Microfluidics Using Spatially Defined Arrays of Droplets in One, Two, and Three Dimensions

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Keywords

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Abstract

Spatially defined arrays of droplets differ from bulk emulsions in that droplets in arrays can be indexed on the basis of one or more spatial variables to enable identification, monitoring, and addressability of individual droplets. Spatial indexing is critical in experiments with hundreds to millions of unique compartmentalized microscale processes—for example, in applications such as digital measurements of rare events in a large sample, high-throughput time-lapse studies of the contents of individual droplets, and controlled droplet-droplet interactions. This review describes approaches for spatially organizing and manipulating droplets in one-, two-, and three-dimensional structured arrays, including aspiration, laminar flow, droplet traps, the SlipChip, self-assembly, and optical or electrical fields. This review also presents techniques to analyze droplets in arrays and applications of spatially defined arrays, including time-lapse studies of chemical, enzymatic, and cellular processes, as well as further opportunities in chemical, biological, and engineering sciences, including perturbation/response experiments and personal and point-of-care diagnostics.

1. INTRODUCTION: MICROFLUIDIC COMPARTMENTALIZATION

This review describes the microfluidic generation and manipulation of droplets in spatially defined arrays to simplify and enable high-throughput and multiplexed experiments for a variety of applications. By focusing on arrays of droplets, this review aims to complement a number of excellent reviews on formation, manipulation, and applications of droplets (1–8).

Microfluidics is frequently used to compartmentalize fluids, solutions, or suspensions of particles such as beads or cells. General advantages of compartmentalization (3, 4) include high-throughput processing of many small volumes, isolation of interfering species into separate compartments, and raising of the local density of dilute compounds or particles. Pico- or nanoliter volumes facilitate high-throughput work with precious or hazardous samples for applications such as protein crystallization (9) and analysis of blood (10, 11), tissue homogenate (12), or cerebrospinal fluid (13); submicrometer volumes may also be used for work with pharmaceutical or nuclear reactors or waste processing. Compartmentalizing a bulk solution into many small volumes to isolate and concentrate rare particles of interest is referred to as stochastic confinement (14); this technique enables detection and analysis of single cells or molecules (15, 16). Compartmentalization can be achieved via multiple methods, including by concentration of analytes in a section of a continuous fluid, such as with capillary electrophoresis and electrophoretic focusing (17–20), or by segmentation of fluids, such as with valves or physical barriers (21) or droplet formation (1–5).

Droplet: a small-volume (submicroliter and less) unit of fluid that is surrounded by an immiscible fluid and typically does not wet the walls of the container

Droplet array: a set of droplets whose identity is indexed by one or more spatial variables

Stochastic confinement: isolation and concentration of individual particles from a bulk solution by compartmentalization into sufficient small volumes that mostly contain either one or zero particles, according to a random probability distribution

PCR: polymerase chain reaction

RfOEG: triethyleneglycol mono(1H,1H-perfluorooctyl) ether, a fluorous surfactant with an oligoethylene glycol head group

1.1. Compartmentalization in Droplets

Droplets are a simple way to achieve rapid and robust compartmentalization of fluids and their contents. For the purposes of this review, we define droplets as small-volume units of fluid that are surrounded by another immiscible fluid, such as an oil, that typically do not wet the walls of their container. Droplets provide all of the general advantages of compartmentalization discussed above and provide several additional advantages. Droplet formation (1–5) and dynamics (22) are well characterized and robust over a range of fluid viscosities and surface tensions (23, 24). The rate of mixing of reagents compartmentalized inside droplets is well controlled and reproducible, which facilitates the study of time-resolved reactions (25). Droplets also offer the flexibility to choose a carrier fluid that complements the system. Droplets of aqueous or organic solution can be protected with immiscible and inert fluorinated fluids (e.g., 3M's Fluorinert^{TM}), and droplets containing polymerase chain reaction (PCR) reagents can be surrounded with mineral oil to match standard operating conditions. Surrounding the droplet with carrier fluid mitigates problems of dispersion in the fluid and adsorption onto surfaces, allows the surface chemistry to be controlled at a liquid-liquid interface instead of at a liquid-solid interface (13, 26–32), reduces evaporation, and can regulate gas supply for oxygen-sensitive reactions or anaerobic cultivation. Droplets are naturally suited to studies of interfacial and two-phase interactions, including (*a*) fluid extraction and gas-liquid equilibria for catalysis (33) and (*b*) protein crystallization by vapor diffusion (9, 34–36).

Although the creation of an additional interface is fundamental to the use of droplets, it does carry potential disadvantages. For example, molecules can potentially be lost by partitioning into the surrounding fluid, or they can be lost to the interface itself. These disadvantages can be minimized by using inert carrier fluids, compatible surfactants, and/or preequilibration. Aqueous droplets in fluorocarbon are a rare example of a truly immiscible system with negligible solubility between phases (37); other systems usually allow some exchange of solvent and/or solute (38). Fluorinated surfactants such as RfOEG and related molecules can reduce or prevent loss of compounds such as proteins to the interface (30–32, 39). The straightforward synthesis of RfOEG

was described in Reference 30. Finally, the carrier fluid (14) or the droplets themselves (33) can be preequilibrated with a desired gaseous atmosphere or aqueous solution to control exchange of gases and small molecules. Another potential disadvantage is that droplets may coalesce if they contact one another. This problem can be avoided by stabilizing the interface with surfactants (32) or by preempting contact between droplets by using spacers of a different phase (40) or ordered arrays. Finally, small volumes can be problematic if chemical reactions inadvertently become substrate limited or if the metabolism of living cells depletes the oxygen or nutrients before the experiment is complete (41, 42). To avoid such issues, droplet volumes should be carefully chosen; alternatively, fresh solution may be merged with the droplets to refresh them. The pros and cons of droplets and their many chemical and biological applications have been covered in several excellent reviews (2–4, 43).

Bulk emulsion: an emulsion (i.e. an assembly of droplets) without a specific spatial organization

1.2. Bulk Emulsions and Arrays of Droplets

Droplet-based compartmentalization can involve spatial organization as in an array, or not, as in a bulk emulsion. Bulk emulsions are simpler to produce than arrays; thousands of droplets can be quickly produced by mechanical disruption or by extrusion through a filter. Bulk emulsions are well suited for high-throughput analysis of initially equivalent droplets to obtain statistical data, such as for directed molecular evolution (44, 45), screening for cellular behaviors by fluorescence-activated droplet sorting (46), or emulsion-based PCR (8, 47, 48). For multiplexed experiments involving droplets of different compositions, such as large-scale screening of reaction conditions, indexing is required. Bulk emulsions can be indexed by using chemistry or beads to identify each droplet; this approach has been employed to analyze single-cell responses to different concentrations of one or more drugs (49–51) and to classify single cells by using surface biomarkers (52). The generation and application of bulk emulsions have been carefully reviewed elsewhere (3, 7, 8) and so are not discussed here.

In an array, droplets are indexed by their spatial layout. Spatial indexing is convenient when a large number of discrete conditions must be distinguished. Furthermore, spatially defined arrays are ideal when individual droplets need to be monitored over time, physically or chemically addressed, retrieved, or reproduced. Arrays also provide a means of controlling physical or chemical interactions between multiple droplets in a highly parallel fashion. These advantages were first utilized in one-dimensional (1D) arrays and then, more recently, in two-dimensional (2D) and three-dimensional (3D) arrays. Here, we review available methods to generate spatially defined arrays of each type, and we discuss current and potential applications of these arrays in the chemical, biological, and engineering sciences.

Table 1 briefly summarizes operations to manipulate droplets in arrays (nicely reviewed for droplets in general in Reference 1), and the sidebar summarizes methods to analyze droplets. These methods are generally applicable to arrays in all three dimensions.

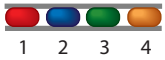
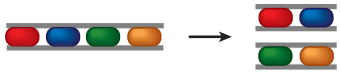
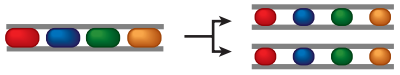
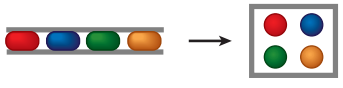
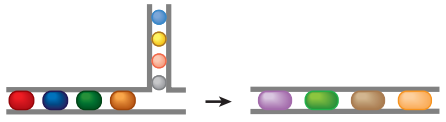
2. ONE-DIMENSIONAL DROPLET ARRAYS

Droplets in 1D arrays can be indexed by one variable. For example, droplets in a straight or winding channel, a piece of tubing, or a line of wells can be indexed by distance or by droplet count from the beginning point of that sequence of droplets.

2.1. Generation of One-Dimensional Droplet Arrays

Methods to generate droplets that are uniform in composition have been intensively reviewed (1–5). The two classic methods to form a series of droplets from a single solution are the merging

Table 1 Droplet array operations

Droplet operation	Representative schematics	Analog to array operations	References
Identify and index: index or retrieve/delete the droplets in the array; measure the size of the array		A = {1 2 3 4} Count = 4; A2 = 2	37, 57, 95, 120, 148
Divide array		{1 2 3 4} → {1 2} + {3 4}	
Rearrange: alter sequence; code/decode by rearrangement or changing spacing		{1 2 3 4} → {1 4 3 2}	68, 93
Sort: select droplets by size, density, viscosity, or contents		{4 1 2 3} $\xrightarrow{>2}$ {4 3}	46, 149–151
Split: fragment individual droplets into smaller daughter droplets either in series or in parallel		In series: {1 2 3 4} → {1 1 2 2 3 3 4 4} In parallel (shown): {1 2 3 4} → $\begin{Bmatrix}1&2&3&4\\1&2&3&4\end{Bmatrix}$	37 (in series); 78, 81, 87, 88 (in parallel)
Change dimension: capture a one-dimensional array of droplets into a two-dimensional array, etc.		{1 2 3 4} → $\begin{Bmatrix}1&2\\3&4\end{Bmatrix}$	78, 81
Merge a chemical with all droplets		{1 2 3 4} $\xrightarrow{+1}$ {2 3 4 5}	10, 58, 93
Perform pairwise merging of arrays of droplets		{1 2 3 4} + {0 1 2 3} → {1 3 5 7}	120

of fluids in a T junction (53) and flow-focusing (54). Both of these techniques generate droplets in a continuous stream. Another method to generate an array of uniform droplets is to induce spontaneous breakup of a fluid stream by using the geometric constraints of the device, such as narrow necks (55) or side chambers (56, 57) along the channel. When taking advantage of stochastic confinement, droplets generated via these methods vary in the number of captured particles, although they are generated from a single solution or fluid.

