolyte conductivity of 2.8 mS/m. Figures 7a and 7c shows that the minimum field magnitude (E_0) required to induce particle deviation increases with fluid velocity (U), similar to the high frequency regime. The induced deviation also decreases sharply below a given value of the electric field magnitude.

In both cases the three curves collapse when plotted as a function of $E_0 a/\sqrt{U}$ (see Figures 7b and 7d). Comparison between Figures 4b and 7b shows that a significantly lower electric field magnitude is required to achieve full particle deflection at low frequencies. The results indicate that although DEP cannot be responsible for particle deviation in this frequency range, the electrical driving force that competes with the Stokes drag (which scales with U) must also scale with E_0^2. This suggests that the low frequency deviation does not depend on the amplitude of the particle oscillation (that scales with E_0).

Figure 8 shows results for 1 µm microspheres but at a higher electrolyte conductivity of 15.7 mS/m. Comparison with Figure 7d shows that a higher electric field magnitude is necessary to achieve the same deflection. The mechanism responsible for the deviation becomes weaker as the conductivity of the electrolyte increases, even though the nDEP force for these small particles increases with increasing conductivity (up to a limit, until $\mathrm{Re}[f_{CM}]$ becomes -0.5).

The size dependence of the force was characterised by measuring the low frequency deviation for three different particle sizes, summarized in Figure 9. This shows the measured deviation angle as a function of $E_0 a/\sqrt{U}$ for 3, 2 and 1 µm diameter particles in low conductivity electrolyte (2.8 mS/m). As for the high frequency case, the curves for the 3 µm and the 2 µm beads collapse but not the 1 µm particles, which implies that the low frequency deviation mechanism scales with particle radius squared as for nDEP induced deviation, even though the mechanisms are different. The difference in behaviour between the 1 µm and the larger particles could be the same as at high frequencies since, again the 3 and 2 µm particles were observed to speed up along the flow direction when the electric field was applied, while the velocity of the 1 µm particles was unchanged.

To elucidate the physical mechanism underlying the low frequency phenomena, particle behaviour was studied in the absence of pressure-driven flow. Quadrupolar electrohydrodynamic flows were observed around insulating pillars, with flow patterns similar to those created by induced charge electroosmosis (ICEO) around conducting surfaces [44]. The flow patterns were observed using 500 nm polystyrene fluorescent nanospheres flow tracers. For such small particles the DEP force is negligible and the motion is dominated by the fluid drag force.

These fluid flows were only seen in the presence of a low frequency AC signal, and qualitative observations show that they decay rapidly with the frequency of the signal, and also with increasing conductivity of the electrolyte. These trends mirror the behaviour of particles at low frequency suggesting that the electrohydrodynamic rolls play an important role in controlling particle trajectories. A picture of the flows is shown in Figure 10, and videos of these observations are provided in the supplementary material.

Although the scaling laws for the low frequency particle deviation have been experimentally mapped, the underlying physical mechanism is not yet fully understood. The origin and role of the electrokinetic quadrupolar flows requires further investigation, as does the influence of the EP oscillation on particles. In addition, phenomena such as electrokinetic particle-wall repulsion [45] and changes in the low frequency dielectrophoretic behaviour may also be involved.

5 Numerical simulations of the nDEP induced deviation

5.1 Unit cell and basic equations

Numerical simulations were performed using the finite element method (COMSOL Multiphysics 5.3a). A working model was developed in an attempt to predict the electrokinetically induced deviations. Since the physics behind the low frequency induced deviation are not fully understood, the model only considers the effect of nDEP on particles. Following the method of Kim *et al.* [8], particle trajectories were simulated using a single unit cell. The total deviation was then calculated using a transfer function that relates the initial and final positions.

