A programmable microfluidic platform for multisample injection, discretization, and droplet manipulation

Cite as: Biomicrofluidics 14, 014112 (2020); doi: 10.1063/1.5143434

Submitted: 26 December 2019 · Accepted: 26 January 2020 ·

Published Online: 5 February 2020

Hesam Babahosseini,^{1,2} Supriya Padmanabhan,³ Tom Misteli,¹ and Don L. DeVoe^{2,3,a)}

AFFILIATIONS

¹National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

²Department of Mechanical Engineering, University of Maryland, College Park, Maryland 20742, USA

³Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, Maryland 20742, USA

^{a)}Author to whom correspondence should be addressed: ddev@umd.edu. Tel.: +1-301-405-8125

ABSTRACT

A programmable microfluidic platform enabling on-demand sampling, compartmentalization, and manipulation of multiple aqueous volumes is presented. The system provides random-access actuation of a microtrap array supporting selective discretization of picoliter volumes from multiple sample inputs. The platform comprises two interconnected chips, with parallel T-junctions and multiplexed microvalves within one chip enabling programmable injection of aqueous sample plugs, and nanoliter volumes transferred to a second microtrap array chip in which the plugs are actively discretized into picoliter droplets within a static array of membrane displacement actuators. The system employs two different multiplexer designs that reduce the number of input signals required for both sample injection and discretization. This versatile droplet-based technology offers flexible sample workflows and functionalities for the formation and manipulation of heterogeneous picoliter droplets, with particular utility for applications in biochemical synthesis and cell-based assays requiring flexible and programmable operation of parallel and multistep droplet processes. The platform is used here for the selective encapsulation of differentially labeled cells within a discrete droplet array.

Published under license by AIP Publishing. <https://doi.org/10.1063/1.5143434>

I. INTRODUCTION

Sample discretization is a required functionality for a wide range of diagnostic and bioanalytical systems.^{1,2} For example, conventional well plates are widely employed as reactors for cell screening assays, with microliter-scale reagent volumes isolated in discrete wells by manual or robotic pipetting. Miniaturization of sample discretization through the application of droplet microfluidic technology has the potential to significantly reduce sample consumption, while simultaneously increasing throughput, reducing cost, and improving assay sensitivity.³ Droplet-based microfluidic technology has proven to be particularly useful, with the ability to readily confine reagents within picoliter volume reactors.⁴ Droplet-based microfluidic systems can enable rapid reaction of reagents with minimum contamination in an automated and inexpensive fashion for various chemical or biochemical applications.⁵

Microfluidic droplet generation is commonly achieved using a T-junction^{6,7} or flow-focusing^{8,9} configuration, in which a dispersed

phase fluid is sheared into discrete volumes by an immiscible continuous phase fluid. To facilitate on-demand injection of the sample with control over droplet timing and volume, pneumatic membrane microvalves fabricated in polydimethylsiloxane (PDMS) can be integrated at the aqueous inlet of a T-junction.^{10–12} Alternatively, other mechanisms enabling active droplet formation based on acoustic,¹³ magnetic,¹⁴ or electrical¹⁵ forces have also been used, and sample volumes can also be self-discretized into microwells to generate an array of trapped droplets with uniform droplet size.¹⁶ Following droplet formation, manipulation steps as trapping, storage, and release can provide additional functionality for many chemical and biological assays. Passive droplet trapping has been widely explored using a hydrodynamic approach in which the fluidic resistance through a given trap increases following droplet capture, thereby guiding any subsequent droplets toward downstream traps,^{17–19} and active microfluidic trapping based on flow-controlled wetting,²⁰ Laplace pressure,²¹ and electrohydrodynamics²² has also been developed.

Beyond droplet generation and trapping, a number of platforms have been developed to provide programmable droplet manipulation, including storage, monitoring, merging, release, and multistep processing of individual droplets.^{23–26} For example, trapped droplets can be simultaneously released by flow reversal,²⁷ and on-demand release of individual droplets has been reported using hydrodynamic,^{26,28–30} electrical,³¹ acoustic,³² pneumatic,³³ or optothermal^{34,35} methods. Controlled droplet formation and release have been shown to enable complex operations, such as the formation of a trap array containing individual cells.^{26,30} Droplet manipulation has also been combined with parallel droplet or plug generation to support the processing of multiple samples in a single device. For example, a microfluidic platform containing a set of T-junctions was reported for droplet formation from multiple samples, with generated droplets isolated in a trap array.³⁶ Although this device uses a simple strategy to form and store a droplet array, the trapped droplets cannot be retrieved for further operations. In a related system, metered volumes of up to eight different samples were sequestered within individual traps or combined within a single trap, with the recovery of selected droplets from a large-scale array achieved by hydrodynamic flow with on-chip multiplexed valving.²⁰

