Dynamic radial positioning of a hydrodynamically focused particle stream enabled by a three-dimensional microfluidic nozzle

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The ability to confine flows and focus particle streams has become an integral component of the design of microfluidic systems for the analysis of a wide range of samples. Presented here is the implementation of a 3D microfluidic nozzle capable of both focusing particles as well as dynamically positioning those particles in selected flow lamina within the downstream analysis channel. Through the independent adjustment of the three sheath inlet flows, the nozzle controlled the size of a focused stream for 6, 10, and 15 μm polystyrene microparticles. Additional flow adjustment allowed the nozzle to dynamically position the focused particle stream to a specific area within the downstream channel. This unique ability provides additional capability and sample flexibility to the system. In order to gain insight into the fluidic behavior of the system, experimental conditions and results were duplicated within 4.75 μm using a COMSOL Multiphysics[®] model to elucidate the structure, direction, proportion, and fate of fluid lamina throughout the nozzle region. The COMSOL Multiphysics model showed that the position and distribution of particles upon entering the nozzle have negligible influence over its focusing ability, extending the experimental results into a wider range of particle sizes and system flow rates. These results are promising for the application of this design to allow for a relatively simple, fast, fully fluidically controlled nozzle for selective particle focusing and positioning for further particle analysis and sorting. © 2015 AIP Publishing LLC.

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I. INTRODUCTION

Over the past several decades, the miniaturization and development of biological and chemical assays in microfluidic devices have grown from a small focused effort into a well-established field of research.^{1–4} The application of microfluidics often has a number of advantages in biological analyses, including increased sensitivity, reduced cost and sample requirements, and the ability to easily manipulate cells and particle streams.^{3,5} As it is desirable to analyze particles and solutions in a uniform fashion as well as precisely and predictably manipulate fluid in channels, flow confinement and particle stream focusing have become an important part of the design and implementation of microfluidic systems, including diffusion-

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based mixers,⁶ cell and surface chemical patterning,^{7–9} microchemical synthesis,¹⁰ and flow cytometers for cell sorting and analysis.^{11,12} A number of methods have been employed to focus particle streams, including acoustics,^{13–17} electroosmosis,^{18–26} dielectrophoresis,^{18,27–29} and magnetophoresis.^{30,31} However, hydrodynamic methods remain the most widespread method in microfluidics for confining flow and have been well established in commercial flow cytometers.

Initial attempts at hydrodynamic focusing were aimed at confining sample flow to a narrow column, which is generally referred to as two dimensional (2D) flow focusing.³² Knight *et al.* demonstrated in 1998 the possibility of significant microfluidic focusing in 2D by confining the sample flow from a 10 μm nozzle to a width of only 50 nm,⁶ while other early focusing techniques sought additional ways to make the sample flow more concise, including the use of air as the sheath fluid³³ and a syringe pump pulling on the outlet to create focusing.³⁴ However, these techniques were generally limited to planar (2D) focusing. As a result of the small diameter and relatively low fluid velocity in microfluidic channels, the fluid in these systems has a low Reynolds number and exhibits laminar flow. Thus, the fluid stream will have maximum velocity in its center and zero velocity at the wall, if approximated with the parabolic flow profile characteristic of a low Reynolds number.³⁵ 2D hydrodynamic focusing is therefore a limited solution for microfluidic flow cytometry, as the inconsistent velocities of particles across the stream will be constantly shuffling the order of those particles, which could impede sorting. Irregular particle velocities could also bring multiple particles within the interrogation area simultaneously, thereby confounding a device's ability to accurately obtain data about each particle. Applications involving small and/or precious amounts of sample could also be limited by 2D focusing, as variation in the unfocused dimension might result in a loss of sample or measurement fidelity.

