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Study of inertial hydrodynamic focusing in sheath-driven flows for lab-on-a-chip flow cytometry

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ABSTRACT

Miniature flow cytometer models enable fast and cost-effective management of diseases in vulnerable and low-end settings. The single-line focusing of cell or particle samples is achieved using hydrodynamic forces in the microfluidic channels. The two common configurations among them are the single-sheath and dual-sheath flows wherein the sample is directed through the main channel, and the surrounding sheath fluids are directed into the main channel through inlets on either side of the main channel. Most models predict the width of the focused sample stream based on hydrodynamic focusing in the low Reynolds number regime ($Re \ll 1$), where the viscous forces dominate the inertial forces. In this work, we present comparative analysis of particle focusing by single-sheath and dual-sheath configurations for focusing of micron-sized cells/particles in the range 2 to 20 μm in the higher Re ($10 < Re < 80$) laminar regime. A quantitative analysis of the relative focused stream width (w_f/w_{ch}) as a function of flow rate ratio (FRR = Sample flow rate/Sheath flow rate) for the two configurations is presented. The particle tracing results are also compared with the experimental fluorescent microscopy results at various FRR. The deviations of the results from the theoretical predictions of hydrodynamic focusing at $Re \ll 1$, are explained analytically. These findings clearly outline the range of flow parameters and relative particle sizes that can be used for cytometry studies for a given channel geometry. This is a highly predictive modeling method as it provides substantial results of particle positions across the microchannel width according to their size and FRR for single-line focusing of particles. Such information is crucial for one to engineer miniaturized flow cytometry for screening of desired cells or particles.

Keywords: miniature flow cytometer, inertial microfluidics, hydrodynamic focusing, Reynolds number, cell focusing, single-sheath flow, dual-sheath flow.

1. INTRODUCTION

Flow cytometry has been used as a powerful tool for identification, characterization, enumeration and classification of single cells or particles in areas of biology and medicine like pathology, immunology, gene sequencing, chromosome sorting, biodefence, and diagnosis of diseases. Particularly, flow cytometry is instrumental in the qualitative and quantitative estimation of drug/gene delivery to cancer cells, which are useful for theranostics applications [1]. It uses a laser beam to interact with cells, one at a time, and extract quantitative and qualitative information about a sample population based on size, shape, texture and fluorescence [2]. Conventional lasers, complex optical and mechanical circuitry render the flow cytometer a bulky, complicated and expensive instrument, limiting its use in state-of-the-art research laboratories and high-end clinical settings by skilled personnel. Moreover, the sample needs to be subjected to multiple pre-treatment steps before it can be fed to the fluidic system of the flow cytometer. For performing flow cytometry measurements, the sample cell population needs to be focused to a narrow stream of single cells which are then illuminated and their photo-signals are recorded to yield measures of cell population, and specific cell characteristics (size, fluorescence, etc.) [3]. For this, large volumes of sheath fluids, generally buffer solutions, need to be made available at the time of taking measurements, because the pressure driven-sheath fluid in the focusing nozzle of the flow cytometer helps in narrowing down the sample stream to a single-line of moving cells or particles through the hydrodynamic focusing effect. Overall, it is tedious and expensive to use such macroscale, bulky flow cytometers for frequent use in diagnosis and management of infectious diseases in widespread low-end settings. Thus, portable flow cytometer models have been proposed wherein focusing of cell or particle samples is achieved using hydrodynamic forces in the microfluidic channels. These microfluidic focusing devices are integrated with optical fibers to form a portable optofluidic lab-on-a-chip flow cytometry framework for management of diseases which can reachout to the larger community in need as part of affordable healthcare. Although these miniature lab-on-a-chip flow cytometers do not yield the enormously high throughput as the conventional ones, they can well be designed for particular applications

that yield reliable and subjective information which can serve as important markers for effective disease management. The development of commercial miniature lab-on-a-chip flow cytometers can enable fast, and cost-effective method of management of diseases in vulnerable, and low-end settings. Theoretical and experimental studies related to microfluidics-based particle focusing configurations have been proposed that can enable design of lab-on-a-chip flow cytometry devices [4-6]. The most common configurations among them are the single-sheath [7-9] and dual-sheath [10-13] rectangular micron-sized fluidic channels wherein the sample is directed through the main channel, and the surrounding sheath fluids are directed into the main channel through inlets on either side of the main channel. Most models predict the width of the focused stream based on hydrodynamic focusing in the low Reynolds regime ($Re \ll 1$), where the viscous forces dominate the inertial forces. However, there have been no comparative studies between the single-sheath and dual-sheath configurations related to optimization of microchannel design parameters with respect to the cell or particle size at higher Re .

