

# A Continuous Flow Microdroplet “Lysis” System

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## ABSTRACT

Microdroplet-based technologies are ideally suited for a wide range of chemical and biological applications. Although researchers have developed highly effective methods for handling droplets within microfluidic systems, difficulties remain for retrieving the inner contents of droplets (*e.g.*, biomolecules, reagents, cells, and microbeads). To achieve this goal, here we demonstrate a versatile, continuous flow methodology for “lysing” microdroplets. Our microfluidic platform passively: (i) guides droplets between different liquids (*i.e.*, oil flow and water flow), (ii) washes away the surfactant of microdroplets, and (iii) lyses the droplets to release their contents into the water flow. The presented system was employed to wash and lyse water-in-oil droplets (31.6  $\mu\text{m}$  in diameter) as well as separate the contents from the oil within a few minutes.

## KEYWORDS

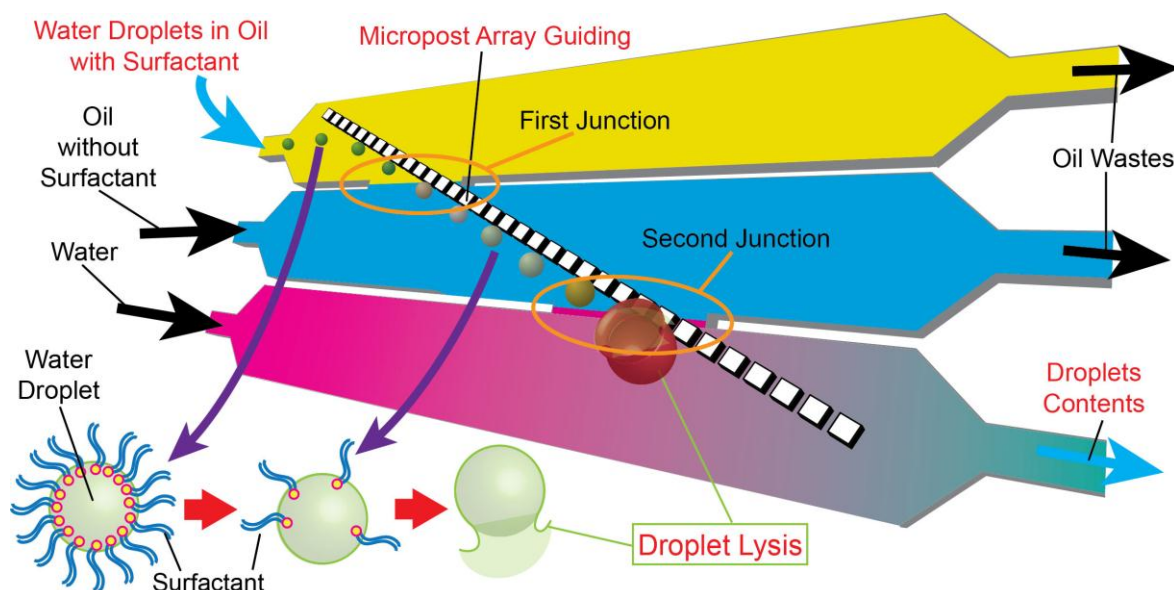
Railing, Microdroplet, Lysis, Microfluidics, Lab-on-a-Chip

## INTRODUCTION

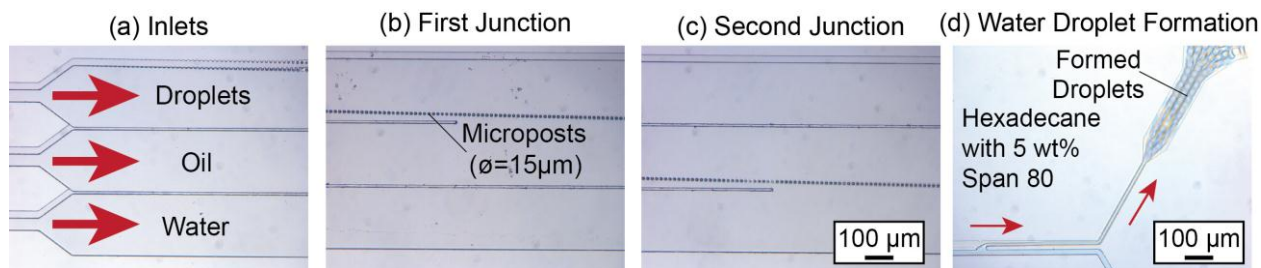
For advanced biological applications, such as genomics, quantitative cellular studies, and point-of-care diagnostics, microdroplet-based platforms offer significant advantages, including rapid mixing and low background noise [1-4]. In particular, continuous flow methodologies could provide high-throughput processing capabilities for microdroplets [5]. Previously, continuous flow hydrodynamic railing systems have been successfully employed to passively handle microdroplets [6-9], microbeads [10-13], and living cells [13]. Although the formation of droplets is relatively simple, methods for retrieving the contents inside droplets remain complex and time-consuming, mainly due to the existence of the surfactant in the oil solution. To overcome this issue, here we use a continuous flow micropost array railing system to rapidly separate, wash, and lyse microdroplets, and retrieve the contents inside the droplets.

