Static array of droplets and on-demand recovery for biological assays

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ABSTRACT

Microfluidics has revolutionized several research areas by providing compact yet powerful microanalytical devices that in many cases outperform conventional systems. Among different microfluidics technologies, droplet microfluidics has emerged as a powerful platform to enable analyses of biological samples and phenomena because of its simplicity and versatility. Droplet microfluidics enables high-throughput encapsulation, manipulation, and analysis of single cells while drastically reducing the cost and time required by conventional technologies. For many of these microanalysis systems, manipulation of individual droplets is extremely important as it enables multiplexed high dimensional phenotyping of the targets, going beyond surface phenotyping. One of the key manipulation steps that needs to be implemented with high precision is enabling long-term observation of droplets and recovery of a subset of these droplets for further analysis. This Perspective highlights the recent advances and provides an outlook on future developments that will enable highly complex analyses of biological samples.

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The field of microfluidics has evolved rapidly over the last few decades in an ongoing effort to transform the bulky, expensive, complex analytical systems into coin-sized, inexpensive, and simple devices that can separate, concentrate, induce reactions, add reagents, and allow observations to enable analyses of biological samples.1,2 Droplet microfluidics, which takes advantage of the formation of highly uniform emulsion droplets, has emerged as a powerful platform for biological research. It can dramatically facilitate high-throughput single-cell manipulation and analysis while using a small volume of reagents.

In the last few decades, the cell biology community has recognized that a genetically identical population of cells from the same lineage can display a wide range of heterogeneity in their phenotype, gene expression, and fate. Such recognition has led to a strong desire to develop and use advanced methods to observe single cells and perform multi-omics profiling and analyses at the single cell level. Conventional methods of cell observation and manipulations were originally developed to handle large volumes of samples or manipulate a very small number (<10) of cells. Such systems include multi-well plates, laser capture micromanipulator, and microcapillary pipette manipulator, all of which involve laborious and time-consuming procedures that limit their throughput.

In response, new technologies to enable single cell analyses have been developed and commercialized. A prime example is the 10x Genomics® platform, which allows for preparation of multiple sequencing libraries of single cells without the need for micromanipulators or laser capture manipulators.3 Although high-throughput single-cell sequencing is possible, this system does not allow direct observation for phenotyping and sorting of cells based on their phenotype. A common practice to improve specificity of this technology is to pre-sort the single cell population, often through conventional surface marker phenotyping equipment such as fluorescence activated cell sorter (FACS) and magnetic activated cell sorter (MACS). Although these methods offer extremely high throughput and speed, they are typically limited to the use of known surface markers, which can be problematic because heterogeneous single cells are known to possess identical markers, and populations with unknown surface markers cannot be sorted using these methods.4

One of the biggest advantages of microfluidics is their flexibility and modularity. Microfluidic devices can be specifically tailored
and designed to meet the needs for a particular experiment. Droplet microfluidics, in particular, has emerged as a powerful platform that can be engineered to allow extended dynamic phenotyping. Droplets can function as closed reaction chambers that allow generalized phenotyping of individual cells such as metabolite synthesis rate, cell morphology, cell division kinetics, etc. For dynamic observation over extended time, it is critical to trap and observe cell-containing droplets and to recover a subset of the captured droplets for subsequent analyses and processing. The selection of a trapping method for droplet array formation must be carefully considered, as the releasing method and the selectivity of droplets are largely restricted by which type of trapping method is used. Static arrays can be largely divided into two-dimensional traps and three-dimensional traps. Typically, arrays utilizing two-dimensional traps rely on hydrodynamic force, electric field, magnetic field, or capillary force, whereas arrays using three-dimensional traps rely on pneumatic valves and density difference between continuous and dispersed phases to trap droplets.

This perspective will provide a brief review of selected microfluidic methods for droplet capture, static array formation, and recovery, as well as provide suggestions for potential improvements. In particular, this paper will focus on droplet trapping and manipulation methods that are designed to facilitate dynamic, long-term observation and selective recovery of encapsulated samples of living cells. Although numerous methods currently exist, there is still ample room for improvement in selectivity, throughput, and ease-of-use. Key features of highlighted methods are summarized in Table I.