In an effort to address some of the shortcomings of 2D focusing, a number of methods have been developed for shaping and focusing microfluidic flow in three dimensions. Sundararajan *et al.* used six sheath channels to best approximate a complete cylindrical sheath flow in order to focus a Rhodamine solution,³⁶ while later efforts used cascaded streams to focus the sample stream first vertically, then horizontally.^{37–39} These studies did achieve hydrodynamic focusing, but through relatively complex machining that could be difficult to reproduce on a larger scale. Other examples include the induction of 3D focusing by altering the interior of the sample flow channel in various ways, first through the addition of protruding stripes⁴⁰ and then through chevron-shaped grooves,⁴¹ as well as microfluidic drifting to create 3D focusing in a single-layer, planar device.⁴² More recent iterations have achieved sub-micron accuracy through the use of computational fluid dynamics to refine and improve chip design.⁴³ Other novel approaches taken include the use of a chimney, which is used to release sample fluid perpendicularly into the sheath stream,⁴⁴ and the employment of a contraction-expansion array which utilizes Dean flow to hydrodynamically focus in 3D with only one sheath inlet.⁴⁵ This type of focusing has allowed for a reduction in the complexity of manufacturing such that improved focusing can be achieved within a single channel structure. Due to the reduced manufacturing complexity and improved focusing, several groups have been focused on the evolution of this approach.^{42,43,46–49} While it is a robust method to focus a particle stream, there are higher flow and thus higher Reynolds number requirements making this techniques unfeasible for Reynolds numbers <1 . It is also unable to change the position of a focused stream in 3-dimensions within the channel without changing the entire channel structure. Another design uses a two layer microfluidic chip to overcome some of the limitations of inertial focusing methods, such as a limited range of flow and particle type.⁵⁰ A number of other designs have been implemented, ranging from complex multilayered structures to an “off the shelf” nozzle fabricated from readily available parts.^{36,37,39,42,47,51–55} However, many previous designs are limited to specific flow regimes, particle sizes, or both, and none allow the addressing of the focused particle stream to a specific and arbitrary location in the analysis channel.

Here, we present the design, fabrication, implementation, and analysis of a microfluidic nozzle that allows for not only the precise and tunable hydrodynamic focusing of a particle stream but also the unique ability to dynamically position particles in precise flow lamina in a downstream channel. The design of the nozzle incorporates three independent sheath inlet flows

and one sample flow for the introduction of particles. The ability to focus and position three different sizes of polystyrene particles was investigated both experimentally and computationally using COMSOL Multiphysics finite element modeling. Using a sufficiently high ratio of total flow to sample flow (greater than 100:1), a monodisperse stream of 6, 10, or 15 μm particles could be focused to a total size of less than 10 μm in a 250 μm diameter channel. Particle streams could also be addressed to four independent locations within the channel by adjusting each of the three sheath flows. The performance of the nozzle was verified using a COMSOL Multiphysics model, which revealed the geometry of the fluidic lamina in the nozzle and the behavior of the particles positioned throughout the sample inlet flow. The unique ability to dynamically position particles within different lamina could be used for several applications, ranging from the sorting of cells and particles to the development of novel assays that rely on non-centric particle positions.

II. EXPERIMENTAL

A. Chip construction

The microfluidic flowcell consisted of five plates of fused silica and was similar in fabrication to flowcells used in the previous work.⁵⁶ Each square plate had a side length of 25.4 mm and was 0.5 mm thick, with the exception of the plate furthest downstream, which was 1.782 mm. Two 250 μm diameter circular channels were formed by the thru-holes in the third and fourth plates, each with a total length of 500 μm , while a focusing nozzle was fabricated on the second plate. The features on each individual plate, including the nozzle, were first patterned with a femtosecond laser and then wet etched with hydrofluoric acid (HF). Areas exposed to the laser etch at a rate approximately 100 times faster than unexposed material, allowing precise channels and features to be fabricated. The individual plates were then thermally bonded together to form the final chip (Translume, Inc., Ann Arbor, MI, USA). Once bonded, the sample inlet channel (75 μm in diameter) entered the analysis channel through the base of the nozzle, where it was surrounded by 3 sheath flow inlets that were rectangular channels measuring $150 \times 100 \mu\text{m}$. The three sheath flows were evenly distributed around the circle. Five 350 μm diameter ports were drilled into the flowcell to serve as the inlet (3), outlet (1), and sample injection (1) locations. Fluid connectors (Nanoport, Upchurch Scientific, Inc., Oak Harbor, WA) were screwed into a custom 3D-printed plastic holder (FineLine Prototyping) that aligned the fluidic connections with the ports on the microfluidic chip.

