PAPER

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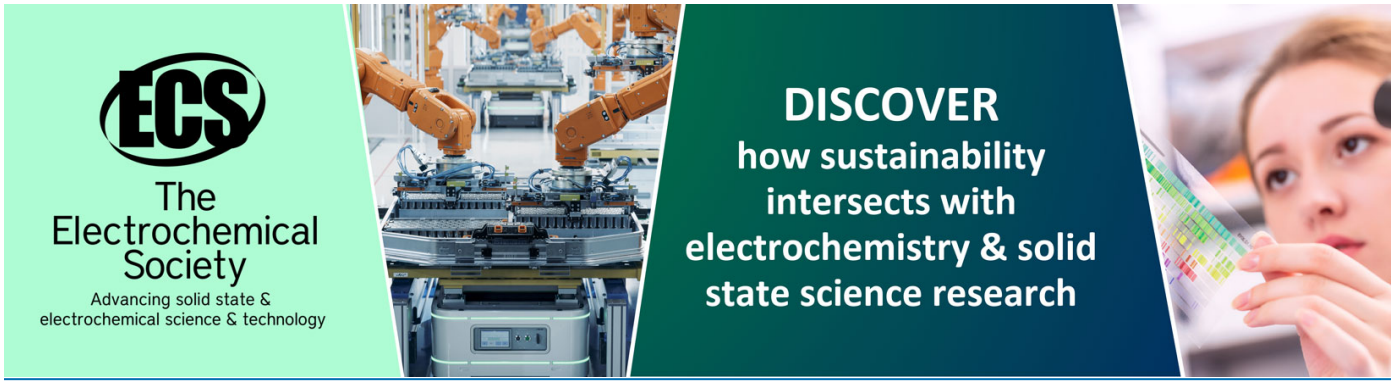

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A random-access microarray for programmable droplet storage, retrieval and manipulation

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Abstract

This article presents an integrated microfluidic system that is capable of programmably metering, entrapping, coalescing, addressably storing, retrieving and manipulating emulsion droplets. A multilayer, flexible PDMS chip with specially designed fluidic channels dynamically reconfigured by pneumatically actuated diaphragms is utilized to integrate a variety of droplet manipulation schemes. Once droplets are formed, their motions are coordinated by a 2D multiplexing scheme, which exploits the bidirectional movement of diaphragms to implement a random-access microarray. In the prototype demonstration, a PDMS molding and bonding process is used to fabricate the proposed microfluidic system. Emulsion droplets with desired volumes and compositions are produced, addressably stored, manipulated and retrieved from a 4×4 array, which employs just 4 ($= 2 \times \log_2 4$) control inputs for the operation. It has been demonstrated that (1) the integration of droplet manipulation and 2D multiplexing schemes can be achieved readily using bidirectional diaphragm valves, (2) multiplexing of an $N \times N$ array could be realized utilizing only $2 \times \log_2 N$ control inputs and (3) a multifunctional, random-access microarray can be accomplished employing a multilayer PDMS chip. As such, the demonstrated random-access microarray could potentially serve as a platform for continuous tracking and multistep processing of emulsion droplets, which is desired for various biological and chemical applications.

(Some figures may appear in colour only in the online journal)

Introduction

Among various types of microfluidic systems, emulsion-based systems have recently attracted significant interest because of their potential impact on diverse chemical and biological applications [1–5]. Inside an emulsion, the droplet interface encloses its contents, and therefore enables a droplet to act as a convenient vessel for transporting materials and carrying out chemical reactions [6]. Furthermore, the interface offers the significant benefit of preventing sample dispersion and fouling on the channel surface. By compartmentalizing reactions into micrometer-sized droplets, the miniaturization and large-scale parallel processing of reactions, which are desired for applications involving the investigation of huge parameter spaces, could eventually be realized. The resulting low sample consumption and high reaction throughput are expected to significantly accelerate the progress in drug discovery, protein crystallization and various chemical and biological screening and synthesis [7–10]. In addition to the control over stoichiometry, which is achieved by the ability to form droplets with diverse volumes and compositions, the ability to parallelly manipulate a large number of droplets over certain periods of time could further realize the controllability desired for continuous tracking and multistep processing [11–13].

As the number of samples dramatically increases, traditional automation schemes such as robot-controlled pipettes and multiwell plates become too complicated and expensive. By contrast, microfluidic systems, which consist of micromachined or molded channels and integrated

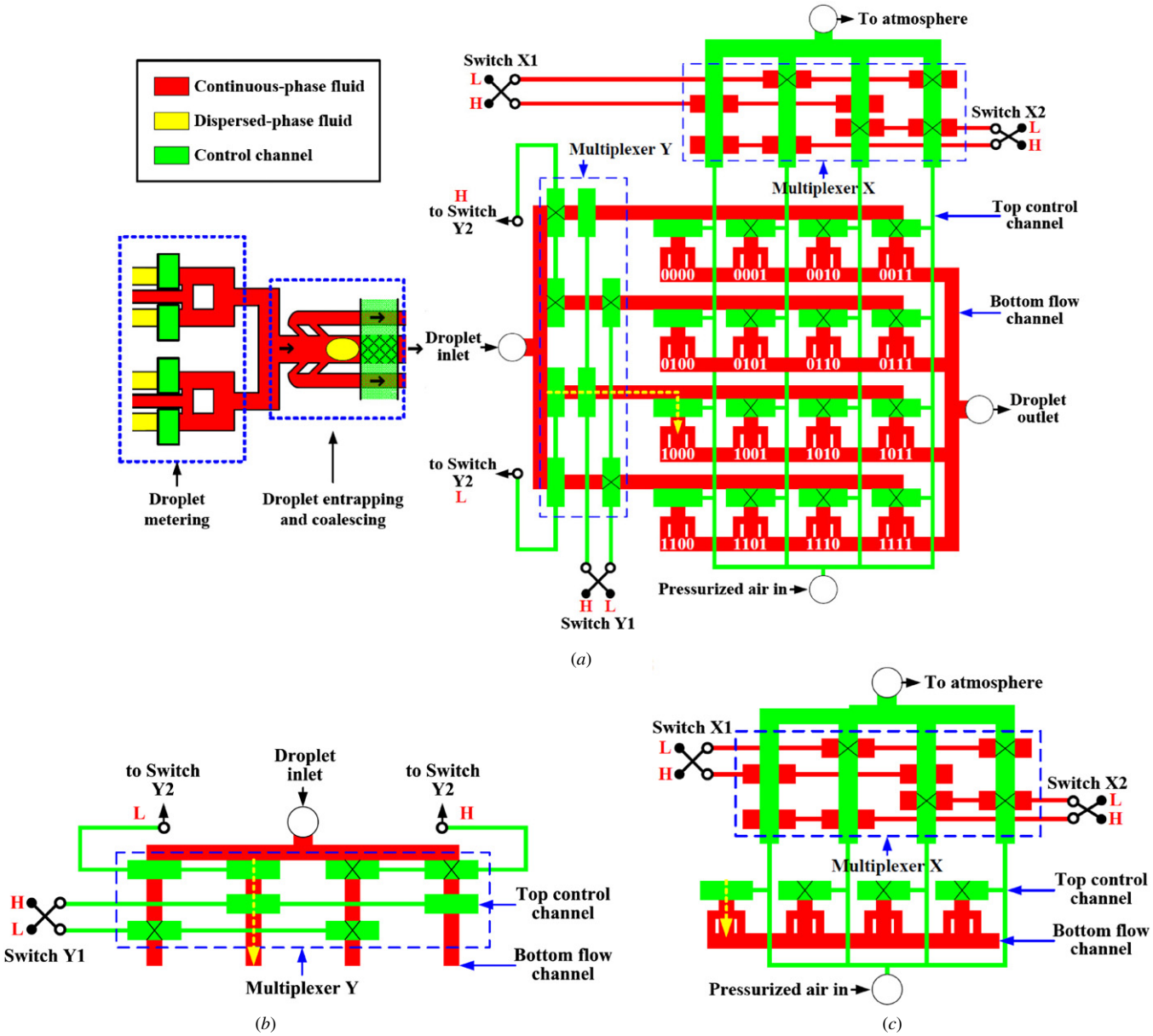


Figure 1. Schematic illustrations of (*a*) the proposed 4 × 4 random-access microarray, (*b*) multiplexer Y and (*c*) multiplexer X.