T junction: a microfluidic junction used to generate droplets from one fluid stream by introducing it into a flowing cross-stream of immiscible fluid

Methods to generate 1D arrays of droplets that are nonuniform in composition (i.e., from multiple solutions or fluids) include laminar flow and aspiration. When many different combinations of a few solutions are needed, laminar flow is an attractive method. Laminar flow can generate droplets with controlled and variable compositions by varying the relative flow rates of incoming solutions (25) with any type of junction. For example, a multi-inlet junction coupled with a T junction can generate an array of droplets containing solutions with a gradient in concentration when

EXAMPLES OF ANALYSIS METHODS FOR DROPLET CONTENTS

Droplets can be analyzed by any method that is adaptable to submicroliter volumes. Imaging of droplets and their contents by bright-field, phase-contrast, differential interference contrast, chemiluminescence, or fluorescence microscopy is especially convenient and has been widely used. Examples include monitoring the growth of crystals (9), protein aggregation, (13) or liquid-liquid extraction (146); assays for live cells and microorganisms (93, 103, 106) or enzymes (98, 133, 134); and so on. Fluorescence spectroscopy—including laser-induced fluorescence (135), fluorescence correlation spectroscopy (78, 136), Förster resonance energy transfer (137), and cylindrical illumination confocal spectroscopy (138)—has also been employed.

Chemical identification is facilitated by methods such as mass spectrometry, which includes electrospray ionization mass spectrometry (142, 143) and matrix-assisted laser desorption/ionization mass spectrometry (65). Other spectrometric techniques include Raman spectroscopy (57), surface-enhanced Raman scattering (139), nuclear magnetic resonance (140), and X-ray crystallography (9, 34, 35, 58, 59, 95–97, 141). Capillary electrophoresis methods have also been employed in continuous-flow droplet microfluidic systems (144) and digital microfluidic systems (121), as have electrochemical analysis methods including amperometry (71) and chronoamperometry (145).

Standard biological analysis methods are compatible with droplets. Heterogeneous immunoassays on a microchannel surface (62) and on beads within the droplet (98) have been established, as have PCR (45, 99, 100) and related methods for nucleic acid amplification.

Finally, imaging by cell-phone camera has been demonstrated with paper-based array colorimetric analyses (147), and it may also be applicable to the analysis of 2D droplet arrays in resource-poor conditions.

the flow rates are varied at each inlet for individual components (**Figure 1*a***) (58). A cross-junction can generate alternating droplets of specified compositions with optimal capillary number and water fraction (**Figure 1*b***) (59). Integrating valves with T junctions can more arbitrarily control the size and the composition of the droplets in the generated array (**Figure 1*c***) (60, 61). When a large number of different solutions or fluids is needed, aspiration is appropriate. Aspirating each droplet sequentially in a drop-on-demand mode (62) is a straightforward way to generate an array of droplets with an arbitrary sequence in terms of composition and size of droplets (**Figure 1*d***). Robotics and miniaturized aspirating tips have been used to precisely program complex 1D arrays of picoliter- to nanoliter-volume droplets with flexible and controllable sizes, combinations of reagents, and sequences (63). Cartridges of many different reagents preloaded in an array of droplets can be made by this method and used for a range of applications (64).

2.2. Characteristics and Applications of One-Dimensional Droplet Arrays

The main difference between spatially organized droplets and bulk emulsions is that the droplets in an array can be indexed by spatial position, which facilitates the identification, monitoring, and manipulation of individual droplets. Many different components can be screened by linking their identity to the index, and droplets of interest can be identified and recovered.

Flow-focusing: a method to generate droplets from one fluid stream by pinching it off using two converging streams of immiscible fluid at a microfluidic junction, usually with a narrow neck

Cartridges preloaded with a 1D array of droplets containing varying reagents have been used to screen conditions for protein crystallization (34) and to test or evolve catalysts (33, 65). Aspiration of droplets in carrier fluid has been automated with a robotic platform and used to generate an array of microliter-sized droplets to screen for cells with tumor markers via high-throughput PCR (66, 67). A microfluidic hybrid method was developed to increase the number of parameters that could be tracked by spatial indexing. A microdevice was used to combine a cartridge of larger droplets with additional solutions of reagents from side channels using varying injection rates,

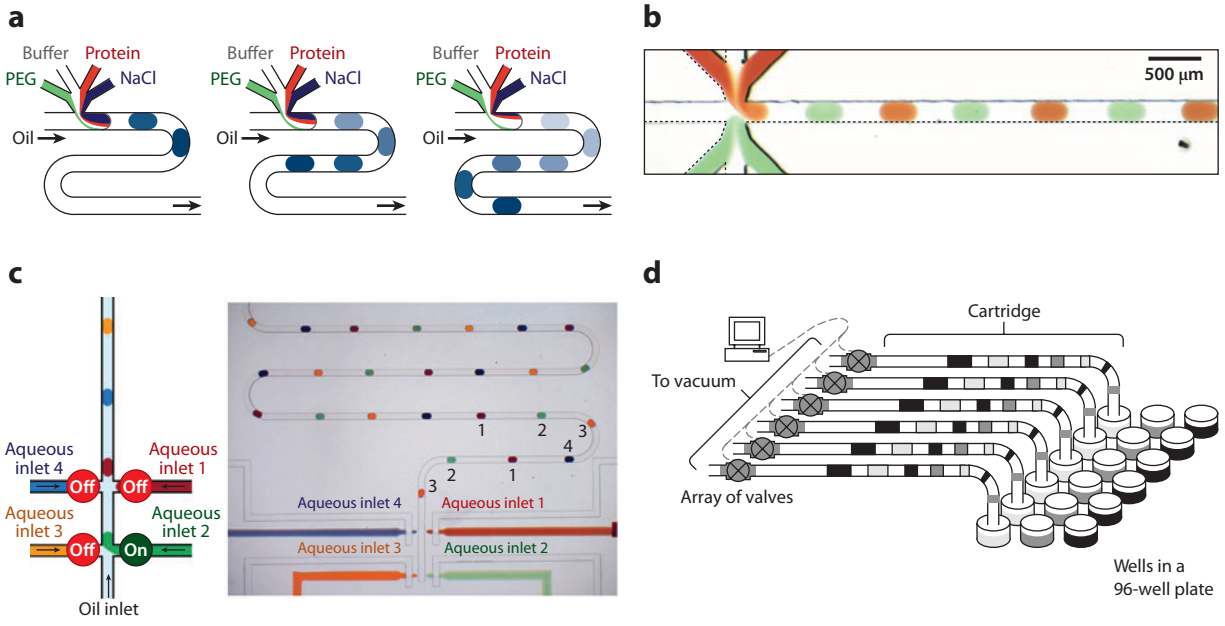


Figure 1

Methods to generate one-dimensional arrays of droplets with varying composition or size. (*a*) An array of droplets with varying composition was formed on a microfluidic chip through variation of the flow rates of individual input streams. The flow rate of the NaCl stream (*blue*) was decreased over time, and the flow rate of the buffer stream (*white*) was increased, which formed a gradient of NaCl concentration between droplets. Abbreviation: PEG, polyethylene glycol. Reprinted with permission from Reference 58. Copyright 2003, American Chemical Society. (*b*) An array of droplets with alternating composition was formed through the use of a microfluidic cross-junction. Reprinted with permission from Reference 59. Copyright 2004, American Chemical Society. (*c*) Droplet arrays with arbitrary composition were produced by sequentially switching on and off the microvalves corresponding to each solution. Reproduced with permission from the Royal Society of Chemistry (61). (*d*) Arbitrary arrays of droplets were generated in cartridges by aspiration. Droplets of reagent were aspirated into the cartridges sequentially by dipping the ends of the cartridges into the appropriate reagents. Reprinted with permission from Reference 62. Copyright 2005, American Chemical Society.

while simultaneously separating each large droplet into a series of smaller droplets. In the initial array, the index represented only the identity of the reagents, whereas in the outgoing array the index represented both the identity and the concentration. The hybrid method was validated by screening and optimizing membrane protein crystallization (**Figure 2*a***) (37) and by screening the antibiotic sensitivity of methicillin-resistant *Staphylococcus aureus* (14). When the index of droplets in a 1D array is based on their spatial position, codes can be encrypted into the array through these spatial patterns. For example, the relative spatial position of droplets in a 1D array was transmitted into a particular pattern through the use of a microchannel network (**Figure 2*b***) (68). Relative position was also utilized to generate a microdroplet-based shift register, which groups droplets to generate interfaces between them and updates serially as droplets flow through the register (69).

Although the index of a 1D array is normally coded by its spatial position, the spatial index is easily converted to a temporal value if the droplets are formed in a continuously flowing stream with known velocity. In this case, assuming that droplets are moving at the average velocity of the flow, then $t = d/U$, where t (in seconds) is the time point, d (in millimeters) is the distance traveled, and U (in millimeters per second) is the constant flow velocity. The zero time point is set by combining reagents at a defined position in the device, and droplets are analyzed downstream

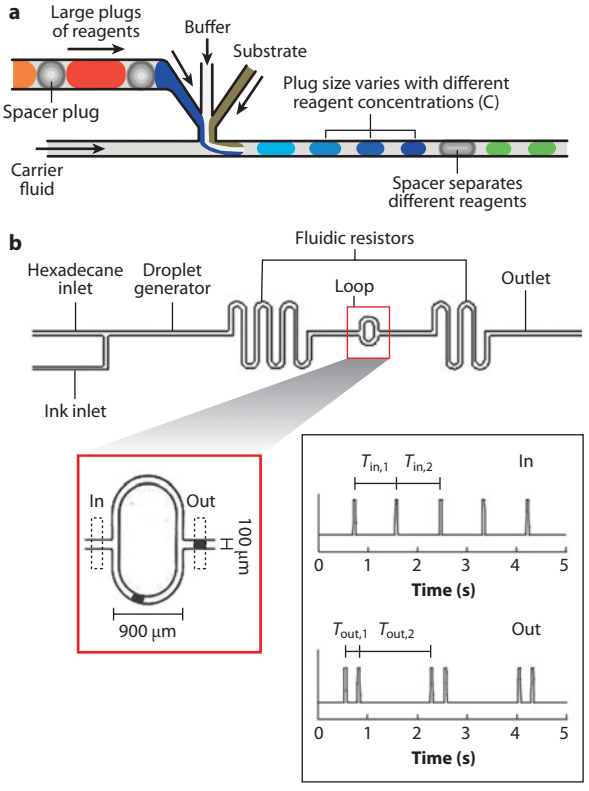


Figure 2

Methods based on indexing of a one-dimensional droplet array. (*a*) Schematic illustration of the microfluidic hybrid approach. A preformed cartridge of ~140-nl reagent droplets (plugs) separated by spacers was flowed into a microfluidic channel and combined with streams of buffer and substrate. The combined solutions were flowed into a stream of a fluorinated carrier fluid, generating ~50 smaller (10–15-nl) plugs for each reagent plug. The flow rates were varied to generate droplets with different concentrations of the reagent, and indexing was sustained by correlating the size of the plug with concentration. Reprinted with permission from Reference 37. Copyright 2006, National Academy of Sciences. (*b*) A microfluidic network used to pattern the relative spatial position of an array of droplets. (*Top*) A generalized schematic diagram of the microchannel network. Droplets generated at a T junction proceeded to a loop, where they took one of two paths. (*Bottom left*) An optical micrograph of the loop showing one droplet in the lower branch and one droplet in the outlet channel. (*Bottom right*) Plots of droplets being detected as they moved through the "In" and "Out" windows shown in the lower left panel; the spike indicates the presence of a droplet. The upper plot shows uniform time intervals ($T_{in,1} = T_{in,2}$) between droplets as they reached the loop. The lower plot depicts the two different time intervals ($T_{out,1} \neq T_{out,2}$) that separated the droplets as they emerged from the loop. From Reference 68. Reprinted with permission from the American Association for the Advancement of Science.

from this point. Thus, droplets analyzed according to their spatial position can provide temporal information on the progress of a reaction or event (70). This approach has been used to measure kinetics, including enzymatic kinetics (70, 71), coagulation (10, 11, 72), autocatalytic amplification (73), cell-based enzyme assays (74), streptavidin-biotin binding kinetics (75), DNA hybridization (76), and fluidic mixing (77).

