On-chip density mixer enhanced by air chamber

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(Received 6 April 2018; accepted 26 June 2018; published online 11 July 2018)

This paper proposes an on-chip density mixer that can achieve even density in a target chamber with a swirling flow enhanced by an air chamber. The system is composed of a main channel, a target chamber where two liquids with different densities are included, an isolated air chamber, and an external vibration pump driven by a piezo actuator at the entrance of the main channel. The air chamber is expected to amplify the vibration owing to structure softening. The amplification would be more pronounced at the resonance frequencies of the structure. We developed the system and conducted experiments. We showed that the swirling motion in the target chamber with an air chamber is stronger than that without an air chamber. We also confirmed that the time resulting in even density is shorter when the pump is driven at a resonance frequency. An air-based virtual valve is introduced for maintaining a constant density in the target chamber. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5033482>

I. INTRODUCTION

Lab-on-a-chip systems have become a popular approach for cell culture and are conventionally utilized as a platform for biological research (Zhang *et al.*, 2009; Okuyama *et al.*, 2010). For example, Forry and Locascio (2011) developed an on-chip CO₂ control system and tested cell cultures with different CO₂ concentrations on a microfluidic chip. Compared with conventional cell culture in Petri dishes, on-chip cell culture has the advantages of being contamination-free and having a low sample volume and better consistency of culture environment (Inoue *et al.*, 2001; Kobel *et al.*, 2010; and McGuire *et al.*, 2009). For example, Di Carlo *et al.* (2006) developed a microfluidic array that can monitor a single cell culture on a single chip.

There are challenges to on-chip cell culture, and in this study, we particularly aim to solve the issue of uneven mixing on a chip. Figure 1(a) shows an example of concentration control of two chambers connected to the same channel. Chambers 1 and 2 have two different conditions in terms of the quantity of a representing chemical, shown in dark green. Chamber 1 has less of a chemical injection than does chamber 2, indicated by the size of the green areas shown in Fig. 1(a). After a proper duration of diffusion, the two chambers would result in even distribution, as shown in Fig. 1(b). The concentration in chamber 2 is higher than in chamber 1 owing to the difference in the initial chemical injections, as shown in Fig. 1(a). We have developed a technique to achieve this injection control (Takayama *et al.*, 2017), and here we focus on speeding up the mixing to replace the spontaneous diffusion, which often takes several minutes, or even hours, in practice.

Mixing in a microfluidic system has been a well-known issue, primarily owing to the low Reynolds number in such a confined space. In contrast, mixing in a macro-scale container, such as a glass flask, is relatively easy because of the high Reynolds number. Such a high Reynolds number enhances the efficiency of mixing by flow turbulence and vortices in the container,

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FIG. 1. Conceptual image showing why swirling flow is necessary in an on-chip chamber where (a) and (b) are just after new medicine is injected into two chambers at a different quantity and after natural diffusion, respectively.

while flows are mostly laminar in an on-chip system with a low Reynolds number. One of the approaches for mixing two liquids on a chip is to merge two liquids inside one channel by breaking the layer boundaries (Hsiao *et al.*, 2014; Lam and Li, 2012; Camesasca *et al.*, 2006; Singh *et al.*, 2008; Kang *et al.*, 2008, Lin *et al.*, 2005; and Liu *et al.*, 2008). Conventional approaches include adding bumps inside the channel or changing the shape of the channel from straight to curved. While these approaches work well, in practice, they require continuous flow and cannot achieve a localized mix, i.e., mixing liquids inside a chamber without injecting new liquids.

Figure 2 shows three types of on-chip mixers capable of achieving localized mixing. Figures 2(a)–2(c) are the actuator built-in mixer, the actuator separated mixer, and the proposed enhancer built-in mixer, respectively. With the type of built-in actuator shown in Fig. 1(a), one disadvantage is that the cost of the chip makes it too expensive to be disposable. For example, AC electroosmotic flow has been used for mixing and electrodes need to be fabricated on the chip (Sasaki *et al.*, 2006). Vibrations generated by a piezo actuator attached to the chip significantly increase the cost of the chip (Shang *et al.*, 2016). For example, with the type of separated actuator shown in Fig. 2(b), acoustic vibration by a separated voice coil motor has been utilized for mixing (Oberti *et al.*, 2009). The main issue with this type is the efficiency of mixing (Ahmed *et al.*, 2009; Franke *et al.*, 2009; and Arifin *et al.*, 2007).