components, enable the rapid analysis of very small quantities of samples in a portable, automated and inexpensive format [14–17]. A variety of microfluidic schemes have been proposed to achieve large-scale integration [18], which enables a large number of assays with multiple reagents to be performed parallelly in an automated manner. For example, it has been demonstrated that miniature fluidic valves can be integrated in a manner similar to transistors employed in integrated circuits [19–21]. With no need to account for the detailed properties of the manipulated fluids, novel 2D microarrays for multistep processing and studying continuous kinetics have been realized with great success. However, most existing microarrays use more than enough control inputs for multiplexing [22, 23], or employ fewer control inputs but achieve merely static operation [24–27] or sequential access of droplets [28, 29]. For random-access microarrays, the ability to addressably retrieve stored droplets also needs to be included. In addition to multiplexing, metering [30–32], entrapping and coalescing [33–35] of droplets are also required for emulsion-based microarrays to achieve stoichiometry control. Previously, these functions have been demonstrated successfully but separately by a variety of novel microfluidic schemes. Regarding the integration of all these functions in one single microfluidic system, currently only limited progress has been accomplished.

To address the need for random-access emulsion-based microarrays capable of metering, entrapping, coalescing, addressably storing, retrieving and manipulating droplets, this paper presents an integration scheme that achieves all the functions utilizing merely microfluidic channels and valves. A multilayer, flexible PDMS microfluidic chip with specially designed fluidic channels dynamically reconfigured by pneumatically actuated diaphragms is utilized to integrate a variety of droplet manipulation schemes. Once droplets with desired volumes and compositions are formed, their

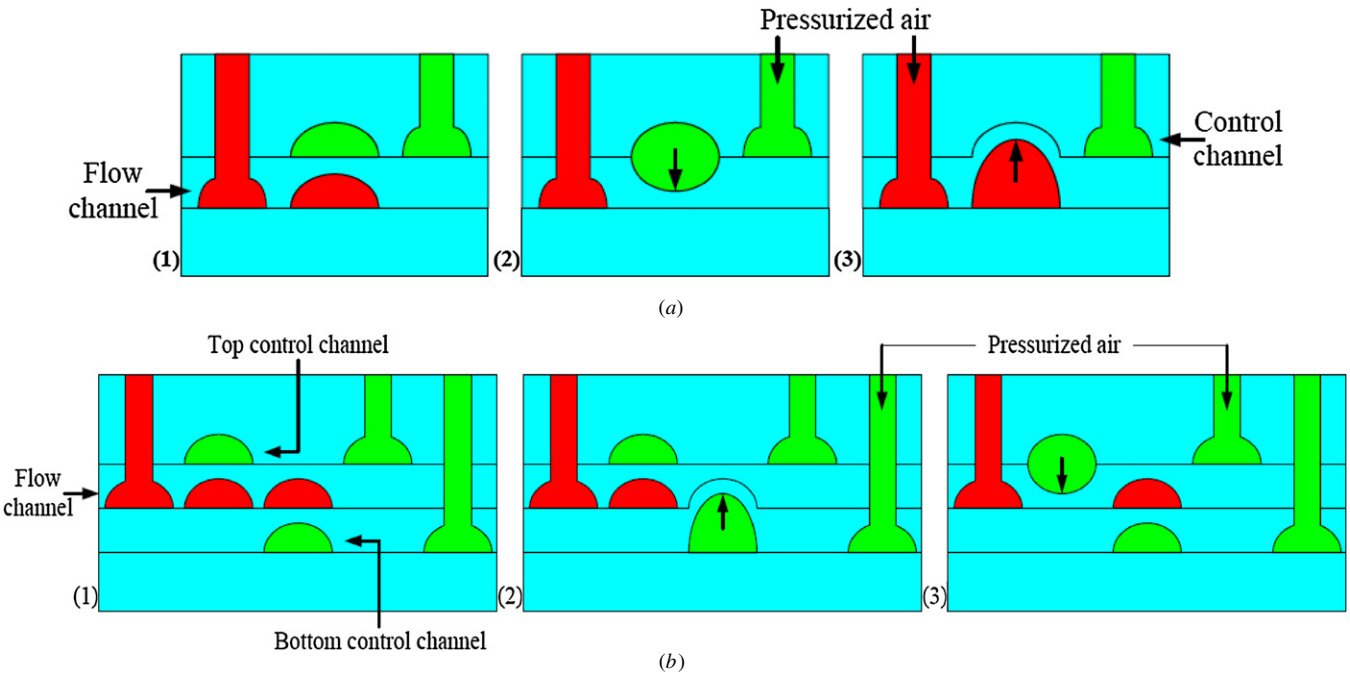


Figure 2. Cross-sectional views of (*a*) three-layer (one layer of control channel) and (*b*) four-layer (two layers of control channels) microarrays.

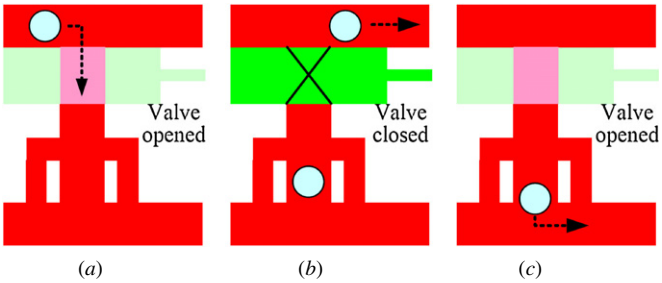


Figure 3. Schematic illustration of a proposed droplet storing scheme: (*a*) targeting, (*b*) entrapping and (*c*) retrieving of droplets.

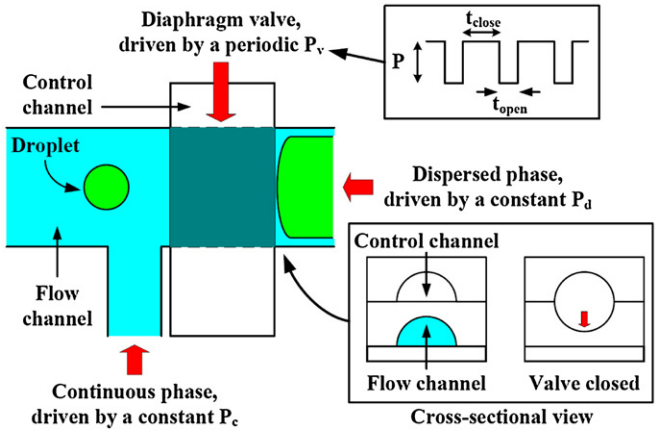


Figure 4. Schematic illustration of the proposed metering scheme.

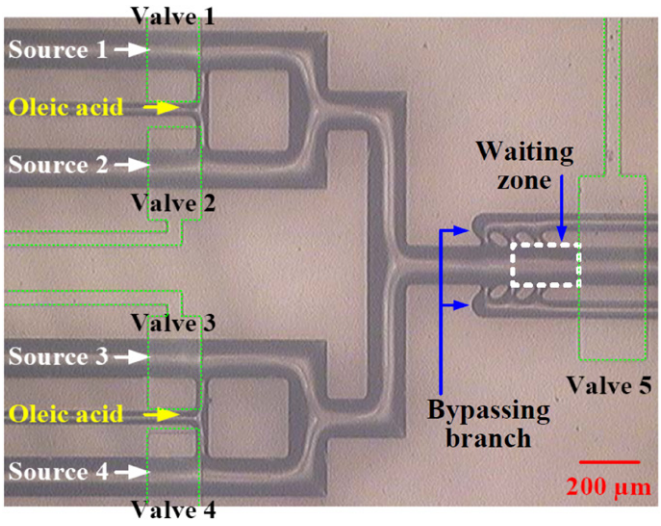


Figure 5. Photograph of a fabricated metering and coalescing PDMS device with four sources.

motions are then coordinated by a novel 2D multiplexing scheme, which exploits the bi-directional movement of diaphragms to implement a random-access microarray. Three accomplishments have been achieved: (1) the integration of droplet manipulation and 2D multiplexing schemes using bi-directional diaphragm valves, (2) multiplexing of an $N \times N$ array utilizing only $2 \times \log_2 N$ control inputs and (3) a multifunctional, random-access PDMS microarray. With droplets functioning as micro-reactors, the proposed random-access microarray could potentially serve as a platform for continuous tracking and multistep processing of emulsion droplets, which is desired for a variety of high-throughput screening and synthesis applications.