The chemistrode, a microfluidic platform based on 1D droplet arrays, combines the two aforementioned characteristics of 1D droplet arrays: (*a*) the ability to preload indexed cartridges and

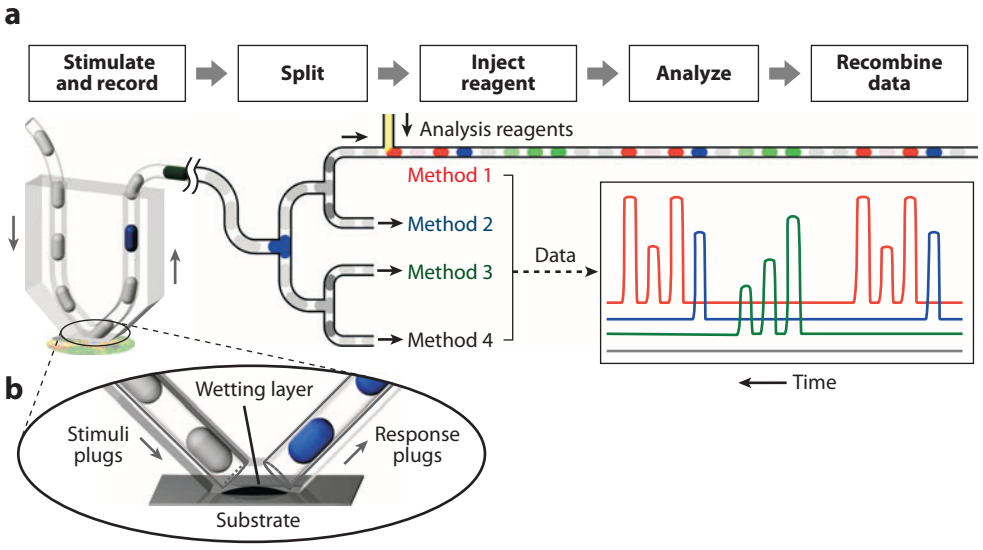


Figure 3

The chemistrode delivers and records multiple molecular signals with high temporal and spatial resolution for off-line analysis by multiple analytical methods in parallel. (*a*) A conceptual schematic drawing of the stimulation, recording, droplet-splitting, and analysis steps. (*b*) Schematic of the chemistrode brought into contact with a hydrophilic substrate, demonstrating how chemical signals are collected into response plugs. Reprinted with permission from Reference 78. Copyright 2008, National Academy of Sciences.

(*b*) the ability to encode and record a temporal sequence (78–81). By controlling the flow rate of a cartridge of droplets that is preloaded with a designed spatial sequence, one can use the chemistrode to deliver a sequence of stimuli with a desired temporal pattern to a stationary substrate. As the array of droplets passes the substrate, the droplets can both stimulate the substrate and collect chemicals that result from this stimulation to record the temporal response of the substrate in its spatial sequence. Analysis of the recovered array of droplets reveals the time-resolved stimulus-response profile of the substrate (**Figure 3**). This approach was validated in conjunction with the chemistrode by recording the stimulus-response profile for murine islets of Langerhans in response to glucose (78). Compartmentalization of a continuous effluent stream into a sequence of droplets has also been used to record the results of other assays, including capillary electrophoresis separation (82) and microdialysis (83–86).

Splitting a 1D array of droplets into multiple parallel daughter 1D arrays (**Table 1**) maintains the indexing such that each daughter array is identical to the original array (87, 88). This feature enables parallel independent analyses (potentially with different methods) for multiple analytes from the original array (see the sidebar for analysis methods) (78, 81). Data from the analysis of each daughter array can be aligned together according to the identical indices to provide a more complete kinetic picture (**Figure 3**). This approach was used to conduct four separate assays to identify various microbial strains that had been stochastically confined in droplets (81).

3. TWO-DIMENSIONAL DROPLET ARRAYS

Droplets in 2D arrays can be indexed by two variables, such as by channel number and axial position for an array of channels or tubing or by x and y position for a planar array of wells or traps.

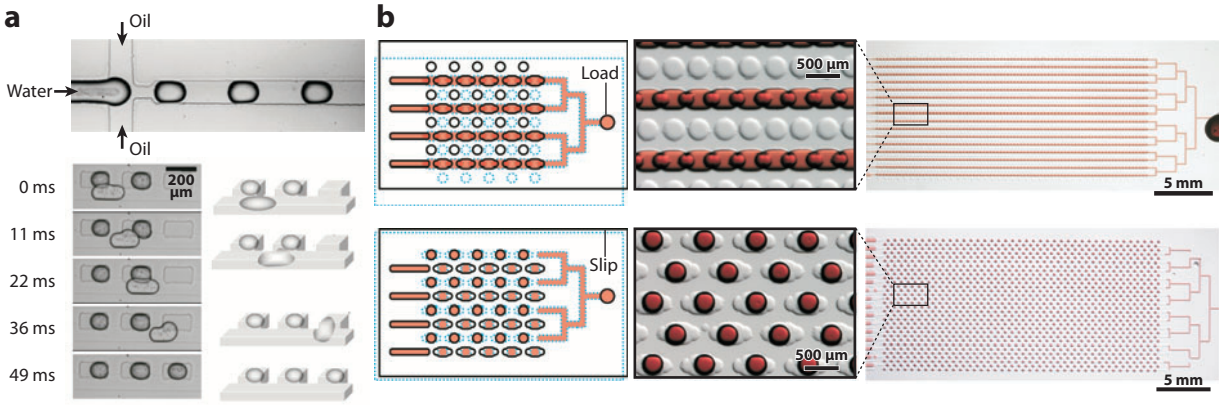


Figure 4

Examples of generation of two-dimensional (2D) droplet arrays. (*a*) Preformed droplets were loaded sequentially into microwells, thereby preserving indexing order. (*Top*) Photograph of droplet generation by flow-focusing on a microfluidic chip. (*Bottom*) Photographs and simplified schematics of droplets spontaneously moving into the first empty well they encounter in a downstream microwell array, driven by a reduction in interfacial energy as they enter the well. The timing of the images is shown to the left of the photographs. Adapted from Reference 105. Copyright 2007, American Chemical Society. (*b*) Hundreds of droplets were formed in situ in a 2D array on a SlipChip. (*Left*) Schematic of a SlipChip. (*Center*, *right*) Photographs of a SlipChip. (*Top*) Fluid (*red*) was loaded into channels formed by overlapping elliptical wells in opposite plates of the chip (*ellipses*). (*Bottom*) The top plate (*dotted blue outline*) was slipped upward in the plane of the image, thereby isolating each elliptical well and overlapping it with a circular well in the opposite plate to generate hundreds of droplets in a regular array. Reproduced with permission from the Royal Society of Chemistry (100).

3.1. Generation of Two-Dimensional Droplet Arrays

Droplets can be arranged in 2D arrays by several methods. One approach is to build on 1D arrays; examples include forming a set of parallel 1D arrays by aspiration (**Figure 1*d***) (62) or routing droplets into a series of parallel channels by controlling the pressure drop with valves or by pinching outlet tubing (89). As discussed above, each droplet of a 1D array can be (*a*) split into multiple identical daughter droplets for 2D storage (87, 88) or (*b*) combined with droplets from another 1D array to form a 2D array of distinct droplets. Another approach is to capture preformed droplets in a 2D array of fluidic traps, either with random placement (90, 91) or with indexing preserved from the inflowing line of droplets (**Figure 4*a***) (82, 92–94). 2D arrays can also be formed in situ, concurrently with droplet formation, by breaking off droplets from the bulk solution at desired locations (56, 94).

A recent example of in situ droplet array formation is the SlipChip, a microfluidic chip made of two layers prepatterned with wells and ducts that are separated by an immiscible carrier fluid such as oil (95). The two layers are initially positioned to form channels by continuously overlapping these wells and ducts. Aqueous solution is then loaded into these channels. When the layers are "slipped" relative to one another, the wells and ducts separate, and the aqueous stream is broken into an array of droplets. This method has been used to form arrays of hundreds or thousands of droplets in a single step (**Figure 4*b***) (95–101).

Microwell and surface arrays are alternative methods of generating 2D arrays of aqueous solution that do not require an immiscible immersion phase. Microwells can be loaded passively (102, 103) or actively by dispensing, and arrays of droplets can be generated on open surfaces by microarray printing. Microwell and surface arrays differ from most droplet arrays in that the solution can wet the substrate. As a consequence, chemicals or cells in the well or surface droplet can interact easily with the surface. Several excellent recent reviews (3, 16, 104) further discuss the

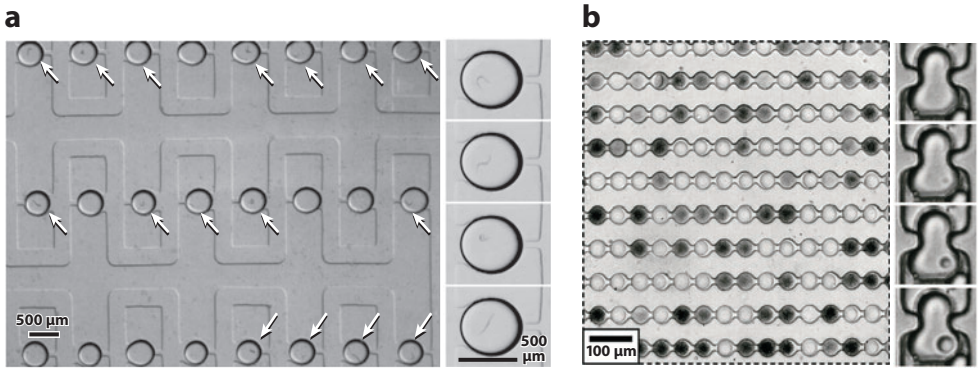


Figure 5

Two-dimensional arrays facilitate high-throughput time-lapse imaging and analysis. (*a*) Photograph of high-throughput single-animal experiments that were performed over long time periods through the use of an array of droplets containing zero or one *Caenorhabditis elegans* worm per droplet. Worms (*in droplets marked with white arrows*) were imaged in droplets over a 2-h period to determine mobility in response to a drug. On the right is a magnified view of individual worms in droplets. Reproduced with permission from the Royal Society of Chemistry (93). (*b*) Photograph of subsecond nucleation events that were simultaneously imaged in hundreds of arrayed droplets during solidification of supercooled glycerol solutions. On the right are time-lapse images of nucleation in a selected droplet in a separate device at (*from top to bottom*) 0, 123, 246, and 369 μs. Reproduced with permission from the Royal Society of Chemistry (107).

use and applications of microwell and surface arrays, including high-throughput immunoassays and compartmentalization of cellular samples.

