# In Situ Observation of Diffusion Mixing in a Micro-fluidic Mixer

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Abstract: Mixing of laminar two-liquid flows in micro-mixers was visualized and analyzed by mixing alkaline solution and phenolphthalein. Micro-mixers with flow paths fabricated by optical projection lithography were used. The two liquids were injected using a syringe pump from Y-shape inlets, and states of mixing were observed using an optical microscope with a high-resolution digital camera. By using above mentioned two liquids, transparent liquids were colored in red when they were mixed. For this season, the mixing was clearly visualized. Because Reynolds number of the flow was so small as 0.27-17.7, the flow became the laminar one. Accordingly, two liquids were not mixed near the junction where they were joined together. However, they were gradually mixed by diffusion during they flowed in the mixer paths. It was clarified that the mixing ratio varied depending on the flow-path shape, flow rate, and flow-path width.

## 1 INTRODUCTION

Recently, various analyses using micro-fluidic devices are proposed in chemical, bio, and medical fields (Akhtar, et al., 2015) (Inami, et al., 2009) (Pu and Liu, 2004) (Stone, et al., 2004) (Somaiyeh, et al., 2015) (Watts and Wiles, 2007). There are many advantages for using micro-fluidic devices. For example, fluids can be mixed in a narrow area, and even dangerous chemicals can be safely mixed. In addition, because the contact area per unit volume of liquids is increased, high mixing efficiency is obtained, and mixing speed is improved. For this reason, it is expected that such micro-fluidic devices are applicable to analyses of DNAs, genomes, and proteins. They will be especially useful for low-volume wide-variety manufacturing of pharmaceutical devices in the future.

In the field of bio-technology, among various micro-fluidic devices, micro-mixers are conveniently used for various analyses and diagnoses. There are two types of micro-mixers. One is a type in which external mixing energies are supplied. For example, electric fields (Harnett, et al., 2008) (Oddy, et al., 2001) or vibrations (Yang, et al., 2001) are given. Second-type devices are the ones using no external energies (Liu, et al., 2000). It is reported that mixing efficiencies are improved by contriving channel shapes (Wang and Hu, 2010) (Wang, et al., 2002) (Wang, et al., 2012).

Using external energy, efficient mixing is realized. However, the structure of mixer becomes complicated. In addition, in the case of bio-liquids, external mixing energy sometimes gives harmful influence to the liquids. On the other hand, micro-mixers without using external energies are easily used under any circumstances. In addition, because a large number of micro-fluidic devices are often used simultaneously, they had to be easily made with low costs. For this reason, simply manufacturable mixers without using external energies are preferred. Therefore, such type simple micro-mixers are investigated here.

In this research, micro-mixers were fabricated using a newly developed low-cost 1:1 projection exposure system (Morizane et al., 2014) (Morizane and Horiuchi, 2015). The system has a 20-mm square exposure field, and straight-type, snail-type, and meander-type mixers with sizes of 10×15 mm$^2$, 10×15 mm$^2$, and 15×15 mm$^2$ were fabricated. The flow paths with a width of 100 µm were directly fabricated using resist patterns with vertical side walls. The mixers were assembled by binding acrylic lid plate and vessel plate sandwiching the flow path substrates by four screws.

To evaluate the states of mixing clearly, alkaline solution and phenolphthalein are mixed, reffering to the past researches. In the first past research, a snail-type micro-mixer was fabricated in 2-mm square area using 1/19 projection lithography (Horiuchi, et al.,



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2000), and in the second past research, meander-type micro-mixer was fabricated in 6×15 mm$^2$ area using contact lithography (Horiuchi and Yoshino, 2014). In these past researches, mixing performances were observed and evaluated using colored waters. However, it was difficult to quantitatively evaluate the mixing, and clarify what parameters mainly influence the mixing. For this reason, above mentioned liquids were selected. It is considered that because the color of mixed parts are changed from no color to vivid red, they are distinguished clearly.

Two liquids are injected using a syring pump, and the mixing states are in situ observed by an optical microscope with a digital camera. As a results, mixing states are clearly visualized. Mixing ratio and main parameters influencing the mixing are investigated and discussed in detail.

