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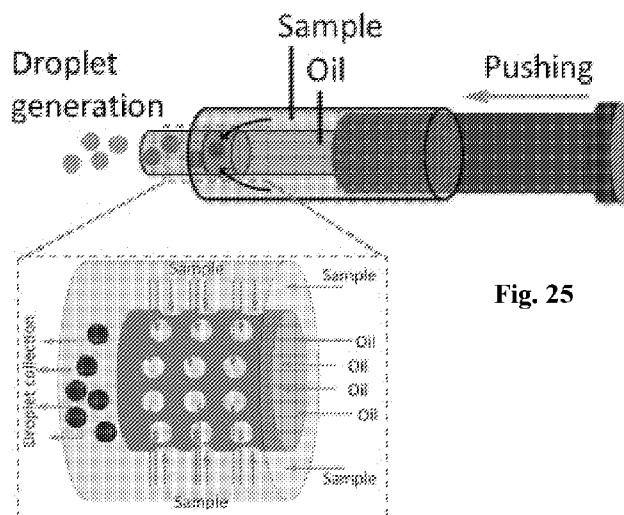


Fig. 25

(57) **Abstract:** Provided are compositions for encapsulated sample trapping, manipulation, analysis, sorting, and screening and methods for making and using the same. Provided are high throughput, multiplexed systems or devices for detecting and/or quantifying a chemical, biological, physiological or pathological analyte, or a single molecule or a single cell, or a chemical or a biochemical reaction comprising use of a floating droplet array (FDA) system integrated with use of a sensing element or reporter or a fluorogenic reaction. In alternative embodiments, high-throughput, multiplexed systems are used as a research or discovery tool to characterize, manipulate, screen, and/or sort immunological agents such as B cells, Car-T cells and antibodies. In alternative embodiments, high-throughput, multiplexed systems are integrated with portable imaging systems. Also provided are high-throughput droplet generation modules that form droplets at high throughput using droplet-generating junctions.



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DROPLET-TRAPPING DEVICES FOR BIOASSAYS AND DIAGNOSTICS

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Patent
5 Application Serial No. (USSN) 62/220,144, filed September 17, 2015. The
aforementioned application is expressly incorporated herein by reference in its entirety
and for all purposes.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant number R01
10 AI117061-01, awarded by the National Institutes of Health (NIH), DHHS. The
government has certain rights in the invention.

TECHNICAL FIELD

Provided are compositions for encapsulated sample trapping, manipulation,
analysis, sorting, and screening and methods for making and using the same. Provided
15 are high throughput, multiplexed systems or devices for detecting and/or quantifying a
chemical, biological, physiological or pathological analyte, or a single molecule or a
single cell, or a chemical or a biochemical reaction comprising use of a floating droplet
array (FDA) system integrated with use of a sensing element or reporter or a fluorogenic
reaction. In alternative embodiments, high-throughput, multiplexed systems are used as a
20 research or discovery tool to characterize, manipulate, screen, and/or sort immunological
agents such as B cells, a Chimeric Antigen Receptor (CAR) T cell (CAR-T cells) and
antibodies. In alternative embodiments, high-throughput, multiplexed systems as
provided herein are integrated with portable imaging systems such as CCD, CMOS,
digital camera, or cell phone-based imaging. In alternative embodiments, the positive
25 droplets are sorted individually or collectively for downstream analysis including
sequencing technologies. Also provided are high-throughput droplet generation modules
that form droplets at high throughput using droplet-generating junctions.

BACKGROUND

High-throughput technologies have found many applications in biology and
30 chemistry such as drug discovery, disease diagnosis, and elucidating biological
mechanisms. These applications often require detection of rare analytes such as nucleic

acids, proteins, metabolites, and cells. In addition, these analytes often exist among a large background of interfering, non-target species. Moreover, real-time analysis is also often required to capture the dynamic nature of biological processes. Therefore, there is a great need for technologies that can isolate, analyze, and quantify individual components of a heterogeneous mixture in a parallel, high-throughput format. Traditional high-throughput technologies such as microwell plates with automated robotic handling systems are widely used in industries such as drug screening. However, these platforms require bulky, expensive machinery, are prone to sample evaporation, and require relatively large sample volumes, which can waste precious reagents or biological samples.

Recently, microfabricated devices have become powerful technologies for high-throughput analysis in many applications such as biological and chemical assays. These technologies often partition a bulk solution into many isolated pico to nanoliter-sized compartments. This compartmentalization confines rare analytes into a small volume, which increases their effective concentration and reduces interference from non-target species. This compartmentalization has been achieved using fluids dispersed into microfabricated wells or in microfluidic chambers which are separated by pneumatically controlled valves. However, retrieving individual samples from these types of devices is difficult to achieve. Moreover, reagent mixing requires complex architecture and microfabrication or is done in bulk before compartmentalization, which may prevent colocalization of initial reaction products from with their initiating target.

Another way to compartmentalize reactions is to partition them into discrete micron-sized droplets surrounded by an immiscible carrier fluid. Droplet-based microfluidics has the advantage of precise control over mixing of fluids, minimal waste of precious reagents, and reduces evaporation and adsorption of molecules at the device walls. Uniform droplets can be generated at kHz frequencies with sizes precisely controlled by fluid flow rates and device geometry. Multiple operations can be performed such as droplet fusion, splitting, cooling, heating, and sorting on- or off-chip as the application requires. Droplet microfluidic devices have been developed for a wide range of applications including micro-material fabrication, directed evolution, mRNA profiling of a heterogeneous population of cells, pathogen detection, and single-cell and single-molecule analysis.

Recently, Hatch and coworkers reported a high-throughput droplet digital PCR (ddPCR) device was developed that analyzed tightly packed droplets in a microfluidic chamber via an integrated CMOS-based wide-field imaging system for absolute quantification of copy number of target DNA. In this development, the dynamic range of ddPCR was increased by 100-fold compared to existing ddPCR systems by increasing the device throughput. However, droplet coalescence was observed for a small fraction of droplets, which was likely exacerbated by their tight-packing. Moreover, neighboring droplets which are close together or overlap complicate image processing, which may result in quantification errors in this type of device. Indexing poses an additional challenge since droplets are free to move throughout the experiment, which hinders real-time monitoring.

Spatially defined arrays of static, immobilized droplets facilitates indexing and monitoring of droplets over time since the array element locations create a natural positioning system. Recently, Huebner and colleagues used droplet traps to immobilize droplets into a 384 element array, which allowed for monitoring of the droplets over time. The droplets could also be subsequently recovered by reversing the flow direction. Similarly, Schmitz and coworkers used channels containing many constrictions to trap up to 8000 droplets. Droplets were subsequently recovered by increasing the flow rate through the channels. However, ultrahigh-throughput analysis is difficult to achieve in these types of devices because the trapping structures are located within the main flow stream and thus a high-density of droplet traps results in a large resistance to flow. Moreover, they are prone to reagent and sample waste since the majority of the droplets pass around the traps. Microfluidic devices have also been previously reported that trap droplets by buoyancy forces between the drops and the carrier fluid. However, these devices require precise alignment of poly-di-methyl-siloxane (PDMS) layers and the highest throughput achieved was only 120 droplet traps, which is comparably low-throughput and impractical for many biological applications. Thus, new devices are needed that can more precisely control droplet trapping, manipulation, analysis, and recovery in an efficient, ultrahigh-throughput manner.

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SUMMARY

In some embodiments, microfluidic droplets are trapped into an array of trapping structures. In some embodiments, droplets are trapped by buoyancy forces between immiscible fluids having different densities. In some embodiments, the droplets can be recovered from the trapping structures by reorienting the device. In some embodiments, various shapes and sizes of trapping structures may be used depending on the application such as droplet trapping, droplet incubation, droplet merging, droplet splitting, sample transfer, and buffer exchange between droplets. In alternative embodiments, these operations are conducted in a massively parallel and high-throughput manner.

In alternative embodiments, provided are guiding structures such as tracks, pillars, or narrow channels which guide droplets to the trapping structures and ensure complete coverage of the trapping structures. In alternative embodiments, inlets or outlets are included to divert droplets away or to the droplet trapping structures and/or other channels or chambers. In some embodiments, products of manufacture as provided herein are integrated with various imaging systems such as a fluorescence microscope, embedded avalanche photodiode (APD), photomultiplier tube (PMT), digital camera, charge-coupled device (CCD), or complementary metal-oxide semiconductor (CMOS) sensor for endpoint or real-time analysis. In some embodiments, products of manufacture as provided herein are integrated with droplet generation modules, droplet trapping modules, droplet manipulation modules, droplet recovery modules, and droplet analysis (e.g., imaging) modules. In some embodiments, products of manufacture as provided herein are packaged in fully integrated, automated, portable systems (see, for example, a non-limiting embodiment depicted in Figure 12). In some embodiments, the device is integrated with data acquisition hardware and software, data processing software, display screens, and a user interface. In some embodiments, products of manufacture as provided herein are synched or integrated with digital communication and computer or mobile device applications. In some embodiments, products of manufacture as provided herein are used in rapid and sensitive assays for detecting and quantifying a chemical, biological, physiological, or pathological analyte, or a single molecule or a single cell.

In alternative embodiments, provided are high throughput, multiplexed systems or devices, or methods, for detecting and/or quantifying a chemical, biological, physiological or pathological analyte, or a single molecule or a single cell, or a chemical

or biochemical reaction or recognition using a floating droplet array (FDA) system integrated with use of a sensing element or reporter or a fluorogenic reaction, comprising:

(a) providing a sensor or sensing reaction that involves a small molecule, peptide, protein, nucleic acid, enzyme, antibody, cell, or chemical agent capable of detecting a target of interest and producing a signal readout;

(b) providing a floating droplet array system, droplet microfluidics system or microdroplet-manipulating device or system;

(d) providing a chemical, biological or an environmental sample containing a target of interest such as a small molecule, metabolite, peptide, protein, nucleic acid, or cell; and

(e) encapsulating the chemical, biological or environmental sample into a plurality of microdroplets, trapping the microdroplets into trapping structures, and processing the microdroplets comprising the encapsulated chemical, biological or environmental sample in the droplet microfluidics system or microdroplet-manipulating device, and detecting the presence of a fluorophore signal, or fluorescence, in each microdroplet (a positive droplet) in the device,

wherein detection of a fluorophore signal or fluorescence in a microdroplet indicates the presence of the target molecule in the microdroplet, and the sample,

and optionally further comprising (f) sorting individually or collectively the positive droplets for downstream analysis including sequencing technologies.

In alternative embodiments, provided are high throughput, multiplexed systems or devices, or methods, for detecting and/or quantifying one or multiple different types of a chemical, a biological, a physiological or a pathological analyte, or one or multiple different types of a single molecule or a single cell, or a chemical or a biochemical reaction, or recognition of a cell or a molecule, using a floating droplet array system in real-time, and optionally one of the signals in the said multiplex assay serves for reference or normalization purposes.

In alternative embodiments, provided are high throughput, multiplexed systems or devices, or methods, for detecting and sorting of single molecules of a chemical, a biological, a physiological or a pathological analytes, or a single molecule or a single cell, using a floating droplet array system.

In alternative embodiments, wherein the cell is a mammalian cell, a circulating tumor cell, a circulating melanoma cell, a B cell, a hybridoma cell, a T cell, a Chimeric Antigen Receptor (CAR) T cell (CAR-T cell), a fungal cell, virus or a bacterial cell, a stem cell, a differentiated cell, an engineered cell; and optionally a heterogeneous cell population can be partitioned and encapsulated into droplets and characterized, manipulated and sorted at a single-cell level; and optionally a droplet can contain one, two, three, four or more of the same type of cell or different types of cells.

In alternative embodiments, the droplet microfluidics system can generate: picoliter droplets or droplets of between about 2 μm to 999 μm in diameter (including any diameter size in between and including these endpoints). In alternative embodiments, between about 100 to 100 billion droplets can be immobilized and analyzed in the droplet array.

In alternative embodiments, droplets are composed of one or many sub-phases as single or multiple emulsions.

In alternative embodiments, a biological sample comprises a blood, serum, saliva, tear, urine, tissue, or CSF sample from an individual, a patient or an animal, as well as non-biological samples including food, water and environmental samples.

In alternative embodiments, the single molecule is a nucleic acid, a nucleic acid point mutation, or a single-nucleotide polymorphism (SNP), ribonucleic acid or a nucleic acid biomarker for, e.g., breast cancer. In alternative embodiments, the single molecule is a protein, a lipid, a carbohydrate, a polysaccharide, a small molecule or a metal. In alternative embodiments, the single cell is a bacteria, a fungi, a virus, a mammalian cell, or a fused cell; and optionally in alternative embodiments, the encapsulated cells can be cultured in droplets without significantly losing their viability from hours to 7 days.

In alternative embodiments, the encapsulated cell(s) or molecule(s) produce a fluorescent signal upon a chemical or biological reaction, and optionally a B cell produces target antibodies or a Chimeric Antigen Receptor (CAR) T cell (CAR-T) kills a cancer cell.

In alternative embodiments, the aptamer is an oligonucleotide, a nucleic acid or a peptide aptamer. In alternative embodiments, the sensor or reaction comprises a DNA strand displacement strategy, a proximity ligation assay, a binding induced DNA assembly assay, a PCR or RT-PCR reaction, an enzyme reaction, a fluorescent dye or

protein, or any fluorogenic reaction. In alternative embodiments, reagents are co-encapsulated with analytes or samples at the beginning or optionally introduced sequentially by, e.g. droplet-droplet fusion.

In alternative embodiments, high throughput, multiplexed systems or devices, or methods, as provided herein further comprise disposable microfluidic “cartridges,” permitting multiplex and rapid detection of multiple types of targets simultaneously, and optionally the high throughput, multiplexed system or device is fully automated, or is fabricated as an all-in-one system or with modular components, or is linked (e.g., by wired or wireless linkage, such as Bluetooth) to an electronic device, e.g., a portable device, e.g., a smart phone and/or a tablet, laptop, for point-of-care applications.