Hydrodynamic force-driven droplet trapping is a commonly used approach for droplet capture and static array formation. In general, this approach takes advantage of differences in hydrostatic resistance to enable droplet capture. Examples include static microdroplet arrays, resettable dynamic microarray, trap-and-release microfluidic microarray, and dropspots.\textsuperscript{6–9}

The static microdroplet array, as shown in Fig. 1(a), utilizes U-shaped traps to arbitrarily capture flowing droplets while the exhaust channels in the traps both ensure single droplet capture and retention and serve as release channels for recovery of the captured droplets.\textsuperscript{5} This is a simple, yet highly effective droplet capture system that is capable of capturing and releasing hundreds of droplets. Depending on the size of droplets, a simple adjustment of device design (e.g., inter-trap distance, trap size) can optimize droplet capture. With optimized inter-trap distance, the device requires approximately 28 500 \( \mu \)m\(^2\) to capture one droplet, somewhat limiting the throughput of this approach. As is, the device offers non-selective capture and recovery of captured droplets. An additional component in the exhaust channel can potentially allow

### Table I. Summary table of selectivity and throughput for each type of droplet capture method in literature.

| Capture method                  | Selectivity | Throughput | Fabrication difficulty | Cell damage | Example          |
|---------------------------------|-------------|------------|------------------------|-------------|------------------|
| Hydrodynamic                    | \( \Delta \) | High       | Low                    | \( \ldots \)/Med | Refs. 6–9       |
| Density-difference              | \( X \)     | X/\( \Omega \) | High                   | Low         | \( \ldots \)/Med | Refs. 10, 12, and 28 |
| Pneumatic valve                 | \( \Omega \) | \( \Omega \) | High                   | Low         | Refs. 14–19      |
| Surface acoustic wave (SAW)     | \( \Omega \) | \( \Omega \) | Low                    | Med         | Refs. 21 and 22  |
| Electrophoresis                 | \( \Omega \) | \( \Omega \) | Low                    | Med         | Refs. 23         |
| Magnetophoresis                 | \( \Delta \) | \( \Delta \) | Low                    | Med         | Refs. 24 and 25  |
| Hydrogel                        | \( X \)     | \( X \)    | High                   | Low         | \( \ldots \)     | Ref. 27 |

**FIG. 1.** Examples of hydrodynamic force-driven droplet trapping and recovery methods [(a)–(c)]. (a) shows static trapping arrays that trap droplets randomly. Exhaust channels in (a) and (b) are marked with blue arrows. Reprinted with permission from Huebner et al., Lab Chip 9, 692–698 (2009). Copyright 2009 Royal Society of Chemistry. (b) shows sequential droplet trapping into trapping spot and precise recovery of trapped droplet via bubble formation at aluminum pattern, located within the exhaust channel, with laser excitation. Reprinted with permission from Iwai et al., Proc. Natl. Acad. Sci. U.S.A. 104(4), 1146–1151 (2007). Copyright 2008 National Academy of Science. (c) shows a highly efficient droplet trapping and monitoring system, Dropspot. Reprinted with permission from Schmitz et al., Lab Chip 9, 44 (2009). Copyright 2009 Royal Society of Chemistry.
selective recovery. For instance, by using hydrogel that responds to external stimuli such as light, it will be possible to generate traps that can catch and release droplets via hydrogel swelling and collapse. Other stimuli dependent manipulations that can actively or passively drive droplets out of the traps can broaden the utility of this type of device.

The modified resettable dynamic microarray and the trap-and-release microfluidic microarray share an identical device design for droplet capture as shown in Fig. 1(b). Unlike the static microdroplet array, these microarray systems allow droplets to be captured in the order which they are introduced into the device. A droplet traveling through the flow channel enters the first vacant trapping spot. This droplet capture in the trapping spot is due to lower flow resistance in the exhaust channel of the trapping spot compared to that of the main flow channel. A similar phenomenon induces the following droplet to bypass the occupied trapping spot, flow through the loop channel, and enter the next vacant trapping spot. For the modified resettable dynamic microarray system, recovery is simple; back-flowing releases all captured droplets back into the loop channel. In addition, pillars surrounding the exhaust channel ensure smooth recovery of released droplets without clogging the exhaust channel. This is a semi-selective recovery system; as recovered droplets will exit the channel in the order of capture, delicate backflow with timed collection could, in principle, enable selective recovery of the target droplets; however, such recovery has not been demonstrated. This modified resettable array has been shown to capture up to 10 000 droplets in a single chip, which can be easily scaled up to higher numbers. Timed collection of released droplets can potentially be optimized for high-precision selective recovery; however, without extensive optimization, it will likely result in imperfection. Alternatively, the resettable dynamic microarray can incorporate ad-hoc barcode or quantum-dot in the droplets, to improve selectivity; however, this approach would need to add a separation step to enable selective recovery or analysis.