Operating principle

A schematic illustration of the proposed multiplexing scheme for a 4×4 random-access microarray is shown in figure 1(*a*). The integration of metering, entrapping and coalescing functions is also illustrated in the figure. Multiplexers X and

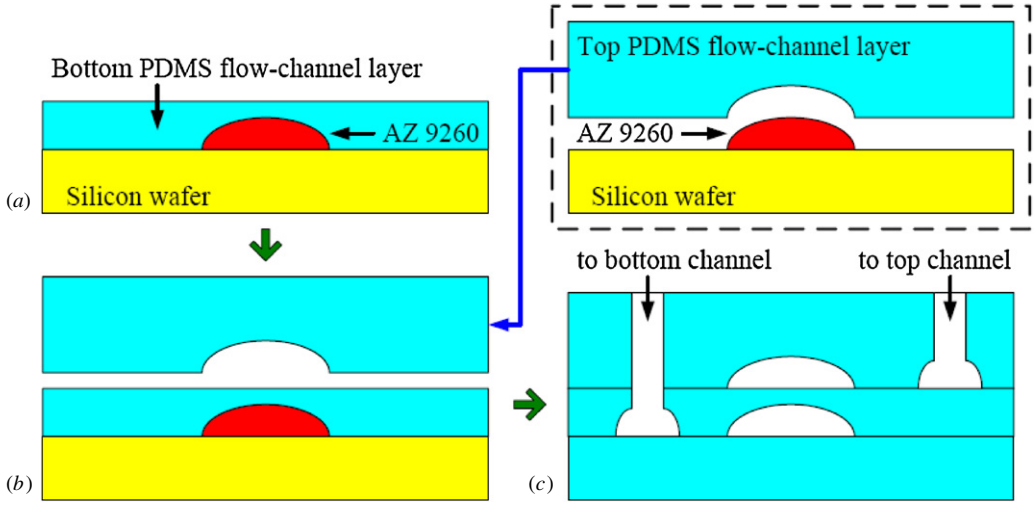


Figure 6. A three-layer, PDMS molding and bonding fabrication process: (*a*) molding, (*b*) bonding and (*c*) packaging of PDMS microstructures.

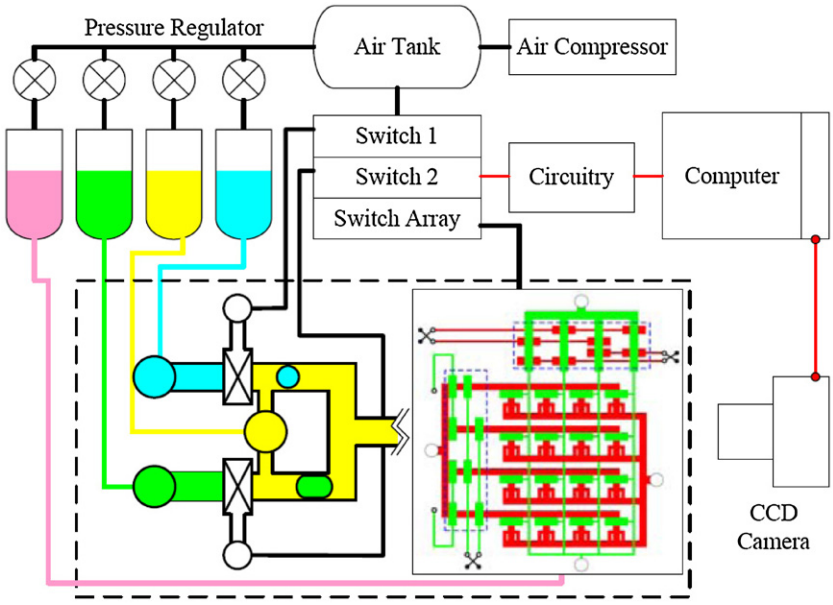


Figure 7. Experimental setup of the proposed random-access microarray.

Y are employed to control the flow path and therefore the destination of an incoming or temporarily stored droplet. An incoming droplet will first arrive at the inlet, and then be directed by multiplexer Y to one of the four flow paths located in the bottom flow channel. Once in the selected flow path, the droplet will be driven further into one of the four storage units determined by multiplexer X. A cross-sectional view of the three-layer microarray is illustrated in figure 2(*a*). An elastic PDMS diaphragm is sandwiched between top control channel and bottom flow channel. Since both channels are round, either one of them could be pressurized to block the other one. It functions as a bidirectional diaphragm valve, which could greatly simplify the integration of multiple logic components on one single microfluidic chip. For multiplexer Y (as illustrated in figure 1(*b*)), pressurized air is filled into the top control channel, so the diaphragm moves downward to block the underneath droplet flow in three out of four flow paths. For multiplexer X (as illustrated in figure 1(*c*)), pressurized air is filled into the bottom flow channel, so three out of four flow paths in the top control channel are blocked, which leads to pressurization of these three flow paths. Furthermore, the pressurization also causes the diaphragm along these flow paths to move downward, which results in the blockage in three out of the four columns located in the bottom flow channel. Therefore, the incoming droplet will be driven into the only column through which flow is allowed to pass. As such, a pneumatic inverter is realized utilizing the bi-directional diaphragm valve. By employing M control inputs in a multiplexer, 2^M different flow paths can be accomplished. Overall 4 ($= 2 \times \log_2 4$) control inputs (Y2Y1X2X1) are utilized to operate the 4×4 microarray, and the corresponding destinations of all input combinations are also labeled in figure 1(*a*). For the case indicated in figure 1(*a*), Y2Y1X2X1 = 1000, the incoming droplet will be driven into the storage unit

labeled 1000. For an $N \times N$ array, only $2 \times \log_2 N$ control inputs are required for its operation. In the case a four-layer microstructure (as illustrated in figure 2(*b*)) is employed, the integration of multiple multiplexers in one single microfluidic chip can be further simplified. Instead of utilizing just the inverter design presented in figure 1(*c*), multiplexer X can be implemented in exactly the same manner as multiplexer Y. Once the droplet arrives at the target storage unit, the flow path is blocked to stop and entrap the droplet, as illustrated in figure 3(*b*). Meanwhile, a trapped droplet could be retrieved from a storage unit simply by redirecting the continuous phase flow to pass through the storage unit, so the droplet will be dragged along the flow path and finally to the outlet.

In addition to 2D multiplexing and corresponding storing and retrieving of droplets, manipulation schemes such as metering, entrapping and coalescing are also integrated in one single microfluidic chip as already shown in figure 1(*a*). Further illustrated in figure 4, a microfluidic T-junction with a diaphragm valve mounted on its top is employed to control the metering process [32]. Whenever sufficient pressure is applied to the top control channel, the diaphragm deflects downward and blocks the bottom flow channel. The dispersed and continuous phase fluids are driven independently by pressures with constant magnitudes of P_d and P_c, respectively, and fed into the corresponding microfluidic channels. Without diaphragm actuation, emulsion droplets with certain dimensions can be produced at specific frequencies, and the result can be adjusted by varying the fluid driving pressures P_d and P_c. The diaphragm valve is located across the flow channel and right upstream of the T-junction. It is driven by a constant pressure P, which is sufficient to deflect the diaphragm and completely block the underneath flow channel, and switched on and off using an electromagnetic actuator. Periodically, the diaphragm valve is pneumatically enabled (or pressurized) for t_{close} and then disabled for t_{open}. Normally, the valve blocks the dispersed phase flow completely, while a limited volume of the dispersed phase fluid can flow through the path once the valve is opened. The collapse of the diaphragm breaks the dispersed phase flow and produces a discrete droplet, which is then driven away from the T-junction by the continuous phase flow. With the utilization of the diaphragm valve, the resulting droplet volume and breakup frequency could be adjusted independently by varying the open time (t_{open}) and cycle time ($t_{\text{close}} + t_{\text{open}}$) of the valve, respectively. Intotal, four control parameters (t_{close}, t_{open}, P_d and P_c) are utilized to regulate the metering process, so droplets could be produced in an on-demand manner.

