

Focusing-enhanced mixing in microfluidic channels

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A focusing-based microfluidic mixer was studied. The micromixer utilizes the focusing process required for cytometry to reduce the diffusion distance of molecules to be mixed in order to facilitate the passive diffusion-controlled mixing process. It was found that both the high flow rate ratio of the sheath flow to the flows to be mixed and the low flow rate of the mixing fluids resulted in the short mixing length required within the microfluidic channel. It was shown that a complete mixing was achieved within a distance of 4 mm in the micromixer for the focused mixing fluids at a flow rate of 2 $\mu\text{l}/\text{min}$ and a flow rate ratio of the sheath flow to the flows to be mixed at 4:1. The mixer described here is simple and can be easily fabricated and controlled. © 2008 American Institute of Physics. [DOI: [10.1063/1.2894313](http://dx.doi.org/10.1063/1.2894313)]

I. INTRODUCTION

Microfluidic systems feature minimized consumption of samples and reagents, high throughput, and the amenability for process integration and parallelization. As a result, they are playing increasingly important roles in life science research and medical diagnostics.^{1,2} Depending on specific targeted applications, the microfluidic systems can be configured to perform various functions through combinations of microfluidic structure designs and external accessories. Among these, fluid mixing within microchannels is one of the most common and important functions since many biological and chemical processes, such as protein folding, enzyme reaction, protein-ligand interaction, cell activation, and chemical synthesis require complete mixing of the reagents.³ Unfortunately, efficient fluidic mixing is very difficult to achieve because fluid flows within microfluidic channels are usually laminar—with Reynolds number of the order of unity—due to the small dimensions of the channels and slow flow rates of the fluids.¹⁻³ Therefore mixing in microfluidic channels is purely dictated by passive molecular diffusions. This problem is more pronounced in biological applications where large biomolecules, such as proteins (eg., antibodies and enzymes) that have significantly smaller diffusion coefficients, are mixed or reacted within the microchannels. This normally requires a significantly longer time and greater channel length to bring different biomolecules together through pure diffusion.

To overcome the difficulty and to achieve a complete mixing at reasonable time and channel length scales, many active and passive mixing technologies have been developed. Active micromixers use extra energy, in addition to fluid pumping energy, to stir the fluids to be mixed in microscale within microfluidic channels and have been shown to be just as efficient in mixing reagents as in macromixers in macroscales.⁴ Examples of this type of active micromixers include

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ultrasonic wave agitation,⁵ magnetic bead transportation and magnetohydrodynamic pumping,^{1,6} chaotic advection,⁷ and electrokinetic pumping.⁸ While this approach is successful in solving the problems associated with ineffective mixing in the micromixers, device fabrication, device packaging, and system integration involved in this approach are complicated, thus limiting its implementations in practical applications that are driven by cost and time savings. Passive micromixers, on the other hand, only use fluid pumping energy and employ physical structures in microfluidic channels to reduce the length that molecules have to diffuse to mix (i.e., diffusion distance), thereby facilitating the mixing. Such devices are relatively easy and inexpensive to fabricate and package. The reduction in diffusion distance is usually achieved by simply decreasing the fluid stream dimension through fluid splitting,^{9,10} fluid focusing,¹¹ stretching and folding volume of fluid over the cross section of channel through a series of grooves designs on the channel floors,^{12,13} splitting and recombining fluid streams,^{3,14} and introducing segmented gas-liquid (slug) or liquid-liquid (droplet) flow.¹⁵⁻¹⁷ Some of the passive micromixers, such as those based on grooved floors, have successfully been used in various applications.

While there are many ways to achieve efficient mixing in microfluidic channels, decisions as to which method to use to achieve complete mixing are application specific. Factors such as compatibilities in device functions, structures, processing, testing, and cost savings are critically important for choosing a particular mixing mechanism. In designing a microchip based flow cytometer (i.e., microcytometers) that integrates cell labeling (interaction with specific antibodies) and cell detection into one chip, we have developed a new focusing-based mixing mechanism for microcytometers. Since fluid focusing is a process required in cell detection in microcytometers, a system can be significantly simplified if the same focusing process can generate efficient mixing required for cell labeling prior to the detection. In this paper, we report an effective focusing-based mixer to be used in microcytometers.

