High throughput particle analysis: Combining dielectrophoretic particle focussing with confocal optical detection

David Holmes, Hywel Morgan*, Nicolas G. Green

School of Electronics and Computing Science, University of Southampton, Highfield, Southampton SO17 1BJ, UK

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Abstract

A micro flow cytometer has been fabricated that detects and counts fluorescent particles flowing through a microchannel at high speed based upon their fluorescence emission intensity. Dielectrophoresis is used to continuously focus particles within the flowing fluid stream into the centre of the device, which is 40 \( \mu \)m high and 250 \( \mu \)m wide. The method ensures that all the particles pass through an interrogation region approximately 5 \( \mu \)m in diameter, which is created by focusing a beam of light into a spot. The functioning of the device was demonstrated by detecting and counting fluorescent latex particles at a rate of up to 250 particles/s. A mixture of three different populations of latex particle was used, each sub-population with a distinct level of fluorescent intensity. The device was evaluated by comparison with a conventional fluorescent activated cell sorter (FACS) and numerical simulation demonstrated that for 6 \( \mu \)m beads, and for this design of chip the theoretical throughput is of the order of 1000 particles/s (corresponding to a particle velocity of 10 mm s\(^{-1}\)).

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1. Introduction

Integrated microfluidic devices capable of processing, sorting and counting cells and other micron- or sub-micron-sized particles will have diverse applications in fields such as medicine, biology, chemistry and particle science. One application of this type of device is analysis of human blood, where, for example, differential white blood cell count is used as an early indicator of disease. Other applications include high speed detection of rare cells, for example, small numbers of malignant cells for early diagnosis of cancer, or the detection and isolation of foetal cells from the maternal circulation for prenatal genetic analysis. In each of these cases, the specificity of the device must be extremely high: foetal cells, for example, are only found at approximately one in a million white cells. Some of the challenges in this type of analysis have been addressed by modern flow cytometers: machines that can serially analyse cells using light scattering and fluorescence. The best machines can process up to 50,000 cells/s and are capable of sorting the cells into different outlets according to user defined criteria, such as a fluorescent antibody tag or the degree of light scattering (Ibrahim and van den Engh, 2003). However, these instruments are extremely expensive and complicated, require trained dedicated staff, and therefore, tend only to be found at hospitals and research institutes.

Microfabrication technology is now being used to produce microfluidic systems for chemical and biological analysis and several research groups have reported different designs of micro-devices for particle counting and sorting. In general, particles are detected and analysed using optical methods (scattering and/or fluorescence), or electrically using direct current (as in the case of the Coulter counter) or AC for impedance analysis. For efficient detection, the particles and/or fluid are focussed into a narrow stream, usually 20–30 \( \mu \)m wide, ensuring that all particles pass serially through a relatively small detection region. In this work, we describe the design and measurement of an integrated micro-device that can detect and count fluorescent particles at high speed based on fluorescence emission intensity. The micro-device has two components: a focussing section which uses dielectrophoresis to centre particles in the channel as they enter the second section, where a confocal optical detection method is implemented. The operation of
the device was experimentally characterised by detecting and counting fluorescent latex particles at a rate of 250 particles/s. The device was benchmarked against a commercial flow cytometer using a calibration mix of three different populations of latex particles; each sub-population with a distinct level of fluorescent intensity. The system performance was also numerically simulated and the maximum theoretical throughput determined.

2. Background and theory

2.1. Focussing in micro-devices

The operation of a micro flow cytometer requires confinement of a sample stream into an accurately controlled detection/measurement region to ensure reliability and reproducibility. One technique that is often used to confine a sample into a narrow beam is hydrodynamic focussing, the principle of which is shown in Fig. 1(a). The central sample stream (containing the cells) is pinched in one dimension by the two side streams. Kröger et al. (2002) recently showed how hydrodynamic focussing could be used in a prototype cell sorting chip. Following detection by fluorescence emission, these authors used an "off-chip valve" switching technique to sort latex particles. Lee et al. (2001) compared experiments of sheath flow focussing systems with numerical simulation, characterising the relationship between focussed beam diameter and the ratio of sample to sheath velocities. Recently, Pamme et al. (2003) showed how hydrodynamic focussing could be used to detect particles and particle agglomerates in a flow-through chip using light scattering at fixed angles.

