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(71) Applicant: **10X GENOMICS, INC.** [US/US]; 6230 Stoneridge Mall Road, Pleasanton, California 94588-3260 (US).

(72) Inventors: **ZIRALDO, Solongo B.**; 6230 Stoneridge Mall Road, Pleasanton, California 94588-3260 (US). **MCDERMOTT, Geoffrey**; 6230 Stoneridge Mall Road, Pleasanton, California 94588-3260 (US). **LANCE, Shea Thompson**; 6230 Stoneridge Mall Road, Pleasanton, California 94588-3260 (US).

(74) Agent: **LEE, Na Kyung**; Wilson Sonsini Goodrich & Rosati, PC, 650 Page Mill Road, Palo Alto, California 94304 (US).

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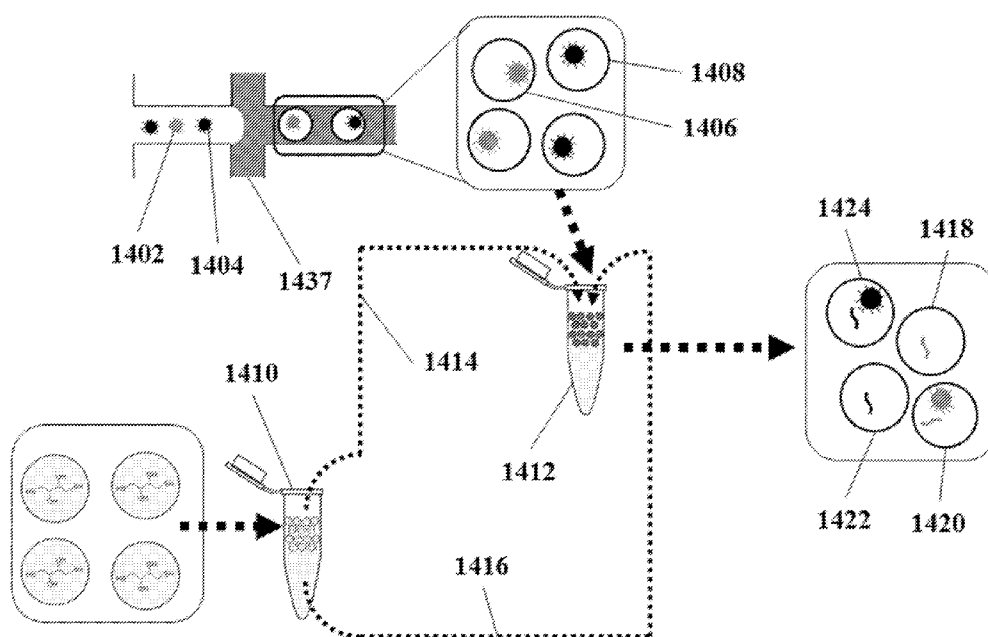


FIG. 14

(57) Abstract: The disclosure provides systems and methods for droplet processing. For example, a method can include providing a first droplet and a second droplet. In some cases, the first droplet may have a first concentration of a reagent and the second droplet may have a second concentration of the reagent. The second droplet may comprise a bead or a biological particle. The method can also include subjecting the first droplet and the second droplet to conditions sufficient to transfer the reagent from the first droplet to the second droplet, thereby decreasing the first concentration in the first droplet and increasing the second concentration in the second droplet.

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SYSTEMS AND METHODS FOR TRANSFER OF REAGENTS BETWEEN DROPLETS**CROSS-REFERENCE**

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/934,256, filed November 12, 2019, and U.S. Provisional Patent Application No. 62/804,633, filed February 12, 2019, each of which is entirely incorporated herein by reference.

BACKGROUND

[0002] A sample may be processed for various purposes, such as identification of a type of moiety within the sample. The sample may be a biological sample. Biological samples may be processed, such as for detection of a disease (e.g., cancer) or identification of a particular species. There are various approaches for processing samples, such as polymerase chain reaction (PCR) and sequencing.