In alternative embodiments, the throughput, multiplexed system is engineered to comprise one or any of: desirable portability (for example, packaged as backpacks), automating fluid handling (e.g., droplet generation and auto sampling), and integrating electronics including a diode laser, LED panel, light source, operating, and/or data analyzing software, display with fluorescence microscopy, embedded APD (avalanche photodiode), photomultiplier tube (PMT), digital camera, charge-coupled device (CCD), complementary metal-oxide semiconductor (CMOS) sensor.

In alternative embodiments, high throughput, multiplexed systems or devices, or methods, as provided herein further comprise disposable microfluidic “cartridges,” permitting multiplex and rapid detection of multiple types of targets simultaneously, and optionally the high throughput, multiplexed system or device is fully automated, or is fabricated as an all-in-one system or with modular components, or is linked to an electronic device, e.g., a portable device, e.g., a smart phone and/or a Bluetooth, or is integrated with a portable temperature controller (e.g. Peltier-based thermocycler), for point-of-care applications, for point-of-care applications.

In alternative embodiments, high throughput, multiplexed systems or devices, or methods, as provided herein further comprise, or comprise, trapping structures of various sizes/shapes for immobilizing droplets of various compositions in a spatially controlled, defined, and parallel format. In alternative embodiments, the high-throughput system traps droplets into trapping structures, whereby droplets float or sink into trapping structures due to density differences between the dispersed and continuous phases. In alternative embodiments, the droplets may be recovered by reorienting the device. In

alternative embodiments, the droplets may be fused or merged or split in step-wise processes to accommodate chemistries or stimulations or media change in sequential steps.

In alternative embodiments, a high-throughput system as provided herein
5 comprises a multilayer microfluidic device whereby droplets are trapped in a region above or below the main flow stream. In alternative embodiments, the high-throughput system can comprise guiding structures such as tracks, pillars, or narrow channels which guide droplets to the trapping structures and ensure complete and efficient coverage of the trapping structures. In alternative embodiments, the high-throughput system comprises
10 inlets or outlets to divert droplets away or to the droplet trapping structures and/or other channels or chambers.

In alternative embodiments, a high-throughput system as provided herein is integrated with sorting elements for retrieving many or individual droplets, whereby the sorting elements may be electrode, pneumatic valve, laser, microneedle, or acoustic-based
15 droplet retrieval systems. In alternative embodiments, a high-throughput system as provided herein can index droplets based on one or many spatial or temporal variables. In alternative embodiments, droplets are indexed based on uniquely barcoded beads, a nucleic acid barcode, a fluorophore, an organic or an inorganic dye barcode, or a colorimetric barcode.

20 In alternative embodiments, a high-throughput system as provided herein is integrated with data acquisition hardware or software, data analysis software, a user interface, or computer or mobile device applications.

In alternative embodiments, a high-throughput system as provided herein is integrated with a sorting element or elements for retrieving a plurality of or individual
25 droplets, whereby optionally the sorting element comprises an electrode, a pneumatic valve, a microfluidic controlled valve, a laser, a microneedle, a magnetic field or an acoustic-based droplet retrieval systems.

In alternative embodiments, a high-throughput system as provided herein is sorted or retrieved droplets are analyzed using downstream methods for their contents, whereby
30 optionally the downstream methods comprise sequencing, next-generation sequencing (NGS) (optionally pyrosequencing or massively parallel signature sequencing) and single-cell sequencing techniques.

In alternative embodiments, a high-throughput system as provided herein serves as a research or discovery tool to characterize, manipulate, screen, and/or sort immunological agents including B cells, plasma cells, hybridomas, antibodies, monoclonal antibodies, nanobodies, antibody-drug conjugates, T cells, a Chimeric Antigen Receptor (CAR) T cell (CAR-T), native or engineered cells; optionally the presence or production of the said immunological agent(s) or when the said immunological agent(s) activate, inhibit or modulate a biological molecule, signal or event in the droplets produce a detectable signal readout; optionally the said signal readout can indicate or quantify the presence, or binding or biological functions of the said immunological agent(s).

In alternative embodiments, provided are high-throughput droplet generation modules, whereby droplets are formed at high throughput using droplet-generating junctions comprising:

- (a) a dispersed phase which flows through a plurality of channels in a radial direction prior to dispersion;
- (b) a carrier phase that flows through one or many channels in a direction perpendicular to the radially flowing phase that is to be dispersed; and
- (c) droplets generated as the carrier phase comes into contact with the immiscible dispersed phase in the arrangement described in (a) and (b).

In alternative embodiments, a high-throughput system as provided herein comprises a stacked, 3D arrangement of droplet-generating junctions such that droplet generation can occur at many junctions simultaneously.

In alternative embodiments, a high-throughput system as provided herein comprises droplet-generating module integrated within a portable handheld fluidic device, such as a syringe.

In alternative embodiments, a high-throughput system as provided herein comprises a driving pressure for fluid flow, which can be generated by hand using a force-transferring device such as a plunger.

The details of one or more embodiments as set forth in the accompanying drawings and the description below. Other features, objects, and advantages of alternative embodiments will be apparent from the description and drawings, and from the claims.

All publications, patents, patent applications cited herein are hereby expressly incorporated by reference for all purposes.

DESCRIPTION OF DRAWINGS

5 Figures are described in detail, below. Like reference symbols in the various drawings indicate like elements, unless otherwise stated.

FIG. 1A-B are schematic illustrations for the workflow of the Floating Droplet Array; FIG. 1A illustrates a general workflow involving droplet generation, trapping for analysis, and subsequent droplet recovery; FIG. 1B illustrates: an exemplary step-by-step
10 operation: (i) generation of droplets, which flow into the trapping chamber and float into the wells; (ii) after all the wells have been filled, (iii) the remaining droplets are purged; and (iv) the trapped droplets are then analyzed (v) droplets are recovered by flipping the device so that droplets float out of the wells -and (vi) droplets are sent for downstream handling on- or off-chip.

15 FIG. 2A-B are schematic illustrations depicting the workflow for the Floating Droplet Array: FIG. 2A illustrates photographic image of entire microfluidic device; the device was filled with green dye for visualization, scale bar = 1 cm; FIG. 2B illustrates a schematic representation of an exemplary workflow including (i) droplet generation, (ii) droplet loading into the chamber, (iii) droplet trapping, (iv) filling the chamber, (v)
20 purging extraneous droplets, and (vi) droplet recovery by flipping; arrows in (ii)-(vi) represent flow direction; all scale bars for FIG. 2B = 200 μm .

FIG. 3A-C illustrate a computer aided design (CAD) rendering of the Floating Droplet Array; where the top layer of the floating droplet array (FDA) device contains the droplet-trapping microwells while the bottom layer contains the droplet generation and
25 chamber modules (FIG. 3B, middle panel); droplets are generated using a flow-focusing structure (FIG. 3A, Left panel) and trapped into circular microwells (FIG. 3C, Right panel); device geometries were exaggerated in the rendering for visualization purposes.

FIG. 4 illustrates device design parameters for an exemplary device as provided herein for efficient droplet trapping and recovery.

30 FIG. 5A-C schematically illustrate microscopic images of an exemplary ultrahigh-throughput floating droplet array (FDA) using 50 μm wells: FIG. 5A illustrates large-

scale scan of 36 images containing more than 14,000 wells; and FIG. 5B and FIG. 5C illustrate zoomed-in images of trapped droplets; Scale bar = 75 μm .

FIG. 6A-D schematically illustrate controlling the number of droplets per well: FIG. 6A illustrates one droplet per well; FIG. 6B illustrates two droplets per well; FIG. 6C illustrates three droplets per well; and, FIG. 6D illustrates four droplets per well; scale bars (a-b) = 120 μm . FIG. 6E graphically illustrates data showing the controlling of droplet size by manipulating the flow rate ratio (water/oil) for 30 $\times$ 50 μm channel (circles) and 15 $\times$ 50 μm channel (squares).

FIG. 7A-D illustrate droplet crosstalk studies of the diffusion of fluorescein between clustered droplets: FIG. 7A illustrates hydrolysis of FDG by β -gal; FIG. 7B illustrates microscopic image showing overlay of bright-field and FITC channels for FDG droplets with and without a β -gal bead after a 4 hour incubation, scale bar = 50 μm ; FIG. 7C illustrates fluorescent microscopic images showing time course of reaction over a 4 hour incubation to monitor droplet crosstalk, scale bar = 50 μm ; FIG. 7D graphically illustrates data showing the fluorescent intensity of trapped droplets with bead (back row), without bead (middle row), and the oil phase (front row).

FIG. 8A-B illustrate digital quantification of the number of droplets containing a β -gal bead: FIG. 8A illustrates a microscopic image of trapped droplets in a device containing 109,569 microwells of 30 μm size; droplets are generated with 250 μM FDG and a low concentration of β -gal beads so that most droplets do not contain any beads; Insert depicts a zoomed bright field microscopic image of a bead-containing droplet; White circle highlights a β -gal bead (7.8 μm) within a droplet; FIG. 8B illustrates fluorescence microscopic images of droplets within 30 μm microwells; 0 = without target bead (dark droplet without β -gal bead), 1 = with bead (fluorescent droplet with β -gal bead), upper panel: overlay of fluorescent image and brightfield image, middle panel: brightfield image, and lower panel: fluorescent image; all scale bars = 200 μm .

FIG. 9A-C illustrate an exemplary process of encapsulation and single bacteria detection using an exemplary floating droplet array device: FIG. 9A-C illustrate single β -lactamase-producing bacterial cells encapsulated within picoliter-sized droplets (55 μm) along with a fluorogenic substrate for beta-lactamase; FIG. 9A illustrates an overlay of fluorescent image and brightfield image; FIG. 9B illustrates a brightfield image; FIG. 9C

illustrates a fluorescent image; the fluorescent intensity was monitored after a 4 hour incubation; Scale bar = 120 μm .

FIG. 10 illustrates the quantification of fluorescent droplets: the digital single lens reflex (dSLR) camera can be used to quantify fluorescence droplet; LED or LCD panels can be used as an excitation light source.

FIG. 11 illustrates digital quantification of fluorescent droplets from an exemplary floating droplet array device using a CMOS sensor; CMOS sensor can be used to monitor emitted light to analyze droplets for digital quantification; LED or LCD panels can be used as an excitation light source.

FIG. 12 illustrates an exemplary portable floating droplet array system.

FIG. 13 illustrates various exemplary shapes for droplet trapping structures in an exemplary floating droplet array (side view); Example shapes include rectangles, semicircles, triangles, or trapezoids.

FIG. 14 illustrates various exemplary shapes for exemplary droplet trapping structures (top view); example shapes include rectangles, circles, pentagons, stars, triangles, or cross shapes.

FIG. 15 illustrates spatial patterning of exemplary trapping structures; the size of and distance between microwells can be varied; for example, parameters X, Y, and Z can be varied.

FIG. 16 illustrates exemplary parameters for droplet trapping structures and chamber; the size (depth and width) of the microwells and height of chamber can be varied. For example, X, Y, and Z can be varied.

FIG. 17A-D illustrate sized-based clustering of droplets (workflow); different sizes and shapes of microwells can be fabricated to cluster multiple droplets according to their size; large droplets can be generated first and trapped in respective large trapping structures; smaller droplets can then be generated and trapped into respective smaller trapping structures.

FIG. 18A-B illustrate sized-based clustering of droplets (FIG. 18A, side; and, FIG. 18B, top views); different sizes and shapes of microwells can be fabricated to cluster multiple droplets according to their size; large droplets can be generated first and trapped in respective large trapping structures; smaller droplets can then be generated and trapped into respective smaller trapping structures.

FIG. 19A-B illustrate manipulation of mass diffusion between droplets and droplet fusion using chemical means; clustered droplets can be induced to fuse or increase the diffusion of molecules between droplets using chemical reagents such as an alcohol solution (e.g., 2,2,3,3,4,4,4-Heptafluoro-1-butanol); FIG. 19A illustrates clustered
5 droplets before chemical injection, and FIG. 19B illustrates clustered droplets after chemical injection.

FIG. 20A-C illustrate manipulation of mass diffusion between droplets and droplet fusion using physical means; clustered droplets can be induced to fuse or increase the diffusion of molecules between droplets using integrated metal or solution-based
10 electrodes.

FIG. 21A-C illustrate: an exemplary floating droplet array (FIG. 21A); laser-based droplet manipulation (FIG. 21B), and selective droplet recovery (FIG. 21C) using optics; trapped droplets can be precisely manipulated using lasers to release selected droplet from trapping structures; exemplary laser-based manipulation include optical tweezers or
15 microtsunami (laser-based microcavitation bubbles).

FIG. 21D-E illustrate a demonstrated retrieval of a positive droplet containing bacteria expressing beta-lactamase, which reacts with fluorocillin to produce a fluorescent signal and is sorted out by laser manipulation: FIG. 21D and FIG. 21E are illustrations of images recorded before and after laser-mediated release of a positive fluorescent droplet,
20 respectively.

FIG. 22A-C illustrate selective droplet recovery using pneumatic valves, where trapped droplets can be precisely manipulated using pneumatic valves to release droplets from trapping structures: FIG. 22A illustrates an exemplary floating droplet array; FIG. 22B illustrates an exemplary controlled valve; FIG. 22C illustrates an exemplary selective
25 droplet recovery.

FIG. 23 illustrates an exemplary high-throughput droplet generation module showing sample input (upper panel); and a top view and a side view of the high-throughput droplet generation module.

FIG. 24 illustrates an exemplary high-throughput droplet generation module using
30 3D structured droplet generators.

FIG. 25 illustrates an exemplary syringe-based, high-throughput droplet generator.

DETAILED DESCRIPTION

Provided are high-throughput platforms to manipulate, analyze, and screen microfluidic droplets in a parallel format. Droplet trapping is achieved at high efficiency and throughput by trapping droplets in a secondary layer away from the main flow stream. This format allows for the trapping of up to millions or billions of droplets in areas ranging from 1 mm² to 1 m² (or any area between, and including, these endpoints). Droplets trapped in a spatially-defined array facilitates droplet indexing since the array element locations provide a natural positioning system. This is particularly useful for monitoring a process over time or synchronizing reactions from a plurality of droplets to initiate simultaneously. In some embodiments, droplets are passively trapped into the trapping structures by buoyancy forces due to differences in densities between the discrete and carrier phases. In some embodiments, the trapped droplets can be recovered by reorienting the device and sent downstream for further processing on-or off-chip.