While it is relatively easy to implement hydrodynamic focussing of a fluid in one dimension, confinement of a sample stream in two dimensions can be achieved but complex fabrication schemes are required (Wolff et al., 2003; Sundararajan et al., 2002). Accurate control of differential fluid flow rates or pressure on each channel is necessary to ensure efficient focussing, and since the focussing acts on the fluid rather than the particles, small particles and molecules can diffuse from the sample stream into the sheath fluid. Maintaining focussing whilst switching fluids into different channels is also problematic (Kröger et al., 2002).

An alternative method is electrokinetic focussing, which uses DC (at high voltages: ~1 kV) to focus particles and liquids into a narrow stream (Fu et al., 1999, 2002; Schrum et al., 1999; Jacobson and Ramsey, 1997). Typically, the sample fluid stream is driven along the main arm of a cross-shaped channel under the influence of an applied DC field by electroosmosis. As the sample enters the intersection, three fluid streams meet and the sample stream is focussed into narrow beam. Fu et al. (1999, 2002) reported a particle sorting device which used DC fields both to focus and deflect the fluid within a microfabricated channel. Particles were detected in the channels using fluorescence, with the light focussed into a detection volume of a few nanoliters. Jacobson and Ramsey (1997) demonstrated one-dimensional confinement of molecules with applied voltages of 3 kV, whilst Chou et al. (1999) developed a device for detecting DNA molecules. Schrum et al. (1999) showed how electrokinetic focussing could be used to detect 0.97 and 1.94 μm diameter latex particles producing sample stream widths of 7–8 μm. They constructed a particle counter, with a maximum reported sample throughput of 34 particles/s.

2.2. Dielectrophoretic focussing

Another electrokinetic technique, called dielectrophoresis (DEP), uses alternating current (AC) electric fields and can also be used for sample focussing. Dielectrophoresis is the movement of particles due to the interaction of a non-uniform electric field and the electrical dipole moment induced in the particle (Jones, 1995; Morgan and Green, 2003). The advantage of the method is that the focussing force acts on the particles rather than on the fluid, allowing particles to be focussed within a single stream. The principle of this is shown in Fig. 1(b).

![Fig. 1. (a) Schematic diagram showing the principle of 1-D hydrodynamic focussing. The input particle stream is confined on both sides by sheath flow resulting in a focussing of the sample stream, and as a result, the suspended particles. (b) Schematic diagram showing how dielectrophoresis can be used to focus particles in 2-D. Thin microelectrodes on the top and bottom of the channel push the particles into the centre of the channel. The electrodes are typically 100 nm thick and do not influence the fluid flow.](image-url)
The dielectrophoretic force is given by the following equation (Jones, 1995):

$$F_{\text{DEP}} = \frac{1}{2} Re[\mathbf{a} \mathbf{E}] |\mathbf{E}|^2$$

where $\mathbf{a}$ is the volume of the particle, $\mathbf{E}$ the effective polarisability of the particle and $\mathbf{E}$ the electric field. As can be seen from this equation, the force depends on the gradient of the magnitude of the electric field squared (the electrical energy density) and is zero in a uniform electric field (typically used for electrophoresis). Depending on the effective polarisability of the particle, which in turn depends on the frequency of the applied electric field, the force can either push particles towards high strength field points (positive dielectrophoresis) or away from high field points (negative dielectrophoresis). For a simple homogeneous spherical particle such as a latex bead, the polarisability is given by:

$$\alpha = 3 \varepsilon_0 \epsilon_2 \frac{\varepsilon_2 - \varepsilon_m}{\varepsilon_2 + 2 \varepsilon_m}$$

where $\epsilon_2$ and $\epsilon_m$ are the electrical permittivities of the particle and the fluid medium and $\sigma_2$ and $\sigma_m$ are their conductivities. The frequency of the applied electric field is $f$ and $f^2 \approx 1$ is the imaginary unit. For particles with a complicated internal structure such as biological cells, this expression is significantly more involved since each layer has a distinct set of electrical properties (permittivity and conductivity) which have an influence on the polarisability of the particle as a whole. This gives a frequency dependent force (both in terms of magnitude and direction) which can be used to identify the particle and separate different types of particle (Jones, 1995). The frequency dependent part, indicated by the part of Eq. (2) enclosed in square brackets is referred to as the Clausius–Mossotti factor (Jones, 1995).