[0003] Biological samples may be processed within various reaction environments, such as partitions. Partitions may be wells or droplets. Droplets or wells may be employed to process biological samples in a manner that enables the biological samples to be partitioned and processed separately. For example, such droplets may be fluidically isolated from other droplets, enabling accurate control of respective environments in the droplets.

[0004] Biological samples in partitions may be subjected to various processes, such as chemical processes or physical processes. Samples in partitions may be subjected to heating or cooling, or chemical reactions, such as to yield species that may be qualitatively or quantitatively processed.

[0005] Partitioning reagents in droplets can be useful for analyzing various samples.

SUMMARY

[0006] Changing an amount of reagent in a droplet, such as increasing or decreasing the concentration, remains challenging. In an example, an amount of reagent in a droplet may be changed in order to affect the rate or progress of a reaction within the droplet. Such reactions may include cleaving or formation of a bond, reduction, oxidation, hydrolysis, etc. Accordingly, recognized herein is a need for improved systems and methods for processing droplets. In particular, systems and methods described herein are useful in controlling the rate or progress of a reaction inside of a droplet.

[0007] An aspect of the present disclosure provides a method for droplet processing. The comprises: (a) providing a first droplet population and a second droplet population, wherein droplets from the first droplet population have a first concentration of a reagent and droplets

from the second droplet population have a second concentration of the reagent, and wherein the droplets from the second droplet population comprise a bead or an analyte carrier; and (b) subjecting the first droplet population and the second droplet population to conditions sufficient to transfer the reagent between the first droplet population and the second droplet population, thereby changing the first concentration of the reagent in the first droplet population and the second concentration of the reagent in the second droplet population.

[0008] In some embodiments, in (a), the reagent is absent from the second droplet population.

[0009] In some embodiments, the reagent is selected from the group consisting of a reducing agent, lysis agent, sodium ion (Na⁺), magnesium ion (Mg²⁺), potassium ion (K⁺), ammonium ion (NH₄⁺), chloride (Cl⁻), bromide (Br⁻), iodide (I⁻), fluoride (F⁻), adenosine triphosphate (ATP), dinucleotide triphosphate (dNTP), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and n-dodecyl-p-D-maltoside (DBDM). In some embodiments, wherein the reducing agent is dithiothreitol or a functional derivative thereof.

[0010] In some embodiments, the first droplet population comprises a first droplet fraction and a first fluid fraction and wherein the second droplet population comprises a second droplet fraction and a second fluid fraction. In some embodiments, the first fluid fraction or the second fluid fraction comprises a mediator that aids in transfer of the reagent between the first droplet population and the second droplet population. In some embodiments, in (b), the reagent is transferred between the first droplet population and the second droplet population via the mediator. In some embodiments, the mediator is derived from the first droplet population. In some embodiments, the mediator comprises a surfactant. In some embodiments, the mediator is a micelle.

[0011] In some embodiments, (b) comprises subjecting the first droplet population to conditions sufficient to transfer the reagent from the first droplet fraction to the first fluid fraction and subjecting the second droplet population to conditions sufficient to transfer the reagent from the first fluid fraction to droplets of the second droplet population. In some embodiments, the first fluid fraction is a continuous phase in which the first droplet population is dispersed. In some embodiments, wherein the continuous phase comprises an oil. In some embodiments, the first droplet population is provided in a first container and the second droplet population is provided in a second container, and wherein (b) further comprises transferring the first fluid fraction of the first droplet population from the first container to the second container.

[0012] In some embodiments, the method further comprises, subsequent to (a), generating a mixture comprising the first droplet population and the second droplet population, wherein the

reagent is between the first droplet population and the second droplet population within the mixture. In some embodiments, the reagent is transferred between the first droplet population and the second droplet population within the mixture via diffusion.

[0013] In some embodiments, the bead has nucleic acid molecules coupled thereto. In some embodiments, the nucleic acid molecules are nucleic acid barcode molecules. In some embodiments, the reagent dissolves the bead or releases at least a portion of the nucleic acid molecules from the bead.

[0014] In some embodiments, the bead is a gel bead. In some embodiments, the reagent dissolves the gel bead.

[0015] In some embodiments, the reagent is a reducing agent and the bead comprises a disulfide bond that is broken by the reducing agent.