Once droplets (each with specific ingredient and volume) are produced, they are trapped and coalesced to form combined droplets with desired compositions and volumes. The details of these manipulation schemes are illustrated in figure 5. Fluids from up to four sources are metered separately by valves 1–4. A microfluidic channel, with one end temporarily blocked by valve 5 and narrow lateral branches bypassing the incoming flow, is utilized to selectively entrap the dispersed droplets. Since the geometry and therefore the flow in the central channel is symmetrical, it is expected that the droplets would be driven along the centerline of the channel and into the space between valve 5 and the bypassing branches, while the continuous phase fluid bypasses the blockage turning up- and downward. The droplets slow down when moving close to the blockage, and eventually are trapped inside the 'waiting zone', which is actually a dead volume when the channel is blocked. With droplets restrained in the waiting zone, they would collide with each other. Meanwhile, the coalescence of droplets is facilitated by the retreat of the diaphragm, when valve 5 switches from closed to open state. The retreat causes a negative pressure gradient, which sucks in the liquids and in turn accelerates the draining of the liquid film between the trapped droplets. Droplets are coalesced to form combined droplets. As already illustrated in figure 1(*a*), the resulting droplets are fed into multiplexer Y and then guided by multiplexer X to their final destinations.

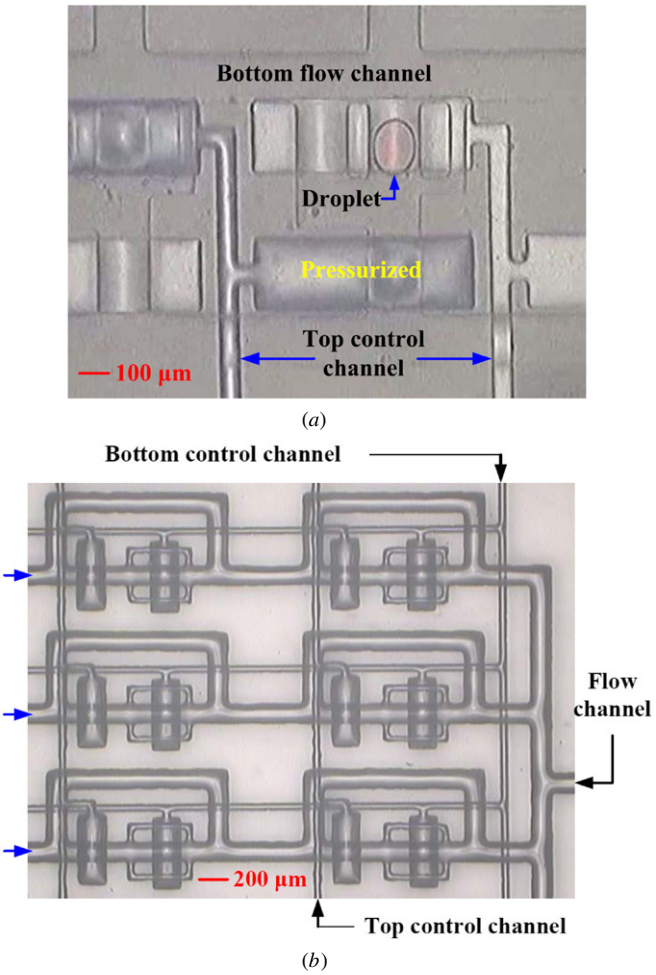


Figure 8. Photographs of fabricated (*a*) three-layer and (*b*) four-layer microarrays.

Fabrication processes

A three-layer, PDMS molding and irreversible bonding process was employed to fabricate the proposed random-access microarray as illustrated in figure 6. First of all, a layer of 25 μm thick positive photoresist (9260, AZ Electronic Materials) was spin coated and patterned on top of a blank silicon wafer to fabricate the mold used for duplicating the

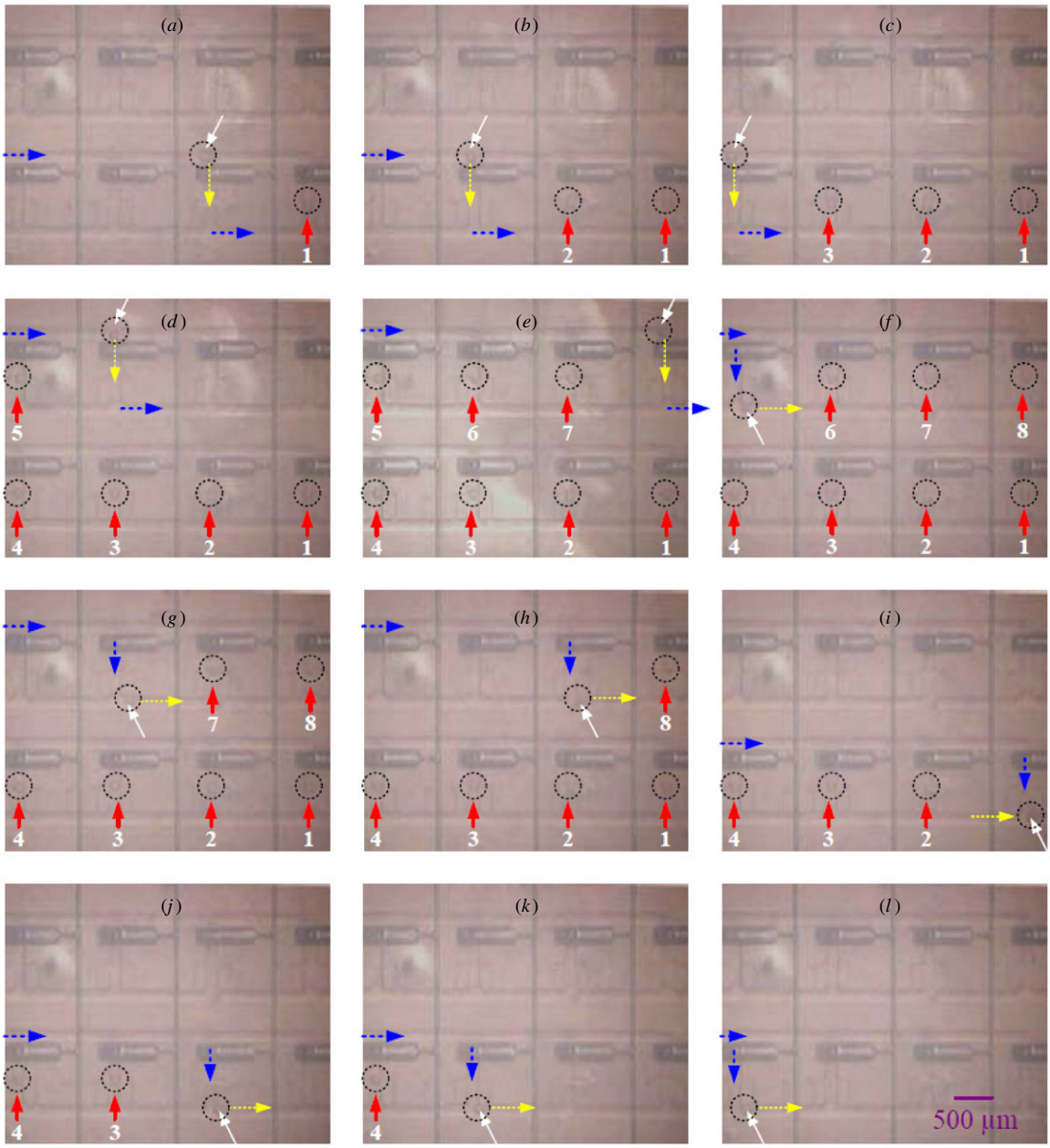


Figure 9. A captured sequence of droplet storing and retrieving utilizing the random-access microarray: (*a–e*) storing and (*f–l*) retrieving of droplets.

