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Passive micromixer using by convection and surface tension effects with air-liquid interface

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Abstract

This article describes a passive micromixer that utilizes an air-liquid interface and surface tension effects to enhance fluid mixing via convection and Marangoni effects. Performance of the microfluidic component is tested within a passive-pumping-based device that consists of three microchannels connected in succession using passive micro-mixers. Mixing was quantified at 5 key points along the length of the device using microscope images of patterned streams of Alexa 488 fluorescent-dyed water and pure DI water flowing through the device. The passive micro-mixer mixed fluid 15–20 times more effectively than diffusion between laminar flow streams alone and is a novel micro-mixer embodiment that provides an additional strategy for removing external components from microscale devices for simpler, autonomous operation.

Keywords

Passive micromixer; Surface tension; Convection and Marangoni effect; Autonomous operation

Introduction

Microscale devices often rely on diffusion to mix reagents due to extremely short length scales; however, there are many microscale applications which require more rapid and efficient means of mixing. The fundamental challenge in the design of microscale mixers (micromixers) is to overcome the nearly reversible nature of low Reynolds number flow and is the subject of a significant area of research in microfluidics^{1,2,3}. In general, micromixers can be classified as passive or active mixers depending on whether an external energy source is applied^{4,5}. Examples of passive micromixers include 3D or multi-vortex mixing^{6,7}, lamination mixing^{8,9}, rotation and injection mixing^{10,11}, chaotic advection¹² and droplet-based mixing^{13,14}. Most passive micromixers have the advantage of low cost, ease of fabrication, flexibility for integration with other microfluidic systems, and eliminate the

need for complex control systems or equipment to supply an external force for mixing besides the fluid pumping mechanism itself (e.g. a syringe pump).^{15,16}

We propose and demonstrate a micromixer design which passively enhances fluid mixing using the open air-liquid interface of a droplet. As fluid enters the droplet, the open air-liquid interface promotes rotational and Marangoni flows to enhance mixing. The passive micromixer design prolongs this mixing process by allowing fluid to temporarily accumulate before being released downstream. The micromixer design has no moving parts and is constructed using standard lithography techniques. Thus, the micromixer can passively produce 3D mixing with a wide range of microchannel designs, is easy to fabricate, and eliminates potentially complicated peripherals for inducing mixing. This new passive micro-mixing embodiment is demonstrated using passive pumping but applies to other methods of pumping as well. Microscopy and fluorescent dye experiments are used to quantify and compare the performance of the micromixer to that of diffusive mixing within microchannel conduits^{12,17}.

Materials and Method

Mixing principle

Previous treatments of mixing in droplets suggest two primary phenomena are the source of the enhanced mixing within the droplet, 3D convection and Marangoni effects. As fluid enters the droplet, the free surface allows the fluid to create complicated patterns, rotating and folding on itself in 3D to reduce the overall diffusion distance for the two fluids to mix. Given the characteristic time for a solute with diffusion coefficient D to diffuse a distance L is given by $L^2/(2D)$, reductions in the diffusion distance, L , can have a dramatic impact on mixing efficiency and times. Marangoni forces arise in a droplet when different parts of the free-surface have different surface-tension. A surface tension gradient can be caused by many different factors. For a mixing droplet, the two most prominent causes of surface tension gradients are evaporation and inherent differences in surface tension between the two fluids that are mixing. As the fluid with one surface-tension enters and reaches or diffuses to different parts of the droplet surface, surface-tension-gradients result in surface-driven-flows that further aid the complex flow patterns that reduce overall diffusion distances for complete mixing. The mixing effects of convection and Marangoni flows are illustrated in many other examples of droplet mixing^{13,14}. The micromixer design further promotes thorough mixing by giving more time for droplet mixing to occur. Flow from one channel to the next through the micromixer is delayed while fluid is allowed to accumulate in the droplet before being released to the next channel. Thus, flow through the device is saltatory, occurring in stages rather than continuously, but does not require any moving components. Thus, the approach used here leverages previously identified phenomena for enhancing mixing in a novel, passive embodiment that can be used to connect microchannels and enhance fluid mixing.