B. Fluidic system and connections

The fluidic control system utilized five pressurized vessels, each filled with approximately 1 ml of PBS buffer (pH 7.4, Sigma, St. Louis, MO, USA), which occupied about 2/3 of the vessel volume. Three of the vessels served as the sheath inlet flows, while the remaining two served as the outlet and sample injection, respectively. Each vessel was fluidically connected to the flowcell via 100 μm inner diameter PEEK capillary tubing (IDEX, Lake Forest, Illinois, USA). By controlling the pressure above the liquid in each vessel, pulse-free, stable, and reproducible fluid flow could be achieved.⁵⁷ In this system, a custom printed circuit board (PCB) was fabricated to both supply 24 V power and convey the control signal from a computer through the use of a 16-bit, 8 channel, high-drive, data acquisition, analog output device (Measurement Computing, Norton, MA, USA) to each of the 5 electronic pressure controllers (EPCs) from Parker-Hannifin (Cleveland, OH, USA). The multi-reservoir technique isolated the flow system, increasing the stability and adding the capability to control the direction of flow. Flow direction and flow rate, in both the analysis channel (representing the combined flow rates of the sample inlet and sheath flow inlets) and the sample inlet channel (representing only the sample inlet flow), were precisely measured to a resolution of ± 1.5 nl/min using a calibrated commercial liquid mass flow meter (Sensirion, Inc., Westlake Village, CA, USA). Flow sensors were not separately installed on each of the sheath flow inlets. Custom LabVIEW software

monitored the flow sensors and adjusted the pressure of the EPCs in order to maintain a specific flow rate input by the user, allowing for independent adjustment of the pressure for each of the 5 EPCs.

C. Imaging and video collection

The microfluidic flowcell was observed from two viewpoints in this study: an infinity corrected long working distance objective (Edmund Optics, Barrington, NJ, USA) and a lens tube system (Infinity Photo-Optical, Boulder, CO, USA) connected to a CCD camera (Basler, Inc., Exton, PA, USA) that was illuminated using a PL-800 fiber optic light (Edmund Optics, Barrington, NJ, USA). The first viewpoint looked at the side of the outlet flow channel and was used to initially characterize the system and determine the desired focal depth for the second viewpoint. The second viewpoint, which was also the observation plane for the experimental and computational data (Figure 1(a)), was aligned with the center of the channel such that flowing particles were advancing toward the objective. In this specific orientation, sheath flow inlet 1 enters at the 12 o'clock position, while sheath flow inlets 2 and 3 enter at the 8 and 4 o'clock positions, respectively (Figure 1(a)). Both camera/objective setups and their associated illuminators were mounted independently of the flow cell and each other on separate x-y-z translation platforms from Newport (Irvine, CA, USA). Data collection was performed using StreamPix 5 (Norpix, Inc., Quebec, Canada). Experimental data were analyzed using a custom script in MATLAB that automatically tracked the location of each particle as it passed through the observation plane using an image analysis routine based on background subtraction and brightness

FIG. 1. Nozzle and system schematic. (a) A 3D dimensional drawing of the focusing nozzle. Blue arrows indicate fluid flow direction and the red disk represents the observation and analysis plane used in both the experiments and simulation. (b) A micrograph of the constructed nozzle. (c) System schematic. The pneumatic pumping system, including all fluid vials, tubing, electronic pressure controllers (EPCs), nanoliter mass flow meters (NFM), and electronic connections (dotted lines) are shown. Solid black lines represent liquid tubing while thick gray lines represent air tubing. (d) Illustration of the focusing and addressing of the sample inlet flow to a specific off-center location within the analysis channel.

threshold. Both the frame rate of the camera and image analysis parameters were adjusted such that each particle was counted only at its most focused point.

D. Microparticle injection

Bare polystyrene microparticles (microParticles GmbH, Berlin, Germany) of three sizes were used in this study: 6 μm (6.110 ± 0.063), 10 μm (10.57 ± 0.11), and 15 μm (14.94 ± 0.13). For both the focusing and addressing experiments, each particle size was analyzed separately and diluted to an appropriate concentration based on the desired sample flow rate such that the particle throughput at the observation plane was approximately 0.2–0.5 particles/s. This relatively low throughput generally ensured that only one particle was in the observation plane at one time, increasing the accuracy and efficiency of the data analysis.

