

A microfluidic platform for parallel synchronization of multiple droplets by ladder-like fluidic network

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ABSTRACT

We propose a microfluidic platform where a dilution module to generate concentration gradient and a ladder-like fluidic network for a parallel synchronization of a droplet were integrated. The device consists of two layers: upper layer is for oil phase and lower layer for water phase. The desired concentration at the lower layer was achieved by means of controlled volumetric mixing ratios of two merging solution in a T-junction. By controlling channel length, fluidic resistances were adjusted. The water-in-oil droplets generated at lower layer were injected to the synchronization region where a top, middle, and bottom channel were interconnected among the three main channels. By a pressure difference caused from the droplets among main channels, the oil, carrier fluid, flows through the interconnection channels until the pressure in each channel was balanced automatically. The proposed device showed the feasibility of the synchronization of three droplets with 92% efficiency at the optimized flow rate condition. As the device enables passive synchronization of droplets with different concentrations, it has a potential to be integrated with a conventional droplet-based microfluidic platform for a reliability of mixing.

Keywords: droplet, synchronization, dilution

1 INTRODUCTION

Droplet-based microfluidic device can be considered as a promising platform for high-throughput applications in biological and chemical studies [1-5]. Unlike the continuous flow system, each droplet of water can be surrounded by immiscible carrier oil playing an important role in eliminating cross-contamination between droplets [6]. This approach allows all molecular reactions to be confined to the volume of a single droplet as a self-contained microreactor [7-8]. Moreover, it provides a strong reduction in reagent volume and reaction time because the dimensional scaling effect allows for rapid heat and mass transfer in droplets [9].

One of the key unit functions in droplet-based microfluidics is to mix two or more reagents within droplet for performing reactions. In order to merge different reagent, it is necessary to precisely manipulate droplets in space and time [10]. For example, one droplet should be

close enough to touch the other droplet. Otherwise, it is possible to generate droplets containing multiple reagents by co-infusing several fluid streams at the location where droplets form [11-12]. However, the configuration is restricted by reactions between the interfaces of streams. Sometimes, the pre-formed droplets are necessary to incubate reagents until reaction conditions are ready. In addition, when performing biological reactions in droplet-based microfluidic devices, it requires multiple steps with new reagents added at each step. Many research groups have published how to merge the droplet with various methods. As a active method, Gupta *et al.* demonstrated a droplet synchronization by using impedance change across a pair of electrodes [6]. It provides on demand droplet generation by switching on and off electrically. As a passive method, Praksh et al. have showed air bubble synchronization by using a ladder structure where the bubbles caused a pressure difference between two main channels, leading to velocity gradient of bubbles [13]. As a result, they could achieve paired bubbles in the main channels.

We present a method for a parallel synchronization of multiple droplets with different concentrations without external equipment for the synchronization in a continuous flow manner. Our previous device [14](e.g., a synchronized droplet pairing ($n=2$) using a ladder-like fluidic network) has modified for a synchronization of three droplets ($n=3$) with different concentrations where concentration gradient generator is integrated. In order to obtain the optimized condition of synchronization, a flow rate ratio (Q_{oil}/Q_{water}) was investigated from 2 to 8. For the quantitative evaluation of concentration generator, aqueous fluorescent dye was used and measured by captured images. We expect that this proposed method will be useful for many droplet-based microfluidic applications. Especially, the fusion of the droplets may easily be integrated by depositing electrodes at the end of channel in the proposed device.

2 WORKING PRINCIPLE

Our device consists of a dilution module to mix a fluorescence dye stream and water stream, a hydrodynamic flow focusing structure to generate water-in-oil droplets, and a ladder-like channel network for the synchronization of multiple droplets (**Fig. 1**). The lower layer includes the dilution module and the three flow focusing junctions for a

Table 1 Flow rate condition to measure the length of droplet and generation frequency.