## CONCEPT

**Figure 1** illustrates the basic concept of the microdroplet lysis system. The system consists of arrayed microposts and three microfluidic flow channels, corresponding to continuous loading of water-in-oil droplets with surfactant (*yellow*), oil without surfactant (*blue*), and water flow (*magenta*). As shown in prior reports, the arrayed microposts enable suspended microparticulates to be guided without disrupting the primary flow directions of the continuously loaded fluids [6, 11-13]. At the first junction (*see* Fig. 1), microdroplets are guided from the oil flow with surfactant to the oil flow without surfactant, which serves to remove the surfactant from the droplets. At the second junction, the shear force of the water flow combined with the surface tension of the droplets result in the collapse of the droplets, thereby releasing the contents of the droplets into the water flow. Thereafter, the water solution containing the contents of the droplets is collected at the outlet while the remaining oil phase flow is directed to the waste ports.



**Figure 1.** Conceptual illustrations of the continuous flow microdroplet “lysis” system. Fluids including water-in-oil droplets with surfactant (*yellow*), oil without surfactant (*blue*), and water flow (*magenta*) are continuously inputted in parallel. The arrayed microposts passively guide the droplets into the adjacent flow streams to wash the droplets (and remove the surfactant) and then lyse the droplets. Microdroplet lysis enables the contents inside the droplets to be retrieved for subsequent analysis and/or use in additional experiments.



**Figure 2.** Microphotos of a fabricated microdroplet lysis system. (a-c) The system has three independent inlets and outlets for the continuous inputs of droplets, oil, and water solutions, as well as arrayed microposts for passively guiding the movement of the microdroplets. (d) Water droplets are generated separately via an H-shaped junction.

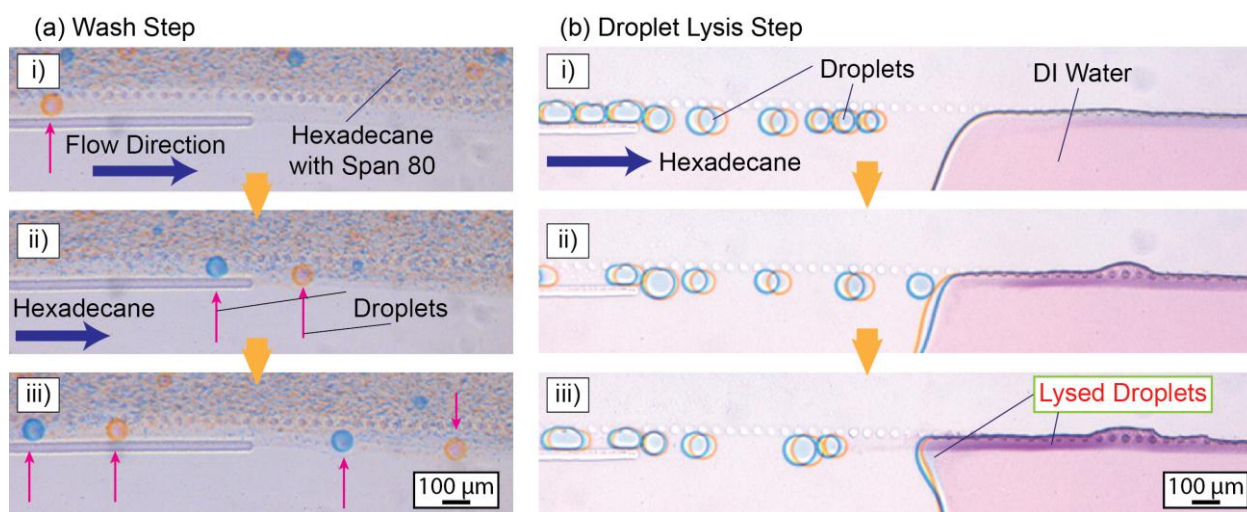
## FABRICATION

Figures 2(a-c) show microphotos of a fabricated device. In contrast to our prior works [11-13], here we used a two-layer soft lithography process to maintain a gap between the microposts and the substrate. The thickness of the microposts and the main channel were  $10\ \mu\text{m}$  and  $20\ \mu\text{m}$ , respectively. For the successful guidance of the droplets, the microposts ( $15 \times 15\ \mu\text{m}^2$  squares;  $5\ \mu\text{m}$  separation gap) were arrayed at a  $1^\circ$  angle with respect to the direction of the fluid flow. These structures were transferred from a SU-8 mold to poly(dimethylsiloxane) (PDMS), and then permanently bonded to glass substrates *via* oxygen plasma treatment.