The unit cell geometry and the calculated electric field distribution, flow velocity field and $\nabla|\boldsymbol{E}|^2$ are shown in Figure 11. The geometry of the unit cell was the same as the geometry of the experimental device, with a symmetric array of circular posts where $D_p = G_x = G_y$ and the same offset $\theta = 3.18°$. To calculate the fluid velocity field (Figure 11b), the 2D Stokes equation was solved (the Reynolds number (Re) is typically very low; $Re \sim 10^{-3}$ for $U = 100\ \mu m/s$):

$$\eta\nabla^2\boldsymbol{v}_f - \boldsymbol{\nabla}p = 0 \quad (9)$$

where p is the pressure. A no-slip boundary condition was imposed on all four posts walls. Periodic boundary conditions were used for boundaries H-A and D-E to obtain the same velocity and pressure profile, and a pressure difference was established to simulate the fluid flow (from left to right in Figure 11b). Periodic boundary conditions without a pressure difference were also set between B-C and F-G:

$$\begin{gathered}\boldsymbol{v}_{f,A-B} = \boldsymbol{v}_{f,C-D} = \boldsymbol{v}_{f,E-F} = \boldsymbol{v}_{f,G-H} = 0 \\ \boldsymbol{v}_{f,H-A} = \boldsymbol{v}_{f,D-E}\ ;\boldsymbol{v}_{f,B-C} = \boldsymbol{v}_{f,F-G} \qquad (10) \\ p_{H-A} = p_{D-E} + \Delta p\ ; p_{B-C} = p_{G-F}\end{gathered}$$

These conditions result in a non-zero vertical flow in the y direction which is a non-realistic situation and is only valid when considering ideal infinite DLD arrays. To take into account the effect of the enclosing walls in a real DLD device, a second fluid flow component was added with a pressure difference between B-C and F-G such that the net fluid velocity in the y direction was zero. The resulting average velocity was determined in the central region of the unit cell (black line in Figure 11a) and then adjusted to the typical values of U measured experimentally.

An electric field was imposed perpendicular to the fluid flow with a magnitude E_0 equal to the experimental results. The electric field distribution inside the unit cell $\boldsymbol{E}$ was calculated as a sum of the uniform field in the y direction (perpendicular to the fluid flow) and a perturbation created by the posts $\boldsymbol{E}'$:

$$\boldsymbol{E} = \boldsymbol{E}' + E_0\boldsymbol{u}_y \quad (11)$$

Then the electric potential is given by:

$$\phi = \phi' - E_0 y \quad (12)$$

To calculate $\boldsymbol{E}'$, the Laplace's equation for the electric potential ϕ' was solved with periodic conditions in boundaries H-A/D-E and B-C/F-G:

$$\nabla^2\phi' = 0 \quad (13)$$

To model the posts as perfect insulators, a zero normal current density was set on the post walls:

$$\frac{\partial \phi}{\partial n} = 0 \rightarrow \frac{\partial \phi'}{\partial n} = E_0 n_y \quad (14)$$

To compute particle trajectories, the total field $\boldsymbol{E}$ (plotted in Figure 11c) was used. This field distribution gives the DEP spatial dependence showed in Figure 11d.

5.2 Particle trajectories in the DLD unit cell and transfer function

The method used to quantify particle deviation from the simulated particle trajectories is based on Kim *et al* [8]. In the absence of an electric field, given that the inertial effects can be neglected, the particles are assumed to always have the same velocity as the fluid velocity field, $\boldsymbol{u} = \boldsymbol{v_f}$, i.e. they follow the fluid streamlines. The trajectories can then be calculated using particle tracing: in every time step, the initial position is set to be the final position of the previous step and the particle velocity set to that of the fluid flow at that point. From the calculated particle trajectories in the unit cell it is possible to extract a function that relates the initial vertical position y_i where the particle enters the unit cell (through wall H-A) and the final exiting position y_f where the particle leaves the unit cell (through wall D-E). This is called the transfer function f of the system. Then:

$$h_{i+1} = f(h_i) \quad (15)$$

where $h = y/G$. Note that y_i and y_f are defined as the distance to the posts, i.e. the distance to points A and D in Figure 11a respectively. Since the particle enters the adjacent unit cell at the position it leaves the previous cell, this function completely determines particle trajectories through a DLD array. It is then possible to calculate the final position of a given particle after passing an arbitrary number n of unit cells simply by applying n times the transfer function to the initial position:

$$h_{i+n} = f^n(h_i) \quad (16)$$

Thus, in the case of a symmetric array (as in this work) with a periodicity N, $h_{N+i} = f^N(h_i) = h_i$. Given the transfer function f it is possible to calculate the total lateral displacement Δy after a particle has passed through n cells:

$$\Delta y = n\Delta\lambda + G\sum_{k=i}^{n+i}(h_{k+1} - h_k) \quad (17)$$

and then the deviation angle θ is given by:

$$\theta = \operatorname{atan}\left(\frac{\Delta y}{n\lambda}\right) \quad (18)$$

Note that the difference $h_{k+1} - h_k$ becomes negative when the particle zigzags and passes below the post. Examples of particle trajectories in the unit cell, along with the corresponding transfer function are given in Figure 12.