Provided are compositions and methods for trapping and analyzing droplets containing a sample at high-throughput and in a parallel format. This may be used in chemical and biological assays for the detection of metabolites, small molecules, proteins, lipids, nucleic acids, viruses and cells. In some embodiments, detection of analytes can be achieved by using sensor elements. In some embodiments the sensor elements are comprised of oligonucleotides, peptides, proteins, aptamers, antibodies, and cells. In some embodiments, signal amplification reactions such as polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), loop-mediated amplification reaction (LAMP), exponential amplification reaction (EXPAR), rolling circle amplification (RCA), strand displacement amplification (SDA), hybridization chain reaction (HCR), nucleic acid sequence based amplification (NASBA), helicase dependent amplification (HDA), nicking enzyme amplification reaction (NEAR), recombinase polymerase amplification (RPA), and enzymatic reaction may be used. In alternative embodiments, the devices are integrated with temperature-controlling systems (from 4°C to 95 °C) and with heating and cooling functions so reactions in droplets can be controlled at desirable temperatures.

In alternative embodiments, exemplary platforms or systems as provided herein enable rapid and simple droplet manipulation using a floating droplet array system (e.g., as schematically illustrated in Figure 1, a schematic illustration for the workflow of the Floating Droplet Array. (a) General workflow involves droplet generation, trapping for

analysis, and subsequent droplet recovery (b) Step-by-step operation: (i) generated droplets flow into the trapping chamber and float into the wells; (ii) after all the wells have been filled, (iii) the remaining droplets are purged and (iv) the trapped droplets are then analyzed (v) droplets are recovered by flipping the device so that droplets float out of the wells and (vi) droplets are sent for downstream handling on- or off-chip.

We demonstrated the effectiveness of an exemplary system as provided herein by generating, trapping, and recovering droplets. Figure 2 shows the workflow for the Floating Droplet Array. (a) Photographic image of the entire microfluidic device. The device was filled with green dye for visualization, scale bar = 1 cm; (b) Schematic representation of the workflow including (i) droplet generation, (ii) droplet loading into the chamber, (iii) droplet trapping, (iv) filling the chamber, (v) purging extraneous droplets, and (vi) droplet recovery by flipping. Blue arrows in (ii)-(vi) represent flow direction. All scale bars for (b) = 200 μm

A computer aided design (CAD) rendering of the Floating Droplet Array (FDA) is shown in Figure 3. The top layer of the FDA device contains the droplet-trapping microwells while the bottom layer contains the droplet generation and chamber modules (middle panel). Droplets are generated using a flow-focusing structure (Left panel) and trapped into circular microwells (Right panel). Device geometries were exaggerated in the rendering for visualization purposes.

In alternative embodiments, geometric parameters as provided herein, such as the diameter of the well, d_{well} , depth of the well, h_{well} , height of the chamber, h_{chamber} , and inter-well spacing, x , can be chosen accordingly to efficiently trap, manipulate, analyze, and release a droplet in a well (Figure 4). All the parameters can be varied according to the application.

Figure 5 shows an ultrahigh-throughput floating droplet array. (a) Large-scale scan of 36 images containing more than 14,000 wells and (b, c) zoomed-in images of trapped droplets (scale bar in (c) is 75 μm). Moreover, the device is highly efficient in trapping droplets as can be seen in Figure 5 with 100% of >14,000 wells analyzed containing a single droplet. We found that with the device dimensions used in this study (which are merely a representative example and non-limiting, as other dimensions can readily be used), we can consistently fill 100% of the microwells with single droplets when they are generated to be 10-20 % smaller in diameter compared to the microwells.

In alternative embodiments, exemplary platforms or systems as provided herein can be used for multiple droplet clustering into a single trapping structure in a simple, robust, and well-controlled manner. This can be achieved by varying the size of the droplets so that more than one droplet could fit into each well. As seen in Figure 6, we demonstrated to precisely manipulate one, two, three, and four droplets per well by controlling the droplet size. This ability of an exemplary Floating Droplet Array (FDA) device can be used for clustering multiple droplets that contain different samples or reagents within the same microwell for various complex biological studies such as enzymatic assays, drug screening, and cell-cell communication. This can be achieved by controlling diffusion (crosstalk) between droplets or merging droplets within the same microwell, in a highly parallel manner. Figure 6 shows the manipulation of the number of droplets per well by varying droplet size. (a) one droplet per well, (b) two droplets per well, (c) three droplets per well and (d) four droplets per well. Scale bars (a-b) = 120 μm . (e) Controlling droplet size by manipulating the flow rate ratio (water/oil) for $30 \times 50 \mu\text{m}$ channel (circles) and $15 \times 50 \mu\text{m}$ channel (squares).

In alternative embodiments, systems as provided herein can be used to monitor diffusion of agents between droplets within the same microwell as shown in Figure 7. To study this phenomenon using an exemplary Floating Droplet Array (FDA) device, we chose β -galactosidase (β -gal) and its fluorogenic substrate (FDG) as a model system (Figure 7a). We encapsulated 250 μM FDG and a low concentration of β -gal-conjugated microbeads, which resulted in only a few droplets containing a β -gal-conjugated bead and most droplets containing FDG without any β -gal beads.

In alternative embodiments, systems as provided herein can be used to cluster multiple droplets of differing contents (i.e. cells, reagents, or samples) and merging or splitting them using a chemical reagent or externally applied electric field.

In alternative embodiments, exemplary platforms or systems as provided herein can be used for digital quantification of single molecules. We demonstrated digital quantification of analytes with spatially indexed droplets. This was achieved by encapsulating FDG along with a very low concentration of β -gal beads (10 beads/ μl) so that the majority of droplets contain no β -gal bead and only a few droplets contain only one bead. Streptavidin-conjugated beads (7.8 μm) were used since they can be easily visualized and can also immobilize a large number of β -gal molecules, to yield strong

enzymatic activity. As can be seen in Figure 8a, there is only one fluorescent droplet, and it is the only one that contains a β -gal bead, among 1008 droplets in the image.

In alternative embodiments, products of manufacture as provided herein are used for monitoring single cells. In alternative embodiments, products of manufacture as provided herein are used for monitoring antimicrobial-resistant bacteria. We demonstrated encapsulation and detection of single bacteria using the floating droplet array device in Figure 9. β -lactamase producing bacterial cells were encapsulated within picoliter-sized droplets (55 μ m) at the single-cell level along with fluorogenic substrate for β -lactamase. The fluorescent intensity was monitored after 4 hour incubation (Figure 9). Scale bar = 120 μ m.

In alternative embodiments, products of manufacture as provided herein can immobilizing droplets in a manner that yields facile indexing of droplets that is needed for real-time monitoring over an extended period of time. For example, it can be used for many applications such as single-cell or molecule analysis, genetic sequencing, biochemical profiling, cell culture, pathogen detection, and drug discovery.

In alternative embodiments, a floating droplet array system as provided herein can be integrated with various functions for further manipulating droplets such as droplet splitting and fusing in parallel or sequential formats.

In alternative embodiments, a floating droplet array system as provided herein can be used for portable, point-of-care technologies when combined with CMOS, CCD, or cell phone-based imaging systems. Figure 10 and Figure 11 shows a schematic illustration of digital quantification of fluorescent droplets from the floating droplet array device. A digital single lens reflex (dSLR) camera (Figure 10) or CMOS sensor can be used to quantify fluorescence droplets. LED or LCD panels can be used as an excitation light source. Figure 12 is a non-limiting illustration of one embodiment of a portable floating droplet array system that can be used for point-of-care or portable diagnostics and integrated with a smartphone or tablet PC.

In alternative embodiments of a floating droplet array system as provided herein, the shape of the droplet trapping structures can be varied to form any shape such as a rectangle, semicircle, triangle, or trapezoid (Figure 13 and Figure 14).

In alternative embodiments, of a floating droplet array system as provided herein, the size and spacing of the droplet trapping structure can be varied as can be seen in Figure 15 and 16. Example parameters of X, Y and Z can be varied.

In alternative embodiments, of a floating droplet array system as provided herein
5 can be used for sized-based clustering of multiple floating droplets in an array format, see, e.g., Figure 17. Different-sized microwells can be fabricated to cluster droplets in a well-controlled, parallel manner according to their size. Bigger droplets are generated first and can be trapped in their respective, relatively large microwells. Smaller droplets are then generated and are immobilized into their respective microwells. This process can be
10 continued in this manner to precisely control the arrangement and content of clustered droplets.

For example, sample A can be encapsulated into bigger droplets, sample B can be encapsulated in middle-sized droplets, and sample C can be encapsulated into small droplets as in Figure 17 or Figure 18.

15 In alternative embodiments, droplet diffusion and droplet fusion as provided herein can be manipulated through physical (e.g., an applied electric field) and/or chemical (e.g., reagents such as an alcohol solution; e.g., 2,2,3,3,4,4,4-Heptafluoro-1-butanol)) means. Figure 19 shows manipulation of droplet diffusion and droplet fusion. Clustered droplets can be induced to fuse or increase mass diffusion between neighboring
20 droplets using chemical reagents.

In alternative embodiments, a clustered floating droplet array as provided herein can be manipulated by an externally applied electric field. Figure 20 shows manipulation of droplet diffusion and droplet fusion by externally applied electric fields. Clustered droplets can be induced to fuse or increase mass diffusion between neighboring droplets
25 using an electric field applied via metal or solution-based electrodes.

In alternative embodiments, relatively large droplets are encapsulated with one or more cells and trapped into respective large microwells. Smaller droplets containing cell nutrient media, chemical reagents, biomolecules, beads, or cells can then be generated and clustered with the large droplets by trapping into respective microwells. Diffusion or
30 fusion between droplets may or may not be manipulated using a chemical reagent, electric or magnetic field, or thermal or optical radiation.

In alternative embodiments, a product of manufacture as provided herein can be used for fabrication of complex heterogeneous composite materials. For example, monomer A can be encapsulated into bigger droplets, monomer B can be encapsulated in middle-sized droplets, and monomer C can be encapsulated into small droplets. The droplets can then be precisely assembled through size-based clustering. Subsequently, the droplets can be polymerized by the addition of a chemical reagent, light or thermal radiation to yield a composite material with isotropic or anisotropic properties.

In alternative embodiments, a clustered floating droplet array as provided herein can be used to selectively sort/isolate and correspondingly recover droplets. Figure 21 shows manipulation of selective droplet recovery. Trapped droplets can be precisely manipulated using optics to release selected droplet from trapping structures. Example laser-based manipulation include optical tweezers or microtsunami (laser-based microcavitation bubbles). Figure 22 illustrates valve-based recovery of droplets. The droplets can also be barcoded by, for example, using a co-encapsulated bead to facilitate sorting and recovery of the corresponding droplets.

In alternative embodiments, microencapsulated emulsions or droplets can be made using a 2D (Figure 23) or 3D (Figure 24)-based high-throughput droplet generation system. For portable systems, microencapsulated emulsions or droplets can be made using a syringe-based high-throughput droplet generator (Figure 25).

In alternative embodiments, the droplets are formed from a discrete phase with a density greater than the carrier phase and thus droplets are trapped by sinking into the wells.

Alternative exemplary embodiments will be further described with reference to the following examples; however, it is to be understood that these exemplary embodiments are not limited to such examples.

EXAMPLES

Example 1: Droplet microfluidics fabrication and setup: Device fabrication

The microfluidic device was designed using AutoCAD™ (Autodesk, San Rafael, CA, USA) and printed to high-resolution transparency photomasks (CAD/Art Services, Bandon, OR, USA). The devices were fabricated from poly-di-methyl-siloxane (PDMS) using standard soft lithography techniques [36]. Four inch silicon wafers were briefly rinsed with 5% hydrofluoric acid (Sigma-Aldrich, St. Louis, MO, USA) and

deionized (DI) water. Prior to spin coating (6NPP-LITE™, Laurell Technologies Corporation, USA), wafers were dehydrated in an oven at 95°C for 10 minutes. Negative photoresist (approximately 3 g, SU-8 50™, MicroChem, Chestech, UK) was then spin-coated (500 rpm for 10 seconds then 3000 rpm for 30 s) onto the wafer. The SU-8™ layer
 5 was then cured on a hotplate at 65 °C for 5 minutes and at 95 °C for 30 minutes. The cured SU-8™ layer was then exposed to UV radiation (14 seconds (s), 20 mW/cm², AB&M INC UV Flood Exposure System) through the photomask and the wafer was subsequently post-baked at 65°C for 1 minute and 95°C for 5 minutes. Unexposed SU-8™ was removed by soaking in SU-8™ developer for 5 minutes. The wafer was then
 10 cleaned using isopropyl alcohol, blow dried with filtered nitrogen gas and silanized with perfluorooctyl-trichlorosilane (Sigma-Aldrich, St. Louis, MO, USA) under vacuum for 3 hours. For fabrication of the devices, PDMS base and curing agent were mixed in a ratio of 10:1 w/w, degassed, poured onto SU8™-on-Si wafer masters and fully cured overnight in an oven at 65°C. After thermal curing, the PDMS layer was peeled off the master. Inlet
 15 and outlet holes were made with a 1 mm-sized biopsy punch (Kay Industries Co. Tokyo, Japan). PDMS layers were bonded immediately following oxygen plasma treatment and stored overnight before use.