[0016] In some embodiments, the analyte carrier is a cell. In some embodiments, the reagent lyses the cell. In some embodiments, the analyte carrier is a cell bead.

[0017] In some embodiments, the droplets from the second droplet population comprise the bead and the analyte carrier.

[0018] In some embodiments, in (a), the first concentration is greater than the second concentration.

[0019] In some embodiments, the droplets from the second droplet population comprise an antibody coupled to a nucleic acid molecule. In some embodiments, the reagent is capable of breaking a linkage between the antibody and the nucleic acid molecule.

[0020] In some embodiments, during or subsequent to (b), the droplets from the first droplet population decrease in size or the droplets from the second droplet population increase in size.

[0021] In some embodiments, prior to (a), the droplets from the first droplet population or the droplets from the second droplet population are generated using a microfluidic device.

[0022] In some embodiments, prior to (a), the first droplet population or the second droplet population is generated upon agitation of a mixture of immiscible phases.

[0023] In some embodiments, the method further comprises prior to (a), generating the second droplet population from (i) a first fluid volume comprising a population of beads and a population of analyte carriers and (ii) a second fluid volume immiscible with the first fluid volume, wherein a droplet from the second droplet population comprises the bead from the population of beads and the analyte carrier from the population of analyte carriers.

[0024] In some embodiments, the second droplet population is generated by applying energy to the first fluid volume, the second fluid volume, or both the first fluid volume and the second

fluid volume. In some embodiments, the second droplet population is generated by providing a mixture comprising the first fluid volume and the second fluid volume and agitating the mixture.

[0025] In some embodiments, the second droplet population is generated in absence of using a microfluidic device. In some embodiments, the second droplet population is generated using a microfluidic device. In some embodiments, the first fluid volume comprises an aqueous fluid. In some embodiments, the second fluid volume comprises an oil. In some embodiments, the first fluid volume does not include the reagent.

[0026] In some embodiments, the droplet comprises a single bead and a single analyte carrier. In some embodiments, the analyte carrier is a cell. In some embodiments, the analyte carrier is a cell bead. In some embodiments, the analyte carrier is a cell nucleus. In some embodiments, the population of analyte carriers comprises a plurality of transposed nuclei.

[0027] In some embodiments, the method further comprises subjecting a population of cell nuclei to transposition in bulk to yield the plurality of transposed nuclei. In some embodiments, the method further comprises subsequent to (b), using a nucleic acid molecule derived from the analyte carrier and a nucleic acid barcode molecule coupled to the bead to generate a barcoded nucleic acid molecule. In some embodiments, the method further comprises sequencing the barcoded nucleic acid molecule, or derivative thereof.

[0028] In an aspect, the present disclosure provides a method for nucleic acid processing, comprising: (a) agitating (i) a first fluid volume comprising a population of beads and a population of analyte carriers, wherein the population of beads comprises a plurality of nucleic acid barcode molecules, wherein the population of analyte carriers comprises a plurality of nucleic acid molecules, and (ii) a second fluid volume immiscible with the first fluid volume, to generate a plurality of droplets, wherein a first droplet from the plurality of droplets comprises a bead from the population of beads and an analyte carrier from the population of analyte carriers, wherein the bead comprises a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules, wherein the analyte carrier comprises a nucleic acid molecule of the plurality of nucleic acid molecules, wherein the first droplet has a first concentration of a reagent, and wherein the reagent is configured to release the nucleic acid barcode molecule or release the nucleic acid molecule in the droplet; (b) subjecting the first droplet and a second droplet having a second concentration of the reagent to conditions sufficient to transfer the reagent between the first droplet and the second droplet, thereby changing the first concentration of the reagent in the first droplet and the second concentration of the reagent in the second droplet; and (c) generating a barcoded nucleic acid molecule using at least the nucleic acid barcode molecule and

the nucleic acid molecule in the first droplet. In some embodiments, agitating comprises vortexing.