E. COMSOL Multiphysics® modeling

The microfluidic channel geometry used for simulation (Figure 1) originated from the Solidworks® 2011 file used for the experimental fabrication; however, the microfluidic channels leading to and from the nozzle and analysis channel area were truncated. The length of the sample inlet channel was 200 μm from the face of the sample inlet to the nozzle opening. Next, the lengths of each of the sheath flow inlet were 162 μm to the opening for the speedway channel. Finally, the length of the analysis channel was 210 μm . The orientation of the model follows the same conventions detailed above for the experimental conditions. The Solidworks® 2011 models were imported into COMSOL Multiphysics v4.3b using the CAD import module. The observation window for particle behavior was $\sim 210 \mu\text{m}$ down the speedway from the nozzle, which mirrors the position where the particles were monitored, experimentally. The nozzle geometry was discretized using the user-defined, free tetrahedral, mesh size fine, calibrated for fluid dynamics with a minimum element equality of 0.761 and 34 191 total elements. The walls were modeled as no slip boundaries and the inlets/outlets as either open boundaries or with the laminar flow rates, detailed in Table I.

The simulated sample particles mimic the physical properties of the polystyrene particles used for the experimental inquiry. The simulated particle density used was 1.05 g/cm³; the particle diameters matched the average manufacturer reported experimental particle diameters (6.11, 10.57, and 14.94 μm); and the particles were not charged. The simulated particles were introduced along the face of the sample inlet to generate a tightly focused, centralized stream of particles (input function $\frac{1}{(x^4+y^4)}$). The approach was used to approximate the tightly focused sample stream observed experimentally. However, the significance of sample introduction was further explored with several other input methods being compared. In addition to the tightly focused, centralized stream created by $\frac{1}{(x^4+y^4)}$, the following sample input functions were investigated: $\frac{1}{(x^2+y^2)}$ (less tightly focused, centralized stream), spf.U (particles were distributed across the sample inlet based on lamina velocity, wherein a higher number of particles were located in the faster central lamina versus the slower wall-adjacent lamina), projected plane grid (a uniform distribution of particles across a square grid encompassing the sample inlet), and mesh based with a refinement factor of 1 (a uniform distribution of a single particle across each mesh element at the sample inlet). The fluid component of the simulation was solved separately as a stationary solution, while the particle tracking component was dependently coupled to the previously solved fluid component and was solved as a time dependent problem.

III. RESULTS AND DISCUSSION

A. Nozzle design and functionality

As pictured in Figure 1(a), the focusing nozzle was comprised of a 75 μm diameter circular sample inlet channel that joined 3 rectangular sheath flow inlet channels ($150 \times 100 \mu\text{m}$) upon its entry into the 250 μm diameter circular analysis channel. The base of the nozzle was a truncated cone with a diameter of 250 μm at the base and 125 μm at the top. Sample entered the system through the injection channel and was surrounded by the 3 inlet channels that served as the sheath

TABLE I. Particle addressing experimental and simulated flow rate and pressure ratio values.