Example 2: Design of the Floating Droplet Array (FDA) device for ultrahigh-throughput
 20 droplet trapping

An example schematic rendering of an exemplary Floating Droplet Array (FDA) device design is shown in Figure 3. The FDA device consists of two layers of PDMS, one for droplet generation and assembly and the other for droplet trapping. The top layer is designed with a microwell array whose well dimensions can be varied according to the
 25 desired droplet size to be trapped. In this work, we used the dimensions (well width × depth) of 30 × 40, 50 × 50, 100 × 50, and 120 × 50 μm, though other dimensions are also readily used according to the embodiments disclosed herein. Fabricated microwells in the top PDMS layer were characterized by scanning electron microscopy (SEM) as shown in Figure 3. The diameter of microwells were determined to be 122.5 ± 6.1, 96.7 ± 4.7, 48.6
 30 ± 2.3, and 27.8 ± 1.4 μm, which correspond to a total well number of 9496, 13320, 34560 and 109569, respectively. The bottom PDMS layer was fabricated with a height of 50 μm and contains two aqueous inlets and a single oil inlet whereby the respective fluids are

directed to a flow-focusing structure for droplet generation (Figure 2b, i). The channel width at the flow-focusing structure is 15 μm when the 30 or 50 μm diameter wells were used and 30 μm when the 100 or 120 μm diameter wells were used. After the flow-focusing structure, we included a widened winding channel, which reduces the velocity of the droplets and aides in droplet visualization. The bottom layer also contains a large chamber (18.5 mm wide $\times$ 37 mm long) which is oriented below the well array. We placed nine large rectangular-shaped resistor structures with long and narrow channels (3 mm long, 200 or 300 μm wide) between them immediately after the entrance of the chamber (Figure 2a). This provides resistance to flow down the length of the chamber and ensures that droplets spread out across the whole width of the chamber before passing through the narrow channels to the well array (Figure 2a). We found this helps to ensure compete coverage of the wells. The chamber also contains four pillar structures (1 mm diameter) placed in the central region of the chamber to prevent undesirable bonding of the well array with the bottom of the chamber due to bowing of the PDMS (Figure 2a). The outlet channels (550 μm wide) are designed at the end of the chamber for collecting excess oil and also to recover the trapped droplets from the FDA device. We also included a waste outlet before the entrance to the chamber to divert undesired droplets such as air, polydisperse, or improperly-sized droplets which often occur at the beginning of device operation from the microwell array. Once generation of the desired droplet size was stable, this waste channel was sealed with a stopper and the droplets were diverted into the chamber for trapping.

Example 3: Droplet generation and manipulation

Figure 2 shows a step-by-step workflow for an exemplary Floating Droplet Array (FDA) device using dye-containing droplets trapped and released in 120 μm microwells. To operate the device, we initially purged the chamber of air by flowing oil (HFE 7500TM without surfactant) through the oil inlet at a flow rate of 10 $\mu\text{l}/\text{min}$ for 5 min. Any residual air trapped in the wells was removed by tilting the device at 45° and gently tapping the device with forceps. Aqueous samples were then introduced for droplet generation with the device oriented so that the wells were above the chamber. We generated droplets using HFE 7500TM + 1.8% PFPE-PEG-PFPE surfactant as the oil phase and 10% food coloring dye as the aqueous phase for generating droplet sizes ranging from 20 to 120 μm

in diameter by varying the oil and aqueous flow rates (i in Figure 2b). Initial droplets were diverted into the intermediate waste outlet until the desired droplet size was stably formed. The waste outlet was then sealed with a stopper and the droplets were consequently guided into the chamber, where they spread across the width of the chamber 5 before passing through the narrow channels between the resistor structures (ii in Figure 2b). The droplets then sequentially filled the wells by floatation due to the density difference between the fluorinated oil and aqueous phase (iii in Figure 2b). Once the array was completely filled (iv in Figure 2b), the aqueous inlets were sealed and oil was introduced at a high flow rate (20-30 $\mu\text{L}/\text{min}$) for 10 min to purge the chamber of any 10 extraneous droplets. The trapped droplets were then incubated and analyzed over time (v in Figure 2b). Subsequently, the droplets were recovered by flipping the device over so that they float out of the wells (vi in Figure 2b). This simple technique is robust and can be applied to a wide range of droplet sizes. Moreover, it is highly efficient in trapping droplets as can be seen in Figure 5 with 100% of greater than ($>$) 14,000 wells analyzed 15 containing a single droplet. We found that with the device dimensions used in this study, we can consistently fill 100% of the microwells with single droplets when they are generated to be 10% to 20 % smaller in diameter compared to the microwells.

Example 4: Fluorophore diffusion between droplets

20 For fluorophore diffusion studies, β -gal beads and 500 μM FDG in PBS were introduced into the microfluidic device via respective inlets at a flow rate of 0.5 $\mu\text{L}/\text{min}$, while the oil phase was injected at a flow rate of 15 $\mu\text{L}/\text{min}$. A 2-mm magnetic stir bar was placed inside a 3 mL syringe and was gently mixed by a portable magnetic stirrer (Utah Biodiesel Supply) to prevent settling of the beads. Uniform 55 μm diameter 25 droplets were generated, such that three droplets could fit within 120 μm diameter microwells. Fluorescence intensity of droplets and surrounding oil phase was analyzed under a fluorescence microscope at various time points to monitor the fluorophore-leaking effect between droplets.

30 Example 5: Digital quantification of β -gal beads

For the digital quantification of β -gal beads using an exemplary Floating Droplet Array (FDA) device, 25 μm sized-droplets, containing 250 μM FDG with or without a

single β -gal bead were trapped within the microfluidic device consisting of 109,569 microwells (30 μ m in diameter). After a 10 minute incubation, microscopic images were taken using a 4 $\times$ objective lens. The experiments were performed in triplicate and the resulting images were analyzed using ImageJ™ software (ver. 1.48) for quantification of
5 fluorescent droplets.

Example 6: Real time monitoring of droplet array.

Fluorescent droplets can be monitored in real-time over the cycling using CMOS sensor or full-frame digital camera.

10

Example 7: On-chip digital PCR and RT-PCR

An exemplary Floating Droplet Array (FDA) device can be used for on-chip and real-time digital PCR (or RT-PCR), that can precisely detect (or quantify) target DNA or RNA sequences, gene mutations and epigenetic modifications, and single-nucleotide
15 polymorphisms (SNP). Droplet-based on-chip and real-time digital PCR can be accomplished using the FDA device by trapping droplets encapsulated with the sample of interest, PCR mixture, and DNA-binding dye or nucleic acid probe (e.g. TaqMan™ probe). The PCR reaction can be conducted using on-chip thermo cycling.

20 Example 8: On-chip, digital isothermal reaction

An exemplary Floating Droplet Array (FDA) device also can be used with nucleic acid isothermal amplification reactions to detect target DNA or RNA sequences, mutant DNA or RNA, and SNP. This can be used for biological analysis and diagnostics. Some examples of isothermal amplification reactions that may be used include loop-mediated
25 amplification reaction (LAMP), exponential amplification reaction (EXPAR), rolling circle amplification (RCA), strand displacement amplification (SDA), hybridization chain reaction (HCR), nucleic acid sequence based amplification (NASBA), helicase dependent amplification (HDA), nicking enzyme amplification reaction (NEAR), and recombinase polymerase amplification (RPA).

30

Example 9: On-chip, digital enzymatic assay

An exemplary Floating Droplet Array (FDA) device can be used for digital quantification assays in real-time. For this purpose, single enzyme molecule can be encapsulated within droplets with fluorogenic or colorimetric substrates. Fluorescence intensity and number of fluorescent droplets can be monitored in real-time using an on-chip detection system.

Example 10: HIV reservoir detection

An exemplary Floating Droplet Array (FDA) device can be used for quantifying HIV reservoirs *in vitro* by quantifying a) the total content of cell-associated viral mRNA markers obtained from mononuclear cells, and b) number of cells composing the reservoir at the single-cell level. Cell-associated (CA) HIV-1 mRNA (specifically multiply spliced (ms) *tat/rev*) can be used here as an indicator of residual viral replication and the size of HIV reservoir because they directly correlate with the reactivation of latent reservoir in vivo. Isolated peripheral blood mononuclear cells (PBMCs) can be encapsulated in droplets at the single-cell level after stimulation with an agent such as phorbol 12-myristate 13-acetate plus ionomycin (PMA/I) to induce viral mRNA expression. Levels of HIV *rev/tat* expression per cell and absolute number of HIV reservoir cells can be determined using the FDA-based digital RT-PCR. In an alternative embodiment, exemplary devices can serve as a HIV outgrowth assay.

Example 11: Circulating tumor cells and tumor free DNA/RNA detection

An exemplary Floating Droplet Array (FDA) device can be used to detect circulating tumor cells (CTCs) and tumor cell-free DNA (or RNA) in the blood. For monitoring CTCs in the blood, red blood cells will be lysed and PBMCs will be encapsulated at the single-cell level per droplet. Single-cell PCR, single-cell RT-PCR, single-cell isothermal DNA (or RNA) amplification (mentioned in example 8), and proximity ligation for isothermal amplification (or DNA strand displacement) can be used to generate a fluorescent signal in droplets that contain single-CTC. For the circulating tumor cell-free DNA (or RNA), plasma sample or isolated DNA (or RNA) can also be analyzed in the FDA device in a similar manner.

Example 12: On-chip, cell culture and detection

Cells can be encapsulated at the single-cell level per droplets and can be grown within droplets to increase the population for:

5 a) On-chip colony forming unit (CFU) assay to quantify the number of cells. The number of droplets that contain a bacteria colony, can be visualized by staining cells via colorimetric or fluorescence dye; or

b) Identification (or profiling) of the cells by monitoring protein secretion. Secreted proteins can be monitored using enzymatic activity assays with fluorogenic/colorimetric substrates or antibody-based proximity ligation to induce isothermal amplification within droplets. The population of cells, secreting a protein of interest, can be quantified in real-time using the FDA device.

Example 13: On-chip, cell-cell interaction and cell-fusion

Using an exemplary Floating Droplet Array (FDA) device, single cells can be encapsulated into droplets and trapped into trapping structures such as microwells. Other types of single cell can be encapsulated into droplets and arranged neighboring previously trapped droplets. By using chemical reagents (such as alcohol solution) or electric fields, droplet fusion or diffusion of molecules between droplets can be controlled for various purposes as described below:

20 a) To monitor cell-cell communication at the single-cell level. Two different types of cells (e.g. a colon cancer cell and mesenchyme stem cell) can be encapsulated in separate droplets and then droplets will be trapped in neighboring trapping structures. Chemical reagents or an electric field can be used to induce permeabilization of molecules between droplets.

25 b) Cell-cell fusion for antibody and hybridoma screening. Two different droplets can be generated, one containing an activated B cell and the other containing target and detecting reagents. B cells can be activated either in vivo (by immunizing and boosting mice with antigen using established adjuvants and administration routes) or in vitro (e.g. using BCR-crosslinking reagents, TLR ligands, CD40L/mAb, cytokines). The other reagent droplet can contain antigen immobilized on beads (e.g. biotinylated antigen immobilized on streptavidin-conjugated beads) as well as a fluorescent secondary antibody (e.g. FITC-anti-mouse IgG). Then, the two different droplets can be trapped in

neighboring trapping structures such that the droplets are in contact. The two droplets can be merged (fused) and cell-fusion can be controlled by osmotic pressure or electric field. The B cells that secrete antibodies specific for antigen will be revealed in droplets that contain positive signal (e.g., a FITC signal in antigen-coated beads).

5 c) In other embodiments, hybridoma cells (generated in previous steps) can be screened using the approach for primary B cells as in b) above.

d) In other embodiments, a screen for antibodies that exert specified functions (e.g., activate, differentiate or kill target cells) can be performed. For example, to detect cells killed by primary B cells, primary CD8⁺ cytotoxic T cells, B cell hybridomas or T
10 cell hybridomas, these cells can be immobilized as single cells in droplets. In a separate set of beads, target cells and detection reagents are immobilized and then the two sets of droplets are fused pairwise to have only one B cell/hybridoma and one target cell.

In alternative embodiments, the detection reagents consist of the following:

- For screening for antibodies that induce cell activation, include fluorescent
15 antibodies in reagent droplet (e.g. anti-CD25, anti-CD69 for T cell activation).

- For screening for antibodies that induce cell differentiation, use genetically modified mice with fluorescent reporters; e.g., for screening for antibodies that induce Tregs (regulatory T cells), use Foxp3-eGFP mice, for screening antibodies that differentiate stem cells to parenchymal cells, use the appropriate genetically modified
20 mice with lineage-determining transcription factors marked by co-expressed fluorescent proteins.

- For screening for cell apoptosis or necrosis, include viability dyes (like 7AAD, PI, and many others at different wavelengths that are available), and an apoptotic marker (like annexin V). In alternative embodiments, only cells that are undergoing apoptosis /
25 necrosis will be stained with these dyes in characteristic patterns.

- Because it takes some time (minutes) for the cell to be killed by holes punched in by cytotoxic T cell, initially nucleus is intact as stained by 7AAD / DAPI, then blebs into apoptotic and necrotic bodies (hours later) at which point the signal becomes pixillated. This is one reason why exemplary high-throughput approaches as provided herein are
30 needed, i.e., to screen events in high-throughput by exemplary FDA.

- As technical controls, both hybridoma/T cells and target cancer/immune/stem cells can be spiked in with cells of same kind but different specificities.

e) The techniques described in b-d) can be applied to engineer and screen T cells including chimeric antigen receptor (CAR) T-cells for a given function such as cancer killing.

f) In alternative embodiments, the positive droplets will then be sorted out with
5 FDA, and subjected to sequencing the BCR or TCR variable regions using primer sets that amplify them, as already established.

Example 14: On-chip screening for receptor-ligand interaction and therapeutic screening.

To monitor receptor-ligand interactions e.g., protein-protein interaction using the
10 FDA device, two different proteins can be encapsulated in separate droplets and then the two droplets can be trapped in neighboring trapping structures. Trapped droplets can be merged by the methods as described in Example 13. For monitoring protein-protein interaction, FRET, life-time imaging, or fluorescence polarization can be integrated in the FDA device.

15 For the inhibitor screening, protein A, protein B, and a library (small molecule, DNA, peptide, antibody or protein) can be encapsulated within separated droplets. Protein A-containing droplets and library-containing droplets can be merged first by activation of an electrode that is located between droplets (see figure 20) and then the other electrode can control merging of the other droplet, containing protein B, with previously merged
20 droplet. Inhibitory effect can be monitored using FRET, life-time imaging, or fluorescence polarization.