In the present work, we describe a microfluidic platform incorporating multisample injection and selective droplet formation combined with random-access droplet manipulation in a flexible and modular system. The system employs integrated membrane valves for flexible on-demand sample injection of picoliter to nanoliter plugs using a parallel T-junction structure with embedded microvalve actuators.²⁹ Unlike prior work, the platform takes advantage of membrane displacement traps for digital manipulation of droplets,³³ enabling the controlled discretization of injected

sample plugs into individual picoliter volume droplets while also supporting direct ejection and recapture of the captured droplets without the need for manipulating bulk hydrodynamic flow within the microfluidic system. To avoid the need for individual control inputs to address each on-chip element, binary pneumatic multiplexers allow an array of 2^N on-chip microvalves to be addressed with only $2N$ control inputs.^{37–39} The multiplexers employ a segmented valve seat design^{40–42} for reliable valve operation, with different multiplexer configurations employed for dynamic sample plug generation and membrane displacement trap operation. The resulting platform supports multiple liquid sampling and handling steps including selective sample plug injection, controlled sample discretization and droplet storage, and on-demand droplet ejection. The system is specifically explored here for selective single-cell trapping and the formation of defined cell ensembles. This technology offers potential for diverse applications including chemical analysis,⁴³ protein crystallization,⁴⁴ organic synthesis,⁴⁴ enzyme assay,^{45,46} cell-based assays,⁴⁶ drug screening,⁴⁷ and organism assays.⁴⁸

II. METHODS

A. Device fabrication

A three-layer PDMS process was employed to fabricate two separate chips that were later combined using tubing for fluidic interconnection between the chips [Fig. 1(a)]. The use of two separate microfluidic devices, rather than a single integrated chip simplifies the fabrication of the multiplexers, which require different channel depths for proper operation. The multichip design also serves to modularize the sample plug generation and droplet manipulation components. Individual PDMS layers were fabricated by soft lithography to create an upper set of microchannels that

FIG. 1. (a) Optical image of an integrated droplet-based microfluidic platform combining (i) an on-demand sample injection chip and (ii) a random-access microtrap array chip, with fluidic interfacing between the chips achieved using Tygon tubing. (b) Schematic view of the combined devices. (c) Cross-sectional views of the PDMS fabrication process, revealing the topologies for (i) a sample injection microvalve or membrane displacement trap actuator and (ii) a blocking microvalve used for multiplexing. Two different multiplexer geometries are used for the sample injection and microtrap array chips.

serve as either fluidic channels or pneumatically actuated control lines, and a lower set of pneumatic channels connected to a constant pressure source, with a thin PDMS membrane separating the upper and lower channel layers [Fig. 1(b)]. The pressure lines are connected to the microvalves used for droplet generation and trapping, and the control lines serve to gate the pressure lines. Master molds for the top fluidic/control layer and the bottom pressure layer were prepared using a negative photoresist (SU-8 2075, MicroChem, Westborough, MA), spin-coated on silicon wafers at 2000 rpm and 3500 rpm for 60 s to yield 70 μm and 40 μm thick structures on silicon wafers, respectively. Master molds for the membrane layer were formed by spin-coating SU-8 at either 9000 rpm or 1000 rpm for 60 s to form 6 μm or 21 μm thick films for the on-demand sample injection chip or random-access microarray chip, respectively. Each photoresist layer was patterned by conventional photolithography. The casting and bonding process was completed layer by layer, starting with the fluidic/control layer. PDMS (Sylgard 184, Dow-Corning, Midland, MI) was mixed at a 10:1 ratio of prepolymer and curing agent, degassed for 15 min under vacuum to remove trapped air bubbles, and poured over the mold masters. Each PDMS layer was incubated at 80 $^{\circ}\text{C}$ for 30 min to partially cure the elastomer, and the mold was peeled off before punching access holes. The membrane layers were formed by spin-coating PDMS at a 20:1 ratio on top of the molds at 1500 rpm or 2500 rpm for 60 s, yielding a membrane thickness of either 37 μm or 21 μm for the on-demand sample injection chip or random-access microarray chip, respectively. The membrane layers were partially cured for 11 min at 80 $^{\circ}\text{C}$. The top fluidic/control layer was then aligned, manually pressed, and bonded on top of the membrane layer and cured overnight at 80 $^{\circ}\text{C}$ before peeling the two-layer structure off the silicon wafer. A bottom pneumatic pressure layer was next patterned and partially cured using the same process as the top fluidic/control layer. Finally, the two-layer bonded PDMS structure was aligned with the pressure layer and incubated at 80 $^{\circ}\text{C}$ overnight for complete bonding. The final PDMS devices were placed in a desiccator under vacuum prior to experiments.