Figure 12a shows particle trajectories in the DLD unit cell in the absence of an electric field, where particles only follow the fluid streamlines. Here, $y_s = h_s G$ indicates the distance of the separatrix streamline to the post (to point A). Hence, the simulated critical diameter corresponds to $2y_s$. Particles entering the unit cell with $y_i < y_s$ will be in the portion of fluid passing below the following post and will zig-zag. The transfer function part located in the top left corner of Figure 12b comes from the zigzagging particles. In the calculations, particles leaving the unit cell through wall B-C then enter through wall G-F. In a real situation, particles would move to the unit cell below, so this needs to be considered when calculating θ.

Assuming a non-elastic hard-wall interaction between particles and posts, the transfer function of a particle with a given radius a can be calculated from the function of point-like particles (see Figure 12c). Since the particles cannot approach closer than one radius to the posts, trajectories with $y_i < a$ are prohibited and then removed from the calculations. Particles that exit the unit cell through wall C with $y_f < a$ or $y_f > G - a$ are then assumed to exit with $y_f = a$ or $y_f = G - a$ respectively. If $h_s < a/G$, i.e. the particle is bigger than the critical diameter, the zigzagging part of the transfer function disappears, and the function cuts the line $h_{i+1} = h_i$ (red line in Figures 12b and 12c). As a result, they will always enter the unit cell at the same vertical position as they exit, i.e. $h_{i+1} = h_i = a/G$, which corresponds to one particle radius away from the post, meaning that the particles hit the posts and displace in bump mode.

The simulations show that the numerically calculated D_c is 4.86 µm, which is lower than the experimentally determined D_c which is between 6 and 7 µm, and lower than estimated from equation 1 (derived empirically by Davis [10]) which is 6.3 µm. However, the calculated value of the D_c is close to the values predicted by the 2D DLD models found in the literature such as that derived by Inglis *et al.* [7].

When an electric field is included in the simulations, particle trajectories in the unit cell are modified (see Figure 13a). The particle velocity is no longer $\boldsymbol{v}_f$ but that given by equation 3, i.e. the sum of the fluid flow and the dielectrophoretic velocities. The deviation angle is then calculated in the same way as explained above in the absence of electric field. The addition of the dielectrophoretic component to the particle velocity modifies the transfer function. If the resulting f cuts the line $h_{i+1} = h_i$, particles leave the unit cell further away from the posts than when they entered, meaning that they are forced to bump on the posts by the action of the electric field, travelling in displacement mode (see Figure 13b).

5.3 Comparison with experimental data

To numerically determine the deviation angles of particles experiencing nDEP with an orthogonal electric field, the trajectories of >2000 particles were simulated inside the DLD unit cell. This enabled the corresponding transfer functions to be calculated by linear interpolation. The particle velocity is given by equation 4 with $\widetilde{\boldsymbol{v}}_f$ and $\widetilde{\boldsymbol{\nabla}}\left|\widetilde{\boldsymbol{E}}_0\right|^2$ and the spatial distributions found in Figures 11b and 11d. Simulations were made for different values of the parameter N. For the simulations, $Re[f_{CM}]$ was set to -0.5 and ε and η values were those of water. To account for particles size, the transfer functions were truncated as explained previously.

Figure 14a shows the calculated deviation angles as a function of $E_0 a/\sqrt{U}$ for three different particle sizes compared to the deviation angles measured experimentally. If the particles are small enough for the hard-wall interaction with the posts to be neglected, i.e. separation is mainly caused by nDEP, the curves for different particle sizes collapse perfectly as expected from the nDEP induced deviation. If the particle size is close to the critical diameter of the device, the interaction with the posts needs to be considered when calculating the trajectories. The simulation results in Figure 14a show the same trend as experiment, with a threshold electric field above which particles are fully deflected at the device tilting angle. Below this field, intermediate angles are found in both simulations and experiments. For even lower field magnitudes the induced deviation disappears, and all the particles travel in zig-zag mode.