Example 15: On-chip in vitro evolution, selection and screening

An exemplary Floating Droplet Array (FDA) device can be used for in vitro
25 evolution, selection and screening. An aptamer library can be compartmentalized within picoliter droplets and trapped within microwell structures. Then the target molecules can also be encapsulated and droplets can be trapped next to the library containing droplets. Two droplets can be merged by the method described above (example 13) and target-aptamer interactions can be used to trigger a fluorescence signal for example by
30 triggering isothermal amplification reaction as describe in example 8.

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A number of embodiments have been described. Nevertheless, it will be
15 understood that various modifications may be made without departing from the spirit and scope of the exemplary embodiments provided herein. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A high throughput, multiplexed system or device, or method, for detecting and/or quantifying a chemical, biological, physiological or pathological analyte, or a single molecule or a single cell, or a chemical or a biochemical reaction; or recognition of
5 a cell or molecule, using a floating droplet array (FDA) system integrated with use of a sensing element or reporter or a fluorogenic reaction, comprising:

(a) providing a sensor or sensing reaction that involves a small molecule, peptide, protein, nucleic acid, enzyme, antibody, cell, or chemical agent capable of detecting a target of interest and producing a signal readout;

10 (b) providing a floating droplet array system, droplet microfluidics system or microdroplet-manipulating device or system;

(d) providing a chemical, biological or an environmental sample containing a target of interest such as a small molecule, an aptamer, a metabolite, peptide, protein, nucleic acid, or cell; and

15 (e) encapsulating the chemical, biological or environmental sample into a plurality of microdroplets, trapping the microdroplets into trapping structures, and processing the microdroplets comprising the encapsulated chemical, biological or environmental sample in the droplet microfluidics system or microdroplet-manipulating device, and detecting the presence of a fluorophore signal, or fluorescence, in each microdroplet (a positive
20 droplet) in the device,

wherein detection of a fluorophore signal or fluorescence in a microdroplet indicates the presence of the target molecule in the microdroplet, and the sample,

and optionally further comprising (f) sorting individually or collectively the positive droplets for downstream analysis including sequencing technologies.

25

2. A high throughput, multiplexed system or device, or method, for detecting and/or quantifying one or multiple different types of a chemical, a biological, a physiological or a pathological analyte, or one or multiple different types of a single molecule or a single cell, or a chemical or a biochemical reaction, or recognition of a cell
30 or a molecule, using a floating droplet array system in real-time, and optionally one of the signals in the said multiplex assay serves for reference or normalization purposes.

3. A high throughput, multiplexed system or device, or method, for detecting and sorting of single molecules of a chemical, a biological, a physiological or a pathological analytes, or a single molecule or a single cell, using a floating droplet array system.

5

4. The high throughput, multiplexed system or device, or method, of any of claims 1 to 3, wherein the cell is a mammalian cell, a circulating tumor cell, a circulating melanoma cell, a B cell, a hybridoma cell, a T cell, a Chimeric Antigen Receptor (CAR) T cell (CAR-T cell), a fungal cell, virus or a bacterial cell, a stem cell, a differentiated
10 cell, an engineered cell; and optionally a heterogeneous cell population can be partitioned and encapsulated into droplets and characterized, manipulated and sorted at a single-cell level; and optionally a droplet can contain one, two, three, four or more of the same type of cell or different types of cells.

15 5. The high throughput, multiplexed system or method of any of claims 1 to 4, wherein the droplet microfluidics system can generate: picoliter droplets or droplets of between about 2 μm to 999 μm in diameter.

6. The high throughput, multiplexed system or method of any of claims 1 to
20 5, wherein 100 to 100 billion droplets can be immobilized and analyzed in the droplet array.

7. The high throughput, multiplexed system or method of any of claims 1 to 6, wherein droplets are composed of one or many sub-phases as single or multiple
25 emulsions.

8. The high throughput, multiplexed system or device, or method, of any of claims 1 to 7, wherein the biological sample comprises a blood, serum, saliva, tear, urine, tissue, or CSF sample from an individual, a patient or an animal, as well as non-biological
30 samples including food, water and environmental samples.

9. The high throughput, multiplexed system or device, or method, of any of claims 1 to 8, wherein the single molecule is a nucleic acid, a nucleic acid point mutation, or a single-nucleotide polymorphism (SNP), ribonucleic acid or a nucleic acid biomarker for, e.g., breast cancer.

5

10. The high throughput, multiplexed system or device, or method, of any of claims 1 to 9, wherein the single molecule is a protein, a lipid, a carbohydrate, a polysaccharide, a small molecule or a metal.

10 11. The high throughput, multiplexed system or device, or method, of any of claims 1 to 10, wherein the single cell is a bacteria, a fungi, a virus, a mammalian cell, or a fused cell; and optionally in alternative embodiments, the encapsulated cells can be cultured in droplets without significantly losing their viability from hours to 7 days.

15 12. The high throughput, multiplexed system or device, or method, of any of claims 1 to 11, wherein the encapsulated cell(s) or molecule(s) produce a fluorescent signal upon a chemical or biological reaction, and optionally a B cell produces target antibodies or a Chimeric Antigen Receptor (CAR) T cell (CAR-T) kills a cancer cell.

20 13. The high throughput, multiplexed system or device, or method, of any of claims 1 to 12, wherein the sensor or reaction comprises a DNA strand displacement strategy, a proximity ligation assay, a binding induced DNA assembly assay, a PCR or RT-PCR reaction, an enzyme reaction, a fluorescent dye or protein, or a fluorogenic reaction; and optionally a reagent or reagents are co-encapsulated with analytes or
25 samples at the beginning or optionally introduced sequentially by, e.g. droplet-droplet fusion.

14. The high throughput, multiplexed system or device, or method, of any of claims 1 to 13, further comprising detecting and/or quantifying the chemical, biological,
30 physiological or pathological analyte, or single molecule or single cell integrated with a detection system comprising the use of an embedded APD (avalanche photodiode),

photomultiplier tube (PMT), digital camera, charge-coupled device (CCD) or complementary metal-oxide semiconductor (CMOS) sensor in a high throughput manner.

15. The high throughput, multiplexed system or device, or method, of any of
5 claims 1 to 14, wherein the throughput, multiplexed system is engineered to comprise one or any of: desirable portability (for example, packaged as backpacks), automating fluid handling (i.e., droplet generation and auto sampling), and integrating electronics including a diode laser, LED panel, light source, operating, and/or data analyzing software, display with fluorescence microscopy, embedded APD (avalanche photodiode), photomultiplier
10 tube (PMT), digital camera, charge-coupled device (CCD), complementary metal-oxide semiconductor (CMOS) sensor.

16. The high throughput, multiplexed system or device, or method, of any of
claims 1 to 15, further comprising disposable microfluidic “cartridges,” permitting
15 multiplex and rapid detection of multiple types of targets simultaneously, and optionally the high throughput, multiplexed system or device is fully automated, or is fabricated as an all-in-one system or with modular components, or is linked to an electronic device, e.g., a portable device, e.g., a smart phone and/or a Bluetooth, or is integrated with a portable temperature controller (e.g. Peltier-based thermocycler), for point-of-care
20 applications.

17. A high-throughput system comprising trapping structures of various sizes/shapes for immobilizing droplets of various compositions in a spatially controlled, defined, and parallel format.

25

18. The high-throughput system of claim 17, comprising trapping droplets into trapping structures, whereby droplets float or sink into trapping structures due to density differences between the dispersed and continuous phases; and optionally the droplets may be recovered by reorienting the device; and optionally the droplets may be fused or
30 merged or split in step-wise processes to accommodate chemistries or stimulations or media change in sequential steps.

19. The high-throughput system of claim 17 or claim 18, comprising a multilayer microfluidic device whereby droplets are trapped in a region above or below the main flow stream.

5 20. The high-throughput system of any of claims 17 to 19, comprising guiding structures such as tracks, pillars, or narrow channels which guide droplets to the trapping structures and ensure complete and efficient coverage of the trapping structures.

21. The high-throughput system of any of claims 17 to 20, comprising inlets or
10 outlets to divert droplets away or to the droplet trapping structures and/or other channels or chambers.

22. The high-throughput system of any of claims 17 to 22, further comprising indexes droplets based on one or many spatial or temporal variables.

15 23. The high-throughput system of any of claims 17 to 22, whereby droplets are indexed based on uniquely barcoded beads, a nucleic acid barcode, a fluorophore, an organic or an inorganic dye barcode, or a colorimetric barcode.

20 24. The high-throughput system of any of claims 17 to 23, integrated with data acquisition hardware or software, data analysis software, a user interface, or computer or mobile device applications.

25 25. The high-throughput system of any of claims 1 to 24, integrated with a sorting element or elements for retrieving a plurality of or individual droplets, whereby optionally the sorting element comprises an electrode, a pneumatic valve, a microfluidic controlled valve, a laser, a microneedle, a magnetic field or an acoustic-based droplet retrieval systems.

30 26. The high-throughput system of any of claims 1 to 25, wherein sorted or retrieved droplets are analyzed using downstream methods for their contents, whereby optionally the downstream methods comprise sequencing, next-generation sequencing

(NGS) (optionally pyrosequencing or massively parallel signature sequencing) and single-cell sequencing techniques.

27. The high-throughput system of any of claims 1 to 26, serving as a research
5 or discovery tool to characterize, manipulate, screen, and/or sort immunological agents including B cells, plasma cells, hybridomas, antibodies, monoclonal antibodies, nanobodies, antibody-drug conjugates, T cells, a Chimeric Antigen Receptor (CAR) T cell (CAR-T), native or engineered cells; optionally the presence or production of the said immunological agent(s) or when the said immunological agent(s) activate, inhibit or
10 modulate a biological molecule, signal or event in the droplets produce a detectable signal readout; optionally the said signal readout can indicate or quantify the presence, or binding or biological functions of the said immunological agent(s).

28. A high-throughput droplet generation module, whereby droplets are
15 formed at high throughput using droplet-generating junctions comprising:

- (a) a dispersed phase which flows through a plurality of channels in a radial direction prior to dispersion;
- (b) a carrier phase that flows through one or many channels in a direction perpendicular to the radially flowing phase that is to be dispersed; and
- 20 (c) droplets generated as the carrier phase comes into contact with an immiscible dispersed phase in the arrangement described in (a) and (b).

29. The high-throughput system of claim 28, comprising a stacked, 3D arrangement of droplet-generating junctions such that droplet generation can occur at
25 many junctions simultaneously.

30. The high-throughput system of claim 28 or claim 29 in which the droplet-generating module is integrated within a portable handheld fluidic device, such as a syringe.

30

31. The high-throughput system of any of claims 28 to 30, whereby the driving pressure for fluid flow can be generated by hand using a force-transferring device such as a plunger.

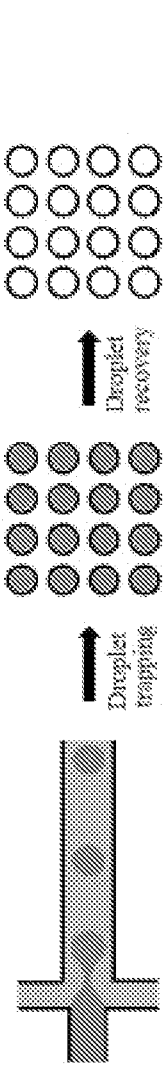


Fig. 1A

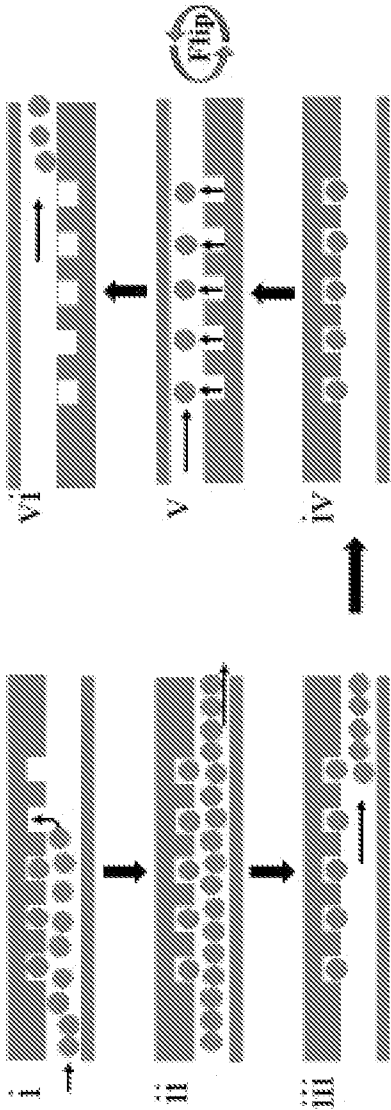


Fig. 1B

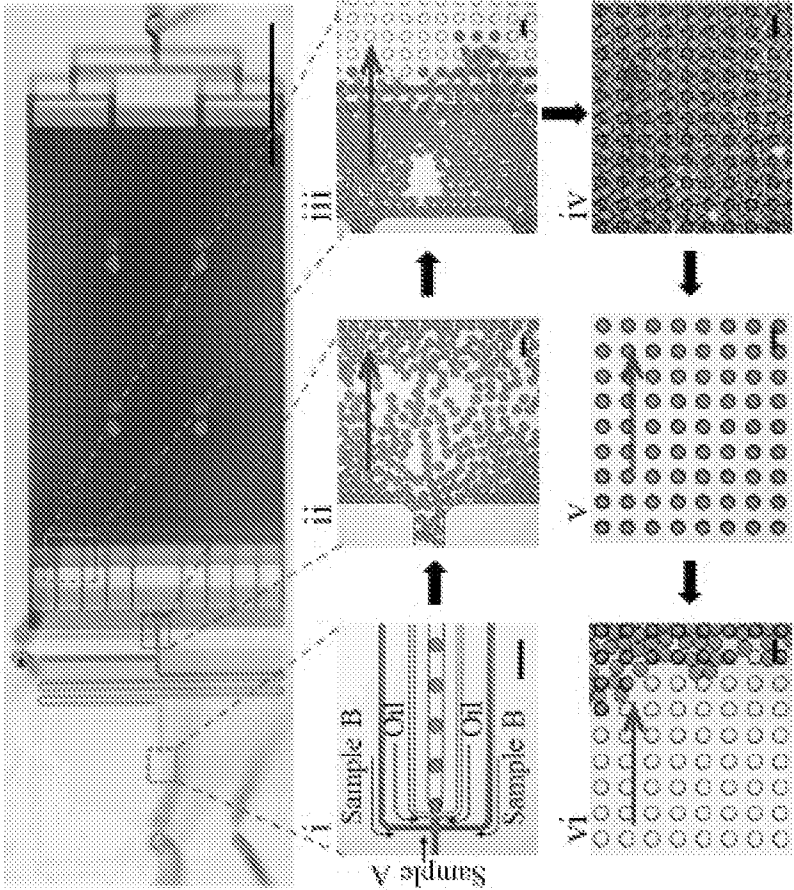


Fig. 2A

Fig. 2B

Fig. 3C

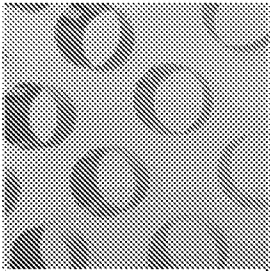


Fig. 3B

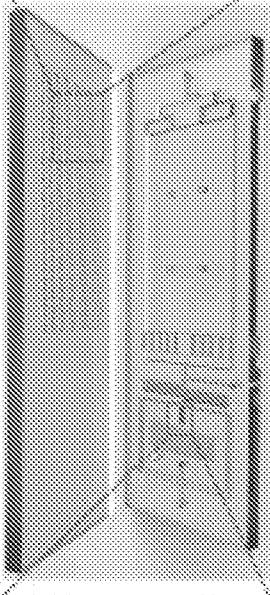
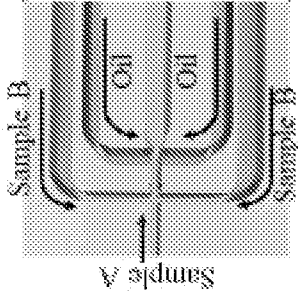