B. System operation

Control over pneumatic inputs was provided using a solenoid valve manifold (Clippard Instrument Laboratory, Cincinnati, OH) connected to a programmable Arduino microcontroller with custom code to coordinate solenoid actuation. For the sample injection microvalves, the pressure line is set at 15 psi and the control line at 20 psi. For the membrane displacement trap microvalves, 10 psi and 20 psi pressures were applied to the pressure and control lines, respectively. To introduce liquids into the fluidic layer, each sample was stored in a custom plastic container and driven through the chip by application of pressurized air to the head of the container. All off-chip pneumatic and fluidic interfaces were made using flexible Tygon microbore tubing (0.51 mm ID, 1.52 mm OD, Cole-Parmer, Vernon Hills, IL) connected to 22 gauge needle segments (Hamilton Company, Reno, NV) inserted in the access ports of the fluidic/control and pressure layers. Diluted food dyes in DI water were used to visualize different aqueous sample solutions. The driving pressure applied to each sample was controlled by an

independent pressure regulator (0–10 psi range, Marsh Bellofram, Newell, WV). Mineral oil with 0.01% SPAN-80 surfactant (Sigma-Aldrich, St. Louis, MO) as a continuous phase was injected into the main channel. All devices were initially primed with continuous phase before sample introduction to assist with plug formation and sample discretization and to minimize unwanted interactions between the sample and PDMS channel or trap walls. All experiments were monitored under an inverted optical microscope (AZ-100, Nikon, Japan) equipped with a CCD camera for imaging.

III. RESULTS AND DISCUSSION

A. Microfluidic device development

1. Multiplexed on-demand sample injection

The integration of an elastomer microvalve with a microfluidic T-junction provides an effective mechanism for precisely controlling the injection of a sample plug into a downstream channel.^{10–12} In this approach, a microvalve positioned over the aqueous inlet channel of the T-junction is actuated by pressurizing the corresponding pneumatic line, deflecting the membrane into the channel to occlude flow. Modulation of the actuator enables the controlled generation of sample droplets or plugs.

To provide control over multiple sample plug generators, an on-chip fluidic multiplexer is employed. The multiplexer design is based upon a repeating unit structure comprised of a series of parallel pneumatic control lines driving blocking valves. The normally open blocking valves close upon the application of pressurized air through the control line, thereby closing off the corresponding channel in the pressure layer. Each pressure layer channel is connected to a constant high pressure source at one end and open to atmosphere on the other. Thus, when a given blocking valve is actuated, the pressure within the channel rises to match the source pressure, while releasing the blocking valve quickly returns the channel to a linear pressure gradient. The pressure channels themselves are used to control flow within the aqueous sample inlets, while control lines connected to membrane valves positioned near the open ends of the pressure channels dictate which lines in the pressure layer are activated. The fluidic and control microchannels are fabricated in the same layer and differ only in their connections to either the aqueous sample inlet or the external software-controlled pneumatic valve manifold. We note that all blocking valves associated with a given control line will open or close together as they share a common air supply pressure. A pressure line will be blocked if any of the blocking valves on it are closed. A total of 2^N control lines are required for individual addressing of 2^N microvalves.^{37–39}

Figure 2 illustrates the multiplexer topology used for sample injection. The multiplexer is designed to open a single sample injection microvalve while keeping all other sample injection valves closed by maintaining high pressure within their corresponding pressure lines. When the control lines are set to allow at least one blocking valve between the high pressure inlet and air outlet port to close, the pressure at the corresponding sample injection valve membrane becomes high, ensuring that the membrane deflects into the fluidic channel and the injection valve remains closed.

FIG. 2. (a) Fabricated sample injection multiplexer and (b) schematic diagram showing the selective opening of an injection valve by venting the corresponding pressure line. (c) Fabricated random-access microtrap array and (d) schematic diagram illustrating the blocking valve states for the actuation of a single membrane displacement trap.

FIG. 3. (a) Cross-sectional schematic of the blocking valve design in open and closed states. In the open state, air flows from the upstream pressure line to the downstream pressure line across the blocking valve seat. Conversely, in its closed state the elastomeric membrane is deflected downward, eliminating the gap and blocking airflow. (b) Hydraulic resistance equivalent circuit diagrams for the pressure lines of the sample injector and microtrap array multiplexers.

Conversely, when all blocking valves along a given pressure line are open, air is shunted to the pressure line outlet and the sample injection valve membrane does not experience significant deflection, resulting in an open injection valve. In this design, the vent lines ensure that the pressure at the injector membrane can be rapidly switched between high and low pressure states for precise control over injection volume. For example, in Fig. 2(b), control lines C1, C3, and C6 are activated such that only the sample injection microvalve connected to pressure line P4 is open, thereby directing the sample into the main channel through the corresponding sample inlet.