The water-in-oil microdroplets were formed using an H-shaped junction in a separated device. The outlet of the device was connected to the inlet of the microdroplet lysis system. We used green-dyed deionized water for the water phase flow and Hexadecane with 5 wt% of Span 80 for the oil phase flow with surfactant. Figure 2d shows the formation process of the water-in-oil microdroplets. The average diameter of the formed droplets was  $31.6\ \mu\text{m}$ .

## EXPERIMENTAL RESULTS

Experimental testing revealed that the microfluidic system facilitated microdroplet lysis, with the successful retrieval and separation of the contents of green-dyed water-in-oil microdroplets from the oil solution. Figure 3 shows experimental results for the droplet lysis process. First, droplets are guided *via* the arrayed microposts from the Hexadecane solution with Span 80 (*top microchannel*) to the pure Hexadecane solution without surfactant (*center channel*) at the first junction. Droplets that are smaller than  $20\ \mu\text{m}$  in diameter as well as aggregated surfactant bypass the arrayed microposts and flow downstream to the oil waste port (Fig. 3a). The Hexadecane flow channel can wash the surface of the droplets to reduce the amount of surfactant – Span 80 – on the surface of the droplets. At the second junction between the Hexadecane and the water microchannel, the boundary of Hexadecane and the water flow is deformed because the Hexadecane is also partially guided by the microposts into the water microchannel (*i.e.*, due to relatively strong surface tension forces). It is observed that the flow velocity of overflowing Hexadecane is slower, causing some droplets to merge together in the overflow region – a phenomenon that is not observed in the Hexadecane solution with Span 80. This suggests that surfactant on the droplet surface has been washed away after the washing step in the pure Hexadecane flow microchannel. When the droplets contacted the red-dyed water flow at



**Figure 3.** Experimental demonstration of the microdroplet lysis system. (a) Introduced water-in-oil microdroplets are guided by the arrayed microposts from the Hexadecane solution with Span 80 in the top microchannel to the pure Hexadecane flow in the middle microchannel for the first wash step. (b) After being washed by the oil solution without surfactant in the middle microchannel, microdroplets are successfully lysed at the second junction between the Hexadecane without surfactant (middle channel) and the water (bottom channel). The droplets and water are dyed in green and red, respectively, for visibility purposes. The water changes color subsequent to droplet lyses.

the second junction, the surface of the droplets merged together at the boundary of the water flow due to the loss of surfactant on the droplet surface. Consequently, the contents inside the droplets were released into the water flow, which enabled collection at the end of the water microchannel. The color of the water flow was observed to change from its original red color to purple when the green-dyed water in the droplets was released and mixed with the dyed water (**Fig. 3b**). These results demonstrate the capability of using the presented continuous flow microfluidic system to: (i) lyse water-in-oil microdroplets, (ii) release the inner contents of droplets into water flow, and (iii) separate the droplet contents from the oil.

## CONCLUSION

In this paper, we have developed a continuous flow microdroplet lysis system to provide a sequential wash and lysis process *via* a microfluidic railing methodology. We have successfully demonstrated the guidance of water-in-oil droplets ( $\phi = 31.6 \mu\text{m}$ ) into different liquids of microchannels by utilizing microposts arrayed at a  $1^\circ$  angle from the flow direction, with a  $10 \mu\text{m}$  gap between the microposts and the glass substrate. Microdroplets were sequentially washed with pure Hexadecane and lysed in the final microchannel with deionized water. Among various types of potential microdroplets, water-in-oil droplets containing only green-dyed deionized water were chosen in this work merely as a demonstrative example. Thus, the same lysis system could be applied to other types of microdroplets. Because it has been previously shown that the micropost array railing system can also be used for other types of microparticles, such as living cells and microbeads [10-13], the droplet lysis principle demonstrated in this work could be employed to rapidly retrieve such particles from within droplets. As such, this quick, robust, and easy-to-operate lysing system could enable the extraction of components generated in microdroplet-based assays for follow-up experiments to expand the potential of microdroplet technologies for advanced biological applications.

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