II. EXPERIMENTS

A. Device fabrication

Molds for microfluidic channels were prepared using photolithography. Briefly, photocurable epoxy SU-8 (MicroChem, Newton, MA) was spin-coated on 4 in. silicon wafers, soft baked and exposed to UV under transparency photomasks to prepare the molds for microfluidic channels. After development and hardening processes, the obtained molds were surface treated with tridecafluoro-1,2,2,2-tetrahydrooctyl trichlorosilane (Sigma-Aldrich) for mold release. Subsequently, poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning, Midland, MI) was cast on top of the molds with a metal retaining ring on each wafer for thickness control. The resin was degassed under vacuum and partially cured at room temperature on a leveled surface first and subsequently cured at 85 °C for 4 h prior to being peeled off the molds. To establish covalent bonding between PDMS with the glass substrate for sealing the microfluidic channels, the surface of the molded PDMS was first activated with air plasma, immediately adhered to a clean glass surface, and subsequently subjected to a brief annealing at 85 °C. The obtained microfluidic devices were composed of rectangular channels with a cross section of 20 μm (height) \times 60 μm (width). Needle punches were used to create small holes at the end of each channel to insert injection ports that formed leak-proof pressure sealing with the surrounding PDMS walls.

B. Device testing

Devices were tested under an inverted microscope (Model IX71, Olympus) using two syringe pumps (Model 33, Harvard Apparatus, Holliston, MA) to independently control the fluid flows. To evaluate the focusing effect in the microfluidic channels, water stained with blue ink and pure DI water were used as main fluid and sheath fluid, respectively. To evaluate the mixing effect, phenolphthalein, a pH indicator, and sodium hydroxide (NaOH), a base, were used as model molecules and referred to as components *A* and *B* to be mixed in the micromixer, respectively. Phenolphthalein was used in this study because it turns from colorless in neutral pH to pink at a pH above 8.2. Therefore by visually examining the color changes, the progress of mixing between

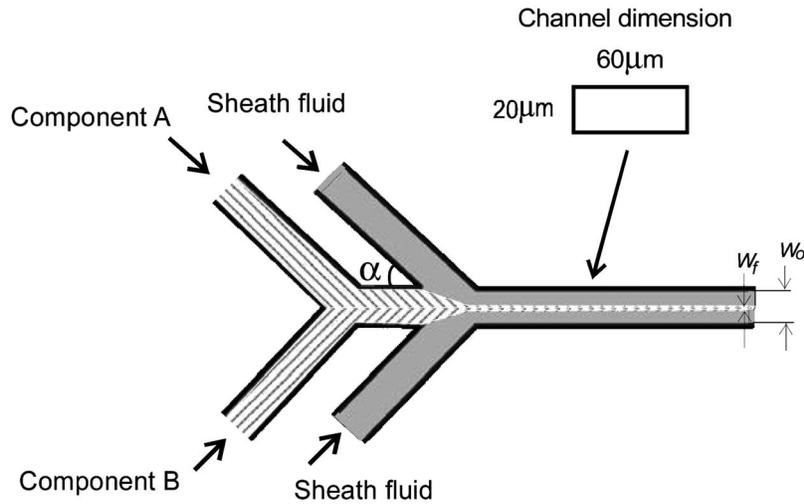


FIG. 1. Schematic diagram of a typical focusing-based microfluidic device.

phenolphthalein (component A) and NaOH (component B) could be easily monitored. Specifically, phenolphthalein ethanol solution at a concentration of 0.033 g/ml and NaOH ethanol solution at a concentration of 0.022 g/ml were introduced respectively into the device at equal flow rates through two injection ports, and were subsequently “squeezed” by ethanol injected from the two sheath channels (as shown in Fig. 1). A small amount of ethanol stained with blue ink was added into the sheath ethanol flows to create a better color contrast. An INFINITY3 cooled charge-coupled device (CCD) color digital camera (Lumenera Corp., Ottawa, ON) was used to capture images of the mixing process, and the images were analyzed using ImagePro Plus imaging analysis software (Qimaging Co., Surrey, BC).