In this work, we present comparative analysis of particle focusing by single-sheath and dual-sheath configurations for focusing of micron-sized cells or particles (2 to 20 μm) in the higher Re ($10 < Re < 80$) laminar regime. Analysis of the 3D Navier-Stokes equations for pressure-driven fluid flow inside microchannels in the laminar regime is performed using the Finite element method (COMSOL Multiphysics 4.3b, MA, USA), and the simulated results are compared with the theoretically predicted velocity and concentration profiles. A quantitative analysis of the relative focused stream width (w_f/w_{ch}) as a function of FRR for the two configurations is presented. The particle tracing results are also compared with the experimental fluorescent microscopy results at various FRRs. The deviations of the results from the theoretical predictions of hydrodynamic focusing at $Re \ll 1$, are explained analytically. These findings clearly outline the range of flow parameters and relative particle sizes that can be used for cytometry studies for a given channel geometry. This is a highly predictive modeling method as it provides substantial results of particle positions across the microchannel width according to their size and FRRs for single-line focusing of particles. Such information is crucial for one to engineer miniaturized flow cytometry for screening of desired cells or particles.

2. SIMULATIONS AND EXPERIMENTS

2.1 Simulations

2.1.1 Modelling of single-sheath and dual-sheath microfluidic design for particle focusing

The microchannel geometry was designed with a channel height of 80 μm . For the single-sheath design, a pair of sheath inlets from either side of the joined the main channel at an angle of 30° . Similar structure was followed for the dual-sheath design where an additional pair of sheath inlets joined the main channel 200 μm from the first pair. The flow was modeled in the laminar flow regime at a sample flow rate of 30 $\mu\text{l/min}$. The sheath flow rates were modulated at different values relative to the sample flow rate to get an understanding of particle focusing at various FRRs. Sample parameters corresponded to polystyrene beads dispersed in DI water and the sheath fluid was DI water. The flow was modeled as incompressible with shallow channel approximation. Simulations were performed at various FRRs (defined in Eqn 1) from 1:1 through 1:5.

$$FRR = \frac{\text{Sample flow rate, } Q_s}{\text{Sheath flow rate, } Q_{sh}} \quad (1)$$

2.1.2 Determination of relative sample focused width (w_f/w_{ch})

The effect of hydrodynamic focusing in the main channel was modeled in case of both sheath flow and no sheath flow. From these results, the width of the sample stream (w_f) as it flowed through the main channel surrounded by the sheath stream was determined through simulations at various FRR values. The simulations were carried out for aspect ratios (AR) of 1.6, 0.8 and 0.4 corresponding to channel widths (w_{ch}) of 50 μm , 100 μm and 200 μm respectively. These studies yielded the relative focused stream width (w_f/w_{ch}) as a function of flow rate ratio for different microchannel geometries.

2.1.3 Determination of particle trajectories with and without sheath flow

Besides the focusing effect as a consequence of sheath flows in the single-sheath and dual-sheath configurations, the particle trajectories were simulated for particle diameters of 2 μm , 10 μm and 20 μm . This is a very informative modeling approach as it provides substantial results of particles positions across the microchannel width according to their size and FRR for single-line focusing of particles for flow cytometry studies.

Figure 1. Schematic illustration of single-sheath and dual-sheath configurations for cell/particle hydrodynamic focusing in a microfluidic chip. The cells/particles get focused to a single line stream in the outlet channel as a result of hydrodynamic focusing.

2.2 Comparison with theoretical model

The relative focused stream width (w_f/w_{ch}) at different FRR modeled using the FEM analysis, was compared with the theoretical focused stream width for a Poiseuille velocity profile for $Re \ll 1$ in a rectangular cross-section microchannel as given in Eqn 2,

$$\frac{w_f}{w_{ch}} = \frac{1}{\gamma(1 + \frac{2}{FRR})} = \gamma \quad (2)$$

where, γ is the ratio of the average velocity of the focused sample v_f and main channel v_{ch} , as described in Eqn 3 [4].

$$\gamma = \frac{v_f}{v_{ch}} = \frac{1 - \frac{192 AR}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \frac{\sinh[\frac{(2n+1)\pi(\phi)}{2AR}]}{\cosh[\frac{(2n+1)\pi}{2AR}]}}{1 - \frac{192 AR}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \tanh[\frac{(2n+1)\pi}{2AR}]} \quad (3)$$

The theoretically calculated values for the relative focused stream width is computed by using the above expression for γ at various FRR and are represented in Figure 2.