Selectivity of the modified resettable array has been improved by adding metal patterns on the exhaust channels to push droplets out of the traps as shown in Fig. 1(b). The trap-and-release microfluidic microarray utilizes a focused laser to heat an aluminum pattern to 132 °C and create a bubble which pushes the droplet out of the trapping spot into the mainstream within 300 ms. The device is relatively simple to fabricate and provides excellent selectivity with a short response time and can process up to 10 000 droplets. However, although heating and bubble generation take place in less than a second, even a brief exposure to high temperature can damage biological samples encapsulated in the droplets. To mitigate this heat-induced damage, the exhaust pattern can be replaced with a hydrogel that swells upon a small temperature increase to minimize heat-induced damage. Alternatively, a buffer spot or a thin stimuli-responsive layer (e.g., hydrogel) can be placed between the trapping spot and the aluminum pattern such that heating generates a bubble, which in turn expands the buffer spot and pushes out the captured droplet.

The dropspot, as shown in Fig. 1(c), is a simple pea-in-a-pod shaped microfluidic device that captures a droplet in an array of circular chambers connected by narrow constrictions. This system mainly depends on droplets pushing preceding droplets to fill the chambers. Due to the simple chamber geometry and trapping algorithm, it can trap a large number of droplets within a single chip. A dropspot that can trap 64 000 droplets has been reported. Similar to the resettable dynamic microarray, the dropspot allows semi-selective recovery of target samples, as droplets are released in the order they were captured. Although manual collection of droplets in target position is possible, it is highly inefficient and error prone.

One of the highest throughput methods for droplet capture uses the intrinsic difference in density between the droplet and the continuous phase. As droplet microfluidics typically uses an aqueous phase and an oil phase, a density difference between the two phases inevitably exists. For example, in an experiment using fluorocarbon oil such as FC-40 or HFE-7500 as the continuous phase, aqueous droplets (density ∼ 1 g/ml) are significantly lighter than the continuous phase (density of FC-40 = 1.85 g/ml; density of HFE-7500 = 1.61 g/ml). Under these conditions, a floating droplet array (FDA) can capture droplets nonspecifically. Although the intrinsic density differences between the phases make the system simple to implement, the system cannot be used if the density difference is negligible.

The floating droplet array (FDA) system, as shown in Fig. 2(a), can operate at a trapping efficiency of approximately 20%, which can be slightly increased by ceasing droplet generation when 40% of the wells are filled to minimize the number of droplets that bypass the wells without getting trapped. The trapping efficiency can be further enhanced by changing the flow rates and droplet size and by varying the dimension of and spacing between trapping wells. In some cases, the buoyancy force, resulting from the density differences, may be too small for droplets to overcome the flow inertia if the continuous flow rate is too high. In such a case, additional oil drainage system may need to be introduced prior to the FDA to reduce the total flow rate and increase the overall volume fraction of droplets in the flow. With optimized droplet flow speed, well size, well depth, and droplet generation time, up to 95% capture efficiency can be achieved. Besides its simple trapping mechanism, another advantage of the FDA is throughput. FDA device design has very small dead space within the device, making it one of the highest throughput systems. The small dead space of the FDA can be attributed to both three-dimensional design and no need for exhaust channels. Although the lack of exhaust channels enables high-throughput capture of droplets, droplet recovery is limited to nonspecific recovery. Flipping the chip releases all droplets from the wells, and since recovery is not order-based like the modified resettable dynamic microarray or the dropspot, the process is completely non-selective. To introduce selectivity, droplets must contain barcodes.

Recently, a UV laser-based system that allows selective recovery of droplets from a FDA has been reported. After nonspecific capture, a focused UV laser with pulse energy above 90 μJ with a pulse duration of a few nanoseconds was used to generate heat-induced cavitation bubbles that expel target droplets out of their trap wells. While this system adds a powerful selective recoverability mechanism, the usage of UV laser could potentially damage the encapsulated biological samples (e.g., cells). As an alternative, the top of the wells can be lined with a hydrogel, which can absorb UV and swell to mitigate such a potential damage. More recently, a new device that uses a less harmful near infrared
A focused NIR laser with pulse energy above 6 mJ and a pulse duration of less than a second was applied on a polystyrene layer containing a photoacoustic dye, which generated heat-induced cavitation bubbles that expelled target droplets out of the wells. Cell viability was not affected by this process. Density difference-driven droplet capture and recovery systems can provide high throughput and selective recovery; however, the approach requires fabrication of three-dimensional devices with multiple layers. Also, an external laser source is needed to enable selective recovery. A system that does not require a high-power laser source and that can be fabricated in a single layer would greatly enhance the implementation of this approach.