III. RESULTS AND DISCUSSION

Figure 1 shows the schematic diagram of the device design principle that can be used to satisfy both rapid mixing and cytometry detection requirements. To achieve this, a set of sheath fluids are introduced to squeeze (i.e., focus) the merged laminar streams of components A and B that are introduced from an upstream Y junction via two separate injection ports into two very narrow streams. Rapid mixing occurs in the focused streams, benefiting from the dramatically reduced diffusion distance of each component in transverse direction to mix, and the well mixed fluids (and focused) will continue to flow through the detection zone. By adjusting the injection channel width and controlling the flow rate of each fluid, a thorough mixing of two components at various volumetric flow ratios can be realized in this current design. This mixing process is different from a previously reported focusing-based mixer that uses one of the components (in great excess) to be mixed in the sheath flow to focus the other component in order to achieve a localized mixing.¹¹

Focusing-based mixing can be easily estimated from a simple diffusion equation as shown below,⁹

$$t = \frac{x^2}{2D}, \quad (1)$$

where t is the average time of a molecule to diffuse over a distance x and D is the diffusion coefficient of the molecule in solution. In this study, x represents the stream width of lamellar flow for each component to be mixed within the microfluidic channels. Evidently, decreases in distance x will exponentially reduce the time it takes for a molecule to diffuse and mix with another (and different) molecule, thus dramatically reducing mixing time and increasing the mixing efficiency.

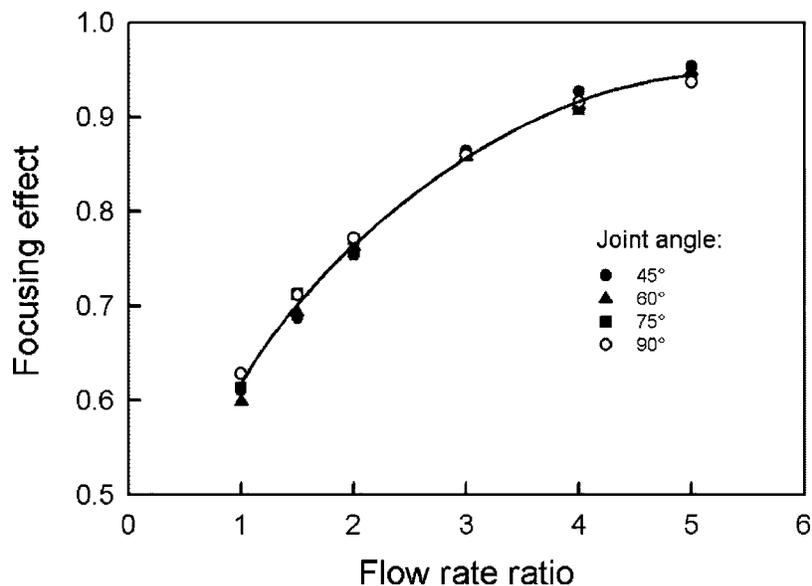


FIG. 2. Focusing effect as a function of flow rate ratio of sheath flow to the main flow at different joint angles. The dimension for the main flow channel was $20 \mu\text{m}(\text{height}) \times 60 \mu\text{m}(\text{width})$.

The focusing mechanism described above takes advantage of the sheath fluid to reduce the stream width of each component to increase the overall mixing. We note that in order to create the desired focusing effect, the flow rate of the sheath flow needs to be greater than that of the main flow consisting of the components to be mixed, and the greater the flow rate difference is the better the focusing effect, i.e., the degree of the width reduction.

To characterize the focusing effect, we defined the degree of width reduction $[1 - W_f/W_o]$, where W_o is the channel width and W_f is the width of the focused stream (as shown in Fig. 1). Upon merging with the sheath flow (DI water), the main flow—composed of blue ink introduced upstream from the Y junction connected to two separate injection ports—is squeezed and focused into the center of the microfluidic channel. The focused stream was found to be able to maintain its stability through the whole channel length with a maximum value of 7 cm in our devices. As clearly shown in Fig. 2, with increasing flow rate ratios of the sheath flow to the main flow, focusing effect increases sharply and tends to level off at a ratio of 5:1. Additional factors, such as device structure, channel surface, and fluidic properties, in theory, may also affect the focusing efficiency. In fact, a device structure-related parameter B , for instance, was introduced into a theoretical equation to describe the focusing process.¹¹ The effects of joint angle (cf. Fig. 1) of sheath fluid channel relative to the main channel were also studied. It was found the angles had almost no effects on the focusing for all the flow ratios studied, as shown in Fig. 2. This result is surprising given that various joint angles ranging from 30° to 90° were reported in the literature.^{9,18,19} Further experiments showed that the angle indeed had effects on the focusing at the early stage (unsteady state) when the fluids initially merged and before entered the single main channel, and that a large joint angle reduced the time and the transition length required to form a stabilized focusing stream (i.e., steady state). Combined, our data suggested that the joint angle only influenced the initial focusing kinetics within a very limited range while the flow rate ratio dictates the equilibrated focusing stream all the way to the channel exit. Since focusing-based mixing is a long process and would mostly happen in the equilibrium flow state, a fixed joint angle of 45° was used in the rest of the study.