[0029] In some embodiments, the first fluid volume comprises an aqueous fluid. In some embodiments, the second fluid volume comprises an oil. In some embodiments, the first fluid volume does not include the reagent. In some embodiments, the first droplet comprises a single bead and a single analyte carrier. In some embodiments, the analyte carrier is a cell. In some embodiments, the reagent lyses the cell. In some embodiments, the analyte carrier is a cell bead. In some embodiments, the analyte carrier is a cell nucleus.

[0030] In some embodiments, the plurality analyte carriers comprise a plurality of transposed nuclei. In some embodiments, the method further comprises subjecting a population of cell nuclei to transposition in bulk to yield the plurality of transposed nuclei. In some embodiments, the method further comprises sequencing the barcoded nucleic acid molecule, or derivative thereof.

[0031] In some embodiments, the reagent is selected from the group consisting of a reducing agent, lysis agent, sodium ion (Na⁺), magnesium ion (Mg²⁺), potassium ion (K⁺), ammonium ion (NH₄⁺), chloride (Cl⁻), bromide (Br⁻), iodide (I⁻), fluoride (F⁻), adenosine triphosphate (ATP), dinucleotide triphosphate (dNTP), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and n-dodecyl-p-D-maltoside (DBDM). In some embodiments, the reducing agent is dithiothreitol or a functional derivative thereof.

[0032] In some embodiments, the plurality of droplets is provided in a first droplet fraction and a first fluid fraction, wherein (b) comprises providing a second plurality of droplets in a second droplet fraction and a second fluid fraction. In some embodiments, the first fluid fraction or the second fluid fraction comprises a mediator that aids in transfer of the reagent between the first droplet and the second droplet.

[0033] In some embodiments, in (b), the reagent is transferred between the first droplet and the second droplet via the mediator. In some embodiments, the mediator comprises a surfactant. In some embodiments, the mediator is a micelle.

[0034] In some embodiments, (b) comprises subjecting the second plurality of droplets to conditions sufficient to transfer the reagent from the second droplet fraction to the second fluid fraction and subjecting the plurality of droplets to conditions sufficient to transfer the reagent from the first fluid fraction to the first droplet fraction. In some embodiments, the first fluid fraction is a continuous phase in which the plurality of droplets is dispersed. In some embodiments, the continuous phase comprises an oil.

[0035] In some embodiments, the method further comprises, in (b), generating a mixture comprising the plurality of droplets and the second plurality of droplets, wherein the reagent is between the plurality of droplets and the second plurality of droplets within the mixture. In some embodiments, the reagent is transferred between the first droplet and the second droplet within the mixture via diffusion. In some embodiments, the bead has a subset of nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules coupled thereto.

[0036] In some embodiments, the reagent dissolves the bead or releases at least a portion of the subset of nucleic acid barcode molecules from the bead. In some embodiments, the bead is a gel bead. In some embodiments, the reagent dissolves the gel bead. In some embodiments, the reagent is a reducing agent and the bead comprises a disulfide bond that is broken by the reducing agent.

[0037] In some embodiments, prior to (b), the first concentration is less than the second concentration. In some embodiments, during or subsequent to (b), the second droplet decreases in diameter or the first droplet increases in diameter.

[0038] Another aspect of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0039] Another aspect of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0040] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0041] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the

disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[0043] **FIG. 1** shows an example of a microfluidic channel structure for partitioning individual analyte carriers;

[0044] **FIG. 2** shows an example of a microfluidic channel structure for delivering barcode carrying beads to droplets;

[0045] **FIG. 3** shows an example of a microfluidic channel structure for co-partitioning analyte carriers and reagents;

[0046] **FIG. 4** shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets;

[0047] **FIG. 5** shows an example of a microfluidic channel structure for increased droplet generation throughput;

[0048] **FIG. 6** shows another example of a microfluidic channel structure for increased droplet generation throughput;

[0049] **FIG. 7A** shows a cross-section view of another example of a microfluidic channel structure with a geometric feature for controlled partitioning;

[0050] **FIG. 7B** shows a perspective view of the channel structure of **FIG. 7A**;

[0051] **FIG. 8** illustrates an example of a barcode carrying bead;

[0052] **FIG. 9** shows a computer system that is programmed or otherwise configured to implement methods provided herein;