lower flow channel. Afterward, the patterned photoresist layer was baked at 120 °C for 30 min, during which the photoresist reflowed and its profile became rounded. Meanwhile, the mold used for duplicating the upper flow channel was fabricated utilizing the same lithography process. After the two photoresist molds were fully stabilized, they were placed in a desiccator under vacuum for 3 h with a vial containing drops of 1H,1H,2H,2H-perfluorooctyl-trichlorosilane (Fluka) to silanize their surfaces for demolding [36]. A mixture of 10:1 PDMS prepolymer and curing agent (Sylgard 184, Dow-Corning) was stirred thoroughly and then degassed under vacuum to remove trapped air bubbles. The casting and bonding process started with the deposition of a thin PDMS mixture layer on top of the lower flow-channel mold. Less than 1/5 of the PDMS mixture was spin coated on the mold at 2000 rpm for 30 s, which yielded a thickness of roughly 50 μm, and cured for 15 min at 85 °C. Meanwhile, about 2/5 of the PDMS mixture was poured onto the upper flow-channel mold, degassed, cured for 15 min at 85 °C and then peeled off from the mold. Afterward, the upper flow-channel layer was pressed and bonded on top of the 50 μm thick lower flow channel layer, and left undisturbed for at least 1 h at 85 °C for the bonding to take effect. The bonded, two-layer PDMS structure was then peeled off from the silicon wafer, and punched through with a sharp metal-tube array to fabricate the holes for multiple inlet and outlet connections. The PDMS structure was then cleaned in an ultrasonic bath to remove residual debris from its surface. Meanwhile, the remaining PDMS mixture was poured onto a blank silicon wafer, degassed, cured for 1 h at 85 °C, and then peeled off from the wafer. The surfaces of the two-layer

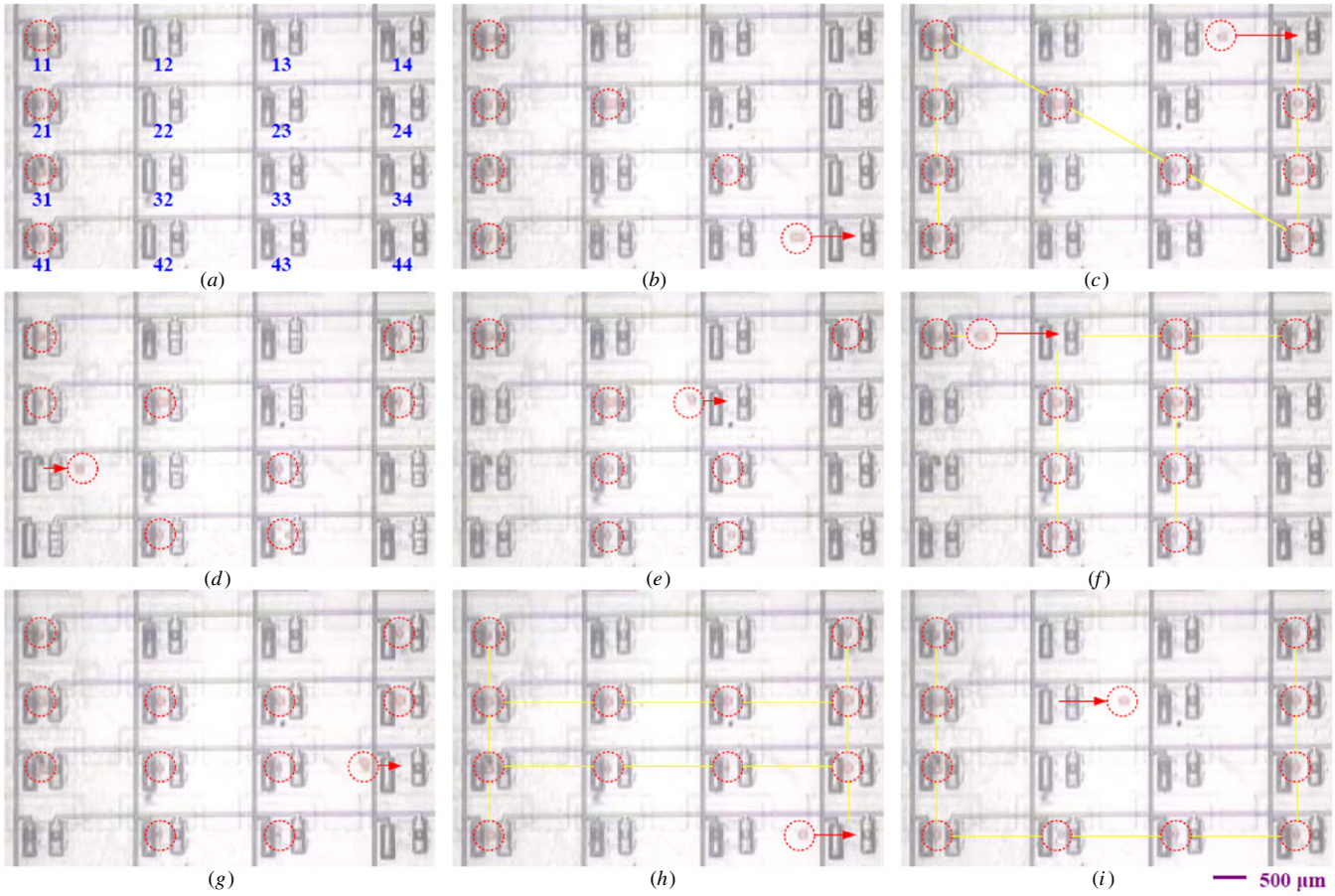


Figure 10. A captured sequence that displays letters in the order of N-T-H-U by selectively and alternately storing and retrieving droplets: (*a*–*c*) N, (*d*–*f*) T, (*g* and *h*) H and (*i*) U.

PDMS structure (on the lower flow-channel side) and the blank layer were then treated with a hand-held corona treater (BD-20AC, Electro-Technic Products), which ionizes surrounding air and creates localized plasma to activate the surfaces for irreversible bonding. The intensity of the corona was set at a relatively low level in order to produce a stable but soft corona with minimal crackling and sparking [37]. The wire electrode was positioned approximately 3 mm above the treated surface, and scanned back and forth for 30 s to 1 min, depending on the size of the surface. The corona-treated surfaces were then pressed together and left undisturbed for at least 1 h at 85 °C for the bonding to take effect. At the end, multiple PTFE tubes were inserted into the punched holes to build the necessary interconnections for compressed air supply and fluidic sample injection and discharge.

Experimental details

In the prototype demonstration, aqueous solutions were dispersed in oleic acid (Aldrich) with 5 wt% polyglycerol polyricinoleate (Grindsted PGPR 90, Danisco) to form stable water-in-oil (W/O) emulsions. All the aqueous solutions were prepared using deionized water as solvent. The experimental setup and operation of the presented random-access microarray is illustrated in figure 7. An air compressor (Model 3–4, Jun–Air) with its output set at 500 kPa was employed as the single pressure source to drive the operation. Each liquid sample was stored in a separate plastic container, which was fed with pressurized air from the top to drive the sample flowing through the bottom tube and into the downstream microfluidic devices. The actual driving pressure applied on each sample was adjusted independently by a separate pressure regulator (IR1000-01G, SMC). Meanwhile, the actuation of each diaphragm valve was controlled independently by a separate electromagnetic valve (VK332-5G-M5, SMC), whose action was governed by computer-controlled relay circuitry. For a 4 × 4 array, four electromagnetic valves were employed in the demonstration. A governing program developed and executed under a software environment (LabVIEW, National Instruments), cooperating with a set of hardware adapter and connector (PCI-6220 + CB-68LP), was employed to coordinate the actuation of the prototype system. As such, the operation can be either preprogrammed or responding to demand in a real-time manner. The storing, retrieval and manipulation of droplets were observed under an optical microscope and the images were recorded using either a standard CCD camera (SSC-DC80, SONY) or a high-speed digital camera (SR series, KODAK Motion Corder Analyzer), which was also connected to the computer.