Mixing within the device shown in Fig. 1 is similar to that observed in a typical T mixer,¹⁸ in which diffusion-controlled mixing takes place at the interface as the two merged fluids (seen as streams under microscope) flow within a microfluidic channel. The process can be characterized by using phenolphthalein as a pH indicator (phenolphthalein) in one fluid and NaOH as the base

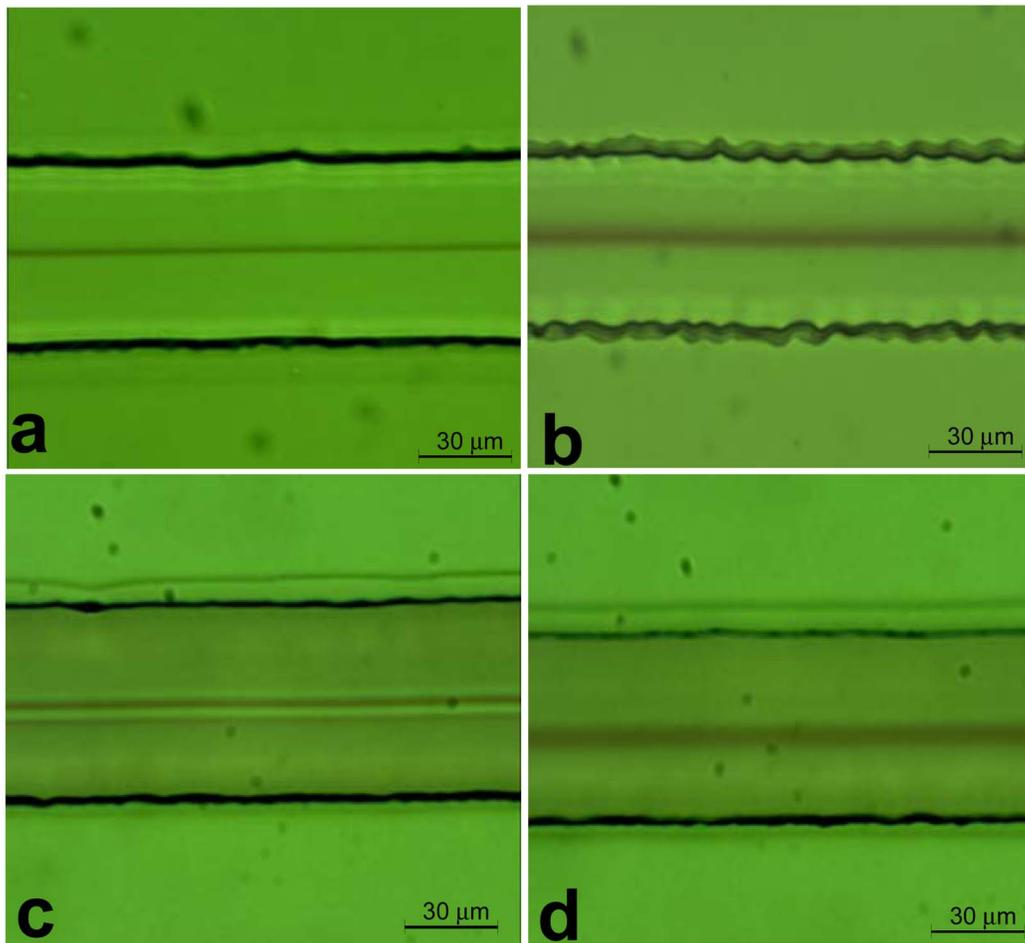


FIG. 3. Mixing of phenolphthalein ethanol solution and NaOH ethanol solution. The dimension for the main flow channel was $20\ \mu\text{m}$ (height) \times $60\ \mu\text{m}$ (width). The two fluids were as follows: (a) Unfocused (no sheath flow introduced) at external sheath flow joint; (b) unfocused (no sheath flow introduced) at a distance of 5 mm downstream from the sheath flow joint; (c) focused at the sheath flow joint; (d) focused 5 mm down stream from the sheath flow joint. Sheath fluid was colored with blue ink for better contrasts in (a) and (b).