The electric field gradients required to move particles are relatively high, but can be generated with relative ease using simple planar micro electrode arrays. These are fabricated with microchannels, generating maximum field gradients at the electrode edges. Particles are attracted from solution to the electrode edges by positive dielectrophoresis or, by changing the frequency of the electric field, repelled from the electrodes.

Microelectrodes have been widely used to move and trap particles by dielectrophoresis, and also to focus particles in two dimensions within a flowing fluid stream (Fiedler et al., 1998; Müller et al., 1999; Schnelle et al., 2000; Gawad et al., 2001; Voldman et al., 2002; Dürr et al., 2003; Morgan et al., 2003, 2004). Fig. 1(b) shows how dielectrophoretic focussing can be implemented within a channel. As shown in the figure, four thin (100 nm) electrodes are fabricated in the channel, which is typically 20–50 μm high. The electrodes therefore do not change the cross-sectional aspect of the channel and have negligible affect on the hydrodynamic pressure-driven flow through the channel.

The electric field produced by the electrodes produces negative DEP of the particles and pushes them into the centre of the channel. As the inter-electrode gap decreases, the width of the particle beam decreases, the fluid remains unperturbed. Therefore, in contrast to hydrodynamic focussing, DEP focussing can be implemented in a single flow stream since it acts on the particles rather than the fluid. However, the disadvantage is that microelectrodes have to be fabricated and then aligned in the microfluidic channel.

DEP focussing has been used as part of a simple microchip cell sorter (Fiedler et al., 1998; Müller et al., 1999; Dürr et al., 2003; Seger et al., 2004). DEP particle focussing has also been used to improve the sensitivity and resolution of impedance based single cell analysis devices (Gawad et al., 2001). DEP can even be used to confine nano-particles (40 nm diameter latex spheres) into a narrow stream (Morgan et al., 2003, 2004).

3. Experimental

3.1. System design and fabrication

Fig. 2(a) shows a schematic diagram of the microchip and the micro electrode design used for the device. A plane and cross-sectional view of the microfluidic channel and the electrode structure is shown. To achieve particle focussing in two dimensions, four electrodes are required, with pairs of electrodes fabricated on the top and bottom surfaces of the channel. The channel was 250 μm wide and 40 μm high. The two substrates were 500 μm thick borosilicate glass wafers.

The triangular-shaped micro electrodes were fabricated on the glass substrates by standard photolithographic patterning of resist, evaporation of metal: Ti/Au/Ti (10 nm/100 nm/10 nm), followed by lift-off in acetone. The electrodes were only 120 μm thick, compared to the channel height of 40 μm and had no effect on the fluid motion through the microchannel.

The channel was fabricated on top of the electrodes using a laminate negative photore sist (Riston MM140, Dupont, UK). Fabrication was as follows: the substrates were baked overnight at 200 °C to remove any water from the surface, and then the temperature was reduced to 120 °C. The laminate resist was deposited on the surface of one substrate by means of a rolling hot press (MegaElectronics A4) at a temperature of 115 °C and a speed of 15 cm/min. The microchannel pattern was exposed through an acetate mask using a HTG mask aligner for 25 s and developed using laminate resist developer (80 mM aqueous sodium bicarbonate), for approximately 60 s. The channel was subsequently closed by placing a second electrode-patterned substrate over the bottom patterned substrate and aligning the two electrode structures in a microscope. UV curing glue was then inserted into the edge of the device, which flowed to fill the gap between the two halves, up to the edge of the channel under capillary forces. After the glue had flowed around the channel walls the device was flood illuminated using a 100 W mercury lamp (from a microscope, Zeiss, EBQ100) to cure the glue.