Q1 (nl/min)	Exp. 6 μm	Exp. 10 μm	Exp. 15 μm	Sim. 6 μm	Sim. 10 μm	Sim. 15 μm
Sample flow	50	50	50	50	50	50
Sheath 1	0.28 ^a	0.25 ^a	0.25 ^a	−1050 ^b	−1350 ^b	−1950 ^b
Sheath 2	1 ^a	1 ^a	1 ^a	9000	9300	9000
Sheath 3	0.22 ^a	0.25 ^a	0.6 ^a	−3000	−3000	−2100
Total flow	5000	5000	5000	5000	5000	5000
Q2 (nl/min)	Exp. 6 μm	Exp. 10 μm	Exp. 15 μm	Sim. 6 μm	Sim. 10 μm	Sim. 15 μm
Sample flow	50	50	50	50	50	50
Sheath 1	1 ^a	1 ^a	1.15 ^a	−450 ^b	−750 ^b	−330 ^b
Sheath 2	1 ^a	1 ^a	1 ^a	−2100	−2400	−2400
Sheath 3	2.4 ^a	2.4 ^a	3.1 ^a	7500	8100	7680
Total flow	5000	5000	5000	5000	5000	5000
Q3 (nl/min)	Exp. 6 μm	Exp. 10 μm	Exp. 15 μm	Sim. 6 μm	Sim. 10 μm	Sim. 15 μm
Sample flow	50	50	50	50	50	50
Sheath 1	2.5 ^a	2.45 ^a	3.05 ^a	10 800	10 800	11 100
Sheath 2	1 ^a	1 ^a	1 ^a	−2925 ^b	−2550 ^b	−3075 ^b
Sheath 3	1 ^a	0.85 ^a	1.1 ^a	−2925 ^b	−3300	−3075 ^b
Total flow	5000	5000	5000	5000	5000	5000
Q4 (nl/min)	Exp. 6 μm	Exp. 10 μm	Exp. 15 μm	Sim. 6 μm	Sim. 10 μm	Sim. 15 μm
Sample flow	50	50	50	50	50	50
Sheath 1	1 ^a	1 ^a	1.9 ^a	6600	6300	9780
Sheath 2	1 ^a	1 ^a	1 ^a	4620	5100	1200
Sheath 3	0.12 ^a	0.1 ^a	0.05 ^a	−6270 ^b	−6450 ^b	−6030 ^b
Total flow	5000	5000	5000	5000	5000	5000

^aA pressure ratio (relative to sheath 2), rather than a flow rate.^bA flow rate determined through the application of an open boundary to balance flow.

flow for the sample. Each inlet channel as well as the injection channel were independently connected to a vial containing either sheath fluid (in this case, PBS buffer, pH 7.4) or, in the case of the injection channel, sample particles in buffer. Figure 1(b) shows a micrograph of the constructed nozzle including the sheath flow inlets and sample inlet channel. Connected to the outlet of the system was another vial that collected the sample and sheath flow (Figure 1(c)—system schematic). By precisely controlling the pressure above the fluid in each of these vials independently, the flow rate of the injection channel as well as each of the 3 inlet channels were independently adjusted, giving the nozzle the ability to both focus a stream of particles as well as address that stream of particles to different locations within the radius of the analysis channel. Specifically, increasing all of the sheath flows with respect to the sample flow would focus the incoming particle stream to a smaller cross sectional area in the analysis channel and vice versa. Also, changing the sheath flows with respect to one another could be used to address the same flow to different locations within the analysis channel, as illustrated in Figure 1(d).

B. Nozzle performance—Experimental particle focusing

Initial experiments focused on assessing the nozzle's ability to focus a stream of incoming particles along the centerline of the analysis channel. To investigate the nozzle's performance across a variety of sizes, three different size polystyrene microspheres were used, nominally

ranging from 6 to 15 μm with 10 μm representing the intermediate size. These sizes were chosen to roughly correspond to the size range of mammalian blood cells.⁵⁸ Experimentally, the total flow of the system (3 sheath flow inlets plus the sample flow) was maintained at 5000 nl/min, while the injection flow rate was varied from 25 nl/min to 1666 nl/min, representing a total flow: sample flow range of 3:1 to 200:1. Particles were imaged approximately 210 μm after their exit from the channel using a camera aligned with the central axis of the analysis channel such that flowing particles are coming toward the objective (Figure 1(a)). This allowed for tracking of both the horizontal and vertical positions of a particle within the channel at the specified analysis plane. As expected, increasing the total flow:core flow ratio resulted in a more tightly focused particle stream.

As illustrated in Figure 2, the average standard deviation from the center of the particle stream for 6 μm particles decreased from 23.9 μm at a ratio of 3:1 to only 1.6 μm at a ratio of 100:1. The average standard deviation represents the average between the horizontal and vertical standard deviation of particles from the center of the particle stream. This average is used because the horizontal standard deviation is larger than the vertical standard deviation for some conditions and smaller for others, with no apparent pattern based on size or flow rate (Table SI-1 in the supplementary material).⁵⁹ Notably, the performance did not appreciably increase when going to the higher ratio of 200:1, likely due to the fact that the core flow was sufficiently small such that other factors, such as initial particle position, particle diffusion, or inertial effects, had a greater impact on the particle's focused position than the width of the injection flow. This trend was similar for both 10 and 15 μm particles, whose particle standard deviations decreased from 21 and 15 μm at 3:1 to 2 and 2 μm at 100:1, respectively, which is less than a particle diameter (Table SI-1).⁵⁹ Assuming a roughly normal distribution of particles, almost all (99.7%) of the particles in the stream would be contained within three standard deviations in either direction from the mean, resulting in a theoretical particle spread of 12 μm . In practice, the maximum distance between particles was less than 10 μm for each of the three particle sizes, resulting in a well-focused particle stream comprising less than 8% of the diameter of the