3.2. Characteristics and Applications of Two-Dimensional Droplet Arrays

2D arrays are naturally capable of higher density than are 1D arrays, in part for physical reasons: The space required to store an array of n droplets usually scales with n in 1D and only $\sqrt{n}$ in 2D, and imaging a planar array of droplets is often simpler than imaging a linear array of droplets in a tube or channel. Furthermore, unlike in 1D droplet arrays, in 2D arrays, droplets can be switched or reorganized easily (analogous to passing cars) without the risk of coalescence, even in the absence of stabilizing surfactants.

Droplets docked in high-density indexed arrays enable high-throughput time-lapse imaging of slow or fast processes. For example, 2D arrays of droplets have been used to monitor the behavior of many individual organisms over minutes or hours. Individual *Caenorhabditis elegans* worms were encapsulated with a neurotoxin metabolite, and an array containing 180 of these droplets was monitored for more than 2 h to quantify the worms' motile response (**Figure 5*a***) (93). *Escherichia coli* cells bearing plasmids for proteins of interest were confined in 239 arrayed droplets to quantify protein expression and enzyme activity for over 20 h (105). Enzyme levels were also monitored in reporter yeast cells by isolating single cells in thousands of droplets on a Dropspots device and monitoring the cleavage of a fluorogenic substrate (106). The 2D array format also lends itself to high-throughput monitoring of protein crystallization, as has been performed on SlipChip (9, 95–97). Droplets that form crystals of interest can be identified by their index and reproduced on a larger scale for analysis by X-ray crystallography. 2D arrays also enable high-throughput measurement of rapid kinetics. For example, subsecond nucleation events were simultaneously monitored in more than 100 droplets during solidification of supercooled glycerol solutions (**Figure 5*b***) (107). In all of the above examples, the droplets were stationary

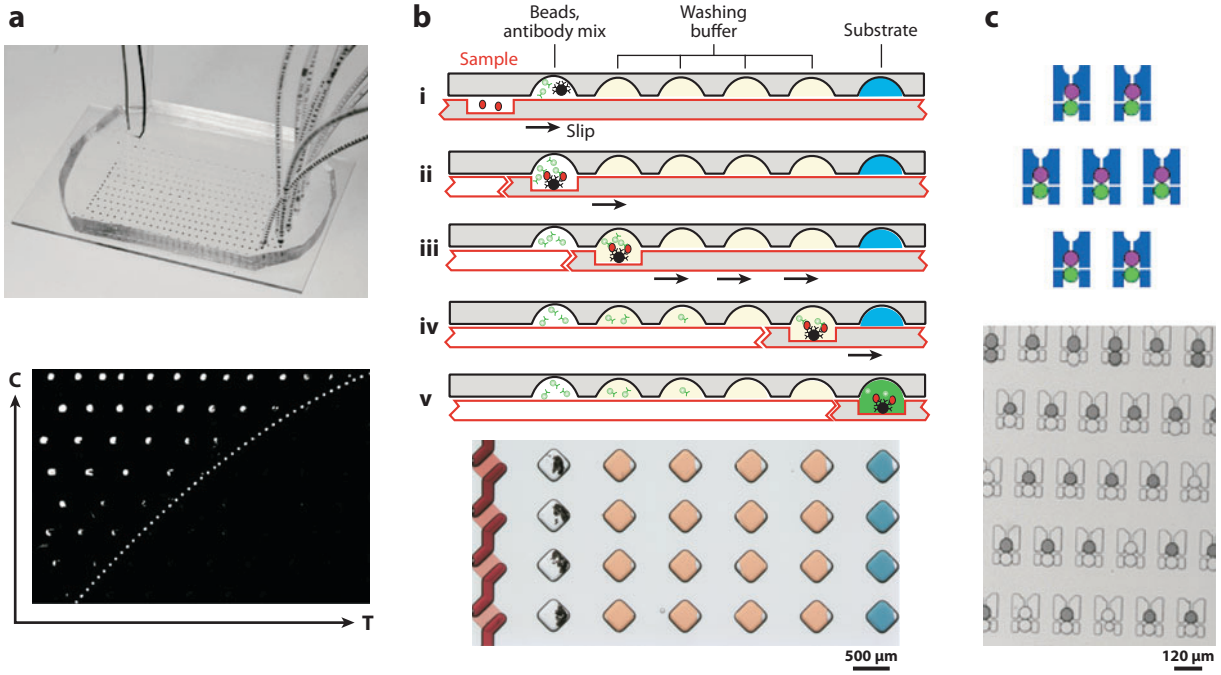


Figure 6

Applications of two-dimensional (2D) arrays of droplets. (*a*) A 2D array was used to read out a concentration-temperature phase diagram on a chip. (*Top*) Photograph of a microfluidic chip with a 2D array of droplets containing colored dye, with a concentration gradient from top to bottom. (*Bottom*) Result of a phase diagram experiment that used droplets of adipic acid with simultaneous concentration and temperature gradients. Crystals of adipic acid appear white, and uncrystallized droplets are not visible. The dotted line gives an estimation of the solubility limit. Reprinted with permission from the Royal Society of Chemistry (89). (*b*) SlipChip was used to perform a multistep heterogeneous immunoassay on each droplet in an array simultaneously. (*Top*) Schematic drawing of the immunoassay (cross-sectional view). (*i*) Solution containing the immunoassay target (*red dots*) was loaded into the bottom well, then (*ii*) slipped right to bring the sample into contact with an assay mixture of beads (*black*) and conjugated antibodies (*green*). (*iii*, *iv*) Additional slips moved the conjugated beads through several wells of washing buffer (*yellow*). (*v*) A final slip brought the beads into contact with a reporter solution (*blue*) in order to quantify the concentration of analyte. (*Bottom*) A photograph of the device (*top view*), with each row of wells filled with colored solution to match the schematic above. Reprinted with permission from Reference 98. Copyright 2010, American Chemical Society. (*c*) Pairs of droplets were trapped in an array in order to investigate diffusion across surfactant bilayers. Droplets were generated and then flowed into the array to be captured. Diffusion of fluorescent small molecules across the interface between the droplets was monitored over time. (*Top*) Schematic of trapped droplets. (*Bottom*) Bright-field microphotograph of trapped droplets in the device. Reproduced with permission from the Royal Society of Chemistry (91).

over time. To image rapidly moving droplets, a microfabricated zone-plate array was recently developed for high-throughput confocal fluorescence imaging of droplets flowing through an array of microfluidic channels. Images were collected at a maximum rate of 184,000 droplets per second (108).

2D arrays make the design and analysis of experiments with two independent variables straightforward. For example, 2D droplet arrays were utilized to construct phase diagrams of the solubility of organic compounds in water. Chemical composition (concentration, *C*) was varied between rows of droplets, and temperature, *T*, was varied along the rows. The solubility diagram, *C* versus *T*, was read out directly from the droplet array (**Figure 6*a***) (89). Similar phase diagrams have been constructed for protein crystallization (60) and for saline-polymer mixtures using the Phase Chip (92).

Another advantage of 2D arrays is that droplets can be manipulated in parallel instead of in linear sequence. This facilitates multistep analysis procedures. For example, the SlipChip can perform multistep and multiplexed analyses on tens to thousands of droplets simultaneously. The SlipChip was used to perform a six-step heterogeneous immunoassay for insulin on 48 independent nanoliter-volume droplets simultaneously (**Figure 6*b***) (98). In another example, the SlipChip was used to generate 384 droplets of a sample containing microbial cells, move the droplets into wells containing 20 different primer pairs, and perform thermal cycling for PCR to identify which microbial strains were present in the sample (99). Researchers have also achieved real-time PCR with 2D arrays by (*a*) fixing emulsion droplets in place in a microfluidic channel (109) or (*b*) printing nonwetting droplets onto a Petri dish surface under oil (110) so that they can be spatially indexed for imaging over time.

In 2D arrays, the relative position of individual droplets can be utilized to control the interactions between droplets. For example, two droplets of differing compositions were trapped after being loaded from both ends of a channel to generate a controlled interface between droplets. These pairs of droplets were used to investigate the diffusion of small molecules across the interface in the presence of different surfactants and carrier fluids (**Figure 6*c***) (91). The formation of lipid bilayers has also been studied at interfaces in an array of droplets (111). For droplets that are not brought into direct contact with one another, bridges between droplets have been used to control communication. For example, a slender neck was used to connect droplets containing protein with droplets containing precipitant on a SlipChip to perform crystallization by nanoliter-scale free-interface diffusion (97). Future work may produce 2D or 3D arrays that control interactions between more than two droplets for complex applications such as intercellular signaling.

Optical trapping, such as that by tweezer arrays and holographic arrays, provides an additional means of controlling the spatial position and interactions between droplets. Although this technology has generally been used to trap particles such as beads (112, 113), it has recently been used to control the position of arrays of droplets such as aerosols. Optical tweezers were used to manipulate, transport, and fuse individual femtoliter-volume aqueous droplets on demand (114). In research leading to manipulation of arrays, aerosol droplets were captured and held in 2D holographic arrays (115), rotated around a carousel for imaging in one position, and coalesced with one another in a controlled manner (116). In the future, this technology may develop into a powerful tool for complex manipulations of 2D or 3D arrays of droplets.

4. THREE-DIMENSIONAL DROPLET ARRAYS

Droplets in 3D arrays are spatially structured or manipulated in space with three variables. Compared with 1D and 2D droplet arrays, 3D arrays are not as well developed. Here, we divide 3D droplet arrays into two categories on the basis of the method of assembly.