2. Multiplexed random-access microtrap arrays

Injected sample plugs are delivered into the fluidically coupled microtrap array chip for discretization using an active digitization strategy. Addressable, selective manipulation of individual droplets is a key operational capability for broad applications of droplet-based microfluidics. To address this need, each trap is integrated with a displacement membrane actuator for controllable ejection of selected droplets.³³ As shown in Fig. 2(c), an integrated multiplexer enables scalable random access to the trap array, allowing selective addressing of individual droplets within the array. Unlike the sample injector multiplexer, in which the multiplexer

FIG. 4. Illustrations of the on-demand sample injectors function. A single aqueous sample is released by shifting the corresponding injection microvalve into an unpressurized state, while all other samples remain blocked using the multiplexer system. (a) Successive injection microvalves are unactuated and multiple on-demand samples are continuously injected into the main microchannel. (b) The injection microvalves are iteratively switched on and off to reliably produce multiple droplets surrounded by the continuous oil phase.

FIG. 5. Effect of valve opening time and inlet pressure ratio for the aqueous and oil phases (P_{aq}/P_{oil}) on sample plug volume.

blocking valves are positioned between the injector membrane and atmospheric pressure venting ports, the trap array multiplexer employs blocking valves that are located directly between a high pressure inlet and the trap membrane elements. When at least one multiplexer blocking valve along a given pressure line is closed, air trapped within the line diffuses through the bulk PDMS, reducing the pressure across the associated trap membrane and preventing the membrane from deflecting. When all blocking valves along a particular pressure line are open, the high inlet pressure is directly transferred to the trap membrane, thereby deflecting the membrane and ejecting any droplet that may have been captured within the trap. An illustration of the random-access trap array multiplexer is shown in Fig. 2. An example depicting the actuation of a single

trap membrane is presented in Fig. 2(d), where control lines C1, C4, C6, and C7 are pressurized such that only pressure line P10 remains open, which leads to the actuation of the corresponding displacement trap membrane.

3. Multiplexer optimization

The injection and trap array multiplexers were optimized to achieve the desired functionality for selective aqueous sample injection and droplet manipulation. The blocking valves consist of three layers including a middle flexible elastomer membrane sandwiched between a top air control channel and a bottom air pressure line segmented with a valve seat [Fig. 3(a)]. Each micro-valve is normally open with a gap that allows air to flow through the segmented pressure line. When actuated via pressurized air in the top control channel, the membrane deflects downward to cover the valve seat and block the airflow in the underlying pressure channel.

Equivalent circuit diagrams depicting the hydraulic resistance in the pressure lines for both multiplexer designs are shown in Fig. 3(b). Each blocking valve acts as a pressure-dependent binary variable resistor on the path of a pressure line. For the membrane displacement trap multiplexer, the hydraulic resistance in the pressure line includes the resistance of the segmented pressurized air delivery channel and the resistance of the blocking valves. When the pressure line is in the closed state, the blocking valves are designed to introduce a high resistance upon actuation which reliably blocks the high pressure airflow through the pressure line. Beyond a certain leakage through the closed blocking valves, airflow can still pressurize the displacement trap microvalve, affecting the stability of stored drops. The blocking valve state can be regulated by the pressure difference across the valve membrane.

FIG. 6. Sample discretization into a microtrap array. After [(i) and (ii)] oil priming of the entire microtrap array and (iii) ejecting bubbles from the traps, (iv) selected traps are actuated, and an aqueous sample plug is introduced into the main channel from the sample injection chip. (v) Sample is pulled into the traps by releasing the displacement trap membranes (vi) and discretized by backfilling the main channel with oil, resulting in (vii) uniform picoliter scale droplets within the microtrap array. (viii)–(x) By sequentially introducing unique sample plugs into the trap array, different droplet compositions are readily achieved.

To ensure that the flexible membrane over the valve seat will block the opening gap, a higher air pressure (20 psi) is applied to the control line above the valve membrane, while a lower pressure (10 psi) is applied to the inlet pressure line. For the trap array multiplexer, a blocking valve gap of $6\mu\text{m}$ was found to be sufficient to seal each valve in its closed state, with higher gaps resulting in leakage and destabilization of trapped droplets. This relatively small gap size leads to a partially open blocking valve during fabrication, with the flexible thin membrane partially bonded onto the valve seat.

For the sample injection multiplexer, the hydraulic resistance in the pressure line includes the resistance of the pressurized air delivery and venting channels and the blocking valves. When the pressure line is in the closed state, the pressure at the injection microvalve is equal to the inlet pressure. However, when the pressure line is in the open state, the high pressure air (15 psi) is vented to the outlet with low hydraulic resistance, resulting in an injection valve pressure close to atmospheric pressure and yielding an open valve state. The low hydraulic resistance was ensured by designing the normally open blocking valves with a larger gap size ($25\mu\text{m}$) and wider and deeper venting channels ($120 \times 70\mu\text{m}$).

B. On-demand multisample plug generation

Each device contains a set of eight independent sample injectors consisting of T-junctions integrated with integrated microvalves, allowing arbitrary sequences of aqueous phase sample volumes to be controllably introduced into the main channel. By adjusting the injection valve opening time, sample volumes ranging from large plugs [Fig. 4(a)] to small droplets [Fig. 4(b)] can be reliably generated with control provided through the attached multiplexer. As seen in Fig. 4(b), small variations in generated plug volumes are observed across the different plug generators. This behavior is due to a combination of limited precision for the pressure regulators used to define the sample inlet pressures and variations in hydrodynamic resistance for the fluidic sample lines. To improve reproducibility, droplet volume should be individually calibrated for each generator by tuning the sample reservoir pressures.