in the other fluid. Mixing of the two fluids would result in a change in pH in the fluids and thus trigger the color change of phenolphthalein within the mixed region, and the intensity of the color can be used to estimate mixing.⁵ As shown in Fig. 3(a), a dark line, which corresponds to the pink color, appeared immediately upon contact at the interface of the two streams and became thicker only around the interface a short distance down stream along the microchannel [Fig. 3(b)], confirming a diffusion controlled mixing in the microfluidic device. The focused streams can be clearly monitored when a small amount of blue ink is added into the sheath fluid to color and differentiate it from the two unmixed streams and the mixed part, which appear brighter and darker respectively under a brightfield microscope [as seen in Fig. 3(c)]. With the completion of mixing, the brighter lines (unmixed streams) are completely replaced by the darker line [mixed part, as seen in Fig. 3(d)]. The mixing process was found irrelevant to the coarse channel wall because of the lamellar flow nature of the fluids.

To further characterize the mixing process during the focused flow, the light intensities across the cross-section of the channel along the microchannel at different locations were analyzed as shown in Fig. 4. It is clear that the mixing only took place at the interface of the two streams when the sheath flow was introduced at the beginning, as the light intensity trace featured two intensity peaks and one sharp valley in the middle, corresponding to the two unmixed streams and the

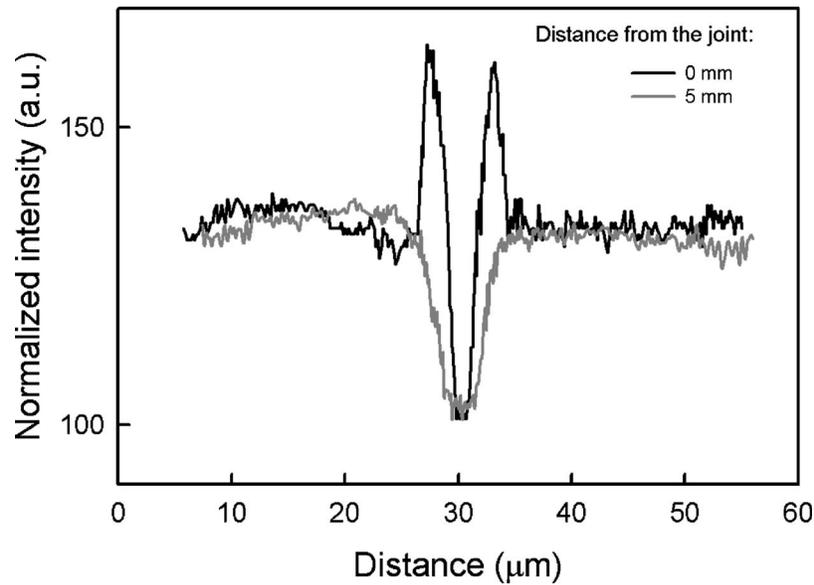


FIG. 4. Light intensity analysis across the channel using focusing-based mixing as shown in Figs. 3(c) and 3(d). The flow rate ratio of sheath flow to the main flow was at 4:1.

mixed region at the interface, respectively. In contrast, an intensity trace 5 mm downstream featured only one clear but broader valley, suggesting that a well mixed and homogeneous solution was obtained. These results agree well with results shown in both Figs. 3(c) and 3(d). Using the intensity of the sheath fluid as a baseline, we respectively integrated the areas underneath the peaks and valley to estimate the percentage of mixing as defined by an expression $[1 - S_p / (S_p + S_d)]$, where S_p is the total areas of the two peaks corresponding to the two unmixed streams, and S_d is the area of the valley corresponding to the mixed components. Figure 5 shows the estimated percentage of mixing as a function of the flow distance downstream from the sheath flow channel joint. It shows that when fluids flow through the channel, the focused streams mixed