Most of the vibration-induced vortices are due to volumetric fluctuations, which cause local fluid flows. For example, the volume of the circular chamber in Fig. 2(b) changes with pumping from the actuator, and such a volumetric change generates vortices for mixing. The proposed empty chambers around the circular chamber in Fig. 2(c) are expected to soften the chamber stiffness, and thus they can enhance the mixing with an increased magnitude of volumetric fluctuation. Experiments were conducted in this study to support the idea.

Experiments were conducted using microfluidic chambers with and without the proposed air chambers to evaluate the enhancement of the mixing. The same set of pumping conditions was applied to both chambers, and a clear difference between the mixing with and without the air chamber was observed. Furthermore, we found a resonance-like response at the pumping

FIG. 2. Classification of the on-chip mixers, where (a), (b), and (c) are the actuator built-in mixer, the actuator separated mixer, and the actuator separated mixer with enhancer, respectively.

frequency of 1 kHz. The performance of the mixing and the possible mechanism of the resonance-like behavior are presented.

This paper is organized as follows. After explaining the basic concepts of how to generate swirling flow in a micro chamber and the design of the chip in Sec. II, the experimental details are introduced in Sec. III. In Sec. IV, we discuss the evaluation method for swirling flow and density in the chamber. In Sec. V, we show the experimental results. After discussions of the experimental results and improvement in the system in Sec. VI, our concluding remarks are summarized in Sec. VII with an indication of future plans.

II. VIBRATION-BASED SWIRLING FLOW ENHANCED BY AIR CHAMBER

A. Neck position

The position of the neck channel plays a critical role in the swirling flow in the chamber, as shown in Fig. 3, with two examples of chip design around the corner of the main channel and circular chamber. Suppose that the pressure vibration is given at the entrance of the main channel. The key is how to transfer the kinetic energy from the flow in the main channel to the swirling motion in the chamber. The neck channels in Figs. 3(a) and 3(b) are connected to the center of the circular chamber and to the bottom part of the circular chamber, respectively. Figure 3(a) has often been used for the conventional work with Virtual Vortex Gears (VVG) (Tsai *et al.*, 2017). However, it is difficult to obtain a strong swirling motion under the neck position, as indicated by the arrows in Fig. 3(a). Meanwhile, in the case of the design shown in Fig. 3(b), a swirling flow in the circular chamber is expected, because a tangential flow along the circular chamber wall will be induced. We would note that while a pressure vibration creates a reciprocating motion of the liquid at the neck channel, it does not cause a net flow in, or out, of the circular chamber through the neck channel. This makes it possible to achieve mixing for an even density through the swirling flow without exchanging the liquid between the main channel and the circular chamber.

B. Enhancement by air chamber

To enhance the effect of the swirling flow in the circular chamber, adding an air chamber to increase the vibration amplitude near the circular chamber may be effective. We conducted preliminary experiments using both microfluidic chips with and without the air chamber to confirm the effectiveness of the air chamber on the strength of the swirling flow, as shown in Fig. 4. The design specifications without and with the air chamber are shown in Figs. 4(a) and 4(b), respectively. The design specifications are the same except for the air chamber. The design parameters besides the thickness of the wall between the circular chamber and the air chamber are determined based on VVG. As for the thickness of the wall, it should be as thin as possible to fabricate, so that we can achieve a large volume change in the circular chamber. Figure 5 shows the preliminary experimental results without and with the air chamber under three different frequencies of vibration with the same amplitude of 3 V. The captured images are taken at $t=0$ and $t=5$ s after the vibration starts, respectively. From Fig. 5, we can see that the swirling flows with the air chamber are generally faster, in terms of mixing rate, than

FIG. 3. Estimated flow patterns with different neck positions when vibration is applied, where (a) and (b) are the channel designs in conventional VVG and the proposed one in this work, respectively.

FIG. 4. Designs of the chamber, where (a) and (b) are without air chamber and with air chamber, respectively.

those without the air chamber. This result well matches what we expect in adding the air chamber. Moreover, the swirling flow speeds up the change depending on the applied frequencies. Based on this preliminary experiment, we then focus on the frequency dependency of the microchip including an air chamber as a swirling flow enhancer.