Bonded chips were mounted onto a PCB. Inlet and outlet holes for the fluids were drilled through one glass substrate prior to bonding, using diamond tipped drill bits (Diama Ltd., UK). Fluid was driven through the device by gravity, giving flow velocities in the region of 1–10 mm s⁻¹. A photograph of the focussing electrodes (fabricated on the bottom of the channel) and the microfluidic channel is shown in Fig. 2(b).
3.2. Optical detection

A confocal optical detection system was implemented using an inverted microscope (Zeiss Axiovert 200), and a schematic of this is shown in Fig. 2(c). A region of the channel, approximately 20 μm downstream of the focussing electrodes was imaged through an objective lens (Zeiss Fluar X20 0.75N.A.). Incident light from a 10 mW HeNe laser (632.8 nm) was coupled into the microscope through an optical fibre and beam expander. The size of the focussed spot in the microchip was approximately 25 μm diameter.

Allophycocyanine loaded 6 μm diameter carboxyl modified latex particles (Molecular Probes, OR, USA) were used as test particles. These particles were supplied as a set of six different fluorescent intensity standards designed for calibration of commercial flow cytometers. Fluorescence emission from the particles was filtered using a Cy5 filter set (Omega Optics) and detected with a photomultiplier (Hamamatsu) and a home-made amplifier with 1 kHz bandwidth. Data was digitised using a 16 bit A/D card (National Instruments), stored on hard disc and processed using N.I. LabVIEW software. The fluorescence emission collected by the objective lens was spatially filtered using a pinhole giving a detection region of approximately 10 μm in diameter, equivalent to a detection volume of the order of 10 fl.

The movement of particles along the channel was simultane-
their terminal velocity, which is given by the ratio of the applied force to the Stokes drag force (White, 1991). For a spherical particle of radius $a$, the Stokes force is $6\pi \eta a$, where $\eta$ is the viscosity of the suspending medium.

The externally applied pressure drives a steady fluid flow in the channel, which was also numerically calculated in FlexPDE. The equation for a two dimensional pressure-driven flow in a rectangular duct is given by Poisson’s equation with the source term derived from the pressure drop per unit length. The channel was assumed to be infinitely long and the Reynolds number calculated to be less than one. Analytical solutions of the fluid flow profile exist for this geometry (White, 1991) but consist of series solutions which do not give an advantage in computation time.

Particle trajectories were simulated using a time step-dynamic particle simulation method implemented in Matlab®. The DEP force and fluid velocities were transferred from the finite element solution and referenced using bs- and tri-linear interpolation. The instantaneous particle velocity was calculated for 400 separate particles. The change in position of each particle after a given time step was calculated as a function of time to simulate the movement of a distribution of particles through the channel.

4. Results and discussion

4.1. Numerical simulation

A similar geometry for focussing particles has been described by Schnelle et al. (1999). These authors used a bar electrode geometry (Schnelle et al., 1999; Dittr et al., 2003), and calculated the DEP force on a particle using an analytical solution for the potential generated by a semi-infinite plate capacitor. By setting the Stokes hydrodynamic force on the particle equal to that produced by the negative DEP repelling force, they were able to estimate the fluid velocity that would push a particle through the DEP barrier, over the electrodes. This gives an approximate maximum fluid velocity at which DEP focussing would be effective.

We have performed a dynamic simulation of the particle behaviour which enables time-dependent particle distributions in the entire channel to be simulated for any electric field configuration and fluid velocity. Fig. 3(a) is a sequence of images showing the effect of DEP focussing on the particles in the microelectrode array for three different voltages: (i) 5, (ii) 10 and (iii) 20 V peak to peak. In these simulations, the pressure-driven flow gives a maximum fluid velocity of 1 mm s$^{-1}$ in the centre of the channel, the particle diameter was set to 6 $\mu$m and the Clausius–Mossotti factor was $-0.5$, giving the maximum negative dielectrophoretic force. Four hundred particles were assigned random starting positions in a box placed at the beginning of the channel (left-hand end of image). The sequence of images in each figure show the progress of particles through the device for the three different voltages at the same flow rate, at time intervals of 0.2 s. The effect of increasing the voltage on the focusing efficiency of the device is immediately apparent. At 20 V peak to peak, focussing occurs over the entire length of the electrode and all the particles are focussed. Simulations at lower voltages show that some focussing of particles occurs but that some particles clearly escape over the front edge of the focussing electrodes. These particles remain unfocussed and do not pass through the detection region.