From the simulation it can be seen that the experimental value of the deviation threshold field for the 1 µm microspheres is closer to the numerical data, unlike the larger 3 and 2 µm particles. This result indicates that the particle size could be responsible for the offset between the calculated and experimental threshold values indicated in Figure 14a. Given that the channels of the experimental DLD devices were only 8 µm tall, the difference in behaviour could be due to electrokinetic particle repulsion from the walls which is strongly dependent on particle size. Repulsion from the top and bottom walls of the channel could force the 3 and 2 µm particles to flow in the central region of the channel section, at the mid-point of the parabolic flow profile where the fluid flow velocity is maximum and 1.5 times the average. A flow velocity U' that is larger than the experimentally derived value U implies that a larger electric field is necessary to achieve the same degree of deflection. Because this effect was not considered in the simulations the best agreement with experiment is found for the 1 µm spheres.

Experimentally, it was observed that the average particle speed for the two larger particles (3 and 2 µm diameter) increased as soon as an electric field was applied, while the 1 µm microspheres kept flowing at the same average velocity. Theoretically, the wall repulsion can be calculated as dipole-dipole repulsion using the method of images. The repulsion force is given by:

$$F = \frac{3\pi\varepsilon a^6 (\mathrm{Re}[f_{\mathrm{CM}}]E_0)^2}{4z^4} \quad (19)$$

where z is the distance to the wall. This is strongly dependent on particle size; the velocity at which the particles move away from the walls is proportional to a^5. This would push the 3 and 2 µm diameter particles into the mid-point of the channel section in a very short time (see Table 1) while the 1 µm diameter particles remain randomly distributed across over the channel section (see Supplementary Material for detailed calculations). Assuming that the larger particles are concentrated into the mid-point of the channel section, Figure 14b shows a comparison of the simulation results with corrected experimental data at a typical flow velocity U' 1.5 times larger than the experimentally derived value of U for the deviation angles of the 3 and 2 µm diameter microspheres. This figure shows much better agreement with the simulations, the theoretical model and the hypothesis that the nDEP induced deviation scales with the parameter N.

Simulation of the high frequency behaviour match the experimental data demonstrating that the particle trajectories are almost entirely due to nDEP. However, simulating the low frequency behaviour is far more complex and requires a different approach. Particle trajectories cannot be simulated in a single DLD unit cell since the oscillation amplitude moves particles to neighbouring unit cells, and the trajectories would depend on the phase of the alternating field. The interaction of particles with the posts would also need to be modelled. Furthermore, the underlying physical principles responsible for the low frequency induced deviation are not completely understood which limits the validity of any model. A full numerical study within this low-frequency regime is beyond the scope of this publication and will be part of future work.

6 Conclusions and outlook

This paper has presented a theoretical, experimental and numerical analysis of the behaviour of particles in a DLD device with an AC electric field applied orthogonal to the fluid flow. The flow rate, electric field magnitude and size dependence of particle behaviour has been characterised, and scaling laws for the AC electrokinetically induced DLD deviation has been derived for the two different frequency regimes identified in our previous publication [34].

At high frequencies, the dependence of the deviation angle has been characterised as a function of flow rate, particle size and magnitude of the electric field. It was demonstrated that the scaling laws agree with theory. Experimental results demonstrate that the induced deviation at high frequency scales with the parameter N, i.e. the ratio of electrical to hydrodynamic forces (see equations 5 and 6). Thus, as expected, the nDEP is the force responsible for deflection at high frequencies.

Following Kim *et al.* [8], a numerical model was developed to calculate the deviation caused by the nDEP force. The simulations results are in good agreement with experiments, proving that nDEP is responsible for the deflection at high frequencies. The model also predicts the existence of the intermediate deviation angles found experimentally.

At low frequencies, the particle deviation scales with the square of the electric field magnitude. This result contradicts our previous conclusion that the induced deviation was caused by electrophoresis where the oscillation amplitude scales with the magnitude of the field. The results demonstrate that other phenomena are responsible for the change in behaviour, although the physical mechanism is not yet established.