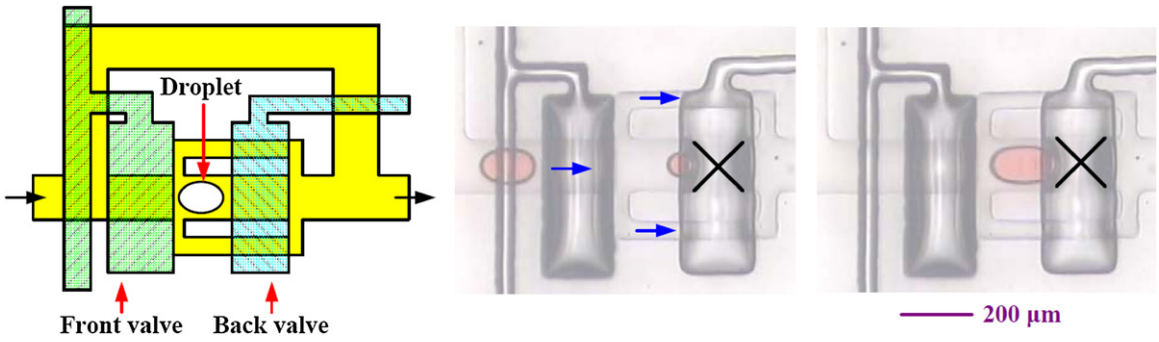


Figure 11. (*a*) Schematic illustration and (*b* and *c*) a captured sequence of droplet entrapment and reinjection of an improved storage unit employing two diaphragm valves.

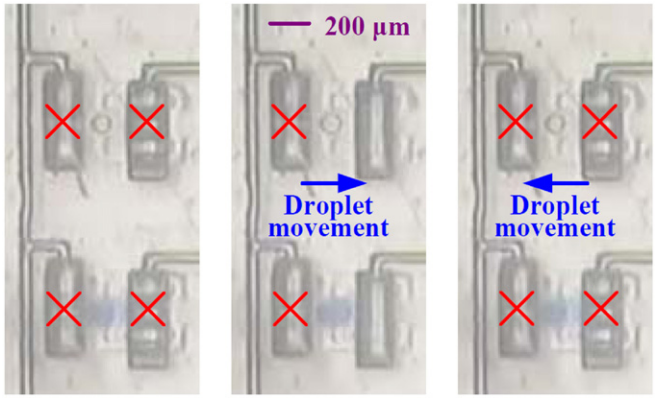


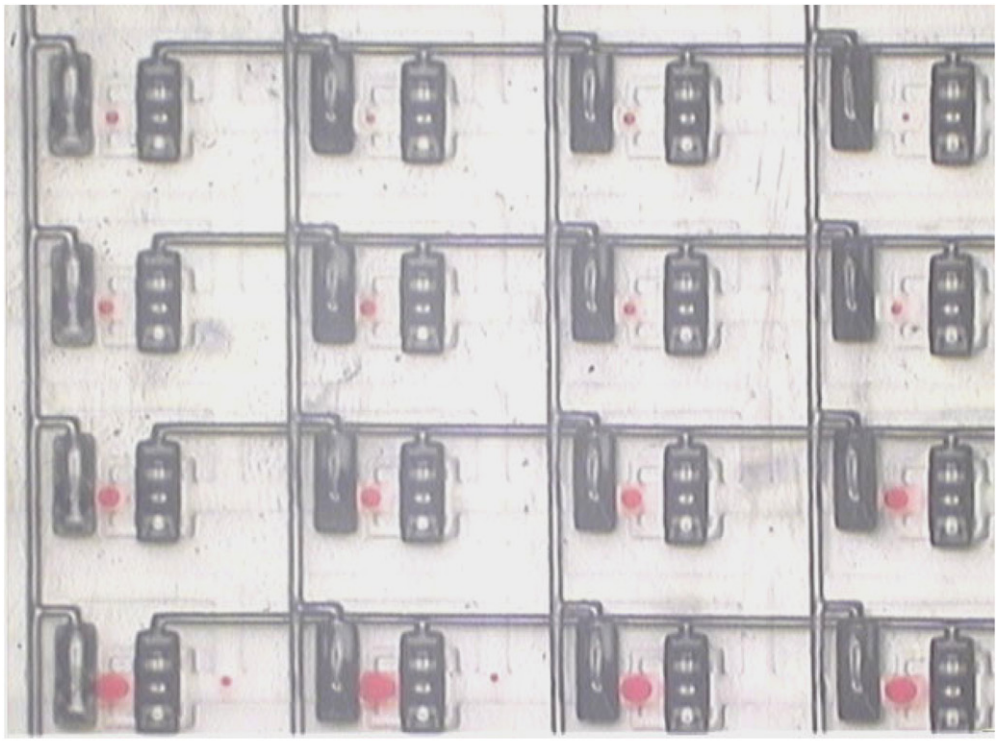
Figure 12. A captured sequence of droplet shaking utilizing the microarray: (*a*) droplet stopped, (*b*) driven forward and (*c*) driven backward.

Results and discussion

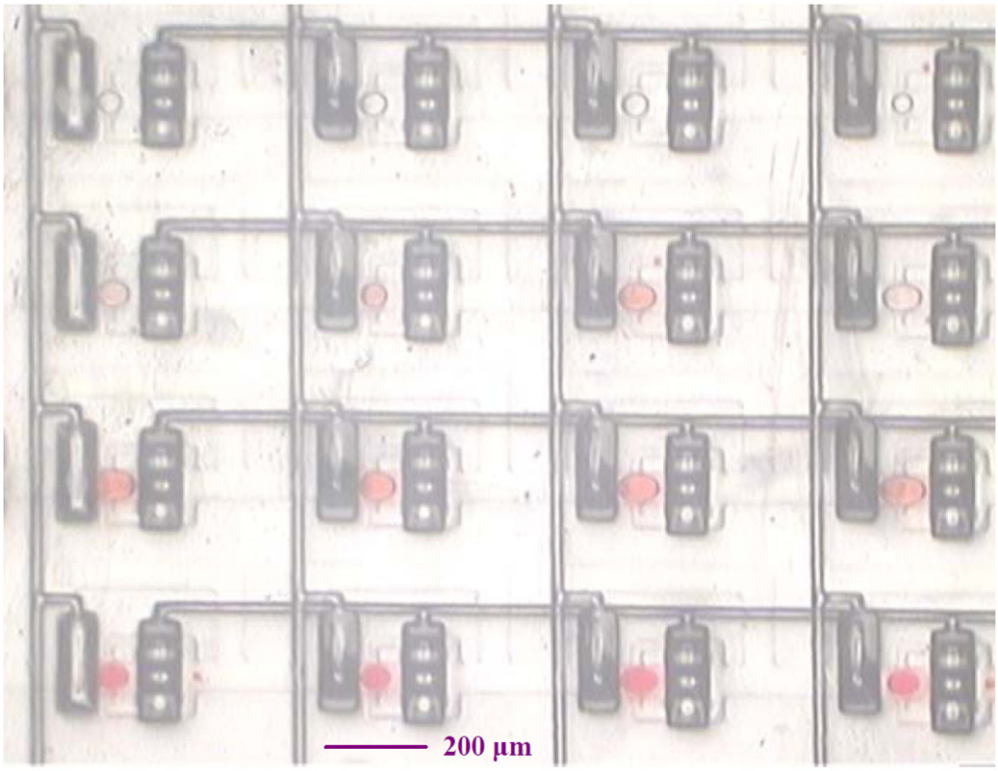
The photographs of fabricated three- and four-layer PDMS microarrays are shown in figures 8(*a*) and (*b*), respectively. Also shown in figure 8(*a*) is the operation of a diaphragm valve. The color of a diaphragm valve turns dark when the diaphragm deflects downward. Otherwise, the diaphragm is transparent and the flow in the underneath channel can be clearly observed. For example, it is seen in figure 8(*a*) that a droplet is passing underneath an undeformed diaphragm. In a four-layer PDMS microarray as shown in figure 8(*b*), control channels are placed both above and underneath a flow channel to simplify the wiring and to prevent intersection. Meanwhile, a fabricated metering, entrapping and coalescing multifunctional device has already been seen in figure 5. Overall four aqueous solutions, with the flow of each of them controlled by one independent diaphragm valve, can be dispersed into the continuous phase oleic acid. In the prototype demonstration, droplets are metered and coalesced to form combined droplets with desired volumes and compositions, and then fed into the random-access microarray for further processing. The minimum valve open (or closed) time actually tested in our experiments is 0.05 s, since the utilized electromagnetic valves are not able to operate correspondingly at frequencies higher than 15 Hz. With a valve open time (t_{open}) of 0.05 s, droplets with volumes less than 1 pL have been successfully generated. Detailed characteristics of this metering and coalescing scheme have been reported by the authors previously [32, 38], so should not be repeated here.