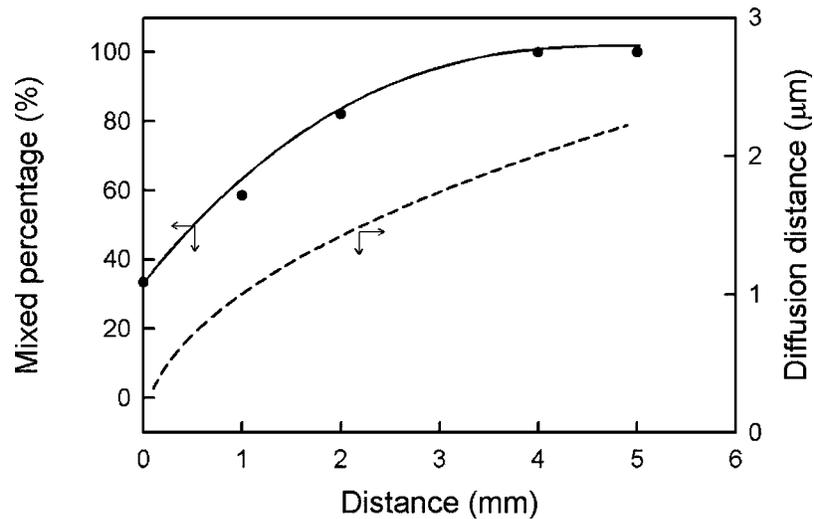


FIG. 5. Percentage of mixing in comparison with the theoretical estimation from Eq. (2). The dimension for the main flow channel was $20 \mu\text{m}(\text{height}) \times 60 \mu\text{m}(\text{width})$. Flow rates of the phenolphthalein and NaOH solutions were at $2 \mu\text{l}/\text{min}$ and the flow rate ratio of sheath flow to the main flow was at 4:1.

quickly and completed the process within only 4 mm of the channel at a flow rate of 2 $\mu\text{l}/\text{min}$ of the streams and at flow rate ratio of the sheath flow to the stream flow at 4:1 (i.e., 8 $\mu\text{l}/\text{min}$ for the sheath flow). The enhanced mixing process, however, was still controlled by passive diffusion, as suggested by the dashed line in Fig. 5 demonstrating similar trends between the enhanced mixing based on the focused fluids using experimental data and predicted passive diffusion between the fluids based on calculations from Eq. (2),

$$x = \sqrt{2Dt} = \sqrt{\frac{2DL}{V}}, \quad (2)$$

where x is the transverse distance over which a molecule with a diffusion coefficient D ($D=2.8 \times 10^{-10} \text{ m}^2/\text{s}$ for a ink diffusing in water²⁰) travels purely by passive diffusion as it flows a longitudinal distance of L at a flow rate of V ($V=2 \mu\text{l}/\text{min}$ for the experiment). As suggested by Fig. 5, the enhanced mixing was still a diffusion-controlled process involving two focused streams pushed against each other by the sheath flows, and it was not in a normal passive diffusion state with uniform pressure in every direction. Naturally, the length of the microchannel required for mixing is directly dependent on both the diffusion distance as measured by the width of the focused streams and the flow rate of the two mixing streams. However, since the width of the focused streams is a function of the flow rate ratio of the sheath flow to the stream flows, the mixing distance in the micromixer is in turn affected by the flow ratios. For instance, by increasing the flow rate ratio from 4:1 to 5:1 at a constant flow rate of 2 $\mu\text{l}/\text{min}$ for the streams or by decreasing the flow rate from 2 $\mu\text{l}/\text{min}$ to 1 $\mu\text{l}/\text{min}$ and keeping the same flow rate ratio (i.e., 4:1), the estimated microchannel length needed for complete mixing was reduced from the 4 mm to less than 3 mm. It should be noted that premixing starts right after the two fluids to be mixed merge and can affect the testing result for the focused stream (cf. Fig. 5). This process can be controlled by adjusting the corresponding channel length.