Other forces act on the particles as they move through the device; most importantly gravity and hydrodynamic lift but these are negligible by comparison with the DEP forces. The net displacement due to gravity is 120 nm over the length of the channel. The hydrodynamic lift force calculation involves a significantly more complicated process. The lift force expressions (Cherukatt and McLaughlin, 1994; Leighton and Actvios, 1985) imply zero net upward forces if the particles move with the fluid, except close to the channel walls, where there is a small difference in the drag force on the particle. In this system, the dielectrophoretic force does not act against the flow of the fluid, and therefore, does little to retard the particle against the fluid, so that the lift force can be neglected.

Fig. 3(b) shows density plots of the final positions of the particles in the $x$–$y$ directions after passing through the microelectrode structure at six different voltages. At low voltages, there is some focussing but the presence of the front edge of the electrode which faces the fluid flow is clearly seen in the number of particles that have escaped and not focussed. As the voltage is increased, the size of the focussed beam shrinks and at 10 V peak to peak, the majority of the particles are inside the detection region delineated by the circle. However, only at 20 V peak to peak is the focussing 100% efficient, with no particles escaping over the electrode edge.

Note that the focussed shape of the beam is elliptical for all voltages. This is due to the fact that in the vertical ($y$) direction the only force acting on the particle is negative DEP, but in the horizontal ($x$) direction the drag force from the fluid moving over the electrode edge has a component which opposes the DEP force. As a result, it is to be expected that there will be some spread in the final distribution in the horizontal ($x$) direction compared to the vertical ($y$) direction as demonstrated by the simulation.

4.2. Experimental DEP focussing

For the focussing and optical detection experiments, latex particles were suspended in dilute KCl solution with a conductivity of 1 mS m$^{-1}$ at a particle number density of $10^{10}$ ml$^{-1}$. Approximately 10 $\mu$l of sample suspension was passed through the device using gravity feed, at a user-defined flow rate up to a maximum particle velocity of 5 mm/s. When a voltage of 20 V peak at 10 MHz was applied to the electrodes, the latex particles experienced negative DEP and were focussed into the channel centre.

For the dimensions of channel shown in Fig. 2, 100% of the particles could be focussed for particle velocities of up to 1 mm s$^{-1}$ at an applied voltage of 20 V. If the particle velocity increased above this limit, it was observed that occasional particles “escaped” over the electrode edges and did not move in the centre of the channel, as predicted by the simulation shown in Fig. 3. Fig. 4(a) shows a single frame from a video showing latex particles in the device exiting from the focussing electrode.
Fig. 3. (a) A sequence of images showing DEP focusing of the particles at three different voltages of: (i) 5, (ii) 10 and (iii) 20 V peak to peak. The maximum fluid velocity was 1 mm s$^{-1}$ and the Clausius–Mossotti factor for the 6 μm diameter particles was set to $-0.5$. The simulation was performed by assigning a random starting position to 400 particles in a box at the beginning of the device (an animation of the simulation results showing particles moving through the device can be seen at www.nano.ecs.soton.ac.uk/bio/videos/sim1.mpg). (b) Density plots of the particle distribution at the end of the focusing electrode for several different applied voltages: the end state of the simulations shown in (a). The circle shown in the center is approximately 6 μm diameter, the size of the bead used in experiments and simulations.
Fig. 4. (a) Photograph of the electrode array showing focussing of 6 μm diameter latex particles. (b) Sequence showing how increasing the applied voltage causes the particles to focus into a tight band at 20 V, with significantly poorer focussing at lower voltages. The dark region on the left of each image is the electrodes, which obscure the particles.