Device design and fabrication

Fig 1 shows a 3D schematic diagram of the device used to characterize the efficacy of the passive micro-mixing strategy. The device was fabricated in poly (dimethylsiloxane)

(PDMS, Sylgard 184 Silicon Elastomer, Dow Corning, USA) from masters prepared by soft lithography using SU8-100 photoresist (MicroChem Corp., Newton, MA)^{18,19}. Briefly, two layers of SU8-100 were spun and exposed individually and developed to generate a mold with fluidic microchannels that are 200 μm high and ports that extend the full depth of the PDMS device (500 μm) on 3 inch silicon wafers. The uncured mixture of PDMS was poured over the SU-8 master and sandwiched between transparency film using weights cushioned with rubber sheets to allow direct molding of the ports into the device²⁰. The device is placed on polystyrene dishes (Omni-Tray, NUNC) and has three channels configured into a T-shape with an overall size of 30 (width) $\times$ 40 (length) $\times$ 0.5 (height) [mm]. The three channels are arranged in sequence with a passive micromixer between each. The passive micromixer consists of a mixing zone (red circle) with adjacent input and output ports as shown in Figure 1. Figure 1(b) provides a more detailed view of the mixing zone and how the droplet, upon accumulation of fluid, connects the two ports. Although the channels of this device were in the same plane (i.e., planar or 2D), the air-liquid interface of the droplet protrudes above the device in the mixing zones to enable 3-dimensional (3D) convection for enhanced mixing. Setup of the device and passive operation of micromixers are described in the experimental procedures.

Experimental procedures

The surface-tension-based passive pumping device was designed to demonstrate and characterize micromixer operation²¹. Setup and operation of the device was as follows (Fig 2). 1) The channels were filled with DI water with an additional droplet of DI water placed at each output port to prepare the device for operation: $50 \pm 10 \mu\text{L}$ at the output port of channel 1, $100 \pm 10 \mu\text{L}$ at the output port of channel 2 and $300 \pm 20 \mu\text{L}$ at the output port of channel 3. These volumes were chosen to ensure sequential passive pumping (i.e., pumping from smaller droplets to larger droplets). 2) To begin to device operation, a 15 μL droplet of fluorescence dyed water (Alexa 488, 1 $\mu\text{g}/\text{ml}$ in DI water, Invitrogen) and a 15 μL droplet of DI water were dispensed simultaneously at the two input ports of channel 1 as shown in Figure 2(a). The T-shape of channel 1 resulted in side-by-side flow of the fluorescently dyed water and DI water to the output port of channel 1 where the temporary droplet reservoir aided mixing and grew in size until it overlapped with the fluid in the input port of the channel 2, creating a connection between channel 1 and channel 2 as shown in Figure 2(b). Upon connecting, the fluid that had previously built up in the mixing zone passively pumped to the output port of channel 2. 4) In a similar way, the fluid of channel 2 accumulated in the output droplet, causing it to grow and cover the mixing zone until it overlapped with the fluid in the input port of channel 3, creating a connection between the channel 2 and the channel 3 as shown in Figure 2(c). Upon connecting, the fluid that had previously built up in the mixing zone passively pumped to the output port of channel 3. Thus, fluid that was initially unmixed passed through three straight channels and two micromixers in a saltatory fashion before it reached the final output of the device (Fig 2d). By accumulating fluid in a droplet with an air-liquid interface before flowing into the next channel, the micromixer allows time for convection and Marangoni effects to enhance mixing before moving to the next channel. The three channel device allowed mixing to be quantified immediately upstream and downstream of each micromixer in order to compare micro-mixing with the diffusive mixing that occurs during transport within the microchannels.