## 2 FABRICATION OF MICRO-MIXERS

Micro-mixers were fabricated, by the procedure shown in Figure 1. At first, flow path patterns were fabricated on a synthetic quartz substrate using projection lithography. Negative resist SU-8 (Micro Chem) with a thickness of 100 µm was used. Next, the substrate was softly baked at 65 °C for 20 min and at 95 °C for 50 min continuously. Then, the resist was exposed to the shape of flow paths using the 1:1 projection exposure system shown in Figure 2. The sizes of the system were 300 mm wide, 600 mm deep, and 1,100 mm high. As a light source, an ultra-violet lamp with a main wavelength of 365 nm (Inflidge, UV-CURE120) was used. A macro lens for a camera (Sigma, 50 mm, F2.8 EX DG MACRO) was used as the projection lens. Numerical aperture was set to 0.089. The exposed substrate was rebaked. The post-exposure bake was done at 65 °C for 10 min and 95 °C for 30 min. After cooling down the substrate to room temperature, the resist on the substrate was developed in SU-8 developer for 10 min. By exposing the resist under appropriately defocused conditions, flow path patterns with almost vertical side walls were obtained, as shown in Figure 3.

Three types of mixers with different flow-path patterns were fabricated, as shown in Figure 4. One was a straight-type mixer with Y-shaped inlets. Second one was a snail-type mixer. Third one was a meander-type mixer. The quartz substrate with the flow path pattern was sandwiched by acrylic vessel and lid plates, and assembled to a micro-mixer by binding the four corners using M3 screws. Teflon tubes with an outer diameter of 1.6 mm and inner diameter of 0.7 mm were attached to two inlets and one outlet using an adhesive. To prevent the distortions of lid and vessel plates, and improve the sealing, a rubber sheet was inserted between the vessel plate and the substrate.

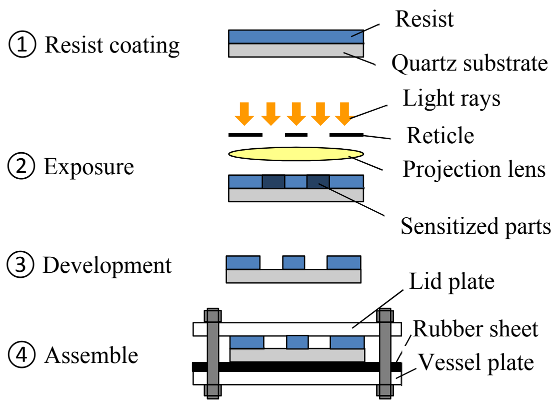



Figure 1: Fabrication method of micro-mixer.

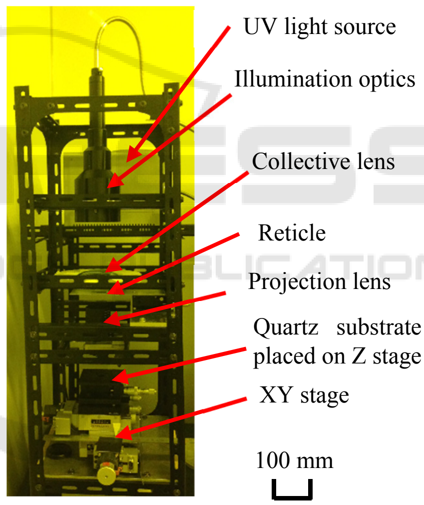
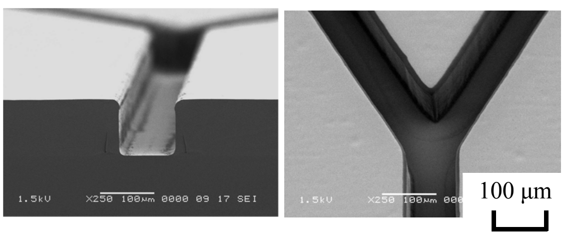
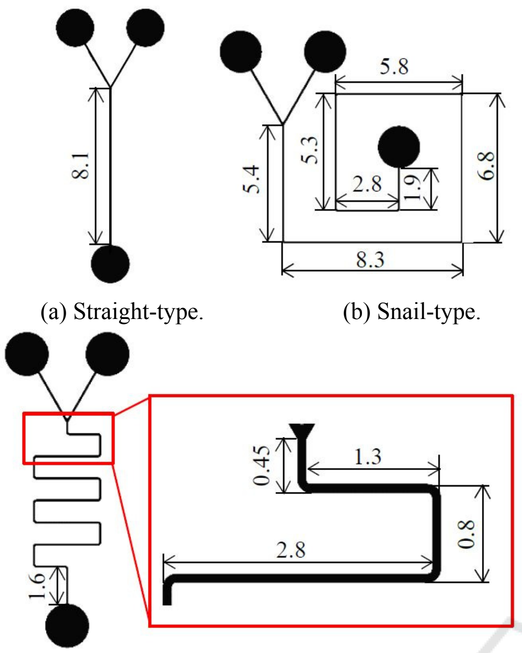