[0053] **FIG. 10** shows an example mode of transferring a reagent from a first droplet to a second droplet using a mediator;

[0054] **FIG. 11A** and **FIG. 11B** show two additional example modes of transferring a reagent between droplets;

[0055] **FIG. 12A** and **FIG. 12B** show example methods of processing a droplet;

[0056] **FIG. 13** shows an example method of processing a droplet;

[0057] **FIG. 14** shows an example method of processing a droplet;

[0058] **FIG. 15A** shows a droplet population comprising gel beads and no DTT; and

[0059] **FIG. 15B** shows the results of an experiment demonstrating the method of processing a droplet as described in Example 1.

[0060] **FIGs. 16A** through **16E** show an example sample processing workflow comprising droplet generation with the aid of agitation, as described in Example 3.

[0061] **FIGs. 17A** through **17D** show an example gene expression workflow comprising droplet generation with the aid of agitation, as described in Example 4.

[0062] **FIGs. 18A** and **18B** show the results of an experiment comprising transporting a reagent after droplet generation and lysing the encapsulated cells, as described in Example 5.

DETAILED DESCRIPTION

[0063] While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

[0064] Where values are described as ranges, it will be understood that such disclosure includes the disclosure of all possible sub-ranges within such ranges, as well as specific numerical values that fall within such ranges irrespective of whether a specific numerical value or specific sub-range is expressly stated.

[0065] The terms “a,” “an,” and “the,” as used herein, generally refers to singular and plural references unless the context clearly dictates otherwise.

[0066] Whenever the term “at least,” “greater than,” or “greater than or equal to” precedes the first numerical value in a series of two or more numerical values, the term “at least,” “greater than” or “greater than or equal to” applies to each of the numerical values in that series of numerical values. For example, greater than or equal to 1, 2, or 3 is equivalent to greater than or equal to 1, greater than or equal to 2, or greater than or equal to 3.

[0067] Whenever the term “no more than,” “less than,” or “less than or equal to” precedes the first numerical value in a series of two or more numerical values, the term “no more than,” “less than,” or “less than or equal to” applies to each of the numerical values in that series of numerical values. For example, less than or equal to 3, 2, or 1 is equivalent to less than or equal to 3, less than or equal to 2, or less than or equal to 1.

[0068] The term “barcode,” as used herein, generally refers to a label, or identifier, that conveys or is capable of conveying information about an analyte. A barcode can be part of an analyte. A barcode can be independent of an analyte. A barcode can be a tag attached to an analyte (e.g., nucleic acid molecule) or a combination of the tag in addition to an endogenous

characteristic of the analyte (e.g., size of the analyte or end sequence(s)). A barcode may be unique. Barcodes can have a variety of different formats. For example, barcodes can include: polynucleotide barcodes; random nucleic acid and/or amino acid sequences; and synthetic nucleic acid and/or amino acid sequences. A barcode can be attached to an analyte in a reversible or irreversible manner. A barcode can be added to, for example, a fragment of a deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sample before, during, and/or after sequencing of the sample. Barcodes can allow for identification and/or quantification of individual sequencing-reads.

[0069] The term “real time,” as used herein, can refer to a response time of less than about 1 second, a tenth of a second, a hundredth of a second, a millisecond, or less. The response time may be greater than 1 second. In some instances, real time can refer to simultaneous or substantially simultaneous processing, detection or identification.

[0070] The term “subject,” as used herein, generally refers to an animal, such as a mammal (e.g., human) or avian (e.g., bird), or other organism, such as a plant. For example, the subject can be a vertebrate, a mammal, a rodent (e.g., a mouse), a primate, a simian or a human. Animals may include, but are not limited to, farm animals, sport animals, and pets. A subject can be a healthy or asymptomatic individual, an individual that has or is suspected of having a disease (e.g., cancer) or a pre-disposition to the disease, and/or an individual that is in need of therapy or suspected of needing therapy. A subject can be a patient. A subject can be a microorganism or microbe (e.g., bacteria, fungi, archaea, viruses).