Precise measurements of the low AC frequency fluid behaviour showed the existence of quadrupolar ICEO-like electrohydrodynamic flows around the insulating DLD pillars. These flows were only found at low frequencies, and have the same qualitative frequency, voltage and electrolyte conductivity dependence as ICEO. These low frequency AC electrohydrodynamic flows play an important role in controlling the particle separation seen at low frequencies. They could also be responsible for the particle equilibrium positions reported in our previous publications, where particles move to intermediate positions between high and low electric field gradients. These equilibrium positions were observed in the presence of a low frequency electric field for particles that did not experience nDEP and in the absence of flow. Other electrokinetic phenomena may thus be involved in driving the low frequency deflection of particles, and a full understanding of this is required in order to develop a theoretical model for the low frequency deviation. Future work will focus on the characterisation of the electrohydrodynamic flows around the insulating pillars, together with the low frequency particle behaviour so that a complete numerical model can be developed.

Acknowledgments

PGS and AR acknowledge financial support by ERDF and Spanish Research Agency MCI under contract PGC2018-099217-B-I00

References

1 T. Salafi, Y. Zhang and Y. Zhang, *A Review on Deterministic Lateral Displacement for Particle Separation and Detection*, Springer Singapore, *Nano-Micro Letters*, 2019, **11**, 77.

2 L. R. Huang, E. C. Cox, R. H. Austin and J. C. Sturm, *Continuous particle separation through deterministic lateral displacement*, *Science*, 2004, no. 5673, **304**, 987–990.

3 J. McGrath, M. Jimenez and H. Bridle, *Deterministic lateral displacement for particle separation: a review, Lab Chip*, 2014, **14**, 4139–4158.

4 J. A. Davis, D. W. Inglis, K. J. Morton, D. A. Lawrence, L. R. Huang, S. Y. Chou, J. C. Sturm and R. H. Austin, *Deterministic hydrodynamics: taking blood apart, Proc. Natl. Acad. Sci.*, 2006, **103**, 14779–14784.

5 S. H. Holm, Z. Zhang, J. P. Beech, G. Gompper, D. A. Fedosov and J. O. Tegenfeldt, *Microfluidic Particle Sorting in Concentrated Erythrocyte Suspensions, Phys. Rev. Appl.*, 2019, **12**, 1.

6 Z. Liu, F. Huang, J. Du, W. Shu, H. Feng, X. Xu and Y. Chen, *Rapid isolation of cancer cells using microfluidic deterministic lateral displacement structure, Biomicrofluidics*, 2013, **7**, 11801.

7 D. W. Inglis, J. A. Davis, R. H. Austin and J. C. Sturm, *Critical particle size for fractionation by deterministic lateral displacement, Lab Chip*, 2006, **6**, 655–658.

8 S. Kim, B. H. Wunsch, H. Hu, J. T. Smith, R. H. Austin and G. Stolovitzky, *Broken flow symmetry explains the*

dynamics of small particles in deterministic lateral displacement arrays, Proc. Natl. Acad. Sci., 2017, **114**, no 26, E5034-E5041.

9 V. Calero, P. Garcia-Sanchez, A. Ramos and H. Morgan, *Combining DC and AC electric fields with deterministic lateral displacement for micro-and nano-particle separation, Biomicrofluidics*, 2019, **13**, 054110.

10 J.A. Davis, *Microfluidic separation of blood components through deterministic lateral displacement*. Princeton University, 2008.

11 S. Ranjan, K. K. Zeming, R. Jureen, D. Fisher and Y. Zhang, *DLD pillar shape design for efficient separation of spherical and non-spherical bioparticles, Lab Chip*, 2014, **14**, 4250–4262.

12 K. Loutherback, K. S. Chou, J. Newman, J. Puchalla, R. H. Austin and J. C. Sturm, *Improved performance of deterministic lateral displacement arrays with triangular posts, Microfluidics and Nanofluidics*, 2010, **9**, 1143–1149.

13 J. chul Hyun, J. Hyun, S. Wang and S. Yang, *Improved pillar shape for deterministic lateral displacement separation method to maintain separation efficiency over a long period of time, Sep. Purif. Technol.*, 2017, **172**, 258–267.

14 K. K. Zeming, T. Salafi, C. H. Chen and Y. Zhang, *Asymmetrical deterministic lateral displacement gaps for dual functions of enhanced separation and throughput of red blood cells, Sci. Rep.*, 2016, **6**, 22934.

15 D. Holmes, G. Whyte, J. Bailey, N. Vergara-Irigaray, A. Ekpenyong, J. Guck and T. Duke, *Separation of blood cells with differing deformability using deterministic lateral displacement, Interface Focus*, 2014, **4**, no 6, 20140011.

