Notes

Confocal Microscopic Evaluation of Mixing Performance for Three-Dimensional Microfluidic Mixer

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We developed a confocal microscopic method for a quantitative evaluation of the mixing performance of a three-dimensional microfluidic mixer. We fabricated a microfluidic baker's transformation (MBT) mixer as a three-dimensional passive-type mixer for the efficient mixing of solutions. Although the MBT mixer is one type of ideal mixers, it is hard to evaluate its mixing performance, since the MBT mixer is based on several cycles of complicated three-dimensional microchannel structures. We applied the method developed here to evaluate the mixing of water and a fluorescein isothiocyanate (FITC; diffusion coefficient, 4.9×10^{-10} m^2 s^{-1}) solution by the MBT mixer. This method enables us to capture vertical section images for the fluid distributions of FITC and water at different three-dimensional microchannel structures of the MBT device. These images are in good agreement with those of mixing images based on numerical simulations. The mixing ratio could be calculated by the fluorescence intensity at each pixel of the vertical section image; complete mixing is recognized by a mixing ratio of more than 90%. The mixing ratios are measured at different cycles of the MBT mixer by changing the flow rate; the mixing performance is evaluated by comparisons with the mixing ratio of the straight microchannel without the MBT mixer.

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Introduction

In microfluidic-based analytical systems, the mixing of samples inside microchannels has been considered to be one of the most important tasks to accomplish well-mixed solutions in a short channel length. Mixing inside microchannels is essentially dominated by the diffusion of samples due to the low Reynolds number inside them. Mixing only by diffusion, however, is a time-consuming process, and a relatively long channel length is needed. In order to solve this inherent problem, a number of active- or passive-type micromixers have been developed.[1] Comparisons of active-type micromixers with the passive type show that the former can realize excellent mixing performance, but with some disadvantages: they are relatively difficult to integrate with other microfluidic components; their cost is relatively high from the standpoint of being a disposable device; and they need complex control units, or an external power source, such as bubble-induced actuation, magnetic stirring, or ultrasonic wave generation. On the other hand, passive-type micromixers have the advantage of integration with other microfluidic components, since they are low cost and easy to use without any external power source.

Chaotic mixing is theoretically known to be the most efficient mixing possible.[2] As a kind of chaotic mixing, we used the baker's transformation,[3] which is one of the ideal mixing methods for the efficient mixing of fluids inside a microchannel, *i.e.* a microfluidic baker's transformation (MBT). A schematic illustration of the baker's transformation processes is shown in Fig. 1. The process consists of 4 steps: fuse, fold, stretch, and cut of fluids. Compared to other chaotic mixing, such as a staggered herringbone mixer,[4] the baker's transformation can keep a constant volume, leading to a benefit that the back pressure of the device is the same as a simple straight channel.[3]

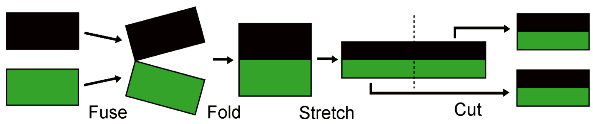

Fig. 1 Schematic illustration of baker's transformation.

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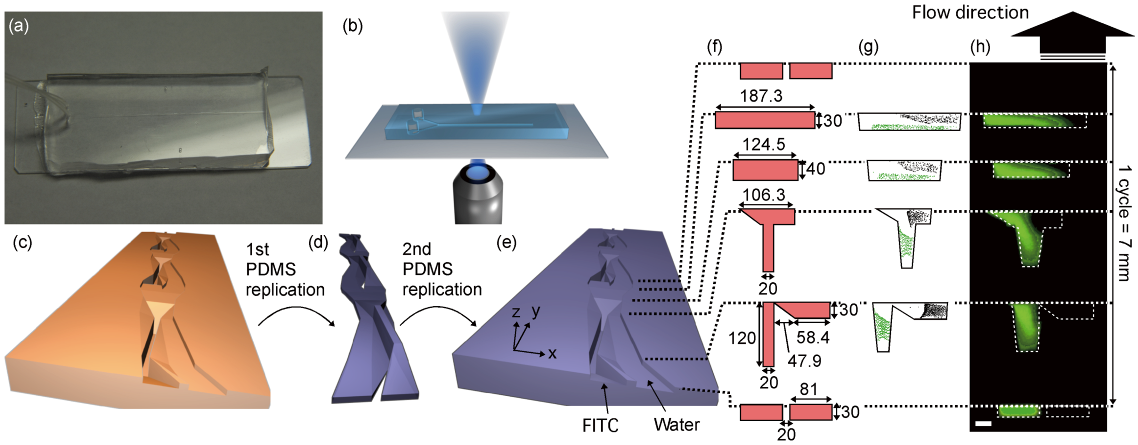

Fig. 2 Overview of the present work. (a) Photograph of the MBT mixer. (b) Schematic illustration of confocal scanning. (c) Schematic three-dimensional diagram of the mold for the MBT mixer. (d) First PDMS replication from the mold. (e) Second PDMS replication from the first PDMS mold. (f) Schematic illustrations of vertical cross sections of the MBT mixer. Numbers show the dimensions as µm. (g) Schematics of microfluidic distributions derived from a numerical simulation of the MBT mixer. (h) Confocal micrographs of vertical cross sections of a microchannel at the 1st cycle. The scale bar is 40 µm. One cycle of the MBT mixer was 7 mm. The dotted lines indicate the replication positions at the PDMS. The flow rate was 20 mm/s. The fluid direction was from bottom to up.