Figure 2: Exposure system used for the research.




(a) Cross section. (b) Bird's-eye view.

Figure 3: Profiles of flow-path patterns.




(a) Straight-type. (b) Snail-type.

(c) Meander-type.

Figure 4: Shapes and sizes of fabricated micro-mixers.

Assembled micro-mixer is shown in Figure 5. By grace of elasticity of the rubber sheet and SU-8, the flow paths of mixers were successfully sealed and no leaks were observed.

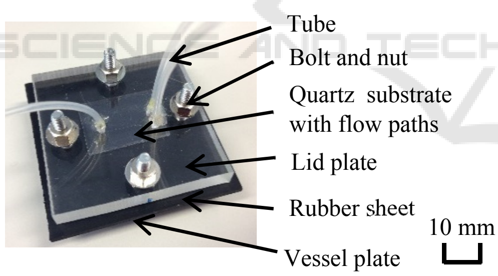



Figure 5: Assembled micro-mixer.

## 3 TWO LIQUID MIXING

### 3.1 Evaluation Method

Mixing of two liquids injected in micro-fluidic paths were in situ observed. Micro-mixers explained in section 2 were used for the experiments. The in situ observation method is shown in Figure 6. The mixers were placed one by one on the stage of optical microscope (Arms system, IMZ-20CU) with a high-resolution digital camera (Arms system, HV-20CU). The two liquids were simultaneously injected using a syringe pump (AS ONE, MSP-3D). Because all of the acrylic lid, resist flow path, and synthetic quartz substrate were transparent, and a white paper sheet was laid under the quartz substrate, liquids were observed as they were in natural colors, if they were colored. In the past researches, waters colored in advance were mixed. However, it was difficult to observe the mixing state clearly. For this reason, as a method for evaluating liquid mixing more clearly, two transparent liquids colored only when they were mixed were chosen. In concrete, strong alkali and phenolphthalein were mixed. As the alkali, 3% TMAH (Tetra methyl ammonium hydroxide) with pH of 13 (Tokyo Ohka Kogyo, PMER P-7G) was used. The phenolphthalein was dissolved in ethanol, and measured pH was 7. When they were mixed, they were colored in vivid red. According, weather they were mixed or not were clearly discriminated. Red and colorless parts were very easily distinguished. Thus, mixing of two liquids was visualized on a monitor display as the in situ image of the digital camera attached to the optical microscope.

To evaluate the mixing ratio quantitatively, the red and colorless areas were binarized to black and white. The threshold was decided by the red color of the fully mixed area. Next, the black and white ratio in the width direction of the flow paths was calculated as the mixing ratio $R_m$. The mixing ratio $R_m$ was defined by eq. (1), as shown in Figure 7, and evaluated at the points shown in Figure 8.

$$R_m = \frac{w_m}{w_f} \tag{1}$$

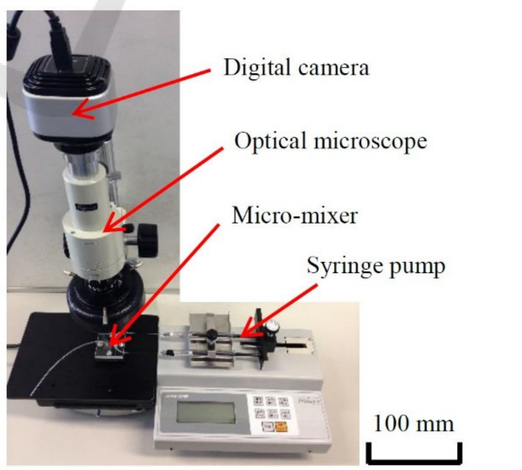



Figure 6: Set up for injecting liquids in the micro-mixer and in situ observation system.