16 M. Xavier, S. H. Holm, J. P. Beech, D. Spencer, J. O. Tegenfeldt, R. O. C. Oreffo and H. Morgan, *Label-free enrichment of primary human skeletal progenitor cells using deterministic lateral displacement, Lab Chip*, 2019, **19**, 513–523.

17 E. Pariset, C. Parent, Y. Fouillet, B. François, N. Verplanck, F. Revol-Cavalier, A. Thuaire and V. Agache, *Separation of biological particles in a modular platform of cascaded deterministic lateral displacement modules, Sci. Rep.*, 2018, **8(1)**, 1-10.

18 J. T. Smith, B. H. Wunsch, N. Dogra, M. E. Ahsen, K. Lee, K. K. Yadav, R. Weil, M. A. Pereira, J. V. Patel, E. A. Duch, J. M. Papalia, M. F. Lofaro, M. Gupta, A. K. Tewari, C. Cordon-Cardo, G. Stolovitzky and S. M. Gifford, *Integrated nanoscale deterministic lateral displacement arrays for separation of extracellular vesicles from clinically-relevant volumes of biological samples, Lab Chip*, 2018, **18(24)**, 3913–3925.

19 R. Devendra and G. Drazer, *Gravity driven deterministic lateral displacement for particle separation in microfluidic devices, Anal. Chem.*, 2012, **84**, 10621–10627.

20 J. P. Beech and J. O. Tegenfeldt, *Tuneable separation in elastomeric microfluidics devices*, *Lab Chip*, 2008, **8**, 657–659.

21 K. K. Zeming, N. V. Thakor, Y. Zhang and C. H. Chen, *Real-time modulated nanoparticle separation with an ultra-large dynamic range, Lab Chip*, 2016, **16(1)**, 75–85.

22 Y. Li, H. Zhang, Y. Li, X. Li, J. Wu, S. Qian and F. Li, *Dynamic control of particle separation in deterministic lateral displacement separator with viscoelastic fluids, Sci. Rep.*, 2018, **8(1)**, 1-9.

23 Pesch, G. R., Du, F., Baune, M., & Thöming, J., *Influence of geometry and material of insulating posts on particle trapping using positive dielectrophoresis*, *Journal of Chromatography A*, 2017, **1483**, 127-137.

24 Jen, C. P., and Chen, T. W., *Selective trapping of live and dead mammalian cells using insulator-based dielectrophoresis within open-top microstructures, Biomedical microdevices*, 2009, **11(3)**, 597.

25 M. A. Zaman, P. Padhy, P. C. Hansen and L. Hesselink, *Dielectrophoresis-assisted plasmonic trapping of dielectric nanoparticles, Phys. Rev. Appl.*, 2017, **95(2)**, 023840.

26 S. D. Ibsen, J. Wright, J. M. Lewis, S. Kim, S. Y. Ko, J. Ong, S. Manouchehri, A. Vyas, J. Akers, C. C. Chen, B. S. Carter, S. C. Esener and M. J. Heller, *Rapid isolation and detection of exosomes and associated biomarkers from plasma, ACS Nano*, 2017, **11(7)**, 6641–6651.

27 P. R. C. Gascoyne and S. Shim, *Isolation of circulating tumor cells by dielectrophoresis*, *Cancers*, 2014, **6(1)**, 545–579.

28 Lapizco-Encinas, B. H., Ozuna-Chacón, S., and Rito-Palomares, M., *Protein manipulation with insulator-based dielectrophoresis and direct current electric fields, Journal of Chromatography A*, 2008, **1206**, 45-51.

29 S. Chang and Y. H. Cho, *A continuous size-dependent particle separator using a negative dielectrophoretic virtual pillar array, Lab Chip*, 2008, **8**, 1930–1936.

30 X. Xing, C. N. Ng, M. L. Chau and L. Yobas, *Railing cells along 3D microelectrode tracks for continuous-flow*

dielectrophoretic sorting, *Lab Chip*, 2018, **18**, 3760–3769.

31 S. K. Srivastava, J. L. Baylon-Cardiel, B. H. Lapizco-Encinas, and A. R. Minerick, *A continuous DC-insulator dielectrophoretic sorter of microparticles, Journal of Chromatography A*, 2011, **1218(13)**, 1780-1789.