By introducing a sheath flow to substantially reduce the width of the streams (i.e., distance to diffuse by passive diffusion) to be mixed, we show that a complete mixing can be achieved within a reasonable time frame and thus with a small usage of device space (e.g., 4 mm). The process has reduced the Péclet number ($Pe=Ul/D$, where U is the average flow rate, l is the cross-sectional dimension, and D is the diffusion coefficient) from 14.9×10^3 for the unfocused two mixing streams to 3.6×10^3 for the focused streams at the flow rate ratio of 4:1 and 2.3×10^3 at 5:1, indicating the increased mixing of the focused streams. In correspondence, value $Pe \times l$, which is related to the channel distance required for mixing to occur in such an uniaxial flow,¹² changes from 450 mm to 26 mm and 11 mm, respectively, in agreement with the trend shown in Fig. 5. The focusing-based mixer is much simpler and easier to fabricate in comparison with many other mixing technologies, which, for instance, require precision fabrication, double exposure and development, or component alignment. Its mixing process can also be controlled easily and conveniently through fluid pumping since the mixing speed is largely dictated by the flow rate ratio and the flow rate of the streams, as described earlier. Channel dimension, unlike in other mixers, is not critical for the mixing since the focusing confines the mixing streams within a very narrow band and the mixing only occurs within the new boundary. As long as the width of focused streams and their flow velocity are kept at the same values, the mixing performance of this type mixer with a rectangular channel should be irrelevant to its channel dimension.

The mixer uses a third stream (e.g., sheath flow) to squeeze the two mixing streams and allow their rapid and complete mixing at various volume ratios. This is better than the reported mixer that uses one of two mixing fluids to squeeze another one,¹¹ which is only suitable to quickly mix one small-quantity fluid with a small amount of another large-quantity fluid and does not allow their complete mixing in a speeded fashion. Nevertheless, the mixer has a major drawback in throughput because it only effectively uses the focused portion of the channel width for most of their mixing, leaving the majority portion of the channel width for the sheath flows. In this study, we used a flow rate of 2 $\mu\text{l}/\text{min}$ for the two mixing streams, and a flow rate of 8 $\mu\text{l}/\text{min}$ for the sheath flow for our PDMS device, which means that only 20% of the channel was used for

transporting the mixing fluids. Here, the corresponding Reynolds number ($Re=Ul/v$, where U is the average flow rate, l is the cross-sectional dimension, and v is the kinematic viscosity of the fluid)¹² for the two mixing streams is only 0.1. While it is possible to increase the channel height to improve its throughput without scarifying its mixing speed, the mixer is still far less efficient than the mixers, such as the grooved-floor mixers, that can utilize the full width of their large channels for mixing without introducing a third fluid.^{12,13} This flow rate limitation is undesirable for the applications where high throughput is required, but it is intrinsically unavoidable for the applications where a downstream focusing is necessary after the mixer functionality. In our targeted integrated microcytometry application, for example, the sample fluid has to be dynamically focused into a very narrow stream by using sheath fluid so that cells would pass the optical detection zone in an aligned and one-by-one fashion.²¹ The good stability of the focused streams as described earlier make it possible to utilize this focusing effect for microcytometry application at a downstream location where the mixing and bioreactions are completed and the labeled cells needed to be detected. By tuning the flow rate and flow rate ratio using only one focusing process, a desired focusing effect can be easily achieved to satisfy the need for both the fast mixing and microcytometry application. The focusing achieved in Fig. 5, in fact, was tested with a consideration of meeting the need for detecting pathogenic cells in downstream. In this case, the channel height as discussed earlier, unfortunately, cannot be increased for higher throughput purpose since its geometry restriction, in combination with the focusing effect, plays a vital role in achieving the desirable cell alignment.

IV. CONCLUSION

Micromixing is an important function but a great challenge for microfluidic devices. Many technologies have been developed to speed up the mixing and most of them use complicated structures that are difficult to microfabricate. We presented a simple mixer that can be easily fabricated and controlled. The mixer was designed for use in microchip-based flow cytometry that integrates both cell labeling and optical detection in a single chip. It shares the hydrodynamic focusing with cytometry and uses the reduced fluid width to reduce the diffusion distance and to promote in-channel mixing. The mixing depends on the focusing effect, which is dictated by the flow rate ratio of the sheath fluids to the mixing fluids but independent to the device's branch angle of the sheath channel to the main channel. It is also related to the mixing fluids flow rate, which controls the diffusion time allowed within a fixed in-channel mixing distance. By increasing the flow rate ratio to improve the focusing effect or decreasing the flow rate of the mixing fluids, the mixing can be completed within a short distance within the microchannel. Using this focusing based mechanism, we show that at a flow rate of 2 $\mu\text{l}/\text{min}$ and a flow rate ratio of 4:1, the mixing of two small molecules can be completed within a distance of 4 mm in a micromixer.

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