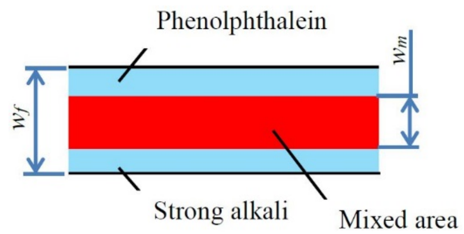
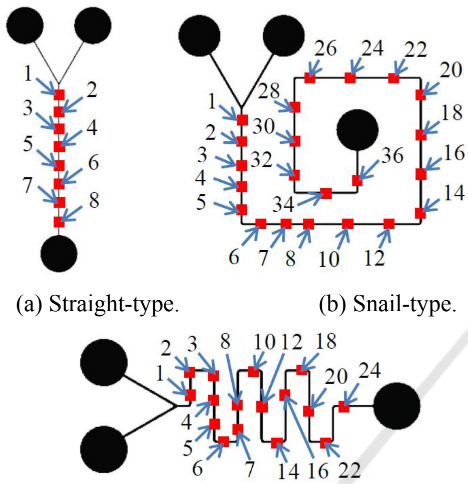



Figure 7: Figure for explaining mixing ratio $R_m$.



(a) Straight-type. (b) Snail-type.

(c) Meander-type.

Figure 8: Points where the mixing ratio $R_m$ was evaluated.

## 3.2 Mixing Ratio Dependence on Flow Rate and Flow-Path Shape

Mixing ratios were measured along each flow path by 1 or 2 mm intervals, and the results are shown in Figures 9-11. It was known that the red part widths gradually increased along the flow paths after the two liquids were joined using the Y-shape inlets. When the flow rate was changed from 0.1 (*Re*=0.27) to 6.4 (*Re*=17.7) ml/h, the mixing ratio changed almost regularly. Here, *Re* is the Reynolds number. It was clarified that the mixing ratio strongly depended on the flow rate. Because Reynolds numbers were very small, and the liquids were mixed by diffusion, the mixing ratio became large for small flow rate.

Next, by comparing the mixing ratios between the snail-type and the meander-type channels, difference of the mixing ratio was investigated. Under all the flow rate conditions of 0.1-6.4 ml/h, mixing ratios of the snail-type mixer were a little higher than that of the meander-type mixer. It was observed that the liquids in the snail-type channel were rapidly mixed after passing corners. Accordingly, it was considered that the right-angled corners like those used in the snail-type channel were effective for mixing.

A panoramic photograph of the visualized meander-type channel (flow rate=0.4) is shown in Figure 12.

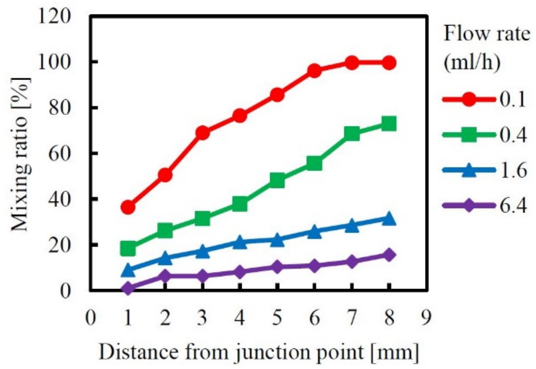



Figure 9: Mixing ratio of straight-type micro-mixer with a flow-path width of 100 µm.