To evaluate sample volume control, the effects of the inlet pressure ratio and valve opening time on plug volume were determined, with the results shown in Fig. 5. The plug volume was found to scale linearly with valve opening time, with the scaling factor dependent on the inlet pressure ratio between the aqueous (P_{aq}) and oil (P_{oil}) phases. Using this approach, the generated plug volume has no upper limit, and long plugs can be generated by increasing the sample injection valve opening time. However, with opening times above 600 ms for $P_{\text{aq}}/P_{\text{oil}} = 1.6$, the generation of multiple small droplets ($<5\%$ of the plug volume) at the tail end of the sample plug was often observed upon microvalve actuation. Controllable formation of uniform droplets as small as 300 pl was achieved by reducing the opening time to 100 ms when $P_{\text{aq}}/P_{\text{oil}} = 1.2$. For smaller opening times, the generated droplets exhibited inconsistent volumes. Multiple properties of the microfluidic device and experimental system are expected to influence this limit, including fluid dynamics for both the sample and the pneumatic lines, compliance of the PDMS channels, and dynamics of the electromechanical components including pneumatic valves

and ancillary control circuitry. Further optimization of the minimum microvalve opening time may be possible by modifying the chip design and experimental apparatus to reduce the time constants associated with each of these elements.

FIG. 7. Illustration of the random-access trap array functionality. (a) Single droplets are sequentially displaced into the main channel by actuating the corresponding membrane displacement element using the multiplexer. (b) A group of droplets are ejected by simultaneously actuating the corresponding membrane displacement elements.

C. Sample discretization and droplet array formation

Following the sample plug formation in the injection chip, each plug is transported into the microtrap array chip through the flexible tubing interconnect for further processing. The delivered plugs are discretized into an array of membrane displacement traps based on the process shown in Fig. 6. After first priming the main channel with oil, all traps are actuated to release trapped air and then released to pull oil into the traps. Specific array elements to be filled with the sample are then actuated to expel oil and maintained in the actuated state, while the aqueous sample plug is hydrodynamically mobilized to the trap openings. The actuated membrane displacement valves were next released to draw the aqueous phase into the traps. The sample is finally discretized by continuing to flow oil through the main channel at a flow rate below $1 \mu\text{l}/\text{min}$ to remove the aqueous phase from the main channel while forming discretized picoliter scale aqueous droplets within the traps. This process can be performed using a subset of selected traps within the array or with all traps filled simultaneously as shown in Fig. 6.

The discretization process depicted in Fig. 6 employs a single sample plug that extends across the full length of the channel in fluid connection with the trap array. Discretization from such a large sample plug is easily automated by adjusting the time

between plug generation and trap actuation. However, as the sample plug volume is reduced, uncertainty in the relative position between the plug and trap openings increases, ultimately demanding direct observation of the plug position during trap actuation to ensure effective sample capture and discretization from small plugs. We have previously demonstrated this approach for the manipulation and capture of single droplets within a low density trap array using microscopy and manual valve actuation. While not explored in this work, we note that this process is amenable to the use of a computer vision system for automated operation.

D. Selective droplet release

After discretization, specific droplets can be released by selectively actuating the membranes associated with the chosen traps. As the membrane deflects into a given trap chamber, the stored droplet is ejected into the main channel where it can be transported by the bulk flow of the continuous oil phase to another location on the chip or to the outlet for off-chip processing. The traps are individually addressed in this manner using the integrated multiplexer by actuating all control lines that do not have a blocking valve on the path of the selected trap such that a pressure of 10 psi is only applied across the displacement membrane associated with the

FIG. 8. (a) Image sequences showing the process for deterministic capture and discretization of an individual cell. (i) An initially actuated microtrap displacement valve is (ii) released when the targeted cell passes the trap entrance. (iii) If the target cell is not trapped, the valve is reset and the process is repeated. (iv) Upon successful capture, the cell is encapsulated into a discrete droplet during oil backfilling of the main channel. (b) Active capture by valve actuation is critical to the process, since cells entering an open trap follow streamlines back into the main channel and cannot be passively retained within the traps. Shading in panel (v) indicates flow velocity magnitude.

desired trap. During the release process, the flow rate of oil in the main channel is set at $0.1 \mu\text{l}/\text{min}$ to yield a capillary number of $\text{Ca} \sim 0.002$, ensuring that droplet splitting during ejection does not occur.³³ During this process, all other droplets remain undisturbed within their respective traps. To release subsequent droplets, the pressure inlet is turned off, and a new pattern of actuated control lines is established to select the next droplet for ejection. Examples of this process are presented in Fig. 8, with individual droplets released from their traps in sequence [Fig. 7(a)] or simultaneously in a group [Fig. 7(b)] to form a pattern of alternating droplets within the array. To avoid unwanted merging between moving and parked droplets, the oil phase used during droplet manipulation contains 0.7% SPAN-80 surfactant.