III. EXPERIMENTAL SYSTEM

A. Overview of the experimental system

Figure 6 shows an overview of the experimental setup, which is constructed by a microfluidic chip including a microchannel filled with pure water containing with microbeads of $1\ \mu\text{m}$, a digital camera mounted on a microscope, a piezo actuator (MESS-TEK: PSt 150/5/40 VS10), a piezo controller (MESS-TEK: M-2655S amplification factor $\times 15$), and a function generator (NF Corporation: WF1944B) for sending a square wave at a specified frequency to the piezo controller. The schematic of the geometrical relationship among the microfluidic channel, the syringe, and the piezo actuator is illustrated on the upper-left corner of Fig. 6.

B. Chip fabrication

The microfluidic chip used in the experiment is made of polydimethylsiloxane (PDMS) and a slide glass for the substrate. The design pattern is replicated on the PDMS chip from a mold. The ratio between PDMS and its curing agent is 9:1. The chip is cured in a 90°C oven for 45 min before bonding to the glass. The mold is made with a standard photolithography process. A silicon wafer and SU8 are used for the mold substrate and photoresist, respectively. The channel design including the air chamber is first printed on the photomask with an LED plotting machine and then patterned on the SU8 by a mask aligner.

FIG. 5. Experimental results to examine the effect of the air chamber where the amplitude of the input square wave signal to the piezo controller is 3 V.

FIG. 6. Overview of experimental setup.

IV. EVALUATION METHOD FOR SWIRLING FLOW AND DENSITY IN THE CHAMBER

A. Evaluation of swirling flow in the chamber

The evaluation of the swirling flow under different pressure frequencies is introduced as follows. The center of the vortex in the main chamber does not always exist at the center of the chamber, and the angular velocity is changing with respect to time. As a result, it is generally difficult to evaluate the effect of frequency by using both the center of rotation and angular velocity, since they are fluctuating with respect to time. To address this issue, we set up an appropriate square window in the chamber and focused on the time that beads pass through from one side to another, and we computed the instantaneous velocity with both the distance and the elapsed time at a particular time. Figure 7(a) depicts the square window, where the window is constructed by 100×100 pixels corresponding to $15.6 \times 15.6 \mu\text{m}^2$. We select the position of the square window as shown in Fig. 7(a), where the center of the window is close to the right wall, so we can expect the stream line to be close to a straight line, and we observe the motion of the micro bead clearly even under a small vortex. Figure 7(b) shows an example of visual images captured by a digital camera. Figure 7(c) shows three possible cases of bead passing. A bead passing through upper and lower edges as shown by the line β is included for data analysis, and two others as shown by the lines α and γ are removed from the data analysis. The tracking of beads is achieved with the images captured at 1000 frames/s.

B. Evaluation of density variation in the chamber

The density of micro beads in the circular chamber varies spatiotemporally. For the uniformization of spatial density, there are two factors, namely, swirling flow and the other is diffusion. As for the time factor, the diffusion is much slower than the swirling flow, while it also contributes to an even distribution of density in the chamber. Meanwhile, a uniform distribution can be achieved much more quickly in a swirling flow than that in a diffusion, since the flow in the chamber can be quickly mixed through a swirling flow. Let us now discuss how to evaluate the density in the chamber. By supposing that there is a strong correlation between the gray level of the captured visual image and density, we evaluate the density in the chamber through the analysis of the visual image of the beads moving in the circular chamber. Figure 8 shows a segmentation model of the microfluidic channel with the circular chamber divided into three

FIG. 7. Square window where (a), (b), and (c) denote the location of the square window, an example of the image captured by the camera, and three possible cases of bead passing, respectively.

regions, where regions A, B, and C are the region in the circular chamber with high concentration area after injecting fluid with beads, the remaining region in the circular chamber without any beads after injecting fluid with beads, and the region at the entrance of neck channel, respectively. The gray level in region C can be used as a reference. It is noted that the numerical values of 255 and 0 in gray levels correspond to white and black, respectively. The luminance value in region B decreases according to the swirling flow with respect to time, which is the tendency toward even density. The luminance value in region A will be eventually coincident with that in region B. If there is no flow mixing between regions A and C, the luminance value should eventually become constant.