Note that the electrodes on the left of the image obscure the particles trajectories. Fig. 4(b) shows a time-lapse sequence of images showing the trajectories of several different particles. This image was formed from the superposition of 200 successive video images. In the absence of any applied voltage, the particles were randomly distributed, as shown in the figure. With the maximum voltage applied, all the particles were pushed into centre of the channel, so that they passed through the small optical detection volume. The velocity of the particles in the centre of the channel in these images was 1050 μm/s. As shown by the images, focussing occurred in two dimensions. Focussing in the vertical direction was demonstrated by moving the microscope objective up and down. The depth of field of the objective is approximately 3 μm, no particles were detected above or below the central plane of the channel. These experimental data and the numerical simulations presented in Fig. 3 are in good agreement, with the simulations tending to predict better focussing than experimental evidence. This could be due to the voltage

Fig. 5. (a) Data showing the PMT output for three different intensities of 6 μm diameter fluorescent particles passing through the device, with a close-up of a section of the data in (b) clearly showing the three intensity peaks. (c) PMT data for beads labelled with fluorescent protein.
on the electrodes being lower than the applied potential. However, the difference is scalable, indicating that the behaviour of the DEP focusing electrodes can be predicted using our model.

4.3. Optical detection and particle counting

Fig. 5(a) shows data for three different intensity fluorescent 6 μm diameter latex particles flowing through the device. Fig. 5(b) shows an expanded section of the data, clearly showing the three different signals from the different particles. Each peak corresponds to one particle passing through the detection volume. The importance of focussing was evident in that when the voltage to the focussing electrodes was switched off, the signal from the photomultiplier fell to zero, recording only the occasional peak as a random particle passed through the detection zone.

Conventional FACS machines analyse fluorescently labelled cells at high speed. Cells are conjugated with fluorescently labelled antibodies, for example, CD+ antibodies for differential analysis of human blood. To determine the sensitivity of our device, plain carboxy-modified 6 μm diameter latex spheres were modified by covalent attachment of proteins onto the surfaces. A monolayer of polyclonal antibody (IgG) was coupled to the surface of the beads using previously published methods (Hermanson, 1996). The monolayer of protein molecules was subsequently fluorescently labelled using the NHS ester of Cy5 (Amersham Biosciences). Coupling was performed using the protocol supplied by the manufacturer. The amount of protein bound to the bead was estimated using the BCA assay (Pierce) following the manufacturer’s protocol, and was found to be approximately one monolayer. Assuming a typical coupling efficiency of two fluorescent molecules per protein, then this gives a bead loading of 10^7 fluoroprobes per bead. The data obtained from these fluorescently labelled particles is shown in Fig. 5(c).

Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcytometer</td>
<td>34.9% (371)</td>
<td>40.0% (425)</td>
<td>25.0% (266)</td>
<td>1062</td>
</tr>
<tr>
<td>%CV</td>
<td>23.7</td>
<td>22.1</td>
<td>12.4</td>
<td></td>
</tr>
<tr>
<td>BD FACScan</td>
<td>34.4% (760)</td>
<td>40.1% (887)</td>
<td>25.5% (563)</td>
<td>2210</td>
</tr>
<tr>
<td>%CV</td>
<td>18.6</td>
<td>19.0</td>
<td>19.1</td>
<td></td>
</tr>
</tbody>
</table>

Columns are data for three different fluorescent intensity 6 μm diameter latex particles. Numbers in brackets refer to total particle counts.

The behaviour of the device was compared with a conventional FACS by quantifying the fluorescence emission from the data shown in Fig. 5. Approximately, 1000 beads were analysed using a peak detection algorithm implemented in LabVIEW. A histogram of the number of particles as a function of fluorescence intensity was constructed and is shown in Fig. 6(a). For efficient peak detection, the data was thresholded to 1% of the maximum, i.e. all values <1% were assumed to be noise and the values set equal to zero. Three distinct peaks can be clearly observed in the data, corresponding to the three distinct populations of particles with different fluorescent emission intensities. The curves on the data are best fit Gaussian.