Using the baker's transformation, as shown in Fig. 1, we can transform fluid layers from one layer into two, and therefore the transformation of n times produces 2^n fluid layers. Consequently, the baker's transformation can shorten the diffusion length exponentially, resulting in rapid mixing. Chaotic mixing has a potential to be the most efficient mixing; however, it is utmost difficult to evaluate its mixing efficiency by the conventional fluorescence microscopy, because it has a three-dimensional mixing behavior. So far, we developed a new design concept for a passive-type three-dimensional micromixer based on baker's transformation, called the MBT mixer.[3] In the present work, we fabricated the MBT mixer and developed a confocal microscopic method to evaluate its mixing performance.

Experimental

Figure 2 shows an overview of the present work. Figure 2(a) shows the MBT mixer fabricated in this study, and Fig. 2(b) is schematic illustration of confocal scanning. To fabricate the mold on an oxygen-free cooper substrate, the basic machining process was planing, which is a cutting process.[5] We used an ultraprecision planing machine, NIC-300 (Nagase Integrex Co., Ltd.), which consists of three feed tables with double hydrostatic oil guide ways on the XYZ axes, two rotary index tables on the BC axes and a five-axis control system. A custom-ordered ultraprecision diamond cutting tool (A.L.M.T., Diamond Corp.), which had a 1-mm nose radius, was used to finish the top surface of the mold. Then, the proposed microchannels were machined with another ultraprecision diamond tool (A.L.M.T., Diamond Corp.), which had a straight cutting edge of 20 µm width (Fig. 2(c)).

PDMS (Dow Corning Inc.) and curing agent (Dow Corning Inc.) were mixed at a ratio of 10 to 1, and then the mixture was poured onto the mold, cured at 65°C for 2 h, peeled from the mold, and baked at 120°C for 30 min (Fig. 2(d)). Because the baked PDMS was sequentially used for the second mold, it was soaked in a commercial detergent solution (5%) for 5 min. Then, it was rinsed with double distilled water and dried in a vacuum chamber. After drying, the PDMS mixture was poured onto the PDMS mold, cured, and peeled from the mold as described above. Before bonding of the second replication PDMS and a commercial slide glass, access holes were punched into the PDMS, and then the PDMS and slide glass were both treated under oxygen plasma.

A numerical simulation was performed using CFD (computational fluid dynamics) software (ANSYS CFX), as shown in Fig. 2(g). The boundary conditions of the numerical simulation were: 20 mm/s flow rate for the inlets; 0 Pa static pressure for the outlets; and 0 mm/s flow rate for the channel wall (the wall was regarded as having no roughness and skidding did not occur). Green and black colors indicate two types of fluids.

A confocal microscope (FV1000, Olympus) was used to observe the mixing behavior through a focus lens of 40×/0.90 (UPLSAPO, Olympus). A fluorescein isothiocyanate (FITC; diffusion coefficient: 4.9×10^{-10} m^2 s^{-1}) solution (5.29×10^{-5} M) and water were introduced into the mixer by a syringe pump at 20 mm/s.[6] Fluorescence was detected at 500 – 600 nm by excitation using an Ar ion laser at 488 nm. Confocal images were captured at a data-acquisition rate of 0.90 frames per second. Stacks of each confocal x-y scan of 512 × 512 pixels were collected with a step of 0.5 µm in the z direction. The scan speed was 200 µs/pixel. Z-series images were loaded into the vendor's software (FV10-ASW) and made into vertical section images, and analyzed.

Results and Discussion

For quantitation of the mixing performance, we measured the fluid mixing three-dimensionally in the MBT mixer by using a confocal microscope, resulting in a quantitative evaluation of the MBT mixer. The fabricated MBT mixer is shown in Fig. 2(e). The vertical cross sections of the mixer along the y axis are illustrated in Fig. 2(f). One cycle of the mixer was 7 mm long, and there were 5 cycles in total. For comparisons between our mixing channel (the MBT mixer) and a simple straight channel, we tested the mixing of water and the FITC