The device described in Figs. 6 and 7 employed an array of 16 trap elements controlled by eight pneumatic inputs through the integrated multiplexer. For applications such as drug screening or digital nucleic acid amplification, significantly higher trap densities with hundreds or thousands of wells are desired. Due to logarithmic scaling of the multiplexer used in this work, a trap array containing $1024 (2^{10})$ elements would require 20 (2×10) pneumatic inputs, suggesting that higher trap densities based on the current device design are feasible. However, world-to-chip interfacing presents a practical limit to array scaling, since each needle port connection presents a potential failure point during device fabrication. To address this limitation, future scaling of the multiplexer-enhanced trap arrays would benefit from the use of a fluidic manifold^{49–51} to obviate the need for individual needle port assembly.

E. Selective single-cell discretization

Droplet microfluidic technology has been widely employed for single-cell assays, allowing properties such as viability,⁵² cytotoxicity,⁵³ or enzymatic response²⁷ to be evaluated from individual cells within defined microenvironments. When using passive droplet generation techniques, the probability of sequestering a single cell within a given droplet is typically defined by the Poisson statistics,^{54,55} resulting in significant variability in the encapsulated cell distribution. To reduce cell count variability, hydrodynamic or inertial sorting of cells prior to droplet encapsulation has been explored,^{56,57} but diversity in the cell properties (e.g., size, shape, compressibility, or density) within a given cell population remains a challenge. Here, we take advantage of the multiplexed membrane displacement traps to controllably isolate individual cells using a deterministic approach. The process is depicted in Fig. 8(a), which includes composite images showing the path of a single cell at different stages of the process. As a target cell approaches the entrance of the desired microtrap, the initially actuated trap membrane is released, thereby opening the trap and pulling the adjacent volume of fluid containing the cell into the trap. The trap contents may then be interrogated to ensure that a single cell of the desired type was captured. If not, the trap contents are released by actuating the membrane, and another capture event is performed. Additional cells can then be captured in additional traps following the same procedure. The active cell capture process based on trap membrane actuation was found to be critical for the successful isolation of single cells. As seen in Fig. 8(b), when flowing past an open trap prefilled with

buffer, cells follow the fluid streamlines within the main channel and do not become entrained in the recirculating flow within the trap itself, resulting in no capture of the cells.

To demonstrate the capability of the microfluidic platform for selective capture of single cells, experiments were conducted with Chinese Hamster Ovary (CHO) cells stained with blue (DAPI; 4',6-diamidino-2-phenylindole), green (HCS CellMask), or red (HCS CellMask) dyes. Solutions with the three differentially labeled cell populations were introduced into the microfluidic chip, and cells were selectively captured within the desired microtraps and isolated after discretization. In these experiments, the average time required for each single cell capture event was less than 3 s. Figure 9 shows examples of droplets with encapsulated single cells in each trap. In Figs. 9(a)–9(c), homogeneous plugs of cells with a single label were injected into the trap array, while in Fig. 9(d), a mixed population of cells was employed. In each case, individual traps were optically monitored under a fluorescence microscope during plug injection to detect the presence of a single cell of the desired population at

FIG. 9. Selective single-cell capture and droplet encapsulation. Each image shows individually encapsulated cells in three sequential traps with either (a)–(c) homogeneous or (d) heterogeneous groupings.

the entrance to the trap, and the appropriate multiplexer control lines were actuated to pull each cell into its corresponding trap.

Unlike passive single-cell capture employing the Poisson statistics, the active cell discretization technique is a cytometric process that requires an optical system for monitoring the trap array. While the monitoring process can be automated to avoid the need for manual control over the process, a disadvantage of this approach is that array density is limited by the optical field of view used for monitoring. The technology is thus better suited for low-volume assays involving rare cells, or experiments requiring precise control over cell numbers within defined populations, than high throughput applications such as drug screening, where large numbers of discretized cells are typically desired.

IV. CONCLUSION

Microfluidic technology enabling programmable generation and manipulation of discrete fluid volumes offers enormous potential for automated and flexible on-chip sample processing. In this work, we combined fluidic multiplexers optimized for different system functions with microvalve actuators enabling sample plugs to be generated on demand and discretized into individual trap elements. The multiplexers allowed the microvalves to be addressed with a minimum number of signal inputs and served to enable programmable operation of all system functions including injection of aqueous samples with tunable volumes, sample discretization into individual traps, and controllable random-access retrieval of the resulting sample packets either individually or simultaneously from a group of selected traps. An important aspect of the active trapping process is its utility for deterministic sequestration of individual cells or other biological elements. Using the random-access microtrap array, the controlled capture of specific single cells within selected traps is demonstrated using a cytometric approach. By enabling flexible sample discretization and programmable control of the resulting sample volumes, the droplet-based microfluidic technology offers particular benefits for low-volume assays involving intracellular communication between discretized populations where precise manipulation of cell ensembles and cellular environment is crucial.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for on-demand sample injections for forming plugs and droplets (Movie S1), random-access microtrap array for selective retrieval of individual or group of droplets (Movie S2), and single-cell selective capture, release, and encapsulation (Movie S3).