Fig. 3A



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Fig. 4

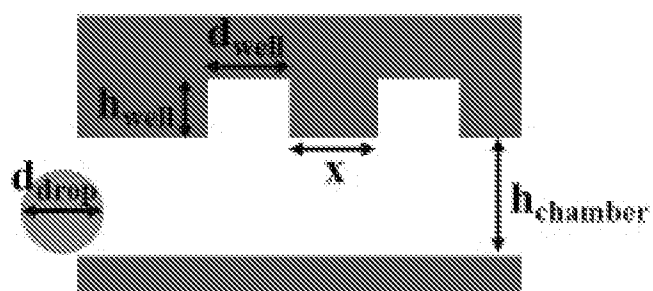


Fig. 5A

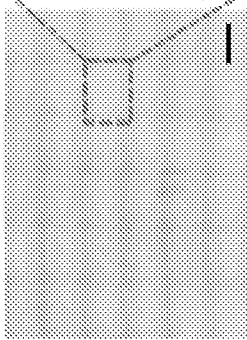


Fig. 5B

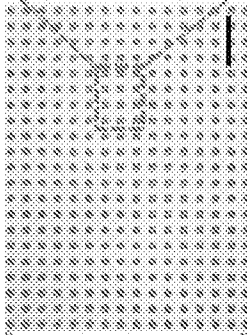
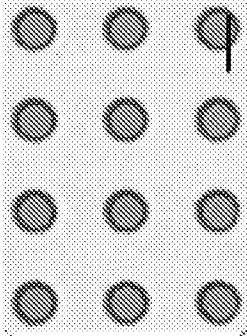


Fig. 5C



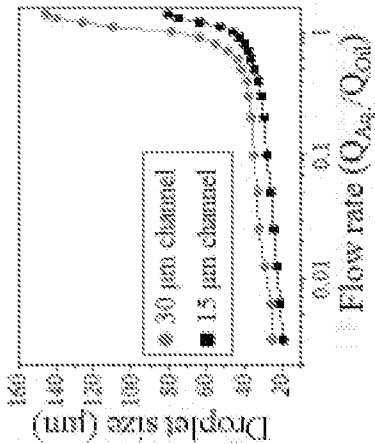


Fig. 6E

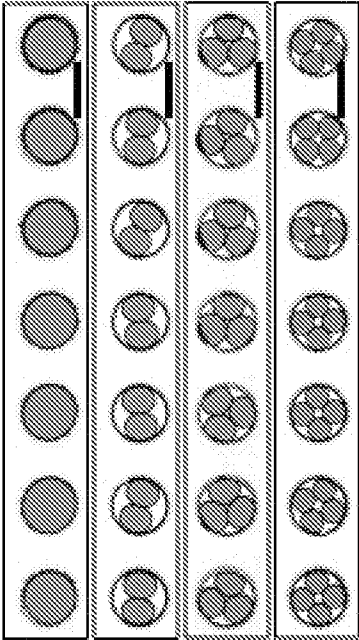


Fig. 6A

Fig. 6B

Fig. 6C

Fig. 6D

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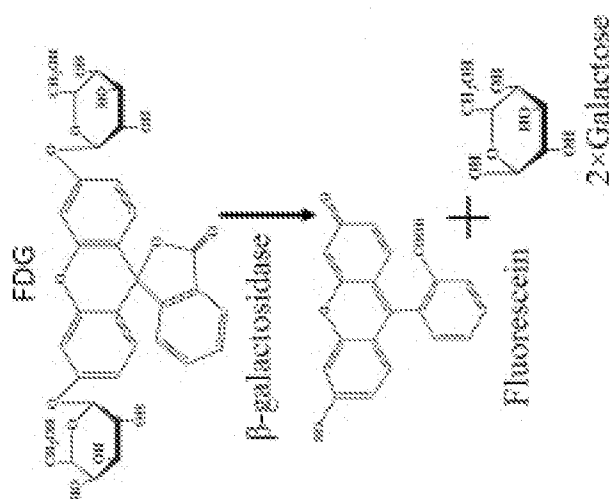


Fig. 7A

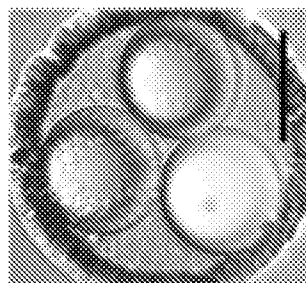


Fig. 7B

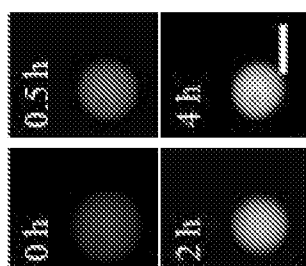


Fig. 7C

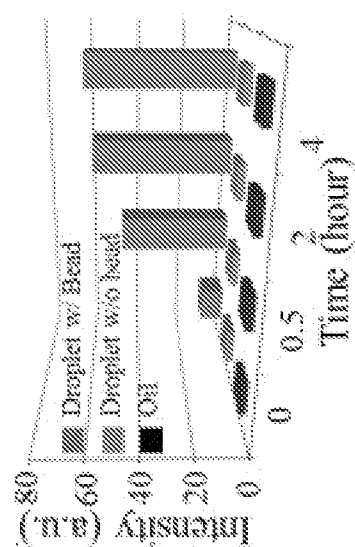


Fig. 7D

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Fig. 8A

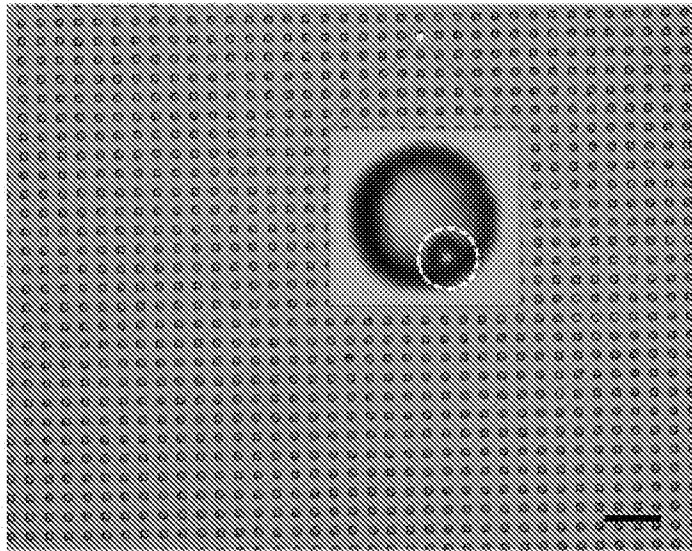


Fig. 8B

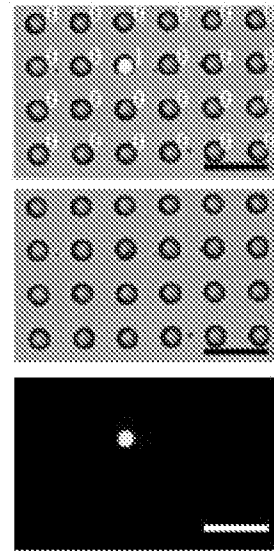


Fig. 9C

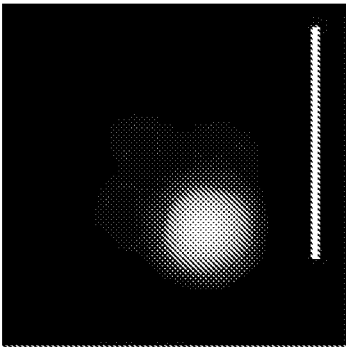


Fig. 9B

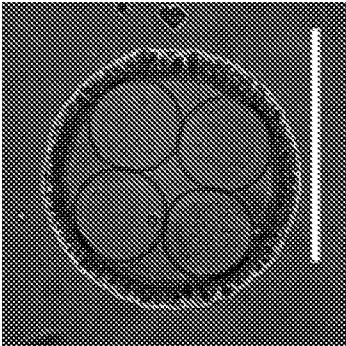
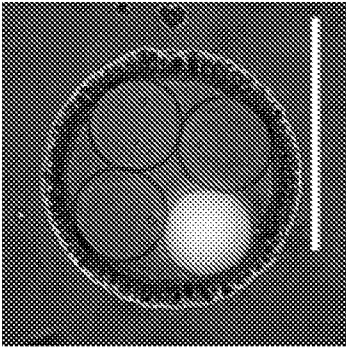
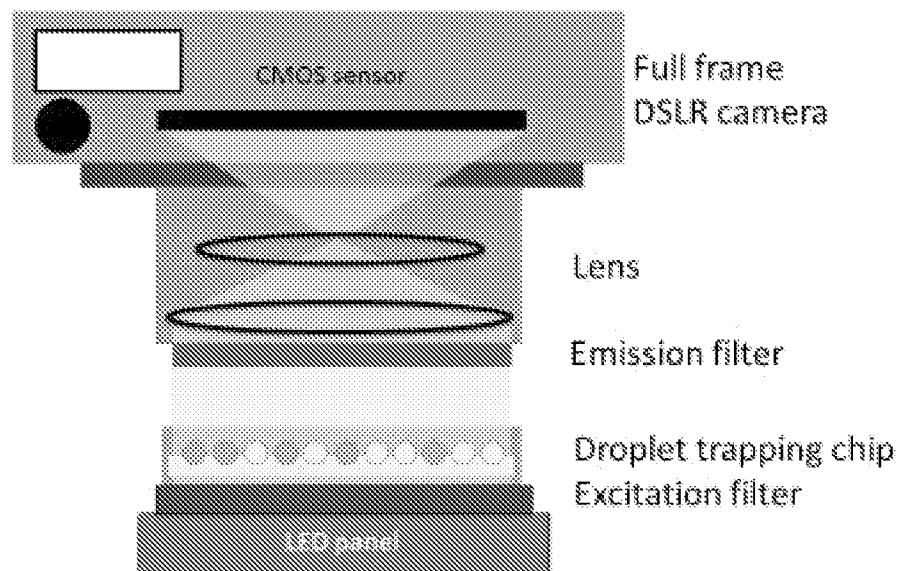