FIG. 2. Nozzle focusing performance. (a) Graph showing the average standard deviation from the mean particle position for 3 particle sizes and 7 flow rate ratios (total flow rate:core flow rate). Total flow rate was constant at 5000 nl/min, while the injection flow rate varied from 25 nl/min to 1666 nl/min. The arrow and box indicate the data used for the plots in (b)-(d). (b) Scatter plot of 6 μm particle locations for a flow rate ratio of 20:1. Each point represents the location of a tracked particle, plotted in μm relative to the center of the channel (0,0). (c) Scatter plot of 10 μm particles under the same conditions. (d) Scatter plot of 15 μm particles under the same conditions.

channel in both the horizontal and vertical direction. In comparison, a previously constructed microfluidic nozzle focused a stream of $1\text{ }\mu\text{m}$ particles to approximately 13% of the channel width.⁵¹

At flow ratios up to $\sim 50:1$, the focusing of the nozzle was less effective for the smaller particles. For example, as highlighted in Figure 2(a) at a ratio of 20:1, the average standard deviation of the $6\text{ }\mu\text{m}$ stream was $7.9\text{ }\mu\text{m}$ vs 5.3 and $3.0\text{ }\mu\text{m}$ for the 10 and $15\text{ }\mu\text{m}$ particle streams, respectively. Such size dependence was not unexpected and has been reported previously in other focusing scenarios.⁶⁰ It is potentially due to a decreased inertial effect on the smaller particles, making the focusing less effective. Figures 2(b)–2(d) show this effect in more detail, as it gives the scatter data for each particle size at the 20:1 ratio. When comparing the plots, it is clear that the stream of $15\text{ }\mu\text{m}$ particles is most focused, covering an area of roughly $144\text{ }\mu\text{m}^2$, followed by the 10 and $6\text{ }\mu\text{m}$ particle streams, which cover areas of 552 and $1080\text{ }\mu\text{m}^2$, respectively. This difference in performance between particle sizes largely disappears at ratios of 50:1 and above, as the streams focused to a point below which the size of the particle does not appreciably impact the particle focusing performance.

C. Particle stream addressing—Experimental and model analysis

After establishing the ability of the nozzle to hydrodynamically focus particles, experiments were conducted to demonstrate the addressing of particle streams to different locations within the channel. To do so, the relative pressures of each of the sheath flow inlet vials were adjusted, which in turn altered the flow rate in each of the channels. In order to maintain a well-focused particle stream, the total flow was again maintained at 5000 nl/min with a sample flow of 50 nl/min . This unique ability is illustrated in Figure 3, in which a single frame from the side (Fig. 3(a)) (Multimedia view) and forward (Fig. 3(b)) (Multimedia view) vantage points each show a $10\text{ }\mu\text{m}$ particle deviated from the center of the channel. Accompanying videos (Figs. 3(a) and 3(b)) show additional particles and demonstrate the addressing ability in real time.

The results of specific particle sizes and locations are shown in Figure 4(a), in which each of the three particle sizes were sent to 4 different Cartesian quadrants (Q1–Q4) in order to demonstrate a range of possible particle locations. The resulting scatter plots for each of the 3 particle sizes are shown in Figures 4(a)–4(c). In general, the particles remain tightly focused for each of the three particle sizes and quadrants, Q1–Q3. One exception is Q4 for each size particle, in which there was a larger spread of particles radiating out from the center of the nozzle. Notably, Q3 and Q4 were the only quadrants that lacked a sheath flow inlet in a directly opposite position. For Q3, this explains the diminished amount of displacement into the quadrant resulting in a near y-axis grouping to maintain a high degree of particle focus. While in Q4 the particle spread was larger when addressing particles to that location further away from opposite of sheath flow inlet 1. Overall, particles could be addressed to a wide area within the channel. For example, particles addressed to Q3 were approximately $80\text{ }\mu\text{m}$ from the center of the channel and about $2/3$ of the distance to the wall, which is sufficiently far to separate particles or