The same suspension of particles was also analysed using a Becton Dickinson FACScan particle analyser (with CELLQuest 3.1 software). This data shown in Fig. 6(b), shows three distinct peaks. A fourth peak close to zero is also evident, which is due to noise in the system. Further analysis was performed on the data in order to determine the number of particles with different intensities. The results are summarised in Table 1, and show almost 100% correlation with the BD FACScan data.

In a recent paper, Wang et al. (2004) reported light scattering data from a microfabricated flow cytometer which used hydrodynamic focussing of the particles ensures that all the particles pass through the small optical detection volume. Examination of the histograms of Fig. 6, allows the coefficient of variation (%CV) to be estimated from the ratio of the standard deviation divided by the mean signal intensity ×100. This data is also summarised in Table 1.

In a recent paper, Wang et al. (2004) reported light scattering data from a microfabricated flow cytometer which used hydrodynamic focussing. They measured the coefficient of variation (%CV) of light scattering from latex particles for sizes in the

![Fig. 6. (a) Histogram of the signals for 6 μm fluorescent latex particles of three different fluorescent intensities shown in Fig. 5(a). (b) Histogram of data for the same beads measured using a Becton Dickinson FACScan particle analyser (data processed with CELLQuest 3.1 software).]
range 2.8–9.1 µm and obtained values between 27 and 30%, figures comparable with other microcytometers utilising DC electrokinetic focussing (Schrum et al., 1999; McClain et al., 2001). They suggested that the %CV for their device was lower than for conventional flow cytometers because of hydrodynamic effects, including low ratio of sheath flow to sample flow, I-D rather than 2-D hydrodynamic focussing, or the pulsatile flow produced by the pumps at low flow rates. Typical light scattering %CV for state-of-the-art flow cytometers depend on the sample and the preparation but can be as low as a few %. However, fluorescent %CVs tend to be higher; for example, in a survey of T-cell analysis methods, Janosy et al. (2000) quoted a best figure of 7%, with typical figures of 14%.

The coefficient of variation for the fluorescence data presented in Fig. 6 is summarised in Table 1 and is in the range of 24–12% for our device, similar to that obtained with the BD FACScan. This indicates that the micro-device is capable of measuring true variations in the properties of the particles and does not introduce additional errors in the data due to inefficient focussing. Compared with conventional flow cytometers, our device has a lower flow-through rate; commercial systems can process samples at up to 100,000 particles/s when optimised. Increased sample throughput for the micro-device can be achieved with a much higher flow rate. To achieve higher flow rates, and therefore, faster sample analysis, the hydrostatic pressure in the device has to be increased. Leakeage from the devices could be a problem; but the limiting factor is likely to be the upper pressure limit that cells can tolerate and the efficiency of the DEP focussing. Shear stress on cells is minimal in these devices because the particles only occupy the central few % of the channel and are therefore not subjected to the high fluid velocity gradients seen near the channel walls.

To evaluate the efficiency of the focussing electrodes at high particle speeds we simulated the device with a central fluid channel and are therefore not subjected to the high fluid velocity gradients seen near the channel walls. For example, focusing 6 µm latex particles into sinusoidal channel structures. Anal. Chem. 69, 3212–3217.

5. Conclusion

We have presented the fabrication of a micro flow cytometer that detects and counts fluorescent particles flowing through a microchannel at high speed based upon their fluorescence emission intensity. We have demonstrated that the combination of dielectrophoretic focussing of particles within a microfluidic channel and confocal optical detection can be used to rapidly analyse populations of fluorescently labelled particles with a resolution equivalent to that of commercial flow cytometers. The functioning of the device was demonstrated by detecting and counting fluorescent latex particles in the micrometre size range at a rate of up to 250 particles/s. With smaller flow channels the possibility of detecting and analysing of sub-micrometre particles will be possible (Morgan et al., 2003). Detection and discrimination of viruses and other nano-particles based on fluorescence, and light scatter is expected to be achievable with such devices.

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