[0071] The term “genome,” as used herein, generally refers to genomic information from a subject, which may be, for example, at least a portion or an entirety of a subject’s hereditary information. A genome can be encoded either in DNA or in RNA. A genome can comprise coding regions (e.g., that code for proteins) as well as non-coding regions. A genome can include the sequence of all chromosomes together in an organism. For example, the human genome ordinarily has a total of 46 chromosomes. The sequence of all of these together may constitute a human genome.

[0072] The terms “adaptor(s)”, “adapter(s)” and “tag(s)” may be used synonymously. An adaptor or tag can be coupled to a polynucleotide sequence to be “tagged” by any approach, including ligation, hybridization, or other approaches.

[0073] The term “sequencing,” as used herein, generally refers to methods and technologies for determining the sequence of nucleotide bases in one or more polynucleotides. The polynucleotides can be, for example, nucleic acid molecules such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), including variants or derivatives thereof (e.g., single stranded

DNA). Sequencing can be performed by various systems currently available, such as, without limitation, a sequencing system by Illumina®, Pacific Biosciences (PacBio®), Oxford Nanopore®, or Life Technologies (Ion Torrent®). Alternatively or in addition, sequencing may be performed using nucleic acid amplification, polymerase chain reaction (PCR) (e.g., digital PCR, quantitative PCR, or real time PCR), or isothermal amplification. Such systems may provide a plurality of raw genetic data corresponding to the genetic information of a subject (e.g., human), as generated by the systems from a sample provided by the subject. In some examples, such systems provide sequencing reads (also “reads” herein). A read may include a string of nucleic acid bases corresponding to a sequence of a nucleic acid molecule that has been sequenced. In some situations, systems and methods provided herein may be used with proteomic information.

[0074] The term “bead,” as used herein, generally refers to a particle. The bead may be a solid or semi-solid particle. The bead may be a gel bead. The gel bead may include a polymer matrix (e.g., matrix formed by polymerization or cross-linking). The polymer matrix may include one or more polymers (e.g., polymers having different functional groups or repeat units). Polymers in the polymer matrix may be randomly arranged, such as in random copolymers, and/or have ordered structures, such as in block copolymers. Cross-linking can be via covalent, ionic, or inductive, interactions, or physical entanglement. The bead may be a macromolecule. The bead may be formed of nucleic acid molecules bound together. The bead may be formed via covalent or non-covalent assembly of molecules (e.g., macromolecules), such as monomers or polymers. Such polymers or monomers may be natural or synthetic. Such polymers or monomers may be or include, for example, nucleic acid molecules (e.g., DNA or RNA). The bead may be formed of a polymeric material. The bead may be magnetic or non-magnetic. The bead may be rigid. The bead may be flexible and/or compressible. The bead may be disruptable or dissolvable. The bead may be a solid particle (e.g., a metal-based particle including but not limited to iron oxide, gold or silver) covered with a coating comprising one or more polymers. Such coating may be disruptable or dissolvable.

[0075] As used herein, the term “barcoded nucleic acid molecule” generally refers to a nucleic acid molecule that results from, for example, the processing of a nucleic acid barcode molecule with a nucleic acid sequence (e.g., nucleic acid sequence complementary to a nucleic acid primer sequence encompassed by the nucleic acid barcode molecule). The nucleic acid sequence may be a targeted sequence or a non-targeted sequence. For example, in the methods and systems described herein, hybridization and reverse transcription of a nucleic acid molecule (e.g., a messenger RNA (mRNA) molecule) of a cell with a nucleic acid barcode molecule (e.g.,

a nucleic acid barcode molecule containing a barcode sequence and a nucleic acid primer sequence complementary to a nucleic acid sequence of the mRNA molecule) results in a barcoded nucleic acid molecule that has a sequence corresponding to the nucleic acid sequence of the mRNA and the barcode sequence (or a reverse complement thereof). A barcoded nucleic acid molecule may serve as a template, such as a template polynucleotide, that can be further processed (e.g., amplified) and sequenced to obtain the target nucleic acid sequence. For example, in the methods and systems described herein, a barcoded nucleic acid molecule may be further processed (e.g., amplified) and sequenced to obtain the nucleic acid sequence of the mRNA.