ACKNOWLEDGMENTS

This research was supported by a CCR FLEX Award and the Intramural Research Program of the NIH, NCI, Center for Cancer Research and by the U.S. National Science Foundation (NSF) through Grant Nos. CMMI1562468 and CBET1844299.

REFERENCES

- ¹W. L. Chou, P. Y. Lee, C. L. Yang, W. Y. Huang, and Y. S. Lin, *Micromachines* **6**, 1249 (2015).

- ²L. I. U. Zhao-miao, Y. Yang, D. U. Yu, and P. Yan, *Chin. J. Anal. Chem.* **45**, 282 (2017).
- ³A. M. Kaushik and K. Hsieh, *WIREs Nanomed. Nanobiotechnol.* **10**, e1522 (2018).
- ⁴P. Zhu and L. Wang, *Lab Chip* **17**, 34 (2017).
- ⁵L. Shang, Y. Cheng, and Y. Zhao, *Chem. Rev.* **117**, 7964 (2017).
- ⁶P. Garstecki, M. J. Fuerstman, H. A. Stone, and G. M. Whitesides, *Lab Chip* **6**, 437 (2006).
- ⁷A. Gupta, S. M. S. Murshed, and R. Kumar, *Appl. Phys. Lett.* **94**, 164107 (2009).
- ⁸L. Yobas, S. Martens, W.-L. Ong, and N. Ranganathan, *Lab Chip* **6**, 1073 (2006).
- ⁹S. L. Anna, N. Bontoux, and H. A. Stone, *Appl. Phys. Lett.* **82**, 364 (2003).
- ¹⁰W. S. Lee, S. Jambovane, D. Kim, and J. W. Hong, *Microfluid. Nanofluid.* **7**, 431 (2009).
- ¹¹X. Sun, K. Tang, R. D. Smith, and R. T. Kelly, *Microfluid. Nanofluid.* **15**, 117 (2013).
- ¹²F. Guo, K. Liu, X. H. Ji, H. J. Ding, M. Zhang, Q. Zeng, W. Liu, S. S. Guo, and X. Z. Zhao, *Appl. Phys. Lett.* **97**, 233701 (2010).
- ¹³A. Bransky, N. Korin, M. Khoury, and S. Levenberg, *Lab Chip* **9**, 516 (2009).
- ¹⁴S. Kahkeshani and D. Di Carlo, *Lab Chip* **16**, 2474 (2016).
- ¹⁵Y. Li, M. Jain, Y. Ma, and K. Nandakumar, *Soft Matter* **11**, 3884 (2015).
- ¹⁶D. E. Cohen, T. Schneider, M. Wang, and D. T. Chiu, *Anal. Chem.* **82**, 5707 (2010).
- ¹⁷W. C. Cheng, Y. He, A. Y. Chang, and L. Que, *Biomicrofluidics* **7**, 1 (2013).
- ¹⁸H. Boukellal, S. Selimović, Y. Jia, G. Cristobal, and S. Fraden, *Lab Chip* **9**, 331 (2009).
- ¹⁹S. S. Bithi, W. S. Wang, M. Sun, J. Blawdziewicz, and S. A. Vanapalli, *Biomicrofluidics* **8**, 1 (2014).
- ²⁰K. Leung, H. Zahn, T. Leaver, K. M. Konwar, N. W. Hanson, A. P. Page, C.-C. Lo, P. S. Chain, S. J. Hallam, and C. L. Hansen, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7665 (2012).
- ²¹M. G. Simon, R. Lin, J. S. Fisher, and A. P. Lee, *Biomicrofluidics* **6**, 1 (2012).
- ²²W. Wang, C. Yang, and C. M. Li, *Lab Chip* **9**, 1504 (2009).
- ²³X. Chen and C. L. Ren, *RSC Adv.* **7**, 16738 (2017).
- ²⁴R. Seemann, M. Brinkmann, T. Pfohl, and S. Herminghaus, *Rep. Prog. Phys.* **75**, 016601 (2012).
- ²⁵S.-Y. Teh, R. Lin, L.-H. Hung, and A. P. Lee, *Lab Chip* **8**, 198 (2008).
- ²⁶H.-H. Jeong, B. Lee, S. H. Jin, S.-G. Jeong, and C.-S. Lee, *Lab Chip* **16**, 1698 (2016).
- ²⁷A. Huebner, D. Bratton, G. Whyte, M. Yang, A. J. DeMello, C. Abell, and F. Hollfelder, *Lab Chip* **9**, 692 (2009).
- ²⁸H. Babahosseini, T. Misteli, and D. L. Devoe, in *Proceedings of Engineering in Medicine and Biology Conference (IEEE)*, (2018).
- ²⁹H. Babahosseini, T. Misteli, and D. L. Devoe, *Lab Chip* **19**, 493 (2019).
- ³⁰S. Jang, B. Lee, H. H. Jeong, S. H. Jin, S. Jang, S. G. Kim, G. Y. Jung, and C. S. Lee, *Lab Chip* **16**, 1909 (2016).
- ³¹F. Ahmadi, K. Samlali, and S. C. C. Shih, *Lab Chip* **19**, 524 (2019).
- ³²R. W. Rambach, K. Linder, M. Heymann, and T. Franke, *Lab Chip* **17**, 3422 (2017).
- ³³S. Padmanabhan, T. Misteli, and D. L. Devoe, *Lab Chip* **17**, 3717 (2017).
- ³⁴W.-H. Tan and S. Takeuchi, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1146 (2007).
- ³⁵W. H. Tan and S. Takeuchi, *Lab Chip* **8**, 259 (2008).
- ³⁶M. A. Czekalska, T. S. Kaminski, K. Makuch, and P. Garstecki, *Sens. Actuators B* **286**, 258 (2019).
- ³⁷J. Melin and S. R. Quake, *Annu. Rev. Biophys. Biomol. Struct.* **36**, 213 (2007).
- ³⁸T. Thorsen, S. J. Maerkl, and S. R. Quake, *Science* **298**, 580 (2002).
- ³⁹Y. M. Tseng, J. J. Wang, and Y. C. Su, *J. Micromech. Microeng.* **22**, 045005 (2012).
- ⁴⁰Z. Hua, Y. Xia, O. Srivannavit, J. M. Rouillard, X. Zhou, X. Gao, and E. Gulari, *J. Micromech. Microeng.* **16**, 1433 (2006).
- ⁴¹H. Takao, M. Ishida, and K. Sawada, *J. Microelectromech. S.* **11**, 421 (2002).
- ⁴²W. H. Grover, A. M. Skelley, C. N. Liu, E. T. Lagally, and R. A. Mathies, *Sens. Actuators B* **89**, 315 (2003).