32 J. P. Beech, K. Keim, B. D. Ho, C. Guiducci and J. O. Tegenfeldt, *Active posts in deterministic lateral displacement devices*, *Adv. Mater. Technol.*, 2019, **4(9)**, 1900339.

33 J. P. Beech, P. Jönsson and J. O. Tegenfeldt, *Tipping the balance of deterministic lateral displacement devices using dielectrophoresis*, *Lab Chip*, 2009, **9**, 2698–2706.

34 V. Calero, P. Garcia-Sanchez, C. Honrado, A. Ramos and H. Morgan, *AC electrokinetic biased deterministic lateral displacement for tunable particle separation*, *Lab Chip*, 2019, **19(8)**, 1386–1396.

35 M. Aghaamoo, A. Aghilinejad, X. Chen and J. Xu, *On the design of deterministic dielectrophoresis for continuous separation of circulating tumor cells from peripheral blood cells, Electrophoresis*, 2019, **40(10)**, 1486-1493.

36 B. H. Lapizco-Encinas, *On the recent developments of insulator-based dielectrophoresis: A review, Electrophoresis*, 2019, **40(3)**, 358-375.

37 E. B. Cummings and A. K. Singh, *Dielectrophoresis in microchips containing arrays of insulating posts: theoretical and experimental results, Anal. Chem.*, 2003, **75(18)**, 4724–4731.

38 A. LaLonde, A. Gencoglu, M. F. Romero-Creel, K. S. Koppula, and B. H. Lapizco-Encinas, *Effect of insulating posts geometry on particle manipulation in insulator based dielectrophoretic devices, Journal of Chromatography A*, 2014, **1344**, 99-108.

39 D. V. Polniak, E. Goodrich, N. Hill, and B. H. Lapizco-Encinas, *Separating large microscale particles by exploiting charge differences with dielectrophoresis, Journal of Chromatography A*, 2018, **1545**, 84-92.

40 N. Hill, B. H. Lapizco-Encinas, *Continuous flow separation of particles with insulator-based dielectrophoresis chromatography*, *Anal. and Bioanal. Chem.*, 2020, 1-12.

41 M. Zehavi, A. Boymelgreen and G. Yossifon, *Competition between induced-charge electro-osmosis and electrothermal effects at low frequencies around a weakly polarizable microchannel corner, Phys. Rev. Appl.*, 2016, **5(4)**, 044013.

42 R. V. Davalos, G. J. McGraw, T. I. Wallow, A. M. Morales, K. L. Krafcik, Y. Fintschenko, E. B. Cummings and B. A. Simmons, *Performance impact of dynamic surface coatings on polymeric insulator-based dielectrophoretic particle separators, Anal. Bioanal. Chem.*, 2008, **390(3)**, 847–855.

43 M. Viefhues, S. Manchada, T. C. Chao, D. Anselmetti, J. Regtmeier and A. Ros, *Physisorbed surface coatings for poly (dimethylsiloxane) and quartz microfluidic devices, Analytical and bioanalytical chemistry*, 2011, *401*(7), 2113.

44 T. M. Squires and M. Z. Bazant, *Induced-charge electro-osmosis, Journal of Fluid Mechanics*, 2004, **509**, 217-252.

45 L. Liang, S. Qian and X. Xuan, *Three-dimensional electrokinetic particle focusing in a rectangular microchannel, J. Colloid Interface Sci.*, 2010, **350**, 377–379.

Figure captions

Figure 1. (a) Geometry of a symmetric DLD consisting of an array of cylindrical posts. (b) Diagram of the mechanism of DLD size based microparticle separation. Reprinted from Calero et al [9], with the permission of AIP Publishing.

Figure 2. Summary of the behaviour of particles undergoing electrokinetically induced deviation in DLD devices. (a) 1 µm microspheres zigzagging in a DLD with $D_c = 6.3$ *µm in the absence of an electric field. (b) Same particles deflected due to the action of nDEP caused by a high frequency (10 kHz) AC electric field. (c) Same particles deflected by a low frequency (50 Hz) AC electric field where particles oscillate along the direction of the electric field lines due to EP. (d) Diagram of the nDEP induced deviation (colours indicate the magnitude of the gradient of the electric field squared). Reprinted from Calero et al [9], with the permission of AIP Publishing. (e) Electric field lines distorted by the insulating posts. Particles experiencing an EP force oscillate along these lines and bump on the posts. The colours represent the electric field magnitude.*

Figure 3. (a) Diagram of the DLD device used for the experiments. (b) Diagram of the angle measured in the experiments compared to the tilt angle of the post array and the particle trajectories (figure is not to scale).