Figure 9 illustrates a recorded droplet storing (*a–e*) and retrieving (*f–l*) sequence of the random-access microarray. Droplets are dragged from left to right, and stored in the array following the order 1 to 8 labeled on the figure. First of all, an incoming droplet is directed by multiplexer Y and driven by the continuous phase flow into either the lower or upper row shown in the figure. Among the four diaphragm valves in the same row, which are regulated by multiplexer X, only one of them remains open at each moment. The flow paths of the continuous phase fluid (indicated by the blue arrows in the figure) and therefore the movement of an incoming droplet (indicated by the yellow arrows) are controlled by the operation of multiplexers X and Y. During the trials, it is noted that all diaphragm valves switch smoothly, and droplets move into target storage units just as planned. Meanwhile, it is also demonstrated that the stored droplets can be retrieved in an arbitrary order. For example, droplet retrieving in the order 5–6–7–8–1–2–3–4 is also illustrated in figure 9. The operation is similar to storing, while droplets are driven out of storage units and to the downstream outlet. The same flow path of the continuous phase fluid is employed to drive a droplet both in and out of a storage unit. In addition, it has also been demonstrated that the storing and retrieving of droplets can operate alternately. Figure 10 demonstrates a random-access sequence that displays letters in the order N–T–H–U by selectively and alternately storing and retrieving droplets. From (*a*) to (*c*), droplets are stored into units 11, 21, 31, 41, 22, 33, 14, 24, 34 and 44 to display letter N. From (*c*) to (*d*), droplets are retrieved from units 41, 44, 31 and 34, and stored into units 42 and 43. From (*d*) to (*f*), droplets are stored into units 23, 12 and 13 to display letter T. From (*f*) to (*h*), droplets are stored into units 41 and 44, and retrieved from units 12, 13, 42 and 43 to display letter H. Finally from (*h*) to (*i*), droplets are stored into units 42 and 43, and retrieved from units 22, 23, 32 and 33 to display letter U.

The original droplet storing scheme (as illustrated in figure 3) employs only one valve for each storage unit, but it could fail if the action of the diaphragm valves is not synchronized with the motion of incoming droplets.



(*a*)



(*b*)

Figure 13. Deposition of 16 droplets with (*a*) the same concentration but various volumes and (*b*) various concentrations and volumes in a 4 × 4 microarray.

In the case a diaphragm valve is closed too early or too late, the incoming droplet would bypass or flow through its target storage unit, respectively. Furthermore, the action of a diaphragm valve could mistakenly cause the breakup of droplets. To further improve the reliability and functionality of the proposed random-access microarray, channel geometries have been redesigned and one additional diaphragm valve has been integrated to each storage unit as illustrated in figure 11(*a*). In the improved design, an incoming droplet will either move straight into a storage unit (if its front valve

is opened) or bypass to the next storage unit. Meanwhile, an additional back valve is located downstream at the exit of each storage unit. To simplify the wiring of control channels, front valves are all placed on top of the flow channel, while back valves are placed underneath in a four-layer microstructure. When the front valve is opened and the back valve is closed, an incoming droplet will move into the storage unit and be securely trapped as shown in figure 11(*b*). As also illustrated in figure 11(*b* and *c*), the reinjection of materials into a stored droplet reactor can be realized by the controlled coalescence of droplets. The opening of the front valve will bring the flow and therefore the coming droplet in, while the closing of the back valve will entrap the droplet but not block the flow. The continuous phase flow bypasses the blockage turning up- and downward. All the back valves are governed by merely one extra control input. When the back valves are closed and opened, the microarray is ready for droplet storing/reinjection and retrieving, respectively. Furthermore, shaking of the stored droplets, which accelerates the mixing inside the droplets, can also be achieved when the front valves are closed and the back valves are opened periodically. As illustrated in figure 12, the retreat and collapse of the diaphragms cause negative and positive pressure gradients, respectively, which in turn drive stored droplets moving forward and backward. As such, droplet reactors could be temporarily stored, reinjected, shaken, monitored and retrieved for further processing in a desired and programmable manner.

The integration of metering, entrapping, coalescing, addressably storing, reinjection, retrieving and parallelly shaking functions all on one single microfluidic chip could greatly enhance the controllability over a variety of screening processes. For example, figure 13(*a*) shows the deposition of 16 droplets with the same concentration but various volumes in a 4 × 4 random-access microarray. The droplets stored in the same rows have roughly the same volumes. This is achieved by the metering, 2D multiplexing and storing of one specific source fluid into the microarray. Furthermore, figure 13(*b*) shows the deposition of 16 droplets with different concentrations and volumes in a 4 × 4 microarray. The droplets stored in the same rows have the same concentrations and volumes. This is achieved by either (1) metering, 2D multiplexing, storing and reinjection, or (2) metering, entrapping, coalescing, 2D multiplexing and storing of two specific source fluids into the microarray. The operation of the prototype system is automated and governed by a program that coordinates the actuation of all the diaphragm valves utilized. Currently, we are working on the integration of droplet monitoring function into the microarray. By measuring the capacitance or impedance across a channel, the appearance of a droplet in a storage unit or even the condition inside the droplet could be detected. Meanwhile, we are also working on the scaling of the microarray. For an 8 × 8 array, $2 \times \log_2 8 = 6$ control inputs are required for multiplexing. Compared to the demonstrated 4 × 4 array, which employs $2 \times \log_2 4 = 4$ control inputs, the area of an 8 × 8 array is roughly quadruple, while the thickness of the four-layer structure remains the same. Since two layers of control channels are employed to simplify the wiring, the complexity of channel network remains manageable when scaling. The overall cost ratio of upgrading from 4 × 4 to 8 × 8 arrays is estimated to be less than the ratio of unit number (or sample size) growth, while the fabrication yield might potentially fall because of the increase in chip size and complexity. As such, this proposed random-access microarray could potentially serve as a platform for continuous tracking and multistep processing of emulsion droplets, which is desired for various high-throughput screening and synthesis applications.

Conclusion

We have successfully demonstrated an integrated microfluidic system that is capable of programmably metering, entrapping, coalescing, addressably storing, reinjection, retrieving and parallelly shaking emulsion droplets, which could potentially function as reactors for continuous tracking and multistep processing. A multilayer, flexible PDMS microfluidic chip with specially designed fluidic-channels dynamically reconfigured by pneumatically-actuated diaphragms is utilized to integrate a variety of droplet manipulation schemes. Once droplets are formed, their motions are coordinated by a 2D multiplexing scheme, which exploits the bidirectional movement of diaphragms to implement a random-access microarray. In the prototype demonstration, a PDMS molding and bonding process is used to fabricate the proposed microfluidic chip. Emulsion droplets with desired volumes and compositions are produced, addressably stored, manipulated and retrieved using a 4 × 4 array, which employs only 4 ($=2 \times \log_2 4$) control inputs for the operation. The operation of the prototype system is automated and governed by a program that coordinates the actuation of all the diaphragm valves utilized. It has been demonstrated that (1) the integration of droplet manipulation and 2D multiplexing schemes can be achieved readily using bidirectional diaphragm valves, (2) multiplexing of an $N \times N$ array could be realized utilizing just $2 \times \log_2 N$ control inputs and (3) a multifunctional, random-access microarray can be accomplished employing multilayer PDMS chip. The integration of metering, entrapping, coalescing, storing, reinjection, shaking and retrieving functions all on one single microfluidic chip could greatly enhance the controllability over a variety of screening processes. With droplets functioning as micro-reactors, the proposed random-access microarray could potentially serve as a platform for continuous tracking and multistep processing of emulsion droplets, which is desired for various high-throughput screening and synthesis applications.