V. EXPERIMENTAL RESULTS

A. Velocity in the chamber under various frequencies

In order to observe how the flow speed changes with respect to frequency, we measure the flow velocity in the square window in the chamber. Figure 9 shows the results where the horizontal and vertical axes denote the pressure frequency at the entrance of the main channel and the average flow speed in the square window, respectively. At every 50 Hz, we measured the

FIG. 8. Areas to analyze the density where regions A, B, and C are the region in the chamber with a high-density area after injecting fluid with the beads, the remaining region in the chamber without any beads after injecting fluid with beads, and the region at the entrance of the neck channel, respectively. For easy analysis, regions A and B are selected so that they may have the same area.

FIG. 9. Relationship between the driving frequency and the mean flow velocity in the square window.

bead speed. The average flow speed and the standard deviation are plotted by the blue filled circles and the horizontal bars, respectively. In Fig. 9, the flow speeds are mostly under $0.05 \mu\text{m/ms}$ at the frequency range under 1200 Hz. We can see two peaks, where one is the peak of $0.09 \mu\text{m/ms}$ at the frequency of 600 Hz and the other is the peak of $0.42 \mu\text{m/ms}$ at the frequency of 1000 Hz. The flow speeds of the peaks are more than 5 times greater than the average flow speed, which is in the range of 0 to 1200 Hz. These two peaks may come from the structural resonance frequency in the system including the main channel, the air chamber, and the tube connecting between the actuator and the microchip. A surprising behavior is that the flow is nearly steady at a frequency of 850 Hz. The average number of frames that it takes the beads to pass through the square window at a frequency of 1000 Hz is 37, which means that high-speed vision at 1000 fps is high enough to measure the beads' speed.

B. Visualization of the growth of swirling flow

We observed the flow pattern in the circular chamber, especially how the beads inside the circular chamber diffuse with respect to time, at three different frequencies. Figure 10 shows the flow visualization, where Figs. 10(a)–10(c) are at a frequency of 800, 1000, and 1200 Hz, respectively (Multimedia view). There are some interesting observations in comparison with the frequency characteristics as shown in Fig. 9. First, the flow velocity reached a maximum at a frequency of 1000 Hz. From Fig. 10(b), we can see that the beads cover the whole area in the chamber at $t = 10$ s under 1000 Hz, while they did not under either 800 or 1200 Hz. Meanwhile, at a frequency of 800 Hz, the flow velocity is nearly zero in the square window. From Fig. 10(a), we can see that the bead density is still far beyond a uniform distribution even at $t = 30$ s under 800 Hz. A similar tendency can be observed at under 1200 Hz as shown in Fig. 10(c), while the speed of density mixing is a bit faster than that under 800 Hz.

C. Diffusion by swirling motion

The initial state in the circular chamber at $t = 0$ is quite distorted in its density distribution pattern, where the density is extremely high at the entrance and extremely low at the outer circle band. Through the swirling motion, the density eventually shows an even distribution. Now let us evaluate the density distribution through luminance. Figure 11 shows the analytical results where Figs. 11(a)–11(c) are at the frequency of 800, 1000, and 1200 Hz, respectively. $\circ$, $\triangle$, and $\square$ denote the luminance values in the regions A, B, and C, respectively, and the dashed-dotted line denotes the average luminance value in both regions A and B, which means the average luminance in the chamber. When all lines constructed by $\circ$, $\triangle$, and $\square$ coincide with each other, it means even density in chamber. Furthermore, when all three lines coincide with a constant value, the density in the chamber becomes an even density and constant without flow-in and flow-out from the neck. Considering this nature of each line, we can say that the density distribution in the chamber becomes even at 60 s after pressure vibration under 1000 Hz

FIG. 10. Visualization of the growth of swirling flow in the chamber, where (a), (b), and (c) are the applied frequencies of 800, 1000, and 1200 Hz, respectively. The origin of time ($t=0$) is defined by the time when initial bead insertion is completed. Multimedia view: <https://doi.org/10.1063/1.5033482.1>

and at 100 s under 1200 Hz, while it takes more than 100 s until an even density is achieved under 800 Hz. From Fig. 11, we can also see another interesting observation where three lines decrease with respect to time after they coincide, which means that beads are gradually flowing into the chamber owing to the density difference between the main channel and chamber. As long as there is no valve at the entrance of the throat, such a diffusion effect will be inevitable. In Sec. VI, we consider how to keep a constant density distribution in the chamber.