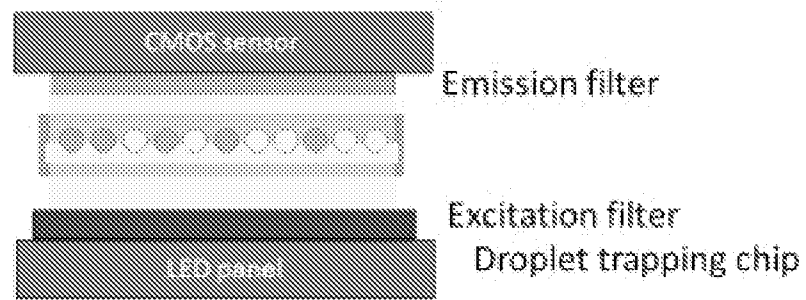
Fig. 9A



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Fig. 10

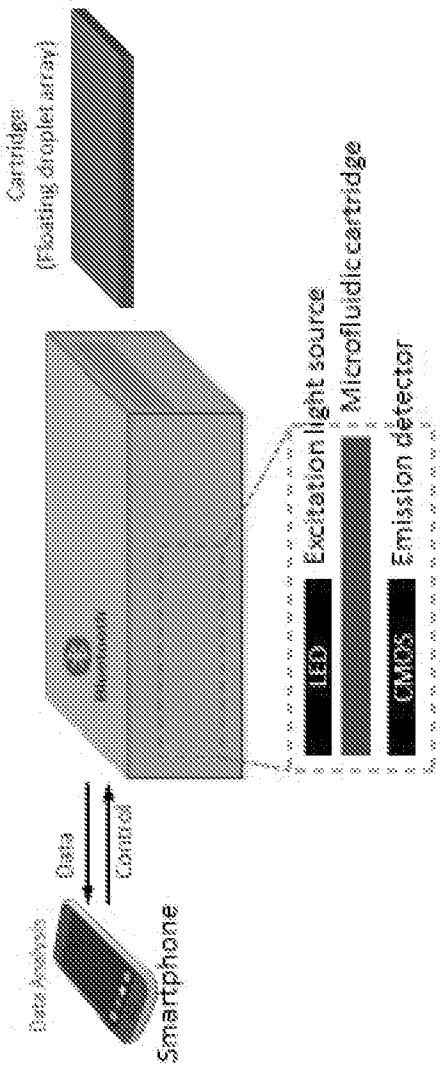


11/25
Fig. 11

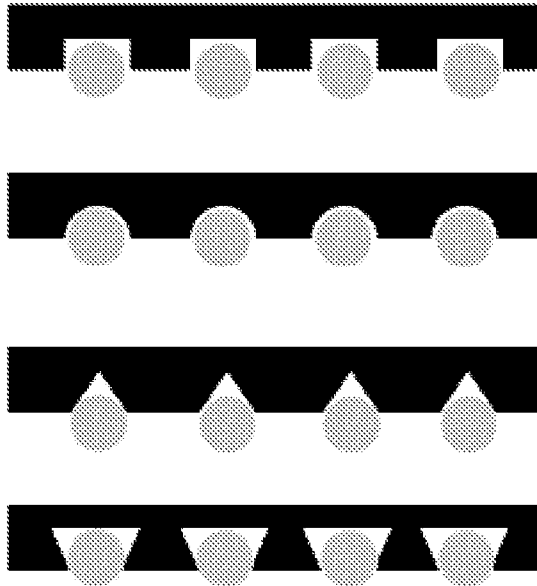


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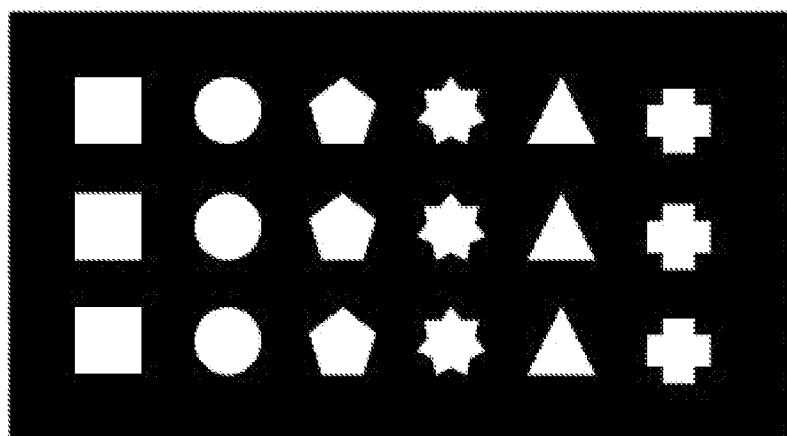
Fig. 12



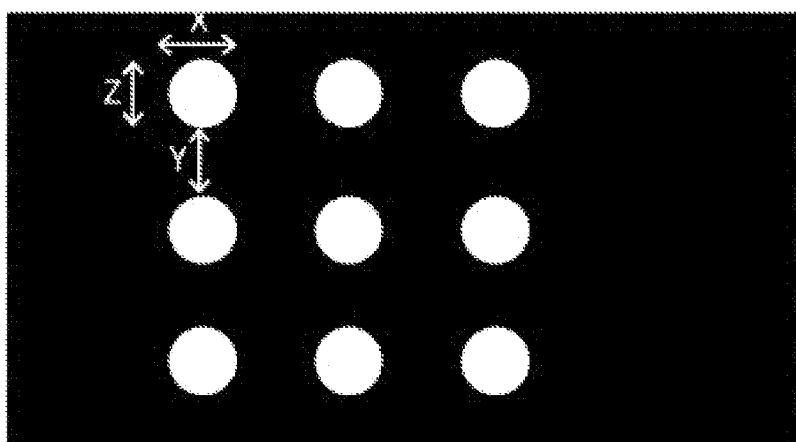
13/25
Fig. 13



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Fig. 14



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Fig. 15



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Fig. 16

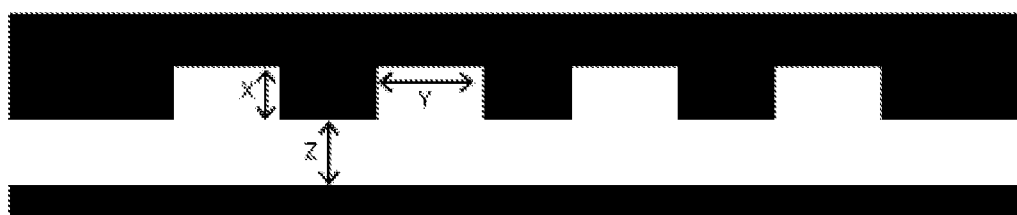


Fig. 17C

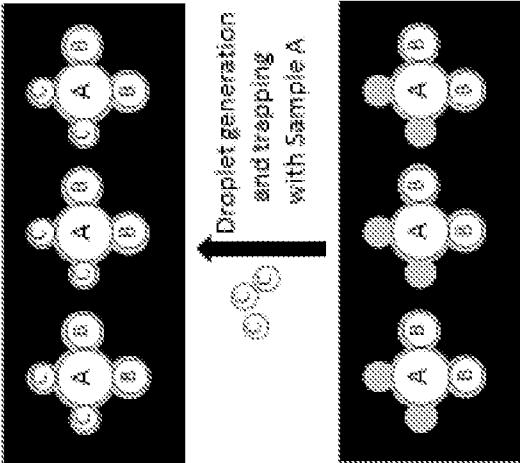


Fig. 17D

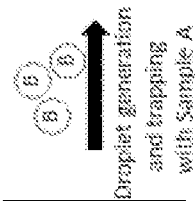


Fig. 17A

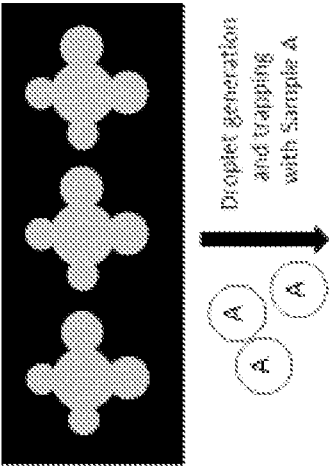


Fig. 17B

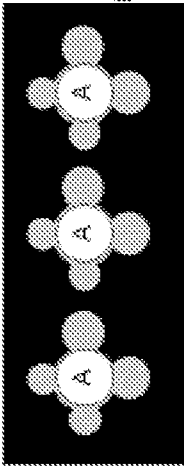




Fig. 18A

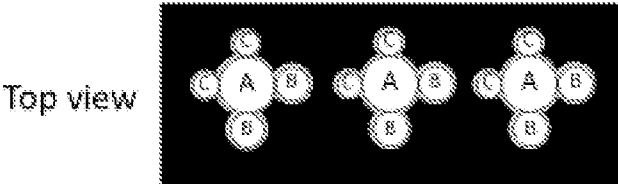


Fig. 18B

Fig. 19B

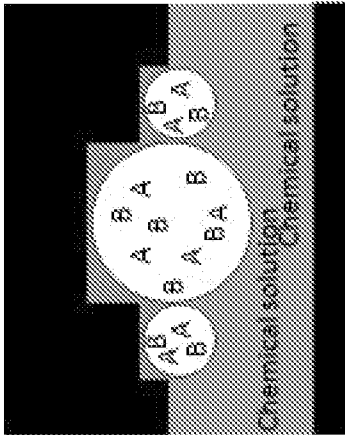


Fig. 19A

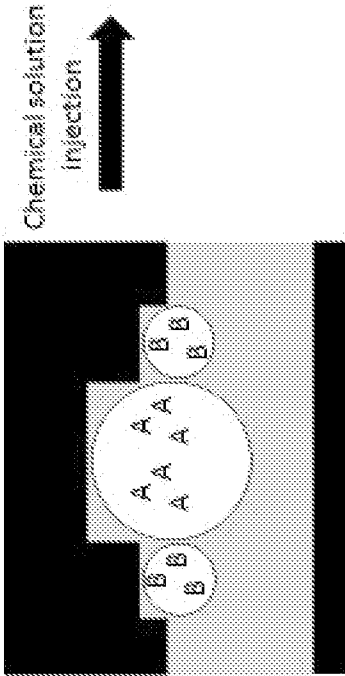
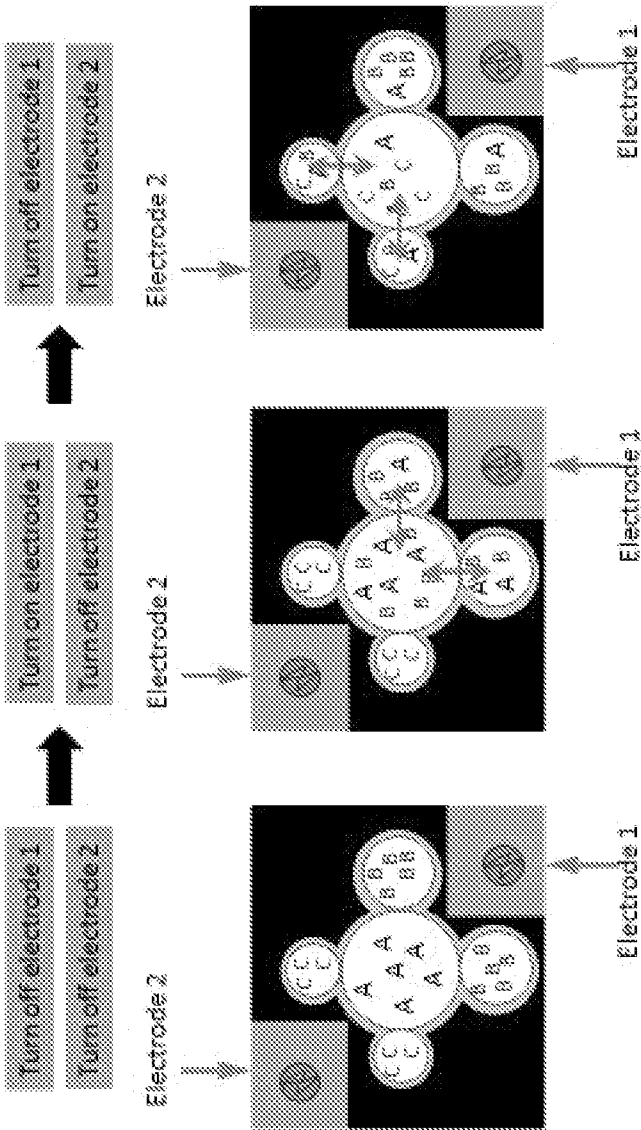


Fig. 20A

Fig. 20B

Fig. 20C



Electrode can be a metal electrode or a liquid electrode (e.g., CaCl_2 solution in the channel)

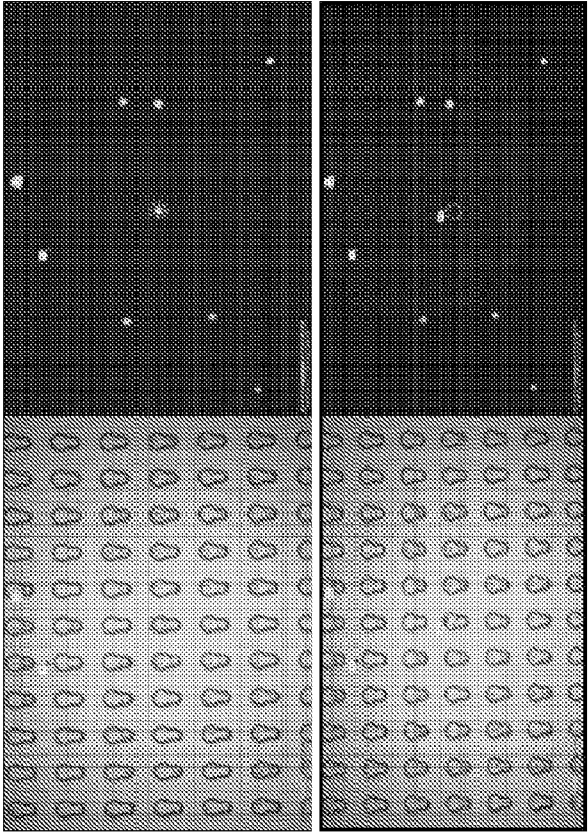
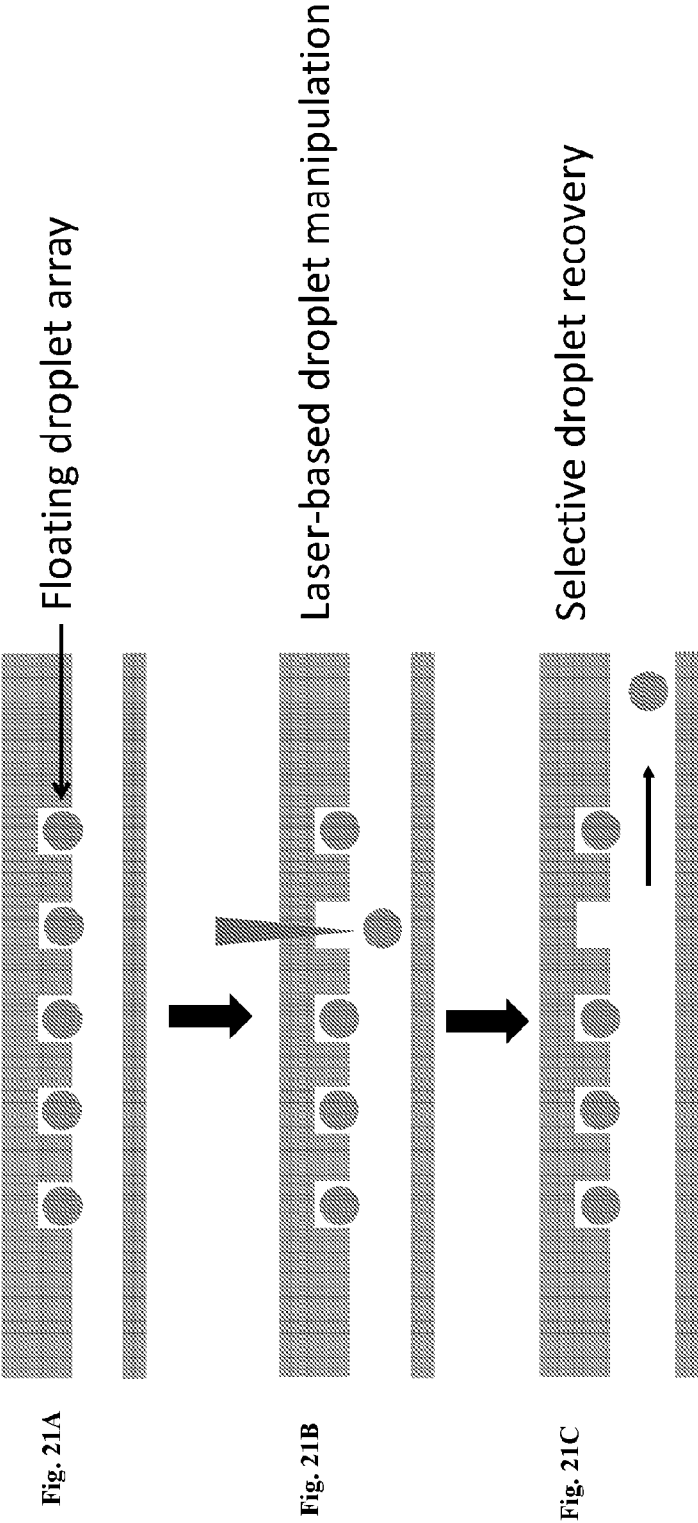