Quantification of mixing efficacy

Microscopy images are used to detect the spatial distribution of water and fluorescently labeled fluid. The microscope provides a top-view of the device; thus, the method is primarily sensitive to planar (x-y) inhomogeneity in the distribution of the fluids as opposed to vertical (z) inhomogeneities. However, given the aspect ratio of the channels, the x-y distribution of the fluids adequately represents the overall mixing of the fluids. Images were taken at 5 locations along the length of the device and analyzed within the channel region (Fig 3). After flat-field correction and background subtraction, the intensity registered at each pixel is linearly proportional to the number of fluorophores within the volume element of each pixel. Flat-field correction was done according to Eq. 1 where CI, OI, DF and FF represent the corrected, original, dark-field and flat-field images, respectively.

$$CI = (OI - DF) / (FF - DF) \quad (1)$$

The degree of mixing, ‘% Mixing’, was quantified in the microscopy images within the channel region based on the average deviation of each pixel (X_i) from the average pixel intensity (MEAN) within the channel region (see Eq. 2). The average deviation, d , was normalized by the mean fluorescence in the region to produce a value similar to a coefficient of variation ($CV = \sigma/\mu$) where d is used in place of σ . Thus, when fluorescence is uniform (i.e., the streams are completely mixed) the average deviation from the mean is 0 while two perfectly segregated streams would produce a value of 1. Eq. (3) is used to convert d to % mixing. The analyzed regions are indicated in Fig 3. The average number of pixels analyzed at each location varied due to channel geometry but averaged ~200,000 pixels. The mean and average deviation was measured using JEX (<http://sourceforge.net/projects/jextools/>), an open source batch image processing software that utilizes algorithms from ImageJ (<http://rsbweb.nih.gov/ij/>).

$$d = \frac{1}{n} \sum_{i=1}^n |X_i - MEAN| \quad (2)$$

$$\%Mixing = 100\% \times \left(1 - \frac{d}{MEAN}\right) \quad (3)$$

Normalization of the average deviation by the mean is necessary to account for dilution at each micromixer. Droplets are pre-staged at each output port to ensure sequential passive pumping but result in dilution of the fluorescent dye as it flows through the device. This approach provides a consistent means for calculating % Mixing regardless of dilution.

Image acquisition and statistical analysis

Fluorescence images were acquired on an inverted fluorescence microscope (IX70, Olympus) using a SPOT RT monochrome digital camera (Diagnostic Instrument, Inc.). Data from the different locations were compared using a student’s t-test and performed using the R software package (<http://www.r-project.org/>). The individual data points and code used to analyze the data is available in the Supplemental Information 1.

Results

Passive micromixer performance was characterized in a passive-pumping-based device using fluorescently dyed water and pure DI water. Microscopy was used to characterize the spatial distributions of the mixing fluids at 5 key points along the length of the device (Fig 3, Supplemental Information 1). It took approximately 2 minutes from the addition of fluid at channel 1 until fluid ceased to accumulate at the output of channel 3 with an estimated Reynolds number of less than 10 in each channel. The fluorescent and non-fluorescent streams were initially separated within channel 1. The % Mixing increased slightly from 20.83% to 25.08% between locations 1 and 2 ($p = 0.085$) due to diffusion-based mixing of the two streams (Fig 3b & 3c)²². The streams then passed through the micromixer, mixing with the pre-staged water droplet at the channel output, increasing the droplet size until it connected with the input of channel 2, allowing it to flow to location 3. The micromixer significantly increased the mixing from 25% to 85% between locations 2 and 3 ($p = 1.2791 \times 10^{-5}$). Diffusion then appeared to increase mixing slightly from 85% to 88% in channel 2 from location 3 to 4 ($p = 0.2379$). The mixed fluid of channel 2 then enters the pre-staged water droplet of the second micromixer. The droplet size increased as fluid from channel entered and mixed in the droplet until it connected with channel 3, allowing it to flow to location 5. The % Mixing was measured to be 76% at location 5, which is less than the 85% at the output of the first micromixer (location 3 vs 5, $p = 0.09$).