- ⁴³S. Mashaghi, A. Abbaspourrad, D. A. Weitz, and A. M. van Oijen, *Trends Anal. Chem.* **82**, 118 (2016).
- ⁴⁴L. Li and R. F. Ismagilov, *Annu. Rev. Biophys.* **39**, 139 (2010).
- ⁴⁵M. P. N. Bui, C. A. Li, K. N. Han, J. Choo, E. K. Lee, and G. H. Seong, *Anal. Chem.* **83**, 1603 (2011).
- ⁴⁶C. Chang, J. Sustarich, R. Bharadwaj, A. Chandrasekaran, P. D. Adams, and A. K. Singh, *Lab Chip* **13**, 1817 (2013).
- ⁴⁷C. Regnault, D. Dheeman, and A. Hochstetter, *High-Throughput* **7**, 18 (2018).
- ⁴⁸W. Shi, H. Wen, Y. Lu, Y. Shi, B. Lin, and J. Qin, *Lab Chip* **10**, 2855 (2010).
- ⁴⁹G. A. Cooksey, A. L. Plant, and J. Atencia, *Lab Chip* **9**, 1298 (2009).
- ⁵⁰E. Wilhelm, C. Neumann, T. Duttonhofer, L. Pires, and B. E. Rapp, *Lab Chip* **13**, 4343 (2013).
- ⁵¹Z. Yang and R. Maeda, *J. Chromatogr. A* **1013**, 29 (2003).
- ⁵²S. Köster, F. E. Angilè, H. Duan, J. J. Agresti, A. Wintner, C. Schmitz, A. C. Rowat, C. a Merten, D. Pisignano, A. D. Griffiths, and D. a Weitz, *Lab Chip* **8**, 1110 (2008).
- ⁵³E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J. B. Hutchison, J. M. Rothberg, D. R. Link, N. Perrimon, and M. L. Samuels, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14195 (2009).
- ⁵⁴D. J. Collins, A. Neild, A. deMello, A.-Q. Liu, and Y. Ai, *Lab Chip* **15**, 3439 (2015).
- ⁵⁵L. Mazutis, J. Gilbert, W. L. Ung, D. A. Weitz, A. D. Griffiths, and J. A. Heyman, *Nat. Protoc.* **8**, 870 (2013).
- ⁵⁶M. Chabert and J. Viovy, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 3191 (2007).
- ⁵⁷J. F. Edd, D. Di Carlo, K. J. Humphry, S. Köster, D. Irimia, D. A. Weitz, and M. Toner, *Lab Chip* **8**, 1262 (2008).