FIG. 3. Micrographs of the side (a) (Multimedia view) and forward (b) (Multimedia view) vantage points showing two different $10\text{ }\mu\text{m}$ particles deviated from the center of the analysis channel. The blue line (a) and cross (b) indicate the approximate center of the channel, while the red arrow shows the particle's deviation from the center. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4914869.1>] [URL: <http://dx.doi.org/10.1063/1.4914869.2>]

FIG. 4. Nozzle addressing experiment and modeling. Scatter plots showing individual particle locations for both experimental ((a)–(c)) and modeling ((d) and (e)) results when each of the three particle sizes are addressed to 4 different locations within the channel (0,0 represents the center of the channel). The total flow rate was 5000 nl/min, while the injection flow rate was 50 nl/min. Each point represents the location of a tracked or modeled particle. Quadrants 1–4 are designated in (a).

particle groups into different downstream lamina. Although particles can be pushed further from the center of the channel, they become less focused when doing so. Furthermore, addressing particles to the outer lamina of the channel may be less desirable, as the low fluid velocities at those locations make downstream handling and collection more difficult. However, a reduction in focusing performance and collection may not be an issue if only unwanted particles are being sent to the edge of the channel.

In an effort to better understand the fluid dynamics within the nozzle as well as the computational limit for addressing particle groups, the experimental conditions were simulated using COMSOL Multiphysics, a finite element model. When comparing the experimental results presented in Figures 4(a)–4(c) to the corresponding particle size simulation results in Figures 4(d)–4(f), the location of the mean particle position of the experimental and simulated particle stream agrees to within $4.75\ \mu\text{m}$. Thus, the computational model is able to accurately reproduce the experimental data. The average x-y standard deviations of the simulated particle positions were approximately 35%, 52%, and 160% tighter in their grouping than the experimental data for the 6, 10 and $15\ \mu\text{m}$ particles, respectively, which approaches the computational limit for minimized scatter spread without resorting to a single point for particle introduction. It follows that the simulated particles could be confined to a tighter location than the experimental particles for a number of reasons, including experimental noise introduced by both the randomness of sample introduction and the possibility of slight disruptions in the system flow rates.

Through the reproduction of the experimental results, the model offers insight into the geometry of the fluidic lamina in the nozzle and better describes the flow rates necessary for the three sheath inlet flows (Figure 5) (Multimedia view). Interestingly, the model reveals that in order to achieve the experimental results, at least one of the sheath flows must actually be negative, as illustrated in Table I. For example, as shown in Figure 5 (Multimedia view), in the case

FIG. 5. Three views of modeled nozzle fluidic lamina. A front, isometric, and side view of the fluid lamina configuration modeled to manipulate $15\ \mu\text{m}$ particles to Q2. The red lines represent magnitude controlled fluid lamina, while the blue arrows also chart the direction of the lamina and their size, its magnitude. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4914869.3>]

of addressing the $15\ \mu\text{m}$ particles to Q2, the simulation results indicated that sheath 3 was flowing at $7680\ \text{nl/min}$, while both sheath 1 and 2 were flowing backward at flow rates of -330 and $-2400\ \text{nl/min}$, respectively. This creates a region of high velocity in sheath 3 as it enters the nozzle, as well as a rotational effect as particles enter the nozzle. There was even more disparity in the flows when addressing particles to Q3 and Q4. In those cases, the total flow going backward through the sheath flow inlets was greater than the total flow in the analysis channel.

This interesting flow behavior was not apparent by examining the flow rate ratios recorded from the experimental data set, as only the sample inlet and total flow rate were monitored. For this reason, only the pressure ratios for the sheath inlet flows are given in Table I. A potential advantage of back-flowing was the ability to apply additional flow from the positively flowing sheath while maintaining the total flow rate exiting from the analysis channel. This gives the operator the ability to direct particles to a greater extent than if the full sum of flow sent through the sheath flows needed to be sent down the analysis channel. Although it is possible, the amount of back flow was never sufficient to allow particles of any size to flow up the sheath inlets in either the experimental or simulated conditions.