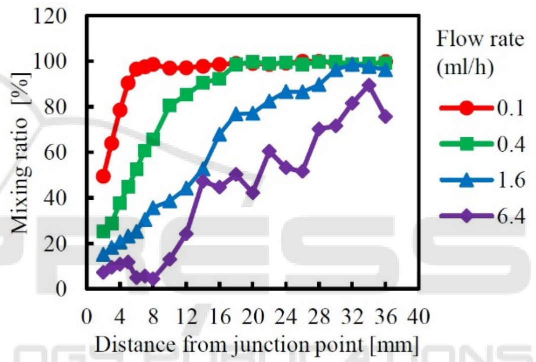



Figure 10: Mixing ratio of snail-type micro-mixer with a flow-path width of 100 µm.

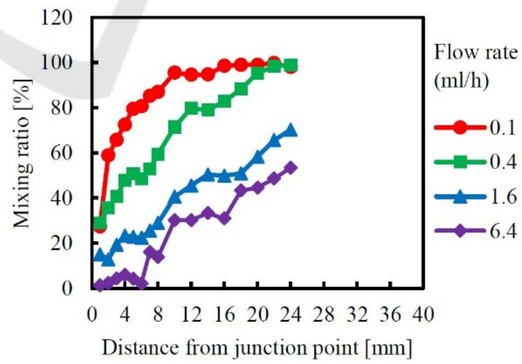



Figure 11: Mixing ratio of meander-type micro-mixer with a flow-path width of 100 µm.

Visual difference of mixed flow are shown in Figure 13. These photographs were taken at the point 3 of the snail-type mixer. It is clearly known that the mixed area or mixing ratio decrease depending on the flow rate.

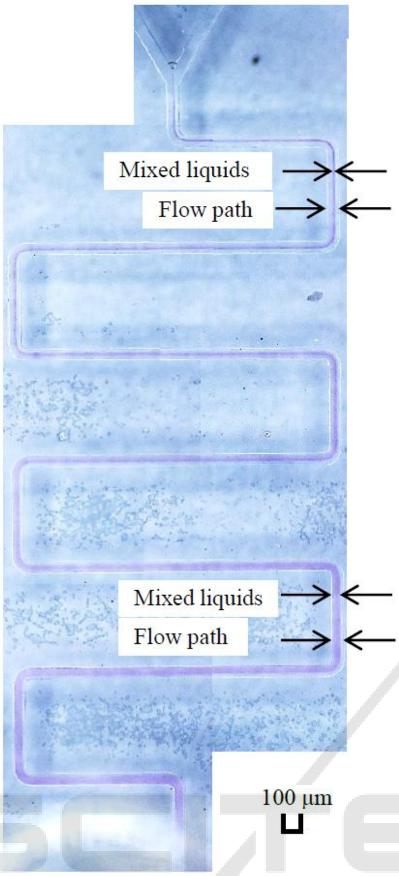



Figure 12: Panoramic photograph of meander-type mixer. It is clearly observed that the flow width of mixed liquids increases gradually from the junction point to the outlet.

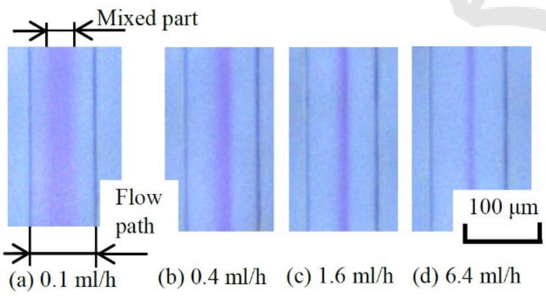



Figure 13: Visual differences of mixed flows observed at point 3 of the snail-type mixer.

### 3.3 Influence of Flow-Path Width

Mixers with a width and depth of 100 µm were used for the experiments shown in section 3.2. In this section, a straight-type mixer with a width of 50 µm and a depth of 100 µm was used, and the mixing performances were compared. Figure 14 shows the results. Comparing Figure 14 with Figure 9, it was found that the mixing ratios of the channel with a narrow flow path were higher than those with a wide flow path.