4.1. Generation of Self-Assembled Three-Dimensional Droplet Arrays

Self-assembled 3D droplet arrays require no microfabricated structure to support the array. Instead, droplets are tightly packed in 3D arrangements, and surfactants are used to prevent coalescence (**Figure 7**). Usually, such arrays consist of droplets of identical composition, and the spatial arrangement is variable and depends on droplet sizes and oil-to-droplet volume ratios (117). Packing has also been controlled by varying the ratio of chamber height to droplet diameter; in an array designed with two overlapping layers, one million individual droplets were imaged and analyzed for high-throughput analysis of digital PCR (118). So far, there have been no reports about indexing 3D self-assembled droplet arrays, but once methods for arranging and addressing specific

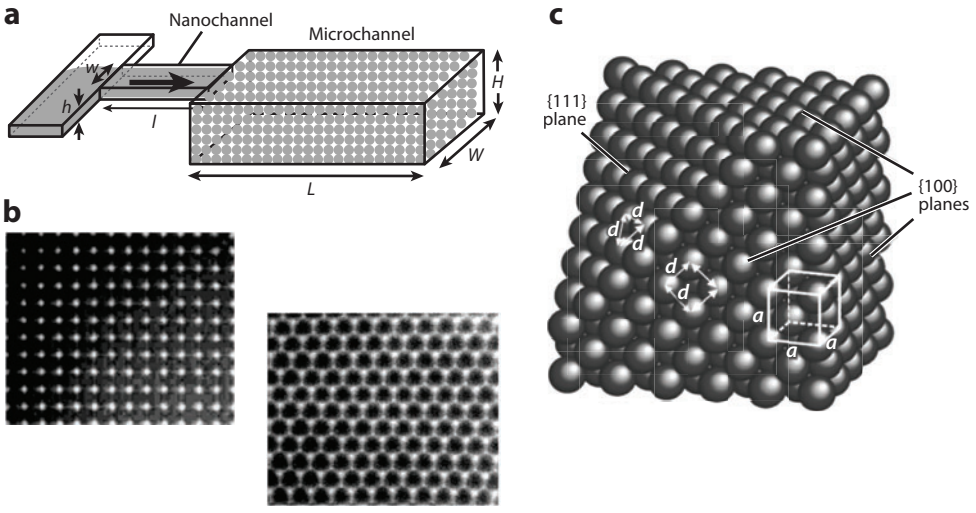


Figure 7

Self-assembled three-dimensional (3D) droplet arrays. (*a*) Schematic of the channel structure used to generate self-organized droplets in 3D arrays. Aqueous droplets suspended in oil are formed at the T junction and flow to a deep microchannel for packing into an organized 3D array. (*b*) Self-organized (*left*) square and (*right*) hexagonal arrangements of homogeneous 3D arrays of droplets observed at the plane adjacent to the channel wall. (*c*) Schematic of the face-centered cubic (fcc) 3D structure, where the square and hexagonal patterns correspond to fcc {100} and {111} planes, respectively. Reproduced with permission from the Royal Society of Chemistry (118).

droplets in such high-density arrays are developed, it may be possible to utilize them for many applications, including stochastic confinement, single-cell analysis, or synthesis of self-organizing colloid materials with specific optical or physical properties.

4.2. Generation of Constructed Three-Dimensional Droplet Arrays

Constructed 3D droplet arrays use specific 3D microstructures such as microwell plates or physical mechanisms such as optical or electrical energies to confine and control the movement and interaction of droplets in 3D space. 3D microfabricated channels have been developed and used for single-phase flow (119); in the future, such methods could be extended to control the flow of droplets from one plane to another. For droplet manipulation without flow, separate microwell plates containing stationary 2D droplet arrays were stacked vertically to combine or separate droplets between the layers, thereby converting multiple 2D arrays into a quasi-3D array (**Figure 8*a***) (120).

The SlipChip also can manipulate multiple 2D droplet arrays in parallel planes. A typical SlipChip contains microstructural features in each plate, and two plates slip relative to one another (95) to vary the microstructure over time (**Figure 8*b***). This motion allows control of simultaneous high-throughput interactions between layered 2D droplet arrays, including mixing, transfer, diffusion, and separation, as well as on-demand rearrangement of the 3D interactions. As discussed above, the SlipChip has been used for applications in protein crystallization (95–97), digital PCR (100), multiplex PCR (99), and nanoliter immunoassays (98). Future development of SlipChip may enable the simultaneous or sequential manipulation of 3D droplet arrays in more than two separate planes, which will allow the programming of more complex multistep interactions and reactions.

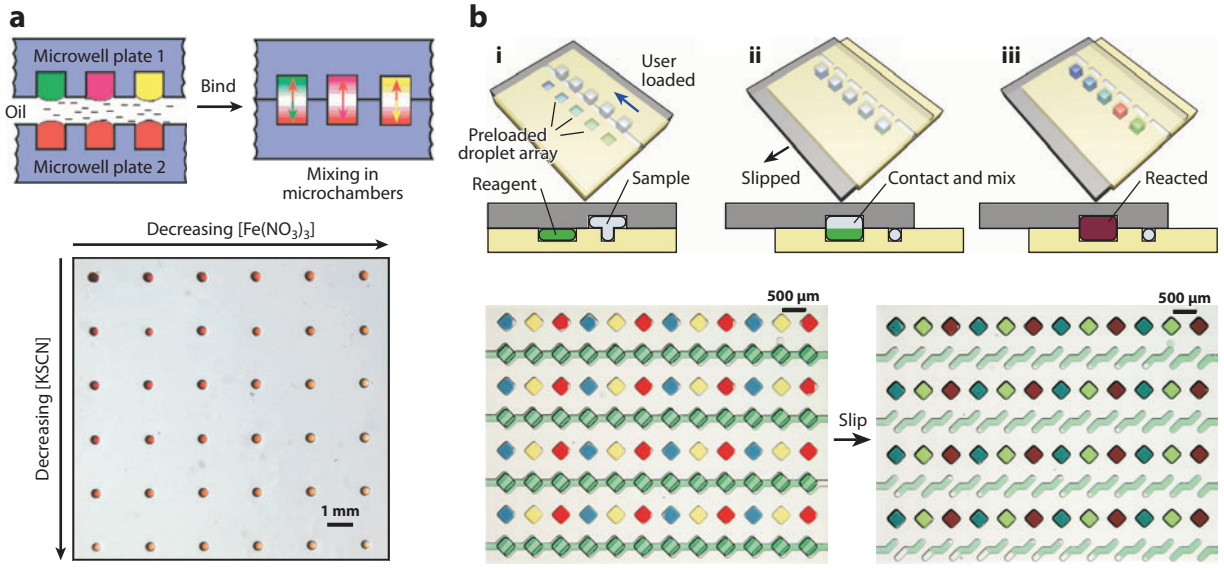


Figure 8

Constructed three-dimensional droplet arrays. (*a*) Two two-dimensional (2D) droplet arrays in microwell plates were assembled under oil to initiate mixing between the arrays. (*Top*) Schematic of the two plates being brought together. (*Bottom*) A micrograph of a 6 × 6 array of microwells containing $Fe(SCN)_x^{3-x}$ solutions with a concentration gradient. Reprinted with permission from Reference 120. Copyright 2007, American Chemical Society. (*b*) Two 2D arrays were moved relative to one another by the SlipChip. (*Top*) Step-by-step operation of a SlipChip consisting of two parallel plates. (*i*) The sample (*white*) was loaded into a series of connected wells and ducts. (*ii*) The plates were slipped to generate individual droplets in the sample wells and combine them with preloaded reagent droplets. (*iii*) The samples mixed with the reagents; the reaction was observed. (*Bottom*) Photographs of an experimental demonstration of mixing between droplet arrays in the SlipChip using food dyes. (*Left*) The bottom plate was preloaded with 48 droplets of blue, yellow, and red dyes. The top plate was loaded with green dye. (*Right*) After slipping, the green-dye droplet array mixed with the preloaded droplet array. Reproduced with permission from the Royal Society of Chemistry (95).

Other methods have been developed to manipulate individual droplets in 3D; these may eventually allow the manipulation of 3D arrays. For example, digital microfluidics driven by electrowetting has been used in a technique termed all-terrain droplet actuation (121). This method enables the manipulation of droplets on nonflat surfaces, including inclined, declined, vertical, twisted, and upside-down geometries (**Figure 9*a***); however, so far the surface can be indexed with only two dimensions. Electrostatic force has also been used to lift an electrically charged droplet vertically between two parallel planar electrode arrays (**Figure 9*b***) (122). Other potential methods to manipulate 3D droplet arrays include optical tweezers (114), magnetic force (123), acoustic trapping (124), ultrasonic–electrostatic hybrid levitation (125), and optically induced dielectrophoresis (126).

3D droplet arrays greatly increase the storage and processing capacity of microfluidic devices and offer the possibility of manipulating droplets in parallel. This area is open to further investigation, and future work will establish the trade-offs between increased performance and complexity and identify applications that will truly benefit from 3D arrays.

5. OUTLOOK

In this review, we discuss the generation and manipulation of droplets in spatially defined arrays in one, two, and three dimensions; we also describe the many novel analyses these arrays have

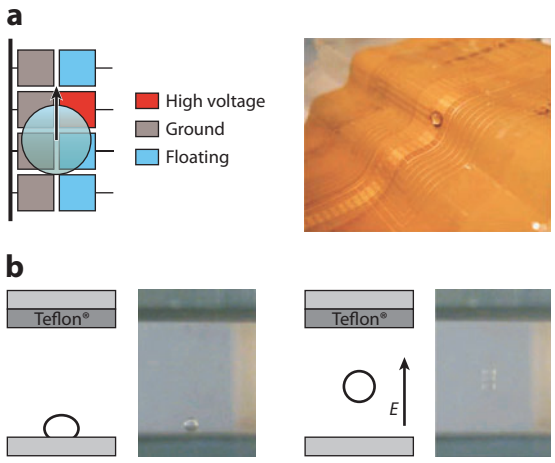


Figure 9

Flexible three-dimensional displacement of droplets using electrowetting and electrostatic levitation. (*a*) Electrode array actuation of a droplet. (*Right*) Photograph of all-terrain droplet actuation on a flexible surface with a staircase geometry. Reprinted from Reference 121 with permission from the Royal Society of Chemistry. (*b*) Water droplet displacement between plates of a plate condenser that were placed 10 mm apart (with a voltage increase from 0 to 1,600 V) in a digital microfluidic system. Reprinted from Reference 122 with permission from Elsevier.

enabled, including time-lapse studies of molecular, enzymatic, and cellular processes. In addition to the applications described in this review, spatially defined arrays of droplets will probably offer many as-yet-untapped opportunities.

When droplets are organized into arrays, they can be perturbed in series or in parallel, and the responses can be easily monitored. Perturbation and response experiments have been fruitful outside of droplets for the elucidation and quantification of cellular phenotypes, such as T and B cell signaling in lymphomas (127, 128), as well as for an enhanced understanding of kinetic mechanisms in chemistry and systems biology (129). Arrays of droplets may provide an excellent tool for high-throughput perturbation and response experiments, especially because droplets also allow for precise control of the timing of mixing and reaction between reagents, thus allowing kinetic measurements to be performed with millisecond resolution (70). Furthermore, controlling the interactions between droplets in arrays may enable the investigation of complex systems such as intercellular signaling.

Preloaded arrays and multistep manipulations may become valuable tools for personal and point-of-care diagnostics. Many diagnostic and personalized medicine applications require multistep processes, and although multistep processes such as immunoassays (62, 98) and PCR (99, 100, 109, 110) have been demonstrated with spatially defined arrays, further research will be required to make these and other diagnostic techniques widely available to clinicians and patients. Another exciting opportunity is for diagnostics based on pattern recognition rather than on analysis of a single biomarker. The importance of testing a panel of multiple biomarkers is particularly relevant to cancer treatment because of the heterogeneity and complexity of cancer (130), but it is also relevant to drug development (131) and treatment of other complex diseases, such as chronic inflammation and autoimmune conditions (132). The advantages of spatially defined arrays of droplets, including indexing, high-throughput analysis, and small sample-volume requirements, are especially pertinent for the analysis and monitoring of multiple biomarkers. We are optimistic

that, as this field develops, droplet arrays will become the basis for novel biomedical devices or innovative tools used to engineer fluid interactions, study chemical kinetics, discover novel catalysts, and improve health care.