Microfluidic devices that use hydrodynamic forces and external stimuli provide the highest throughput and at the same time allow for selective recovery of the captured droplets. Although microfluidics provides great benefits of a closed system, this same isolation makes it difficult to apply an external force to enable on-demand droplet manipulation. Pneumatic valves are useful tools in such a case as they can exert forces locally on-demand to neighboring microfluidic channels. Valves can be used to reduce channel dimensions, which allows for flow of small sized droplets or complete shut-off of channels, or alter the flow directions. Many studies have used pneumatic valves for selective droplet manipulation as they offer the most intuitive and direct control of droplets. Large-scale integration of pneumatic valves, also known as the Quake valve, has shown to enable effective capture
A voltage across thin gold electrodes can exert electrohydrodynamic forces on a droplet, enabling selective capture and release.\textsuperscript{20} The DC voltage generates a non-uniform electric field that pulls droplets via a hydrodynamic inertial force and captures them into individual chambers. To release the captured droplets, voltage is applied to induce two forces, dielectrophoresis force and Coulomb force, which push droplets to the main continuous phase channel. The device provides great selective capture and recovery, but suffers from complex fabrication protocol and low throughput.

Both the SAW- and the electrode-based systems require built-in or peripheral accessories, which limit the throughput; the issue of low throughput can be potentially ameliorated by combining a three-dimensional droplet capture array with vertical incorporation of the IDTs or electrodes, which will require novel design and implementation.

Another method of droplet and particle manipulation is through magnetophoresis, which requires the presence of magnetic beads or ferrofluid within the droplets.\textsuperscript{24,25} This may increase the cost of an experiment or make the system biologically incompatible. Recently, the possibility of manipulating multiple single magnetic particles using a rotating external field has been demonstrated, as shown in Fig. 3(c).\textsuperscript{22} The direction of particle movement can be adjusted from clockwise forward to counterclockwise reverse in semi-circular tracks, and the particle can be forced out of phase-locked condition to phase-slipping regime, skipping it over to the next track. Although magnetophoresis allows multiplexed manipulation of particles or droplets and precise trapping and recovery of samples, the system does not allow for fully selective individual target manipulation and is limited by low speed (hundred microns per second).

A potentially powerful technology, which has not been fully explored or applied to its maximum potential in selective trapping and recovery systems, is hydrogels.\textsuperscript{26} Hydrogels are highly responsive to external stimuli and can be readily tailored to meet many needs in microfluidics. A hydrogel-based, temperature responsive actuator has recently been reported.\textsuperscript{27} Using patterned chromium and gold bilayers to induce local heating, a thermally responsive hydrogel closes one of binary paths on-demand to achieve flow path divergence. Similar to the aforementioned FDA, multiple droplets in the flow channel can be trapped at specific locations by inducing local heating, making this approach a powerful trap and release mechanism. The fast response rate of 250 ms enables efficient trapping and rapid recovery. The current system uses lower critical solution temperature (LCST) hydrogels, which requires cooling below 32 °C to induce swelling and heating above 32 °C to collapse the hydrogel. This condition limits the capturing and observation to below 32 °C, which is not ideal for studies involving living cells. An upper critical solution temperature (UCST) hydrogel that swells and collapses above and below a critical temperature, respectively, may be more useful for studies that involve living cells.

Optimal selective trapping and recovery systems require a balance of selectivity, throughput, and ease of fabrication. Trade-offs among these factors must be considered when choosing an appropriate system for a specific application. As computational and genomic technologies advance, the demands for high resolution, multiplexed phenotyping, and increased sample size will continuously increase. To satisfy these demands while minimizing cost, biocompatibility issues, and design complexity, continued
developments and innovations in droplet microfluidic technology are critical. In particular, adoption and incorporation of advances in materials science and nanotechnology will enable the next generation microfluidic devices for biological assay and analysis applications. For example, stimuli-responsive materials such as hydrogels that can detect the presence of metabolites could potentially enable autonomous selective recovery of droplets, eliminating the needs for external control via light or pressure. Recent advances in artificial intelligence, pattern recognition, and machine learning will also be key to processing large data to better understand complex phenomena that are often associated with biological assays. As the biology community shifts its focus toward understanding the role of subcellular components and organelles, methods to capture, separate, and analyze these delicate components using microfluidics will require new approaches and innovations.
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The authors have no conflicts of interest to declare.

DATA AVAILABILITY

The data that support the findings of this study are available within the article.

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