Flow rate condition	A		B		C		D		E		F		G	
	inlet1	inlet2	inlet1	inlet2	inlet1	inlet2	inlet1	inlet2	inlet1	inlet2	inlet1	inlet2	inlet1	inlet2
Flow rate of water ($\mu\text{l/h}$)	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Flow rate of oil ($\mu\text{l/h}$)	inlet3		inlet3		inlet3		inlet3		inlet3		inlet3		inlet3	
	200		300		400		500		600		700		800	

droplet generation. The dilution module was based on the well-known analogy between microfluidic circuits and electrical circuits. The pressure drop corresponds to a voltage drop, the flow rate to an electrical current, and the flow resistance to an electrical resistance. The hydrodynamic resistance of the microfluidic channel network depends on the channel length with the fact that the cross-sections of all channels are identical [15]. Therefore, a microfluidic network can be analyzed through simple electric circuit analysis. Based on the analysis, the length of all channels was determined ($R_0, R_1, R_2, R_4 = 17$ mm, $R_3 = 8.5$ mm) as shown in **Fig. 1(a)**.

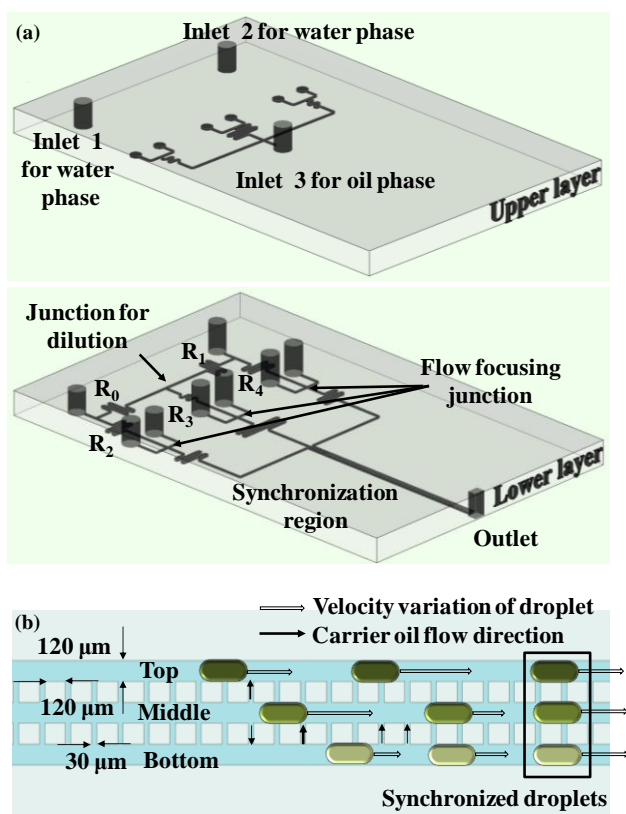


Figure 1: (a) Schematic illustration of the proposed device that can perform the three droplet generations with different concentrations and synchronization. (b) Working principle of synchronization.

To evaluate dilution module aqueous fluorescent dye was injected into inlet 1 and distilled water into inlet 2. As a result, the different concentrations (0%, 50%, 100%) of solution were produced at the flow focusing junction for droplet generation.

The upper layer was used to inject an oil flow to the flow focusing junctions. Once the droplets are generated from the junctions, they enter the ladder structure to be synchronized by a cross-flow of the oil through interconnection channels due to a pressure difference caused by the droplets. They maintain the synchronized position until they meet unmatched droplets (see details in reference [14]).