The deviation from the predicted focused width ratio at higher FRR is due to the existence of inertial lift forces namely, the shear induced-lift forces and the wall induced-lift forces. Both these forces interplay to result in multiple equilibrium positions across the channel cross-section. Interestingly, the wall induced-lift forces superpose with the sheath pressure-driven hydrodynamic force and hence, multiple equilibrium positions are not observed in straight channels in sheath-assisted pressure-driven flows unlike sheathless flows [14], although the width of the focused stream may vary according to channel aspect ratio and the particle size. At higher Re , the extent of the wall-induced lift forces (w_{WL} , equation 4) diminishes and as a consequence, the shear-induced lift forces tend to shift focused particles laterally towards the wall [15].

$$w_{WI} = w_{ch} Re^{-0.5} \quad (4)$$

Nonetheless, higher sheath flow rates at these high Re tend to overcome the dominant shear force, and maintain the focused flow in the main channel, are able to prevent lateral spread of particles even in the presence of inertial lift. Interestingly, the dual-sheath flows minimizes the effect of shear-induced lift as the net sheath flow rate is higher. Hence, high throughput particle focusing can be achieved in dual-sheath configurations at higher Re flows.

Figure 2. Theoretical and simulation predictions of the relative focused stream width (w_f/w_{ch}) for a single-sheath 80 μm wide microchannel at different flow rate ratios.

2.3 Fabrication of microfluidic focusing chip using soft-photolithography

First, a *.gds drawing of the microchannel design is printed on a photomask. Then, SU-8 photoresist is spun on a Silicon (Si) wafer at 2000 rpm for 80 seconds twice to get the desired thickness of 80 μm . Following each recipe, the coated-Si wafer is relaxed at 60°C for 10 minutes following by a 20 minute pre-bake at 120°C. For soft lithography, the SU-8 coated-Si-wafer is subjected to a 25 second UV exposure (40 mW) under the photomask. Thereafter, the wafer is post-baked at 110°C for 20 minutes and thoroughly rinsed in SU-8 developer. Post development, only the channel layout part remains on the Si-wafer while the blank parts get washed off. This wafer is now termed as the 'master'. The master is then hard-baked at 200°C for 2 hours for strengthening the crosslinking between the SU-8 and the wafer.

A PDMS-based molding suspension is prepared by mixing the PDMS elastomer and curing agent together in a 10:1 ratio. The suspension is degassed, and then poured over the master wafer, degassed again, and allowed to cure for about 4 hours at 70°C. The cured PDMS is then peeled off the master wafer, and a PDMS chip patterned with microchannels is obtained. This chip is referred to as the microfluidic chip. Holes are punctured at the inlet and outlet reservoirs for microtubing connections, the microfluidic chip is cleaned with acetone and dried, and finally irreversibly bonded to a clean glass slide by oxygen plasma treatment (40 W, 40 seconds, 270 mTorr) for performing flow experiments.

2.4 Experimental characterization of the two designs

The cell or particle sample is fed through the inlet port of the chip at flow rates controlled by a syringe pump. The waste flows out from the outlet port and is collected in a separate dish. The cells or particles are fluorescently labeled so as to track their focusing positions as they travel through the microchannel length. As the samples are fed at the set flow rate, fluorescent microscopic images of the outlet part of the channels are acquired (Figure 10).

3. RESULTS AND DISCUSSION

Figure 3 shows the design of a single-sheath microfluidic chip with 50 μm wide microchannel for hydrodynamic focusing of particles or cells. For FEM-based computations of the 3-D steady state Navier Stokes equations, the entire microchannel structure is subdivided into microdomains (Figure 3a inset), commonly known as meshing of domains, where the velocity profile, concentration profile and particle tracing calculations are performed. The hydrodynamic focusing effect of single-sheath and dual-sheath flows is presented in the Figure 3d. A higher velocity ratio, γ for dual-sheath flow leads to sharper focusing as shown in the inset of Figure 3d.