SUMMARY POINTS

1. Compartmentalization of a bulk solution into droplets has unique advantages that allow for experiments that cannot be easily performed in the bulk solution.
2. Spatially organized arrays of droplets allow individual droplets to be monitored over time or to be physically or chemically addressed. Droplets of interest can then be retrieved or reproduced. Spatially defined arrays are also a good means of controlling physical or chemical interactions between pairs or multiples of droplets in a highly parallel fashion.
3. 1D arrays of droplets have been used to generate, store, and manipulate cartridges of reagents for screening many reaction conditions; they have also been used to perform time-resolved measurements by converting the spatial order of the droplets to a temporal sequence.
4. Techniques to generate and use 2D arrays of droplets have been widely developed. 2D arrays can manipulate organized droplets at higher throughput than can 1D arrays, and they can easily perform experiments with two independent variables, such as phase diagrams. Droplets in 2D arrays can be manipulated to introduce interaction between droplets.
5. Several methods demonstrate the generation and control of 3D arrays of droplets, but this is still an emerging field. Further improvements to the technology will lead to unique applications.

FUTURE ISSUES

1. Better analytical methods to analyze chemical contents of droplets are needed.
2. Existing methods to access individual droplets of interest in an array should be improved.
3. A simpler and more robust way to generate preloaded 2D or 3D cartridges should be developed.
4. 3D droplet arrays with more than two indexed layers should be developed; indexing with existing multilayer arrays should be integrated.
5. It will be necessary to control communication between more than two droplets at a time so as to study complex interactions between chemical reactions or biological processes.
6. The ability to rearrange droplets in 2D and 3D arrays will be needed for fluidic, physical, and optical trapping mechanisms of array formation.
7. 2D and 3D arrays should be applied to real-world problems, including biomedical devices for multiplexed diagnostics, high-throughput screening of chemical or biological processes for laboratory or industrial use, and detection of rare or stochastic events via compartmentalized samples.

DISCLOSURE STATEMENT

The authors are listed as coinventors on University of Chicago patents or patent applications for some methods presented in this review. All authors may receive royalties from licensing University of Chicago patents or patent applications for some methods presented in this review. R.F.I. has an equity stake in SlipChip, LLC.

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LITERATURE CITED

1. Teh SY, Lin R, Hung LH, Lee AP. 2008. Droplet microfluidics. *Lab Chip* 8:198–220
2. Huebner A, Sharma S, Srisa-Art M, Hollfelder F, Edel JB, deMello AJ. 2008. Microdroplets: a sea of applications? *Lab Chip* 8:1244–54
3. Gorris HH, Walt DR. 2010. Analytical chemistry on the femtoliter scale. *Angew. Chem. Int. Ed.* 49:3880–95
4. Chiu DT, Lorenz RM, Jeffries GDM. 2009. Droplets for ultrasmall-volume analysis. *Anal. Chem.* 81:5111–18
5. Ben-Tzvi P, Rone W. 2010. Microdroplet generation in gaseous and liquid environments. *Microsyst. Technol.* 16:333–56
6. Song H, Chen DL, Ismagilov RF. 2006. Reactions in droplets in microflulidic channels. *Angew. Chem. Int. Ed.* 45:7336–56
7. Griffiths AD, Tawfik DS. 2006. Miniaturising the laboratory in emulsion droplets. *Trends Biotechnol.* 24:395–402
8. Kelly BT, Baret JC, Taly V, Griffiths AD. 2007. Miniaturizing chemistry and biology in microdroplets. *Chem. Commun.* 2007:1773–88
9. Li L, Ismagilov RF. 2010. Protein crystallization using microfluidic technologies based on valves, droplets and SlipChip. *Annu. Rev. Biophys.* 39:139–58
10. Song H, Li HW, Munson MS, Van Ha TG, Ismagilov RF. 2006. On-chip titration of an anticoagulant argatroban and determination of the clotting time within whole blood or plasma using a plug-based microfluidic system. *Anal. Chem.* 78:4839–49
11. Kline TR, Runyon MK, Pothiawala M, Ismagilov RF. 2008. ABO, D blood typing and subtyping using plug-based microfluidics. *Anal. Chem.* 80:6190–97
12. Mousa NA, Jebrail MJ, Yang H, Abdelgawad M, Metalnikov P, et al. 2009. Droplet-scale estrogen assays in breast tissue, blood, and serum. *Sci. Transl. Med.* 1:1ra2
13. Meier M, Kennedy-Darling J, Choi SH, Norstrom EM, Sisodia SS, Ismagilov RF. 2009. Plug-based microfluidics with defined surface chemistry to miniaturize and control aggregation of amyloidogenic peptides. *Angew. Chem. Int. Ed.* 48:1487–89
14. Boedicker JQ, Li L, Kline TR, Ismagilov RF. 2008. Detecting bacteria and determining their susceptibility to antibiotics by stochastic confinement in nanoliter droplets using plug-based microfluidics. *Lab Chip* 8:1265–72
15. Vincent ME, Liu W, Haney EB, Ismagilov RF. 2010. Microfluidic stochastic confinement enhances analysis of rare cells by isolating cells and creating high density environments for control of diffusible signals. *Chem. Soc. Rev.* 39:974–84
16. Walt DR. 2010. Fibre optic microarrays. *Chem. Soc. Rev.* 39:38–50

17. Chiou SH, Wu SH. 1999. Evaluation of commonly used electrophoretic methods for the analysis of proteins and peptides and their application to biotechnology. *Anal. Chim. Acta* 383:47–60
18. Kleparnik K, Bocek P. 2010. Electrophoresis today and tomorrow: helping biologists' dreams come true. *BioEssays* 32:218–26
19. Kremser L, Blaas D, Kenndler E. 2004. Capillary electrophoresis of biological particles: viruses, bacteria, and eukaryotic cells. *Electrophoresis* 25:2282–91
20. Meighan MM, Staton SJR, Hayes MA. 2009. Bioanalytical separations using electric field gradient techniques. *Electrophoresis* 30:852–65
21. Melin J, Quake SR. 2007. Microfluidic large-scale integration: the evolution of design rules for biological automation. *Annu. Rev. Biophys. Biomol. Struct.* 36:213–31
22. Baroud CN, Gallaire F, Dangla R. 2010. Dynamics of microfluidic droplets. *Lab Chip* 10:2032–45
23. Tice JD, Lyon AD, Ismagilov RF. 2004. Effects of viscosity on droplet formation and mixing in microfluidic channels. *Anal. Chim. Acta* 507:73–77
24. Hashimoto M, Garstecki P, Stone HA, Whitesides GM. 2008. Interfacial instabilities in a microfluidic Hele-Shaw cell. *Soft Matter* 4:1403–13
25. Song H, Tice JD, Ismagilov RF. 2003. A microfluidic system for controlling reaction networks in time. *Angew. Chem. Int. Ed.* 42:768–72
26. Baret JC, Kleinschmidt F, El Harrak A, Griffiths AD. 2009. Kinetic aspects of emulsion stabilization by surfactants: a microfluidic analysis. *Langmuir* 25:6088–93
27. Courtois F, Olguin LF, Whyte G, Theberge AB, Huck WTS, et al. 2009. Controlling the retention of small molecules in emulsion microdroplets for use in cell-based assays. *Anal. Chem.* 81:3008–16
28. Martin JD, Hudson SD. 2009. Mass transfer and interfacial properties in two-phase microchannel flows. *New J. Phys.* 11:115005
29. Karande R, Schmid A, Buehler K. 2010. Enzyme catalysis in an aqueous/organic segment flow microreactor: ways to stabilize enzyme activity. *Langmuir* 26:9152–59
30. Kreutz JE, Li L, Roach LS, Hatakeyama T, Ismagilov RF. 2009. Laterally mobile, functionalized self-assembled monolayers at the fluorous-aqueous interface in a plug-based microfluidic system: characterization and testing with membrane protein crystallization. *J. Am. Chem. Soc.* 131:6042–43
31. Roach LS, Song H, Ismagilov RF. 2005. Controlling nonspecific protein adsorption in a plug-based microfluidic system by controlling interfacial chemistry using fluorous-phase surfactants. *Anal. Chem.* 77:785–96
32. Holtze C, Rowat AC, Agresti JJ, Hutchison JB, Angile FE, et al. 2008. Biocompatible surfactants for water-in-fluorocarbon emulsions. *Lab Chip* 8:1632–39
33. Kreutz JE, Shukhaev A, Du W, Druskin S, Daugulis O, Ismagilov RF. 2010. Evolution of catalysts directed by genetic algorithms in a plug-based microfluidic device tested with oxidation of methane by oxygen. *J. Am. Chem. Soc.* 132:3128–32
34. Zheng B, Gerdts CJ, Ismagilov RF. 2005. Using nanoliter plugs in microfluidics to facilitate and understand protein crystallization. *Curr. Opin. Struct. Biol.* 15:548–55
35. Zheng B, Tice JD, Ismagilov RF. 2004. Formation of arrayed droplets of soft lithography and two-phase fluid flow, and application in protein crystallization. *Adv. Mater.* 16:1365–68
36. Zheng B, Tice JD, Roach LS, Ismagilov RF. 2004. A droplet-based, composite PDMS/glass capillary microfluidic system for evaluating protein crystallization conditions by microbatch and vapor-diffusion methods with on-chip X-ray diffraction. *Angew. Chem. Int. Ed.* 43:2508–11
37. Li L, Mustafi D, Fu Q, Tereshko V, Chen DLL, et al. 2006. Nanoliter microfluidic hybrid method for simultaneous screening and optimization validated with crystallization of membrane proteins. *Proc. Natl. Acad. Sci. USA* 103:19243–48
38. He MY, Sun CH, Chiu DT. 2004. Concentrating solutes and nanoparticles within individual aqueous microdroplets. *Anal. Chem.* 76:1222–27
39. Clausell-Tormos J, Lieber D, Baret JC, El-Harrak A, Miller OJ, et al. 2008. Droplet-based microfluidic platforms for the encapsulation and screening of mammalian cells and multicellular organisms. *Chem. Biol.* 15:427–37