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References

[1] Song H, Chen D L and Ismagilov R F 2006 Reactions in droplets in microfluidic channels *Angew. Chem. Int. Ed.* **45** 7336–56

[2] Kelly B T, Baret J C, Taly V and Griffiths D 2007 Miniaturizing chemistry and biology in microdroplets *Chem. Commun.* **18** 1773–88

[3] Leamon J H, Link D R, Egholm M and Rothberg J M 2006 Overview: methods and applications for droplet compartmentalization of biology *Nat. Methods* **3** 541–3

[4] Teh S Y, Lin R, Hung L H and Lee A P 2008 Droplet microfluidics *Lab Chip* **8** 198–220

[5] Theberge A B, Courtois F, Schaerli Y, Fischlechner M, Abell C, Hollfelder F and Huck W T S 2010 Microdroplets in microfluidics: an evolving platform for discoveries in chemistry and biology *Angew. Chem. Int. Ed.* **49** 5846–68

[6] Griffiths A D and Tawfik D S 2006 Miniaturizing the laboratory in emulsion droplets *Trends Biotechnol.* **24** 395–402

[7] Zheng B, Roach L S and Ismagilov R F 2003 Screening of protein crystallization conditions on a microfluidic chip using nanoliter-size droplets *J. Am. Chem. Soc.* **125** 11170–1

[8] Martin K, Henkel T, Baier V, Grodrian A, Schon T, Roth M, Kohler J M and Metze J 2003 Generation of larger numbers of separated microbial populations by cultivation in segmented-flow microdevices *Lab Chip* **3** 202–7

[9] Khan S A, Gunther A, Schmidt M A and Jensen K F 2004 Microfluidic synthesis of colloidal silica *Langmuir* **20** 8604–11

[10] Dittrich P S and Manz A 2006 Lab-on-a-chip: microfluidics in drug discovery *Nat. Rev. Drug Discov.* **5** 210–8

[11] Song H and Ismagilov R F 2003 Millisecond kinetics on a microfluidic chip using nanoliters of reagents *J. Am. Chem. Soc.* **125** 14613–9

[12] Song H, Tice J D and Ismagilov R F 2003 A microfluidic system for controlling reaction networks in time *Angew. Chem. Int. Ed.* **42** 768–72

[13] Mazutis L, Baret J, Treacy P, Skhiri Y, Araghi A F, Ryckelynck M, Taly V and Griffiths A D 2009 Multi-step microfluidic droplet processing: kinetic analysis of an *in vitro* translated enzyme *Lab Chip* **9** 2902–8

[14] Squires T M and Quake S R 2005 Microfluidics: fluid physics at the nanoliter scale *Rev. Mod. Phys.* **77** 977–1026

[15] Duffy D C, McDonald J C, Schueller O J A and Whitesides G M 1998 Rapid prototyping of microfluidic systems in poly(dimethylsiloxane) *Anal. Chem.* **70** 4974–84

[16] Reyes D R, Iossifidis D, Auroux P A and Manz A 2002 Micro total analysis systems: 1. Introduction, theory, and technology *Anal. Chem.* **74** 2623–36

[17] Auroux P A, Iossifidis D, Reyes D R and Manz A 2002 Micro total analysis systems: 2. Analytical standard operations and applications *Anal. Chem.* **74** 2637–52

[18] Melin J and Quake S R 2007 Microfluidic large-scale integration: the evolution of design rules for biological automation *Annu. Rev. Biophys. Biomech.* **36** 213–31

[19] Unger M A, Chou H P, Thorsen T, Scherer A and Quake S R 2000 Monolithic microfabricated valves and pumps by multilayer soft lithography *Science* **288** 113–6

[20] Thorsen T, Maerkl S J and Quake S R 2002 Microfluidic large-scale integration *Science* **298** 580–4

[21] Grover W H, Skelley A M, Liu C N, Lagally E T and Mathies R A 2003 Monolithic membrane valves and diaphragm pumps for practical large-scale integration into glass microfluidic devices *Sensors Actuators B—Chem* **89** 315–23

[22] Lee P J, Hung P J, Rao V M and Lee L P 2005 Nanoliter scale microbioreactor array for quantitative cell biology *Biotechnol. Bioeng.* **94** 5–14

[23] Wang H Y, Bao N and Lu C 2008 A microfluidic cell array with individually addressable culture chambers *Biosens. Bioelectron.* **24** 613–7

[24] Huebner A, Bratton D, Whyte G, Yang M, deMello A J, Abell C and Hollfelder F 2009 Static microdroplet arrays: a microfluidic device for droplet trapping, incubation and release for enzymatic and cell-based assays *Lab Chip* **9** 692–8

[25] Schmitz C H J, Rowat A C, Köster S and Weitz D A 2009 Dropspots: a picoliter array in a microfluidic device *Lab Chip* **9** 44–9

[26] Wang Z, Kim M, Marquez M and Thorsen T 2007 High-density microfluidic arrays for cell cytotoxicity analysis *Lab Chip* **7** 740–5

[27] Shi W, Qin J, Ye N and Lin B 2008 Droplet-based microfluidic system for individual Caenorhabditis elegans assay *Lab Chip* **8** 1432–5

[28] Tan W and Takeuchi S 2007 A trap-and-release integrated microfluidic system for dynamic microarray applications *Proc. Natl Acad. Sci. USA* **104** 1146–51

[29] Tan W and Takeuchi S 2008 Dynamic microarray system with gentle retrieval mechanism for cell-encapsulating hydrogel beads *Lab Chip* **8** 259–66

[30] Thorsen T, Roberts R W, Arnold F H and Quake S R 2001 Dynamic pattern formation in a vesicle-generating microfluidic device *Phys. Rev. Lett.* **86** 4163–6

[31] Anna S L, Bontoux N and Stone H A 2003 Formation of dispersions using 'flow focusing' in microchannels *Appl. Phys. Lett.* **82** 364–6

[32] Lin B C and Su Y C 2008 On-demand liquid-in-liquid droplet metering and fusion utilizing pneumatically-actuated membrane valves *J. Micromech. Microeng.* **18** 115005

[33] Tan Y C, Ho Y L and Lee A P 2007 Droplet coalescence by geometrically mediated flow in microfluidic channels *Microfluid. Nanofluid.* **3** 495–9

[34] Hung L H, Choi K M, Tseng W Y, Tan Y C, Shea K J and Lee A P 2006 Alternating droplet generation and controlled dynamic droplet fusion in microfluidic device for CdS nanoparticle synthesis *Lab Chip* **6** 174–8

[35] Link D R, Grasland-Mongrain E, Duri A, Sarrazin F, Cheng Z, Cristobal G, Marquez M and Weitz D A 2006 Electric control of droplets in microfluidic devices *Angew. Chem. Int. Ed.* **45** 2556–60

[36] Duffy D C, McDonald J C, Schueller O J A and Whitesides G M 1998 Rapid prototyping of microfluidic systems in poly(dimethylsiloxane) *Anal. Chem.* **70** 4974–84

[37] Haubert K, Drier T and Beebe D 2006 PDMS bonding by means of a portable, low-cost corona system *Lab Chip* **6** 1548–9

[38] Lin H H, Chang S C and Su Y C 2010 On-demand double emulsification utilizing pneumatically actuated, selectively surface-modified PDMS micro-devices *Microfluid. Nanofluid.* **9** 1091–102