Figure 3. a) Simulation model of one sheath focusing, inset: meshing domains used for calculation of velocity and concentration of flowing sample. b) Velocity profile obtained for a 50 μm (w_{ch}) wide channel and c) Concentration profile showing the focusing of the samples surrounded by sheath fluid. d) Velocity profile across the microchannel width for FRR = 2. Inset: The hydrodynamic focusing effect due to sheath flow as a function of sample concentration across the microchannel width. A sample could be fluorescent-labeled particles or cells or a dye solution

Figure 4a-f presents an elaborate comparison of the focusing performance with single-sheath and dual-sheath flows in a microfluidic flow cytometry set-up. It is clear from the results (Figure 4g-h) that the dual-sheath model can be used to focus smaller particles (<10 μm), while the single-sheath model can easily focus particles larger than 10 μm , such as white blood cells and tumor cells. While both the single-sheath and dual-sheath models exhibit similar focusing effects at lower FRR (e.g. 1:5), the dual-sheath model shows improved focusing at lower FRR (>1:2). Thus, for a higher throughput, dual-sheath focusing models may be preferred, particularly for smaller particles (e.g. red blood cells, bacteria).

Figure 5 illustrates the particle tracing simulations with and without sheath flow both in single-sheath and dual-sheath configurations. It is an obvious observation that in the absence of sheath flows, there is no focusing of the sample fluid in both the configurations. The highlight of this simulation is the ability of the dual-sheath flow to achieve a single particle line of focused particles even at lower FRR, as opposed to the single-sheath configuration where we still notice multiple streams of particles. This clearly describes

$$\left(\frac{w_f}{w_{ch}}\right)_{DS} \leq \left(\frac{w_f}{w_{ch}}\right)_{SS} \quad (5)$$

Figure 4. Normalized concentration profiles of 15 μm polystyrene beads focused by single-sheath (a-c) and dual-sheath (d-f) flows for different aspect ratio (50, 100 and 200 μm) channels at various FRRs. (g-h) Focused particle stream width as a function of different flow rates; Q_{sh} →Sheath flow rate, Q_s →Sample flow rate, w_f →Width of the focused particle stream, w_{ch} →Width of the main channel.

The as-fabricated single-sheath and dual-sheath microfluidic chips for particle/cell focusing are shown in Figure 6g-h. The chips were fabricated according to the photolithography process outlined in Figure 6a-f. The chips were then investigated for particle focusing using 18.7 μm fluorescently labeled polystyrene beads. The beads sample was fed at a flow rate of 75 $\mu\text{l}/\text{min}$, and the sheath sample (PBS) was fed at 150 $\mu\text{l}/\text{min}$. Thus, at a FRR = 0.5, fluorescent focused streaks, as shown in Figure 7, were imaged using a fluorescence microscope (Eclipse-Ti, Nikon). The experimental results confirm the simulation findings. Since the particle Reynolds number for both cases are of the order of unity, inertial forces dominate and hence the focused stream width deviated from the theoretically predicted values at low Reynolds number.

Figure 5. Role of sheath fluid in achieving hydrodynamic focusing in single-sheath (a, b) and dual-sheath (c, d) configurations. Trajectories of $2 \mu\text{m}$, $10 \mu\text{m}$ and $20 \mu\text{m}$ spherical polystyrene particles in the single-sheath and dual-sheath configurations with (b, d) and without (a, c) sheath flows. Outlet channel width is $200 \mu\text{m}$.

Figure 6. Fabrication process (a-f) of single-sheath (g) and dual sheath (h) microfluidic chips using photolithography process.

Figure 7. Experimental focusing performance of a single-sheath (left) and dual-sheath design. $Re \approx 80$, $Q_s = 75 \mu\text{l/min}$, $Q_{sh} = 150 \mu\text{l/min}$, Diameter of polystyrene microspheres = $18.7 \mu\text{m}$. The micrographs are the $10\times$ magnified views of the outlet channel shown in Figure 1.

4. CONCLUSION

In this paper, we presented a simulation-based and experimental study to demonstrate the difference in focused stream width at higher flow rates compared to simple hydrodynamic focusing using single and dual sheath flows at lower flow rates. The focused stream width deviates from the theoretical predictions as inertial forces come into play at higher Reynolds number. Thus, the focused width predictions for focusing microparticles in channels at higher Re should be inclusive of inertial forces. The advantage of using sheath flows lies in obtaining single equilibrium positions across the channel width as opposed to multiple equilibrium position in straight channels in sheathless inertial flows. These calculations are critical for designing miniature flow cytometers based on sheath flow for focusing particles/cells in microfluidic channels.

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