40. Chen DLL, Li L, Reyes S, Adamson DN, Ismagilov RF. 2007. Using three-phase flow of immiscible liquids to prevent coalescence of droplets in microfluidic channels: criteria to identify the third liquid and validation with protein crystallization. *Langmuir* 23:2255–60
41. Yu HM, Meyvantsson I, Shkel IA, Beebe DJ. 2005. Diffusion dependent cell behavior in microenvironments. *Lab Chip* 5:1089–95
42. Yu HM, Alexander CM, Beebe DJ. 2007. Understanding microchannel culture: parameters involved in soluble factor signaling. *Lab Chip* 7:726–30
43. Schaerli Y, Hollfelder F. 2009. The potential of microfluidic water-in-oil droplets in experimental biology. *Mol. Biosyst.* 5:1392–404
44. Griffiths AD, Tawfik DS. 2003. Directed evolution of an extremely fast phosphotriesterase by in vitro compartmentalization. *EMBO J.* 22:24–35
45. Agresti JJ, Antipov E, Abate AR, Ahn K, Rowat AC, et al. 2010. Ultrahigh-throughput screening in drop-based microfluidics for directed evolution. *Proc. Natl. Acad. Sci. USA* 107:4004–9
46. Baret JC, Miller OJ, Taly V, Ryckelynck M, El-Harrak A, et al. 2009. Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity. *Lab Chip* 9:1850–58
47. Zhang C, Da X. 2010. Single-molecule DNA amplification and analysis using microfluidics. *Chem. Rev.* 110:4910–47
48. Zhang YH, Ozdemir P. 2009. Microfluidic DNA amplification—a review. *Anal. Chim. Acta* 638:115–25
49. Krutzik PO, Nolan GP. 2006. Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. *Nat. Methods* 3:361–68
50. Brouzes E, Medkova M, Savenelli N, Marran D, Twardowski M, et al. 2009. Droplet microfluidic technology for single-cell high-throughput screening. *Proc. Natl. Acad. Sci. USA* 106:14195–200
51. Baret JC, Beck Y, Billas-Massobrio I, Moras D, Griffiths AD. 2010. Quantitative cell-based reporter gene assays using droplet-based microfluidics. *Chem. Biol.* 17:528–36
52. Joensson H, Samuels M, Brouzes E, Medkova M, Uhlen M, et al. 2009. Detection and analysis of low-abundance cell-surface biomarkers using enzymatic amplification in microfluidic droplets. *Angew. Chem. Int. Ed.* 48:2518–21
53. Thorsen T, Roberts RW, Arnold FH, Quake SR. 2001. Dynamic pattern formation in a vesicle-generating microfluidic device. *Phys. Rev. Lett.* 86:4163–66
54. Anna SL, Bontoux N, Stone HA. 2003. Formation of dispersions using "flow focusing" in microchannels. *Appl. Phys. Lett.* 82:364–66
55. Wu L, Li GP, Xu W, Bachman M. 2006. Droplet formation in microchannels under static conditions. *Appl. Phys. Lett.* 89:144106
56. Zhang QQ, Zeng SJ, Qin JH, Lin BC. 2009. Microfluidic droplet trapping array as nanoliter reactors for gas-liquid chemical reaction. *Electrophoresis* 30:3181–88
57. Cohen DE, Schneider T, Wang M, Chiu DT. 2010. Self-digitization of sample volumes. *Anal. Chem.* 82:5707–17
58. Zheng B, Roach LS, Ismagilov RF. 2003. Screening of protein crystallization conditions on a microfluidic chip using nanoliter-size droplets. *J. Am. Chem. Soc.* 125:11170–71
59. Zheng B, Tice JD, Ismagilov RF. 2004. Formation of droplets of in microfluidic channels alternating composition and applications to indexing of concentrations in droplet-based assays. *Anal. Chem.* 76:4977–82
60. Lau BTC, Baitz CA, Dong XP, Hansen CL. 2007. A complete microfluidic screening platform for rational protein crystallization. *J. Am. Chem. Soc.* 129:454–55
61. Zeng SJ, Li BW, Su XO, Qin JH, Lin BC. 2009. Microvalve-actuated precise control of individual droplets in microfluidic devices. *Lab Chip* 9:1340–43
62. Linder V, Sia SK, Whitesides GM. 2005. Reagent-loaded cartridges for valveless and automated fluid delivery in microfluidic devices. *Anal. Chem.* 77:64–71
63. Du WB, Sun M, Gu SQ, Fang Q. 2010. Automated microfluidic screening assay platform based on DropLab. *Anal. Chem.* 9941–47
64. Chen DLL, Ismagilov RF. 2006. Microfluidic cartridges preloaded with nanoliter plugs of reagents: an alternative to 96-well plates for screening. *Curr. Opin. Chem. Biol.* 10:226–31

65. Hatakeyama T, Chen DLL, Ismagilov RF. 2006. Microgram-scale testing of reaction conditions in solution using nanoliter plugs in microfluidics with detection by MALDI-MS. *J. Am. Chem. Soc.* 128:2518–19
66. Dorfman KD, Chabert M, Codarbox JH, Rousseau G, de Cremoux P, Viovy JL. 2005. Contamination free continuous flow microfluidic polymerase chain reaction for quantitative and clinical applications. *Anal. Chem.* 77:3700–4
67. Chabert M, Dorfman KD, de Cremoux P, Roeraade J, Viovy JL. 2006. Automated microdroplet platform for sample manipulation and polymerase chain reaction. *Anal. Chem.* 78:7722–28
68. Fuerstman MJ, Garstecki P, Whitesides GM. 2007. Coding/decoding and reversibility of droplet trains in microfluidic networks. *Science* 315:828–32
69. Zagnoni M, Cooper JM. 2010. A microdroplet-based shift register. *Lab Chip* 10:3069–73
70. Song H, Ismagilov RF. 2003. Millisecond kinetics on a microfluidic chip using nanoliters of reagents. *J. Am. Chem. Soc.* 125:14613–19
71. Han ZY, Li WT, Huang YY, Zheng B. 2009. Measuring rapid enzymatic kinetics by electrochemical method in droplet-based microfluidic devices with pneumatic valves. *Anal. Chem.* 81:5840–45
72. Pompano RR, Li HW, Ismagilov RF. 2008. Rate of mixing controls rate and outcome of autocatalytic processes: theory and microfluidic experiments with chemical reactions and blood coagulation. *Biophys. J.* 95:1531–43
73. Gerdts CJ, Sharoyan DE, Ismagilov RF. 2004. A synthetic reaction network: chemical amplification using nonequilibrium autocatalytic reactions coupled in time. *J. Am. Chem. Soc.* 126:6327–31
74. Huebner A, Olguin LF, Bratton D, Whyte G, Huck WTS, et al. 2008. Development of quantitative cell-based enzyme assays in microdroplets. *Anal. Chem.* 80:3890–96
75. Srisa-Art M, Dyson EC, deMello AJ, Edel JB. 2008. Monitoring of real-time streptavidin-biotin binding kinetics using droplet microfluidics. *Anal. Chem.* 80:7063–67
76. Hsieh ATH, Pan PJH, Lee AP. 2009. Rapid label-free DNA analysis in picoliter microfluidic droplets using FRET probes. *Microfluid. Nanofluid.* 6:391–401
77. Solvas XCI, Srisa-Art M, deMello AJ, Edel JB. 2010. Mapping of fluidic mixing in microdroplets with 1 μs time resolution using fluorescence lifetime imaging. *Anal. Chem.* 82:3950–56
78. Chen D, Du WB, Liu Y, Liu WS, Kuznetsov A, et al. 2008. The chemistrode: a droplet-based microfluidic device for stimulation and recording with high temporal, spatial, and chemical resolution. *Proc. Natl. Acad. Sci. USA* 105:16843–48
79. Liu Y, Ismagilov RF. 2009. Dynamics of coalescence of plugs with a hydrophilic wetting layer induced by flow in a microfluidic chemistrode. *Langmuir* 25:2854–59
80. Chen DL, Du W, Ismagilov RF. 2009. Using TIRF microscopy to quantify and confirm efficient mass transfer at the substrate surface of the chemistrode. *New J. Phys.* 11:075017
81. Liu W, Kim HJ, Lucchetta E, Du W, Ismagilov RF. 2009. Isolation, incubation, and parallel functional testing and identification by FISH of rare microbial single-copy cells from multi-species mixtures using the combination of chemistrode and stochastic confinement. *Lab Chip* 9:2153–62
82. Edgar JS, Milne G, Zhao YQ, Pabbati CP, Lim DSW, Chiu DT. 2009. Compartmentalization of chemically separated components into droplets. *Angew. Chem. Int. Ed.* 48:2719–22
83. Wang M, Roman GT, Schultz K, Jennings C, Kennedy RT. 2008. Improved temporal resolution for in vivo microdialysis by using segmented flow. *Anal. Chem.* 80:5607–15
84. Roman GT, Wang M, Shultz KN, Jennings C, Kennedy RT. 2008. Sampling and electrophoretic analysis of segmented flow streams using virtual walls in a microfluidic device. *Anal. Chem.* 80:8231–38
85. Wang M, Roman GT, Perry ML, Kennedy RT. 2009. Microfluidic chip for high efficiency electrophoretic analysis of segmented flow from a microdialysis probe and in vivo chemical monitoring. *Anal. Chem.* 81:9072–78
86. Wang M, Slaney T, Mabrouk O, Kennedy RT. 2010. Collection of nanoliter microdialysate fractions in plugs for off-line in vivo chemical monitoring with up to 2 s temporal resolution. *J. Neurosci. Methods* 190:39–48
87. Adamson DN, Mustafi D, Zhang JXJ, Zheng B, Ismagilov RF. 2006. Production of arrays of chemically distinct nanolitre plugs via repeated splitting in microfluidic devices. *Lab Chip* 6:1178–86
88. Clausell-Tormos J, Griffiths AD, Merten CA. 2010. An automated two-phase microfluidic system for kinetic analyses and the screening of compound libraries. *Lab Chip* 10:1302–7