[0076] The term “sample,” as used herein, generally refers to a biological sample of a subject. The biological sample may comprise any number of macromolecules, for example, cellular macromolecules. The sample may be a cell sample. The sample may be a cell line or cell culture sample. The sample can include one or more cells. The sample can include one or more microbes. The biological sample may be a nucleic acid sample or protein sample. The biological sample may also be a carbohydrate sample or a lipid sample. The biological sample may be derived from another sample. The sample may be a tissue sample, such as a biopsy, core biopsy, needle aspirate, or fine needle aspirate. The sample may be a fluid sample, such as a blood sample, urine sample, or saliva sample. The sample may be a skin sample. The sample may be a cheek swab. The sample may be a plasma or serum sample. The sample may be a cell-free or cell free sample. A cell-free sample may include extracellular polynucleotides. Extracellular polynucleotides may be isolated from a bodily sample that may be selected from the group consisting of blood, plasma, serum, urine, saliva, mucosal excretions, sputum, stool and tears.

[0077] The term “analyte carrier,” as used herein, generally refers to a discrete biological system derived from a biological sample. The analyte carrier may be or comprise a biological particle. The analyte carrier may be a macromolecule. The analyte carrier may be a small molecule. The analyte carrier may be a virus. The analyte carrier may be a cell or derivative of a cell. The analyte carrier may be an organelle. The analyte carrier may be a rare cell from a population of cells. The analyte carrier may be any type of cell, including without limitation prokaryotic cells, eukaryotic cells, bacterial, fungal, plant, mammalian, or other animal cell type, mycoplasmas, normal tissue cells, tumor cells, or any other cell type, whether derived from single cell or multicellular organisms. The analyte carrier may be a constituent of a cell. The analyte carrier may be or may include DNA, RNA, organelles, proteins, or any combination thereof. The analyte carrier may be or may include a matrix (e.g., a gel or polymer matrix)

comprising a cell or one or more constituents from a cell (e.g., cell bead), such as DNA, RNA, organelles, proteins, or any combination thereof, from the cell. The analyte carrier may be obtained from a tissue of a subject. analyte carrier may be a hardened cell. Such hardened cell may or may not include a cell wall or cell membrane. In some cases, the analyte carrier may include one or more constituents of a cell but may not include other constituents of the cell. An example of such constituents is a nucleus or an organelle. A cell may be a live cell. The live cell may be capable of being cultured, for example, being cultured when enclosed in a gel or polymer matrix or cultured when comprising a gel or polymer matrix.

[0078] The term “macromolecular constituent,” as used herein, generally refers to a macromolecule contained within or from an analyte carrier or biological particle. The macromolecular constituent may comprise a nucleic acid. In some cases, the analyte carrier may be a macromolecule. The macromolecular constituent may comprise DNA. The macromolecular constituent may comprise RNA. The RNA may be coding or non-coding. The RNA may be messenger RNA (mRNA), ribosomal RNA (rRNA) or transfer RNA (tRNA), for example. The RNA may be a transcript. The RNA may be small RNA that are less than 200 nucleic acid bases in length, or large RNA that are greater than 200 nucleic acid bases in length. Small RNAs may include 5.8S ribosomal RNA (rRNA), 5S rRNA, transfer RNA (tRNA), microRNA (miRNA), small interfering RNA (siRNA), small nucleolar RNA (snoRNAs), Piwi-interacting RNA (piRNA), tRNA-derived small RNA (tsRNA) and small rDNA-derived RNA (srRNA). The RNA may be double-stranded RNA or single-stranded RNA. The RNA may be circular RNA. The macromolecular constituent may comprise a protein. The macromolecular constituent may comprise a peptide. The macromolecular constituent may comprise a polypeptide.

