FLOW SPEED PARTICLE FOCUSING IN MICROFLUIDIC IMPEDANCE MEASUREMENTS

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ABSTRACT

We focus particles by hydrodynamic effects in microfluidic channels, in a flow-through impedance spectroscopy (FTIS) system. At low flow speeds the particles are distributed in the channel. By increasing the fluid flow rate, we can cause the particles to focus to localised positions, resulting in tighter measured distributions. The symmetry of the electrodes and channel means that the four expected particle positions results in two signal groups, which can be distinguished by their different shapes. Thus, the apparent size distribution in FTIS can be improved.

KEYWORDS: hydrodynamic focusing, impedance spectroscopy, particle counting

INTRODUCTION

FTIS is a microfluidic technique using impedance sensing to measure particle sizes and other properties [1]. It can be used to perform a 3-part differential analysis of white blood cells [2]. Like all electrical sensing methods, an inhomogeneous electrical field will give a variation in the measured signal, if the particles are distributed in position. Particles may be focused by complex 2D or 3D sheath-flow systems, used in large scale haematology analysers, but on a microfluidic scale a simpler method is to use the hydrodynamic forces on particles to achieve ordering in the channel positions [3].

THEORY

At low flow speeds the particles are distributed in the channel. This combines with the non-uniform electric field of the sensing electrodes, to give a distribution of apparent particle sizes. By increasing the fluid flow rate, hydrodynamic forces cause the particles to focus to localised positions in the channel, resulting in tighter distributions in apparent size. The symmetry of the electrodes and channel means that the four expected particle positions results in two signal groups, which can be distinguished by their different shapes.

EXPERIMENTAL

We performed experiments to see if this focussing could be used to improve the distribution in measured particle sizes for 5μm polystyrene beads in our FTIS system, which is as described previously [2] and summarized in figure 1. In this case the microfluidic channel is rectangular, 35μm high and 40μm wide. The beads were diluted in pocH-pack D diluent (Sysmex, Japan), and measured with 5Vpp 500kHz excitation at a range of flow rates between 5 and 120μl/min. All samples are sufficiently dilute that we do not expect interactions between beads, or overlapping events in the detector to be significant.

Figure 1: (A) Schematic of the FTIS system with a bead passing through it. (B) Magnified photograph of the chip central channel and electrodes, 1 and 3 is one pair of electrodes whereas 2 and 4 is another pair of electrodes. (C) Output signal of the lock-in during the passage of a 5μm bead. The speed is given by v=dx/dt where dx is the distance between the two electrode centres (70μm). The w parameter is the ratio between dt and σ (w=dt/σ), where σ is the standard deviation of the Gaussian model function fitted to each peak.
RESULTS AND DISCUSSION

Figure 2 shows the measured velocity and size distributions for 5µm polystyrene beads at a series of flow rates between 5 and 120µl/min. At low flow rates (figure 2A) the particles are mainly located at the fastest flow speed, corresponding to the centre of the channel’s parabolic flow profile, but with a wide distribution in both speed and apparent size. As the flow rate increases, the particles migrate to two narrow distributions, with slightly differing speed and apparent size (figure 2A - 2E). Figure 2F shows the expected particle distribution [3] in the channel, colour coded into groups that would give the same electrical signal. The slightly rectangular shape of the channel causes the equilibrium positions of the two groups of particles to travel at different speeds.

![Figure 2](image)

Figure 2: (A) The distribution of 5µm beads with a flow-rate of 5µl/min. (B) 10µl/min. (C) 20µl/min. (D) 50µl/min. (E) 100µl/min. (F) Schematic of the expected cross section of the channel at a flow rate of 100µl/min. The shaded beads illustrate the two groups of positions that give identical signals due to symmetry.

![Figure 3](image)

Figure 3: (A) The distribution of the speed v and size and (B) shape parameter w and size of 5µm beads at a flow rate of 100µl/min. We have shaded the events differently above and below a w of 2.1, so that the two groups can be clearly distinguished in the speed / volume plot.

The two groups can be distinguished by a parameter (w) extracted from the shape of the electrical signal detected (figure 3). We find that our detected events have a good fit to a model function composed of a pair of anti-symmetric Gaussian peaks. The ratio of the peak separation (dt) and the peak standard deviation (σ) gives a shape parameter that we call w, where \( w = \frac{dt}{\sigma} \). Essentially, events with a high w have a nearly flat region between the two peaks, as in the exam-
ple event of figure 1C, while events with a low $w$ do not. We do not have sufficient certainty to be sure which shape of events is identified with which group of positions in the channel. However, the separation in $w$ allows us to identify the two groups which overlap in velocity and in apparent volume, thus allowing more accurate measurement of each group separately.

Figure 4 shows the hydrodynamic focussing effect in a sample with a mixture of bead sizes (2, 3, 4, 5μm). As is predicted [3], the degree of focussing is particle size dependant, as well as speed dependant, with larger particles focussing more readily. Thus, the 2μm beads are not focused in this regime, while partial focussing is seen for the 3μm beads, and nearly complete focussing is seen for the 4 and 5μm beads.

**CONCLUSION**

We have shown that hydrodynamic focussing effects can be used to localise the particles flowing in a microfluidic channel. Each group of particles shows an improved size distribution compared to unfocused particles. The two groups of stable channel positions can be distinguished by a shape parameter of the measured signal. Thus, by flowing at high speeds, and measuring populations differentiated by waveform shape, the apparent size distribution in FTIS can be improved.

**REFERENCES**


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