Fig. 21A

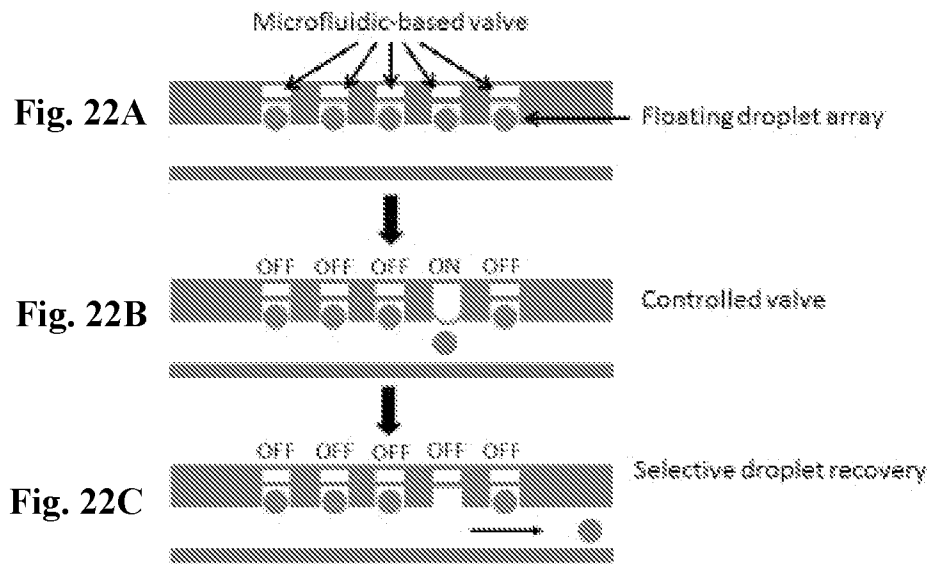
Fig. 21B

Fig. 21C

Fig. 21D

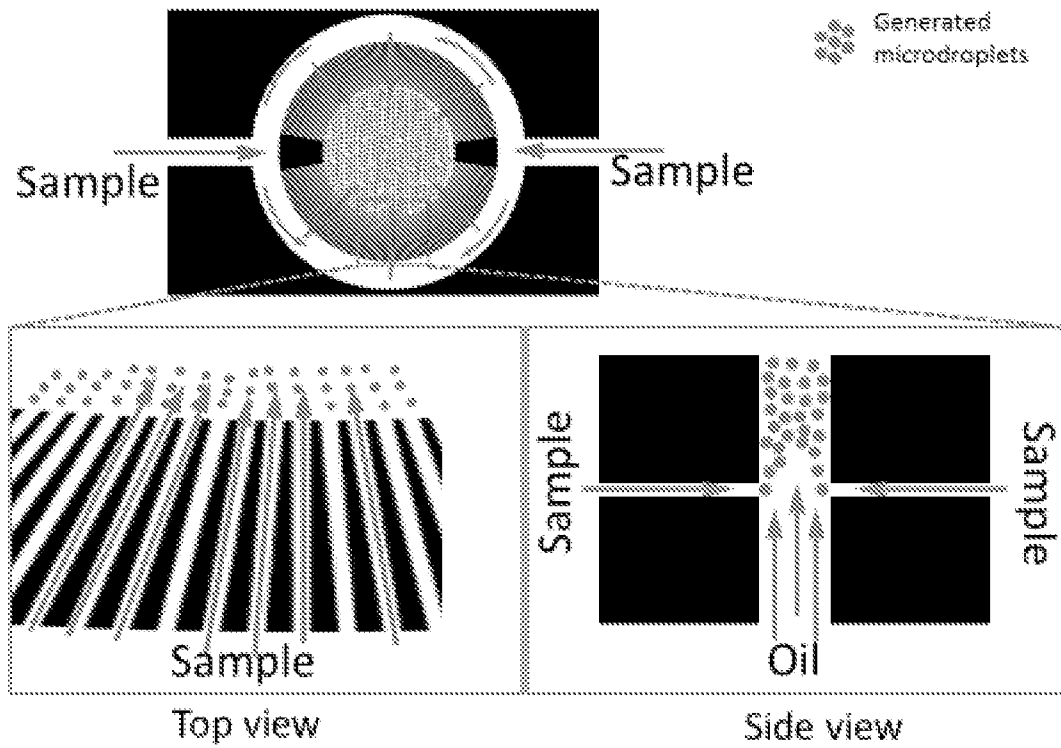
Fig. 21E

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Fig. 23



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Fig. 24

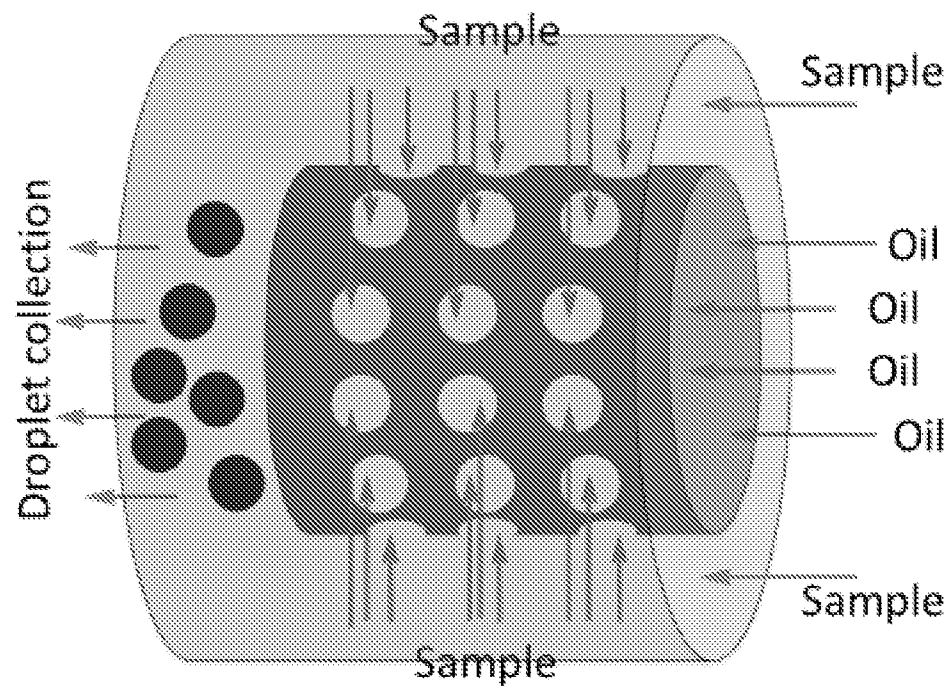
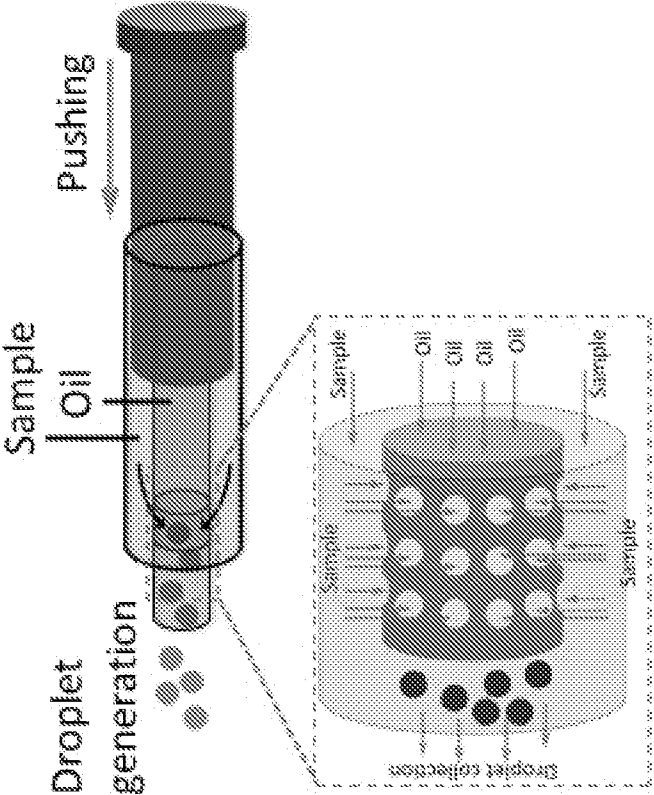


Fig. 25



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/51964

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/533, 33/554, 33/569, 33/53 (2017.01)

CPC - G01N 33/533, 33/554, 33/569, 33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) Classification(s): G01N 33/53, 33/533, 33/554, 33/569, 33/574; C12 1/68 (2017.01)

CPC Classification(s): G01N 33/53, 33/533, 33/554, 33/569, 33/57496; C12Q 1/6816, 2537/143, 2563/159, 2565/629

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; EBSCO

Keywords: multiplexed method, analyte, molecule, cell, floating droplet array, presence of target of interest, real-time, fluorescent signals; sorting, sensing reaction

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.™
X	WO 2015/048173 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) April 2, 2015; column 4, lines 20-28; column 6, lines 24-26; column 7, lines 17-32; column 11, lines 23-27; claims 1, 14	1-4
A	Labanieh, L. et al. 'Floating Droplet Array: An Ultrahigh-Throughput Device for Droplet Trapping, Real-time Analysis and Recovery', Micromachines 2015, 6, 1469-1482; [online], [retrieved on 2017-01-03] Retrieved from the internet: <URL: http://www.mdpi.com/2072-666X/6/10/1431> <doi:10.3390/mi6101431>	1-4
A	US 2013/0040841 A1 (Saxonov, S. et al.) February 14, 2013; entire document	1-4
A	US 2014/0004539 A1 (Simon, A. et al.) January 2, 2014; entire document	1-4

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

04 January 2017 (04.01.2017)

Date of mailing of the international search report

23 JAN 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/51964

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5-16, 20-27, 31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

---Continued from Box III ---

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fee must be paid.

Group I: Claims 1-4 appear to be directed towards detecting and/or quantifying a chemical, biological, physiological or pathological analyte, or a single molecule or a single cell, or a chemical or a biochemical reaction.

Group II: Claims 17-19 appear to be directed towards trapping structures of various sizes/shapes for immobilizing droplets of various compositions in a spatially controlled, defined, and parallel format.

Group III: Claims 28-30 appear to be directed towards droplet-generating junctions.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features.

The special technical features of Group I are at least a high throughput, multiplexed system or device, or method, for detecting and/or quantifying a chemical, biological, physiological or pathological analyte, or a single molecule or a single cell, or a chemical or a biochemical reaction; or recognition of a cell or molecule, using a floating droplet array (FDA) system integrated with use of a sensing element or reporter or a fluorogenic reaction, comprising: (a) providing a sensor or sensing reaction that involves a small molecule, peptide, protein, nucleic acid, enzyme, antibody, cell, or chemical agent capable of detecting a target of interest and producing a signal readout; (b) providing a floating droplet array system, droplet microfluidics system or microdroplet-manipulating device or system; (d) providing a chemical, biological or an environmental sample containing a target of interest such as a small molecule, an aptamer, a metabolite, peptide, protein, nucleic acid, or cell; and (e) encapsulating the chemical, biological or environmental sample into a plurality of microdroplets, trapping the microdroplets into trapping structures, and processing the microdroplets comprising the encapsulated chemical, biological or environmental sample in the droplet microfluidics system or microdroplet-manipulating device, and detecting the presence of a fluorophore signal, or fluorescence, in each microdroplet (a positive droplet) in the device, wherein detection of a fluorophore signal or fluorescence in a microdroplet indicates the presence of the target molecule in the microdroplet, and the sample, and optionally further comprising (f) sorting individually or collectively the positive droplets for downstream analysis including sequencing technologies, which are not present in Groups II or III.

The special technical features of Group II are at least trapping structures of various sizes/shapes for immobilizing droplets of various compositions in a spatially controlled, defined, and parallel format, which are not present in Groups I or III.

The special technical features of Group III are at least whereby droplets are formed at high throughput using droplet-generating junctions comprising: (a) a dispersed phase which flows through a plurality of channels in a radial direction prior to dispersion; (b) a carrier phase that flows through one or many channels in a direction perpendicular to the radially flowing phase that is to be dispersed; and (c) droplets generated as the carrier phase comes into contact with an immiscible dispersed phase in the arrangement described in (a) and (b), which are not present in Groups I or II.

The common technical features are at least a high-throughput system with droplets and trapping structures for the droplets, which are previously disclosed by US 2011/0259742 A1 Li, C et al. (LI).

LI discloses a high-throughput system with droplets and trapping structures for the droplets (an on demand droplet trapping device for through-putting droplets; paragraph [0089]).

Since the common technical features were previously disclosed by LI, they are not special and Groups I-III lack unity.