The model was also used to investigate a wider range of particle sizes, as small as $250\ \text{nm}$ and as large as $60\ \mu\text{m}$. At a total flow rate of $5000\ \text{nl/min}$, these particles behaved similarly in terms of both focusing and addressing, indicating that the performance of the nozzle spans a wide range of sizes, and thus, has broad sample applicability. Higher flow rates, up to $300\ \mu\text{l/min}$, were also simulated with the model. At these flow rates, particles could still be focused and addressed, but their locations spanned a larger range than at $5000\ \text{nl/min}$. Thus, the degree of focusing decreases somewhat at much higher flow rates, but the nozzle would still be quite capable of focusing particles to discrete locations within the channel.

FIG. 6. Graph of various modeled sample inlet methods. COMSOL Multiphysics modeled fate of $15\ \mu\text{m}$ particle trajectories addressed to Q2 from various modeled sample introduction methods. Distance from the center of the channel at the origin of the graph is measured in μm . (a) Particles at the sample inlet modeled using the various sample introduction methods. (b) Particles at the analysis observation plane after their introduction using the various sample introduction methods.

The impact of particle position along the cross-section of the sample inlet was also explored using the COMSOL Multiphysics model. Several particle introduction geometries were simulated for the case of addressing $15\ \mu\text{m}$ particles to Q2 (Figure 6) in an effort to learn how the initial particle position translates to its observed outlet position. The introduction geometries examined range from a square grid to a highly localized central stream (see experimental for more details on the introduction functions and their exact positioning). The various particle introduction methods spanned the entirety of the sample inlet, $75\ \mu\text{m}$ (Figure 6(a)), which was five times the diameter of the particles used. Comparing the inlet position (Figure 6(a)) of the particles for each of the different introduction methods to the outlet position (Figure 6(b)) after correcting for the particles being modeled as points, the nozzle addressing has the capability for reducing the sample scatter spread to two times the diameter of the $15\ \mu\text{m}$ particles used. The nozzle design demonstrates a robust ability to both address and focus particles regardless of the particles origination position in the sample flow. This is an important feature that will reduce dependency on sample flow focusing upstream of the nozzle.

IV. CONCLUSIONS

The ability to confine a stream of particles or cells within a microfluidic channel is important for a number of applications, including flow cytometry, cell sorting, enzyme kinetics, single molecule detection, and individual cell analysis. In order to maximize the applicability of such a focusing system, it should be capable of focusing particles in 3D over a wide range of particle sizes and flow rates. Three dimensional focusing helps to preserve small volume samples and improve measurement fidelity, while a broad range of particle sizes would allow for the analysis of a diverse range of samples using the same device geometry, eliminating the need to customize the channel design to the sample. In this study, a microfluidic nozzle was designed and implemented with the capability to hydrodynamically focus particles through the modulation of three independent sheath flows. This capability was verified experimentally using 6, 10, and $15\ \mu\text{m}$ polystyrene particles, subsequently modeled using COMSOL Multiphysics and extended computationally into an even wider range of particle sizes and flow rates. Also, through the independent adjustability of the sheath flows, the nozzle has the ability to address focused particle streams to a precise and arbitrary location within the channel, a feature which was reproduced with COMSOL Multiphysics and is not present in any other nozzle design. This property allows for the partitioning or sorting of specific samples to different lamina within the flow field simply by adjusting the sheath flow ratios, enabling the potential for facile collection or direction into a specific downstream channel. Independent adjustment of the sheath flows could also be used to precisely introduce a specific chemical, such as an activator or inhibitor to a particle or chemical stream in another sheath flow or the sample flow. The robust focusing and addressing performance of this 3D nozzle is an improvement over current focusing techniques that can benefit from dynamic 3D positioning of the focused flow. As well as being a promising design for a wide variety of microfluidic applications, the somewhat increased design and manufacturing complexity, as compared to planar channel designs utilizing Dean flow, allows for this expanded capability across a wide range of Reynolds numbers covering low flow rates where Dean flow methods are ineffective. Future work will focus on leveraging this technique in more complex microfluidic platforms to analyze biological samples.

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