3 METHOD AND MATERIALS

The device was fabricated by using conventional soft-lithography techniques to form microfluidic channels on a wafer. The 3 inch silicon wafer (University wafers, South Boston, MA, USA) was used as a substrate. To increase the adhesion between SU-8 and the surface of wafer, the wafer was submerged into BHF (buffered hydrofluoric acid) at room temperature for 5 min to remove a thin oxide layer. Afterwards, it was rinsed with acetone, methanol and distilled water. The cleaned wafer was placed on a hot plate at 120 $^{\circ}\text{C}$ for 5 min for complete dehydration. The SU-8 (SU-8 2050, Micro-Chem Corp, Newton, MA, USA) was then spin coated with target thickness (e.g., 50 μm) on the cleaned wafer using a spin processor (WS-650Mz NPP from Laurell Technologies, North Wales, PA, USA). After spin coating process, soft bake, UV exposure, post exposure bake, and development were performed sequentially. The measured thickness of SU-8 pattern was 49 ± 2 μm . A prepolymer of PDMS (Sylgard 184, Dow Corning) and curing agent was thoroughly mixed at a ratio of 10 : 1 (wt/wt). The mixed PDMS was degassed in a vacuum chamber to remove air-bubbles for 20 min. To easily peel off the PDMS from the SU-8 pattern, hexamethyldisilazane (Sigma Aldrich, Saint Louis, MO, USA) was silanized on the SU-8 master in the vacuum chamber at room temperature for 30 min. The PDMS was carefully poured onto the SU-8 master mold and cured at 65 $^{\circ}\text{C}$ for 30 min. The PDMS replica was punched for inlets and outlets and then, the two layers were bonded irreversibly by exposing O_2 plasma on the surface of PDMS. Distilled water, aqueous fluorescein sodium salt (1 mg/mL in water) was used as a water phase and HFE-7500 oil (3M) was used as a oil phase. The ammonium carboxylate of Krytox 157 FSL (Dupont) was added to the oil at 2% by weight as a surfactant.

4 RESULT/DISCUSSION

Our device was tested by varying the flow rates of oil and water as listed in **Table 1**. The flow rate for the water phase was fixed to 50 $\mu\text{l/h}$ and the flow rate for the oil

phase was varied from 200 to 800 $\mu\text{l/h}$ in a step-wise manner. By controlling the flow rate ratio ($Q_{\text{oil}}/Q_{\text{water}}$), we could achieve two different cases in terms of the difference in the droplet size and the generation frequency. As expected, as the oil flow rate (Q_{water}) increased, the size of

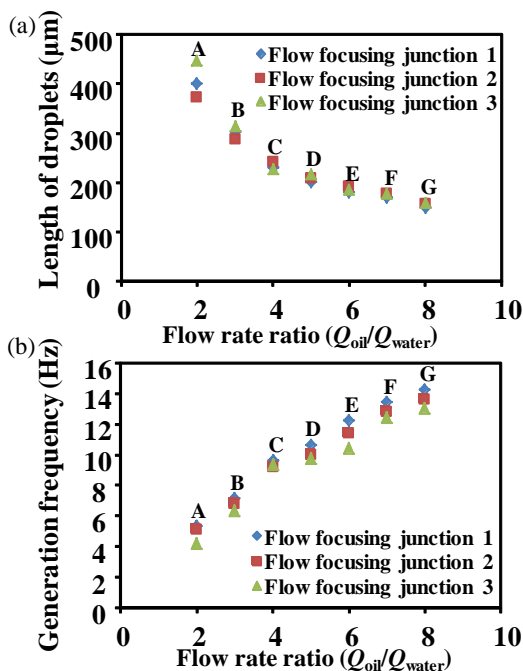


Figure 2: (a) The length of droplets versus flow rate ratio. As flow rate ratio increased, the length of droplet increased. (b) The generation frequency versus flow rate ratio. From each junction, the variation of generation frequency was small.

droplets decreased from 407.54 to 155.87 μm and the generation frequency increased from 4.87 to 13.60 Hz on average as shown in **Fig. 2(a)**. It is critical to control the size of droplets in this device. The size of the generated droplets must be larger than that of the main flow channel to allow strong cross-interaction between the channels. If the droplet size is smaller than that of the channel, the carrier oil can flow across the small droplets, causing weak cross-interaction. For this reason, we investigated the size of droplets as a function of flow rate ratio. The droplet generation frequency difference (**Fig. 2(b)**) is also important parameter for the synchronization because excess droplets make it difficult to be a stable synchronization process [14]. For instance, the case C showed the relatively uniform generation frequency, resulting in 92.1% synchronization efficiency (**Fig. 3**). The synchronization efficiency is defined by the ratio of the number of synchronized droplets and the total number of generated droplets. **Fig. 4(a)** represents the dilution result by measuring fluorescence intensity from the synchronized droplets. All fluorescence images were captured with

fluorescence microscope at the end of synchronization region. The intensity measurement was repeated 10 times