Figure 4. Experimental measurements of the nDEP induced deviation for a 50 kHz AC electric field of (a) 3 µm as a function of the applied voltage amplitude, (b) 3 µm as a function of $E_0 a/\sqrt{U}$*, (c) 2 µm as a function of the applied voltage amplitude and (d) 2 µm as a function of* $E_0 a/\sqrt{U}$*. The suspending medium conductivity was 15.7 mS/m in all cases.*

Figure 5. Experimental measurements of the nDEP induced deviation of 3,2 and 1 µm microspheres for a 50 kHz AC electric field as a function of $E_0 a/\sqrt{U}$*. The suspending medium conductivity was chosen so that all the particles had the same value of* $\mathrm{Re}[f_{CM}] = -0.5$*, as negative as possible (15.7 mS/m for the 2 and 3 µm particles and 50 mS/m for the 1 µm particles).*

Figure 6. Trajectories of 1 µm particles suspended in 1.8 mS/m KCl as they move through the DLD array around the DLD insulating pillars under the action of a low frequency AC electric field orthogonal to the flow. (a) 100 Hz and 400 Vpp signal – bumping on the posts. (b) 50 Hz and 200 Vpp – zigzagging around the posts. Note that the oscillation amplitude is equal in both cases. The images were created by superposition of 200 frames for Figure 6a and 500 frames for Figure 6b from videos recorded with a high-speed camera at 1000 fps.

Figure 7. Experimental measurements of the low frequency induced deviation of 3 and 1 µm microspheres for a 50 Hz AC electric field and a suspending medium conductivity of 2.8 mS/m. (a) For 3 µm particles as a function of the amplitude of the applied voltage. (b) For 3 µm particles as a function of $E_0 a/\sqrt{U}$*. (c) For 1 µm particles as a function of the amplitude of the applied voltage. (d) For 1 µm particles as a function of* $E_0 a/\sqrt{U}$*.*

Figure 8. Experimental measurements of the low frequency induced deviation of 1 µm microspheres for a 50 Hz AC electric field and a suspending medium conductivity of 15.7 mS/m as a function of $E_0 a/\sqrt{U}$*.*

Figure 9. Experimental measurements of the low frequency induced deviation of 3,2 and 1 µm microspheres for a 50 Hz AC electric field and a suspending medium conductivity of 2.8 mS/m as a function of $E_0 a/\sqrt{U}$*.*

Figure 10. Electrohydrodynamic quadrupolar flows around insulating PDMS pillars caused by a 70 Hz AC electric field. The white lines correspond to the trajectories of 500 nm tracer spheres (the picture was created by superposition of 300 frames).

Figure 11. (a) DLD unit cell considered in the simulations. (b) Fluid flow velocity field. The black lines represent the fluid streamlines. (c) Electric field magnitude. The black lines represent the electric field lines. (d) Gradient of the

electric field squared, proportional to the magnitude of the DEP. The black arrows represent the direction of the nDEP force.

Figure 12. (a) Trajectories of point-like particles in the DLD unit cell following the fluid flow. The DLD have the same dimensions as the DLD used in the experiments. (b) Transfer function associated with the trajectories in Figure 12a (zigzagging mode) (c) Transfer function of 6 µm particles (bumping mode) calculated from the transfer function in Figure 12b.

Figure 13. (a) Trajectories of point-like particles when an AC electric field orthogonal to the flow is applied for $E_0 a/\sqrt{U} = 4\, Vm^{-1/2}s^{1/2}$*. (b) Transfer function associated with the trajectories in Fig. 13a for a particle diameter of 1 µm.*

Figure 14. (a) Comparison of the simulated nDEP induced deviation angle θ *as a function of* $E_0 a/\sqrt{U}$ *for 3, 2 and 1 µm diameter particles with the experimental values. (b) Same comparison with corrected values of the experimentally measured deviation of the 3 and 2 µm particles using* $U' = \alpha U$ *where the correction factor* $\alpha = 1.5$ *accounts for the increase in the particle velocity in the centre of the parabolic flow profile.*