The results illustrate the different types of mixing and steps that occur as fluid flows through the device. First, diffusive mixing occurs as fluid flows in a laminar fashion through each channel. Although the streams of fluid from the channel mix with each other as they enter the pre-staged droplet, the streams must also mix with the volume of the pre-staged droplet itself. Thus, the % Mixing at the output of the micromixers is not just a measure of the ability to mix two parallel streams, but the ability of the mixer to mix with the pre-staged droplets (i.e., mix a dispensed drop of one fluid with a previously dispensed volume of another using passive-pumping). The time during which the fluid accumulates at the micromixer provides time for mixing to occur with the pre-staged droplet before moving to the next channel.

Discussion

The study concisely demonstrates the ability to leverage air-liquid interfaces for passive micro-mixing in flow-based devices as well as some of the important parameters to consider when implemented within a passive-pumping-based device. The use of segregated streams entering the first mixer illustrates that fluid does not pass through the mixer in a simple parallel, laminar fashion (i.e. remaining unmixed and simply passing through the fluid junction between the channels). The first micromixer was able to mix the segregated streams of fluorescent and non-fluorescent fluid (initially 25% mixed) with a 50 μL droplet of water and increase mixing to 85%. The second micromixer was able to mix a largely well-mixed fluorescent stream (88%) with a 100 μL water droplet and achieve 76% mixing. These two scenarios illustrate the ability to mix small volumes of fluid ($\sim 15 \mu\text{L}$) of one type with a significantly larger droplet of another type (50 or 100 μL) and achieve high levels of mixing (76% – 85%) via passive methods. Further, the increase in % Mixing achieved through the

first micromixer ($85\% - 25\% = 60\%$) was significantly larger than the increase observed for diffusion-based mixing in both channel 1 ($25\% - 21\% = 4\%$) and 2 ($88\% - 85\% = 3\%$) ($p = 0.0006$). Thus, mixing was enhanced by 15–20 times compared to that which was achieved through mixing during laminar flow in the microchannels. This result illustrates that the ability of the free surface and geometry of the droplet to facilitate and enhance mixing relative to diffusion during laminar flow alone. The reduced level of mixing achieved at the output of the second micromixer compared to the first illustrates the increased difficulty of mixing larger droplets via this passive method and should be taken into consideration during device and process design. However, despite increasing the volume 2-fold from 50 to 100 μL , the % Mixing of the output was not dramatically affected (76% vs 85% , $p = 0.09$). A further consideration of the approach is that Marangoni effects can be affected by factors such as temperature, humidity, droplet geometry, and fluid properties. Thus, micromixer performance will be somewhat dependent upon environmental conditions.

Conclusions

In this study, a passive micromixer was successfully designed, fabricated, and implemented in a passive-pumping-based device. Performance of the micromixer was evaluated using fluorescence microscopy and results demonstrated the ability of the method to increase mixing significantly more than diffusion-based mixing of parallel streams in laminar flow within a microchannel (60% vs $3\text{--}4\%$, $p = 0.0006$). The micromixer relies on saltatory flow and an air-liquid interface to passively enhance mixing of two fluid streams convection and Marangoni effects and is a novel micromixer embodiment applied to a passive-pumping-based device, providing an additional strategy for removing external components from microscale devices for simpler, autonomous operation.

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Figure 1.

(a) Illustration of a passive micromixer. The microchannel input output ports (OP_n) are labeled IP_n and OP_n , respectively, where n is a number of the microchannel. Mixing zones are indicated by a red circle. (a-insert) A photograph of the passive micromixer that is filled with dye water. (b) Schematic of mixing zone between an output port and the input port. The inside line (blue-dashed) indicates the droplet size before pumping (i.e., a prestaged droplet), and the outside line (red-dashed) indicates the droplet size after pumping. The droplet size grows until it connects with the input of the next channel and flows downstream.

Figure 2.
Illustration of sequential pumping and micromixer operation

Figure 3. Microscopy images of micromixer performance. (a) Schematic of device and imaging locations for micromixer characterization. (b–f) Microscope images show typical spatial distributions of fluorescent and non-fluorescent fluid at the 5 key measurement points in the device (top view).

Figure 4. Plot of % Mixing for each of 5 key measurement locations along the micromixer. Error bars are $\pm$ SD.