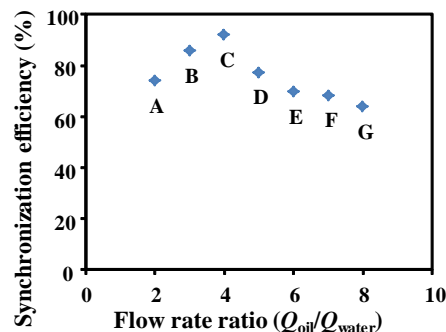


Figure 3: Synchronization efficiency as a function of flow rate ratio. In the case A, the efficiency was relatively low since unstable and non-uniform droplets were generated. As flow rate ratio increases, the size of droplets decrease, leading to unstable synchronization.

and then, the normalized intensity was calculated. **Fig. 4(b)** represents the different concentration of the synchronized droplets. **Fig. 5** shows the movement of unmatched droplet where the following droplet catches up the unmatched droplet and the droplet pairing is changed.

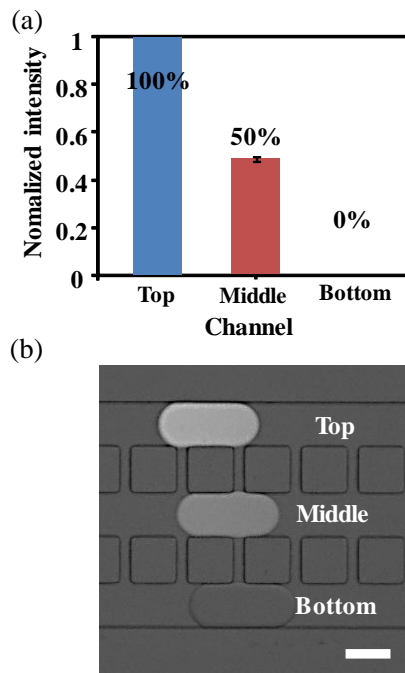


Figure 4: (a) The fluorescence intensity was measured from droplets. All quantitative measurements of the fluorescent intensity were obtained using Olympus Wasabi imaging software package. (b) The fluorescence image of the three droplets with different concentration was captured in the synchronization region. The scale is 100 μm .

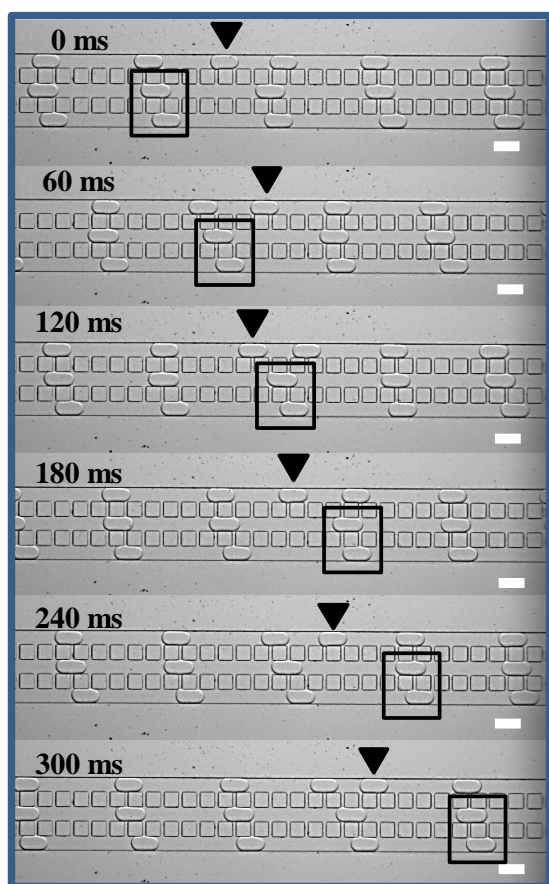


Figure 5: The behavior of unmatched droplet in the ladder structure: the following droplet (rectangular) is chasing the unmatched droplet (triangle) until the pressure difference is zero. The scale is 200 μm .

5 CONCLUSION

We have demonstrated a simple device for the synchronization of three droplets ($n=3$) that can provide different concentrations of droplets simultaneously. We expect that, in principle, more than three droplets with different concentrations would be synchronized using the proposed method and it would be valuable for many droplet-based applications.

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