VI. DISCUSSIONS

The experimental results indicate that it is possible to achieve even density in the chamber after pressure vibration at the entrance, while it is still difficult to keep the density constant with respect to time after stopping the pressure vibration. A future goal of our research is to create multiple cell culture chambers with different conditions. We would note that for a long-term cell culturing the density will become the same between the main channel and the circular chamber owing to the natural diffusion. Here, we discuss how to make a virtual valve for stopping the flow into the chamber through the neck channel. A possible approach is to inject air into the main channel. The air pushes out the liquid from the main channel and isolates the liquid in the circular chamber. If we want to exchange the liquid in the circular chamber, we can

FIG. 11. Experimental results of the change in luminance under a driving voltage of 3 V, where (a), (b), and (c) are the applied frequencies of 800, 1000, and 1200 Hz, respectively.

do it by injecting new liquid into the main channel and pushing out the air. Figure 12 shows the concept of injecting air into the main channel to isolate the chamber. Figure 13 shows the experimental results with respect to time where Figs. 13(a)–13(c) are the same process as shown in Fig. 10(b), and Fig. 13(d) is the picture after an additional step of pushing air into the main channel to choke the throat at $t = 43$ s. Figure 14 shows the change in luminance with respect to time where air is given into the main channel at $t = 24.5$ s. As we can see from Fig. 14, both luminance in regions A and B keep constant at least after the entrance of the neck channel is choked by the air-based virtual valve.

So far, we have mainly focused on how the density changes with respect to time during pressure vibration. Finally, we will focus on the flow patterns in the chamber during the pressure vibration at the entrance of the main channel. Figure 15 shows the visualization of flow patterns during pressure vibration at the frequency of 1000 Hz and the amplitude of 5 V,

FIG. 12. Concept of the air-based virtual valve.

FIG. 13. Experimental results with respect to time where (a), (b), and (c), are the same process as shown in Fig. 10(b), and (d) is the picture after an additional step for pushing air into the main channel to choke the throat at $t = 43$ s, respectively.

where Figs. 15(a) and 15(b) are instantaneous shots during flow-in and flow-out phases, respectively. We would note that the exposure time is not short enough so that we can catch the bead motion by its light trail. Because of this exposure time, we can see which beads are moving quickly, where they are going, and how the wall of the air chamber is moving depending upon the phase. From Fig. 15(a), we can see that during the flow-in phase, beads in the neck channel moves along the circumference while the beads in other areas are nearly stationary. Meanwhile, from Fig. 15(b), we can see that during the flow-out phase, the beads in the stationary area during the flow-in phase move out from the neck channel while the beads in the moving area during the flow-in phase are nearly stationary. Also, we could clearly observe in video that the

FIG. 14. Change in luminance with respect to time where air is introduced into the main channel at $t = 24.5$ to choke the throat.

FIG. 15. Visualization of stream during pressure vibration at the frequency of 1000 Hz and the amplitude of 5 V, where (a) and (b) are instantaneous shots during flow-in and flow-out phases, respectively.

wall in the boundary tends to extend during the flow-in phase, while this is the opposite during the flow-out phase; however, it is difficult to observe this in photos. We believe that this is evidence that the expansion and contraction of the chamber strongly contribute to enhancing the swirling motion in the chamber.

VII. CONCLUDING REMARKS

This paper proposes an on-chip mixer with an air chamber for mixing two different liquids in the chamber connected to the main channel. Our approach is to apply a pressure vibration at the entrance to the main channel. We showed that there is an optimum frequency for the pressure vibration. Through experiments, we found that the mixing effect can be enhanced by arranging an air chamber near the chamber. While two liquids eventually result in an even density, the density still varies with respect to time owing to the small amount of mixing between the main channel and the chamber. Moreover, for a long-term cell culturing, the density will become the same between the main channel and the circular chamber owing to the natural diffusion. To address this issue, we tried an approach of producing a virtual valve by injecting an air column and experimentally confirmed that we can maintain an even density with a constant value. This proposed approach will allow the use of an on-chip bio application in the future.

ACKNOWLEDGMENTS

We appreciate Dr. Mitsuhiro Horade for his great assistance of manufacturing the microfluidic tip. This work was supported by KAKENHI Grant Nos. JP15H05761, JP17K18854, and JP17K18759.

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