LAPLACE TRAP FOR ONE-TO-ONE FUSION OF ASYNCHRONOUSLY GENERATED DROPLETS
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ABSTRACT:
Controlled droplet fusion is a crucial operation for biological and chemical assays on chip. Although several droplet fusion devices have been published, these designs are limited in that they only fuse consecutive droplets from the same inlet. We demonstrate the ability to fuse droplets from different inlets, and generated at different frequencies. The device traps and fuses droplets passively (i.e. without electricity) by balancing the driving hydrostatic pressure with increasing Laplace pressure imposed by the design geometry. Fusion rates between 80-100% were observed, even when the ratio of droplet generation frequencies from different inlets was raised as high as 3:1.

Keywords: droplet, digital, microfluidics, fusion

INTRODUCTION:
As applications for droplet microfluidics have evolved, the need for components that can reliably perform droplet manipulations has also increased.[1] Robust droplet fusion mechanisms in particular are crucial for the execution of a variety of assays on chip. Although many innovative droplet fusion designs have been demonstrated, current fusion mechanisms share a common limitation – fusion events can only occur between consecutive, adjacent droplets in the microfluidic channel.[2] For droplets generated from different sources to be synchronized and fused, normally the generation rates need to be matched and their paths to the fusion region need to be taken into consideration as well. Although recent on-demand droplet generation designs have made this easier, the required control mechanism adds to the system’s overall complexity, which could impede efforts to scale up fusion operations in devices containing many inputs.[3] In this manuscript, we present a novel microfluidic component that enables passive fusion of droplets generated from two distinct sources without the need to synchronize the generation. Furthermore, the device allows droplets that are generated at different frequencies to be fused one-to-one using the slower generation rate as the reference rate.

THEORY:
The Laplace trap achieves one-to-one fusion of droplets by holding the reference droplet at the fusion point until its partner droplet arrives (Figure 1). Two droplets then fuse and proceed downstream. The trap functions by balancing the hydrostatic pressure, given by the Hagan-Poiseuille equation for square channels (1) and the pressure due to surface tension, given by the Young-Laplace equation (2):

\[ \Delta p = \frac{12 \mu Q}{w h^3} \left[ 1 - \frac{h}{w} \sum_{n=1,3,5}^{\infty} \frac{1}{\pi n^2} \operatorname{tanh} \left( \frac{n \pi w}{2h} \right) \right]^{-1} \]  

\[ \Delta p = \frac{2 \gamma}{R} \]  

The hydrostatic pressure propels droplets forward in a channel, while the net Laplace pressure on the droplet pushes against this forward force. By creating a geometry that balances the two forces, a droplet can be held stationary until another force acts upon it. We achieve this balance by designing a narrowing fluidic channel with a by-pass (Figure 1a. dark blue arrow) that routes away continuous phase flow once a droplet is trapped to avoid increasing hydrostatic pressure. As a droplet moves into the Laplace trap, the radius at the front of the droplet becomes smaller than the radius at the back of the droplet, creating a net Laplace pressure that opposes forward motion of the droplet. Droplets of a specific size will develop enough Laplace pressure to equalize the driving hydrostatic pressure and stop completely inside the trap.
Figure 1: a) Photograph of the fusion region of the device. The slower reference droplet enters from the left and is held at the Laplace trap region indicated by the light blue circle. The droplet is prevented from entering the bypass path by a small post located at the reference droplet inlet. It waits for the partner droplet to arrive from the top and the fused droplet exits towards the outlet. The bypass allows the continuous phase to flow without pushing the trapped droplet out of the trap. b) COMSOL simulation of velocity field of the fusion region. The bypass region has normally low flow so droplets will proceed to the trap region.

EXPERIMENTAL:
Standard soft lithography techniques were used to produce the Laplace trap design in PDMS (Sylgard 184, Dow Corning). Fluidic channels were sealed by bonding this layer to a featureless layer of PDMS using oxygen plasma treatment. Picoliter-sized droplets were produced using a simple T-junction configuration, in which a stream of water was sheared into droplets by a continuous phase of oleic acid or mineral oil. Continuous phase was supplied at a constant volumetric flow rate by a syringe pump, while the dispersed phases were driven using a constant pressure source. The size and frequency of the droplets could be varied by altering the flow rate of the continuous phase or the driving pressure of either or both the dispersed phase inlets. Operation of the device was visualized on an inverted microscope, and images and video were captured using a high-speed camera (Fastcam, Photron, Inc.).

RESULTS AND DISCUSSION:
Figure 2 shows a sequence in which one droplet is trapped and waits for a second droplet to arrive at the junction, where fusion then occurs. The droplet entering from the left which enters the trap is termed the reference droplet, since the frequency of droplets from this line sets the frequency for droplet fusion. In order to characterize the efficiency of the system, we examined fusion events occurring between droplets generated at a range of relative frequencies. The frequency of droplet generation from the reference droplet input was always equal to or lower than the frequency of droplets generated in the partner droplet input, which ensured that nearly all of the droplets in the reference droplet line were fused with a partner droplet. In an assay, the reference droplet line could carry contents from samples that are either more scarce or more expensive than the contents of droplets in the partner input. In this manner, little of the reference droplet contents would be wasted due to nonfusion events.

As shown in Figure 3a, fusion efficiencies are very high at higher frequency ratios but drop when the ratio is close to one-to-one. At higher frequency ratios, the requisite wait time of the reference droplet in the fusion trap is smaller than the wait time at lower frequency ratios. For frequency ratios where the droplet waiting time is long, the trapped droplet may be affected by the droplet behind it in the reference line, as this droplet tries to move into the fusion trap. Additionally, the trapping ability of this device depends significantly on droplet size. If droplets are smaller than the optimal size, they will not stop completely in the trap due to the increased Laplace pressure in the backend of the droplet, and the chance of fusion for these droplets is thus lower when the phase shift between the reference droplet line and the partner droplet line is large (i.e. when the
frequency ratio is low). However, with further optimization, including determination of the optimal droplet size for trapping as well as more robust trap design, the efficiency can be raised for all frequency ratios and a wide range of droplet sizes. Figure 3b demonstrates rapid mixing occurring in fused droplets, as indicated by droplets colored with food dye.

CONCLUSION:
This microfluidic system component has the potential to significantly reduce the complexity required for precise droplet fusion as well as enable fusion components to be easily integrated into current droplet-based platforms. Specifically, for reactions with precious reagents, the ability to ensure proper fusion can provide great reduction in cost by allowing all reagents to be utilized.

ACKNOWLEDGMENTS
The authors acknowledge financial support from DARPA through the Micro/Nano Fluidics Fundamentals Focus Center (MF3), and the NSF-IGERT LifeChips program.

REFERENCES

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