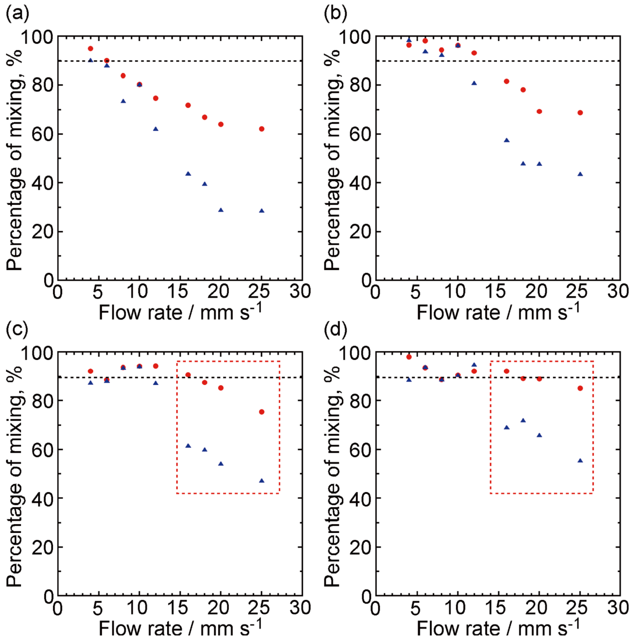

Fig. 3 Mixing ratio of the FITC solution and water *vs.* flow rate at Δy = (a) 14, (b) 21, (c) 28, and (d) 35 mm (*i.e.* after 2, 3, 4, and 5 mixing cycles), respectively. Red circles and blue triangles show the mixing in the MBT mixer and that in a straight channel, respectively. The black dashed line indicates the 90% mixing ratio.

solution. The FITC solution and water were introduced into the mixer by a syringe pump at constant flow rates.

Figure 2(g) illustrates that the fluid distributions of the cross sections showed an inclined lamination of fluids. As shown in the illustration, one of the fluids was stretched vertically, and then mixed with the other to perform the baker's transformation in the MBT mixer. Although we did not expect that the lamination of the fluids would be inclined, we could confirm that some of fluids were stacked. Therefore, we can say that our MBT design has a potential for shortening the diffusion length.

The flames in Fig. 2(h) are confocal micrographs of the vertical cross sections of a channel similar to those in Figs. 2(e) and 2(f) at the 1st cycle. Because of fast scan speed and the step size of 0.5 μm, confocal microscopic imaging revealed the detailed mixing behavior inside the three-dimensional microchannel quantitatively, and therefore we could confirm that the mixing behavior showed good agreement with the fluid distributions of numerical simulations.

The mixing ratios of the FITC solution *vs.* water are shown in Figs. 3(a) – 3(d). The mixing ratio was calculated using the following formula:[7]

$$\left(1-\sqrt{\frac{1}{N}\sum_{\mathrm{i}=1}^{N}(I_{\mathrm{i}}-I_{\mathrm{i}}^{\mathrm{perf.mix}})^2 \Big/ \frac{1}{N}\sum_{\mathrm{i}=1}^{N}(I_{\mathrm{i}}^{0}-I_{\mathrm{i}}^{\mathrm{perf.mix}})^2}\right)\times 100, \qquad (1)$$

where N, I_{i}, I_{i}^{0}, and $I_{\mathrm{i}}^{\mathrm{perf.mix}}$ are the total number of pixels, the fluorescence intensity at pixel i, the fluorescence intensity at pixel i without mixing or diffusion, and the fluorescence intensity of the completely mixed solution at pixel i, respectively. In the MBT mixer, the 90% mixing ratio, which was generally regarded as complete mixing,[1] of the FITC solution and water was attained at 6 mm/s after 2 cycles (Fig. 3(a)), 12 mm/s after 3 cycles (Fig. 3(b)), 16 mm/s after 4 cycles (Fig. 3(c)), and 20 mm/s after 5 cycles (Fig. 3(d)), respectively. For the sake of comparison in Figs. 3(a) – 3(d), we also showed the mixing ratios in the straight channel (100 (width) × 40 (depth) μm). In the straight channel, complete mixing of the FITC solution and water required slower flow rates than those in the mixer; in other words, the mixing efficiency in the MBT mixer was better than that in the straight channel.

Figure 3 clearly demonstrates that the mixing efficiency increased as the number of cycles was increased. For instance, in mixing the FITC solution and water at a 25 mm/s flow rate (the highest flow rate in this work) in the MBT mixer, the mixing ratios were 62, 68, 75, and 85% after 2, 3, 4, and 5 cycles, respectively. A comparison of the mixing ratios in the MBT mixer with those in the straight channel showed that after 2 or 3 cycles of MBT structures, the MBT mixer could not make a big difference of the mixing ratios to the straight channel, but after 4 or 5 cycles, especially in large flow rates, the MBT mixer had an advantage of mixing efficiency (about 20% differences), as indicated in the red dotted boxes of Figs. 3(c) and 3(d).

Conclusion

In conclusion, we developed an efficient method for quantitative evaluation of the mixing performance for a three-dimensional microfluidic mixer. Vertical section images of the complicated three-dimensional microchannel structures were easily captured by this method, and numerical calculations of the vertical images gave us valuable information to compare the mixing performance of the different types of mixers. We applied the confocal microscopic method to optimize the mixing performance of other MBT mixers, and found that the optimized MBT mixer enabled us to mix an FITC solution with water within 51 ms, which is 70-fold faster than mixing by using a straight channel.[3]

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