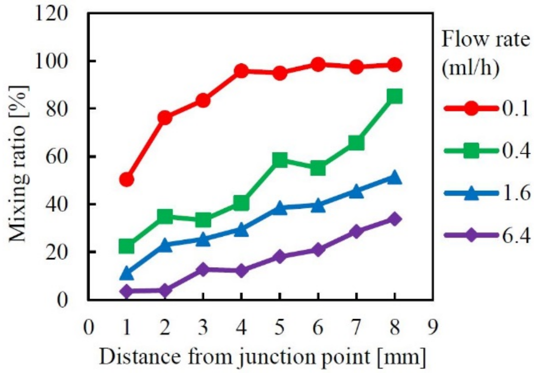



Figure 14: Mixing ratio of straight-type micro-mixer with a flow-path width of 50 µm.

## 4 DISCUSSION

Changes of mixing ratios caused by differences of flow rate and flow path were discussed. In the case of micro-fluidic devices, the width and depth of flow path are very small. For this reason, Reynolds number becomes very small, and the flow becomes laminar. In a laminar flow, mixing by turbulences of flow is not expected, and giving external forces for stirring the flow in the micro-flow path is also difficult. Therefore, molecular diffusion becomes the most probable mixing phenomenon.

In diffusion mixing, diffusion time $t$ is expressed by

$$t \approx L^2 / D \quad \text{(yamamoto, et al., 2013).} \tag{2}$$

Here, $D$ and $L$ are diffusion coefficient and length, respectively. Accordingly, $t$ is proportional to $L^2$, if the diffusion coefficient $D$ is constant. In the case of micro-mixer investigated here, diffusion length $L$ corresponds to ($w_m$/2) because the mixing of liquids expand from the flow-path center to the outside. On the other hand, diffusion time $t$ corresponds to the distance $d$ from the junction point. If the flow rate $Q$ and the cross section $A$ of flow path are constant, $t$ is calculated by the following equation because the flow velocity $v$ is obtained by $v = Q/A$.

$$t = \frac{d}{v} = \frac{d}{Q/A} = \frac{A}{Q} d. \tag{3}$$

Referring to the experimental results shown in Figures 9-14, the mixing ratio gradually saturates and approaches to 100 %, and roughly speaking, the curves seem almost parabolic, as shown in Figure 15. If the curve is parabolic, $d$ is proportional to $R_m^2$. Because $R_m$ equals to $w_m / w_f = 2L / w_f$ and $d = (Q/A)$

$t$, $t$ is proportional to $L^2$. Therefore, it is thought that the experimental results have a tendency approximately agreed with eq. (2).

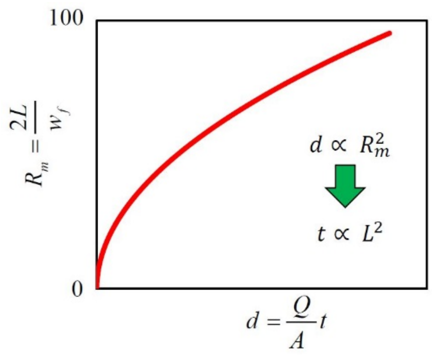



Figure 15: Almost parabolic relationship between the mixing ratio $R_m$ and the distance $d$ from the junction point. If the curve is parabolic, $t$ is proportional to $L^2$.

## 5 CONCLUSIONS

Micro-fluidic mixer patterns were formed on quartz substrates using 1:1 optical projection lithography system, and three types of micro-mixers were fabricated sandwiching the quartz substrates by acrylic lid and vessel plates. Mixing of two liquids were investigated by injecting strong alkali and phenolphthalein solutions simultaneously from Y-shape inlets. The state of mixing was in situ observed on a monitor display using an optical microscope with a high-resolution digital camera. The mixing was clearly visualized caused by the chemical color change from no color to red. Because the flow width colored in red gradually increased along the flow paths, mixing ratio was measured as the colored width devided by the full width of the flow path.

Then, the mixing ratio dependence on flow rate and flow path was clarified. The mixing ratio increased when flow rate was decreased, and narrow flow-path was used. It was demonstrated that the new in situ observation method was effective to clarify the diffusion mixing in micro-fluidic mixers. It was also found that right-angled corners of flow paths were effective for advancing the mixing of liquids.

In this research, flow path depth was fixed to 100 μm. It is considered that the mixing is also influenced by the flow path depth or the aspect ratio of the flow-path cross section. It is necessary to investigate hereafter.

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