89. Laval P, Lisai N, Salmon JB, Joanicot M. 2007. A microfluidic device based on droplet storage for screening solubility diagrams. *Lab Chip* 7:829–34
90. Huebner A, Bratton D, Whyte G, Yang M, deMello AJ, et al. 2009. Static microdroplet arrays: a microfluidic device for droplet trapping, incubation and release for enzymatic and cell-based assays. *Lab Chip* 9:692–98
91. Bai YP, He XM, Liu DS, Patil SN, Bratton D, et al. 2010. A double droplet trap system for studying mass transport across a droplet-droplet interface. *Lab Chip* 10:1281–85
92. Shim JU, Cristobal G, Link DR, Thorsen T, Jia YW, et al. 2007. Control and measurement of the phase behavior of aqueous solutions using microfluidics. *J. Am. Chem. Soc.* 129:8825–35
93. Shi WW, Qin JH, Ye NN, Lin BC. 2008. Droplet-based microfluidic system for individual *Caenorhabditis elegans* assay. *Lab Chip* 8:1432–35
94. Boukellal H, Selimovic S, Jia YW, Cristobal G, Fraden S. 2009. Simple, robust storage of drops and fluids in a microfluidic device. *Lab Chip* 9:331–38
95. Du WB, Li L, Nichols KP, Ismagilov RF. 2009. SlipChip. *Lab Chip* 9:2286–92
96. Li L, Du WB, Ismagilov R. 2010. User-loaded SlipChip for equipment-free multiplexed nanoliter-scale experiments. *J. Am. Chem. Soc.* 132:106–11
97. Li L, Du WB, Ismagilov RF. 2010. Multiparameter screening on SlipChip used for nanoliter protein crystallization combining free interface diffusion and microbatch methods. *J. Am. Chem. Soc.* 132:112–19
98. Liu WS, Chen DL, Du WB, Nichols KP, Ismagilov RF. 2010. SlipChip for immunoassays in nanoliter volumes. *Anal. Chem.* 82:3276–82
99. Shen F, Du WB, Davydova EK, Karymov MA, Pandey J, Ismagilov RF. 2010. Nanoliter multiplex PCR arrays on a SlipChip. *Anal. Chem.* 82:4606–12
100. Shen F, Du W, Kreutz JE, Fok A, Ismagilov RF. 2010. Digital PCR on a SlipChip. *Lab Chip* 10:2666–72
101. Li L, Karymov MA, Nichols KP, Ismagilov RF. 2010. Dead-end filling of SlipChip evaluated theoretically and experimentally as a function of the surface chemistry and the gap size between the plates for lubricated and dry SlipChips. *Langmuir* 26:12465–71
102. Jackman RJ, Duffy DC, Ostuni E, Willmore ND, Whitesides GM. 1998. Fabricating large arrays of microwells with arbitrary dimensions and filling them using discontinuous dewetting. *Anal. Chem.* 70:2280–87
103. Boedicker JQ, Vincent ME, Ismagilov RF. 2009. Microfluidic confinement of single cells of bacteria in small volumes initiates high density behavior of quorum sensing and growth and reveals its variability. *Angew. Chem. Int. Ed.* 48:5908–11
104. Weibel DB, DiLuzio WR, Whitesides GM. 2007. Microfabrication meets microbiology. *Nat. Rev. Microbiol.* 5:209–18
105. Shim JU, Olguin LF, Whyte G, Scott D, Babtie A, et al. 2009. Simultaneous determination of gene expression and enzymatic activity in individual bacterial cells in microdroplet compartments. *J. Am. Chem. Soc.* 131:15251–56
106. Schmitz CHJ, Rowat AC, Koster S, Weitz DA. 2009. Dropspots: a picoliter array in a microfluidic device. *Lab Chip* 9:44–49
107. Edd JF, Humphry KJ, Irimia D, Weitz DA, Toner M. 2009. Nucleation and solidification in static arrays of monodisperse drops. *Lab Chip* 9:1859–65
108. Schonbrun E, Abate AR, Steinvurzel PE, Weitz DA, Crozier KB. 2010. High-throughput fluorescence detection using an integrated zone-plate array. *Lab Chip* 10:852–56
109. Beer NR, Hindson BJ, Wheeler EK, Hall SB, Rose KA, et al. 2007. On-chip, real-time, single-copy polymerase chain reaction in picoliter droplets. *Anal. Chem.* 79:8471–75
110. Kim H, Vishniakou S, Faris GW. 2009. Petri dish PCR: laser-heated reactions in nanoliter droplet arrays. *Lab Chip* 9:1230–35
111. Stanley CE, Elvira KS, Niu XZ, Gee AD, Ces O, et al. 2010. A microfluidic approach for high-throughput droplet interface bilayer (DIB) formation. *Chem. Commun.* 46:1620–22
112. Dufresne ER, Grier DG. 1998. Optical tweezer arrays and optical substrates created with diffractive optics. *Rev. Sci. Instrum.* 69:1974–77
113. Polin M, Ladavac K, Lee SH, Roichman Y, Grier DG. 2005. Optimized holographic optical traps. *Opt. Express* 13:5831–45

114. Lorenz RM, Edgar JS, Jeffries GDM, Chiu DT. 2006. Microfluidic and optical systems for the on-demand generation and manipulation of single femtoliter-volume aqueous droplets. *Anal. Chem.* 78:6433–39
115. Burnham DR, McGloin D. 2006. Holographic optical trapping of aerosol droplets. *Opt. Express* 14:4175–81
116. Butler JR, Wills JB, Mitchem L, Burnham DR, McGloin D, Reid JP. 2009. Spectroscopic characterisation and manipulation of arrays of sub-picolitre aerosol droplets. *Lab Chip* 9:521–28
117. Shui LL, Kooij ES, Wijnperle D, Van Den Berg A, Eijkel JCT. 2009. Liquid crystallography: 3D microdroplet arrangements using microfluidics. *Soft Matter* 5:2708–12
118. Hatch AC, Fisher JS, Lee AP. 2010. 1-million droplet array for high-dynamic-range digital microfluidics. *Proc. Intl. Conf. Miniat. Syst. Chem. Life Sci. 14th, Groningen, Neth.*, pp. 1358–60
119. Wu HK, Odom TW, Chiu DT, Whitesides GM. 2003. Fabrication of complex three-dimensional microchannel systems in PDMS. *J. Am. Chem. Soc.* 125:554–59
120. Zhou X, Lau L, Lam WWL, Au SWN, Zheng B. 2007. Nanoliter dispensing method by degassed poly(dimethylsiloxane) microchannels and its application in protein crystallization. *Anal. Chem.* 79:4924–30
121. Abdelgawad M, Freire SLS, Yang H, Wheeler AR. 2008. All-terrain droplet actuation. *Lab Chip* 8:672–77
122. Roux JM, Fouillet Y, Achard JL. 2007. 3D droplet displacement in microfluidic systems by electrostatic actuation. *Sens. Actuators A* 134:486–93
123. Okochi M, Tsuchiya H, Kumazawa F, Shikida M, Honda H. 2010. Droplet-based gene expression analysis using a device with magnetic force–based droplet-handling system. *J. Biosci. Bioeng.* 109:193–97
124. Lee J, Teh SY, Lee A, Kim HH, Lee C, Shung KK. 2010. Transverse acoustic trapping using a Gaussian focused ultrasound. *Ultrasound Med. Biol.* 36:350–55
125. Chung SK, Trinh EH. 1998. Containerless protein crystal growth in rotating levitated drops. *J. Cryst. Growth* 194:384–97
126. Hung SH, Lin YH, Lee GB. 2010. A microfluidic platform for manipulation and separation of oil-in-water emulsion droplets using optically induced dielectrophoresis. *J. Micromech. Microeng.* 20:045026
127. Irish JM, Hovland R, Krutzik PO, Perez OD, Bruserud O, et al. 2004. Single cell profiling of potentiated phospho-protein networks in cancer cells. *Cell* 118:217–28
128. Lehar J, Zimmermann GR, Krueger AS, Molnar RA, Ledell JT, et al. 2007. Chemical combination effects predict connectivity in biological systems. *Mol. Syst. Biol.* 3:80
129. Ross J. 2008. From the determination of complex reaction mechanisms to systems biology. *Annu. Rev. Biochem.* 77:479–94
130. Pritzker KPH. 2002. Cancer biomarkers: easier said than done. *Clin. Chem.* 48:1147–50
131. Colburn WA. 2003. Biomarkers in drug discovery and development: from target identification through drug marketing. *J. Clin. Pharmacol.* 43:329–41
132. O'Hara J, Richard M, Benoit SE, Groves CJ, Collins M. 2006. Cell-surface and cytokine biomarkers in autoimmune and inflammatory diseases. *Drug Discov. Today* 11:342–47
133. Mazutis L, Baret JC, Griffiths AD. 2009. A fast and efficient microfluidic system for highly selective one-to-one droplet fusion. *Lab Chip* 9:2665–72
134. Ma HC, Horiuchi KY, Wang Y, Kucharewicz SA, Diamond SL. 2005. Nanoliter homogenous ultra-high throughput screening microarray for lead discoveries and IC_{50} profiling. *Assay Drug. Dev. Technol.* 3:177–87
135. Huebner A, Srisa-Art M, Holt D, Abell C, Hollfelder F, et al. 2007. Quantitative detection of protein expression in single cells using droplet microfluidics. *Chem. Commun.* 2007:1218–20
136. Gadd JC, Kuyper CL, Fujimoto BS, Allen RW, Chiu DT. 2008. Sizing subcellular organelles and nanoparticles confined within aqueous droplets. *Anal. Chem.* 80:3450–57
137. Srisa-Art M, deMello AJ, Edel JB. 2007. High-throughput DNA droplet assays using picoliter reactor volumes. *Anal. Chem.* 79:6682–89
138. Rane TD, Puleo CM, Liu KJ, Zhang Y, Lee AP, Wang TH. 2010. Counting single molecules in sub-nanolitre droplets. *Lab Chip* 10:161–64
139. Wang G, Lim C, Chen L, Chon H, Choo J, et al. 2009. Surface-enhanced Raman scattering in nanoliter droplets: towards high-sensitivity detection of mercury (II) ions. *Anal. Bioanal. Chem.* 394:1827–32

140. Akpa BS, Matthews SM, Sederman AJ, Yunus K, Fisher AC, et al. 2007. Study of miscible and immiscible flows in a microchannel using magnetic resonance imaging. *Anal. Chem.* 79:6128–34
141. Li L, Fu Q, Kors C, Stewart L, Nollert P, et al. 2010. A plug-based microfluidic system for dispensing lipidic cubic phase (LCP) material validated by crystallizing membrane proteins in lipidic mesophases. *Microfluid. Nanofluid.* 8:789–98
142. Hager DB, Dovichi NJ, Klassen J, Kebarle P. 1994. Droplet electrospray mass spectrometry. *Anal. Chem.* 66:3944–49
143. Kim SJ, Song Y-A, Skipper PL, Han J. 2006. Electrohydrodynamic generation and delivery of monodisperse picoliter droplets using a poly(dimethylsiloxane) microchip. *Anal. Chem.* 78:8011–19
144. Edgar JS, Pabbati CP, Lorenz RM, He MY, Fiorini GS, Chiu DT. 2006. Capillary electrophoresis separation in the presence of an immiscible boundary for droplet analysis. *Anal. Chem.* 78:6948–54
145. Liu SJ, Gu YF, Le Roux RB, Matthews SM, Bratton D, et al. 2008. The electrochemical detection of droplets in microfluidic devices. *Lab Chip* 8:1937–42
146. Shen H, Fang Q, Fang ZL. 2006. A microfluidic chip based sequential injection system with trapped droplet liquid-liquid extraction and chemiluminescence detection. *Lab Chip* 6:1387–89
147. Martinez AW, Phillips ST, Carrilho E, Thomas SW, Sindi H, Whitesides GM. 2008. Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Anal. Chem.* 80:3699–707
148. Sun M, Fang Q. 2010. High-throughput sample introduction for droplet-based screening with an on-chip integrated sampling probe and slotted-vial array. *Lab Chip* 10:2864–68
149. Mazutis L, Griffiths AD. 2009. Preparation of monodisperse emulsions by hydrodynamic size fractionation. *App. Phys. Lett.* 95:204103
150. Tan YC, Ho YL, Lee AP. 2008. Microfluidic sorting of droplets by size. *Microfluid. Nanofluid.* 4:343–48
151. Abate AR, Agresti JJ, Weitz DA. 2010. Microfluidic sorting with high-speed single-layer membrane valves. *Appl. Phys. Lett.* 96:203509

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