[0079] The term “molecular tag,” as used herein, generally refers to a molecule capable of binding to a macromolecular constituent. The molecular tag may bind to the macromolecular constituent with high affinity. The molecular tag may bind to the macromolecular constituent with high specificity. The molecular tag may comprise a nucleotide sequence. The molecular tag may comprise a nucleic acid sequence. The nucleic acid sequence may be at least a portion or an entirety of the molecular tag. The molecular tag may be a nucleic acid molecule or may be part of a nucleic acid molecule. The molecular tag may be an oligonucleotide or a polypeptide. The molecular tag may comprise a DNA aptamer. The molecular tag may be or comprise a primer. The molecular tag may be, or comprise, a protein. The molecular tag may comprise a polypeptide. The molecular tag may be a barcode.

[0080] The term “partition,” as used herein, generally, refers to a space or volume that may be suitable to contain one or more species or conduct one or more reactions. A partition may be a physical compartment, such as a droplet or well. The partition may isolate space or volume from another space or volume. The droplet may be a first phase (e.g., aqueous phase) in a second phase (e.g., oil) immiscible with the first phase. The droplet may be a first phase in a second phase that does not phase separate from the first phase, such as, for example, a capsule or liposome in an aqueous phase. A partition may comprise one or more other (inner) partitions. In some cases, a partition may be a virtual compartment that can be defined and identified by an index (e.g., indexed libraries) across multiple and/or remote physical compartments. For example, a physical compartment may comprise a plurality of virtual compartments.

Droplet Processing

[0081] Provided herein are systems and methods for processing a droplet. A droplet can be processed such that an amount of a reagent within a droplet may be changed. An amount of reagent within a droplet may be changed by using the principles of mass transport. In an example, an amount of reagent may be changed through differences in the distribution of droplet sizes, for example, Ostwald ripening. In another example, the amount of reagent within a droplet may be changed through heterogeneities in chemical potential between droplets of different reagent compositions, for example, osmosis-driven transport. In another example, the amount of reagent within a droplet may be changed through solubilization of a reagent in an assembly of surfactant molecules, for example, a micelle, which may act as a carrier or mediator of one or more reagents between droplets.

[0082] The present disclosure provides a method for droplet processing. The method may comprise: providing a first droplet and a second droplet, such that the first droplet may have a first concentration of a reagent, and the second droplet may have a second concentration of the reagent. Further, the second droplet may comprise a bead and/or an analyte carrier. In some examples, an analyte carrier may comprise or be a biological particle, such as a cell or part of a cell, a biochemical entity such as a protein, peptide, or other molecule, or other types of analyte carriers, such as analyte carriers provided elsewhere herein. The method may further comprise subjecting the first droplet and the second droplet to conditions sufficient to transfer the reagent from the first droplet to the second droplet, which may decrease the first concentration in the first droplet and increase the second concentration in the second droplet. In some cases, the second droplet may comprise the bead and the analyte carrier. In some examples, the first droplet is part of a first droplet population and/or the second droplet is part of a second droplet population. In other examples, one or more droplets from the first droplet population comprise a reagent at a

first concentration and one or more droplets from the second droplet population comprise the same reagent at a second concentration. In one example, the method includes providing the first and second droplet populations with conditions sufficient to transfer the reagent between the first and second droplet populations which may change the first concentration in the first droplet population and the second concentration in the second droplet population. In another example, the conditions are sufficient to transfer the reagent from one or more droplets of the first droplet population to one or more droplets of the second droplet population which may decrease the first concentration of the reagent in the first droplet population and increase the second concentration of the reagent in the second droplet population. In other examples, the second droplet or second droplet population are initially provided such that the reagent is absent from the second droplet or second droplet population. In another example, the second droplet or second droplet population as initially provided comprise a bead and/or an analyte carrier.

[0083] The first droplet and the second droplet are examples of types of partitions as described elsewhere herein. For example, the droplet may isolate space or volume from another space or volume. The droplet may be a first phase (e.g., aqueous phase) in a second phase (e.g., oil) immiscible with the first phase. The droplet may be a first phase in a second phase that does not phase separate from the first phase, such as, for example, a capsule or liposome in an aqueous phase. A droplet may comprise one or more other (inner) partitions. In some cases, an inner partition may also generally be referred to herein as a core, or a core droplet. An outer partition, such as the area of the droplet outside the inner partition (e.g., core) may also generally be referred to herein as a shell. In some cases, different partitions of a droplet may comprise different phases. In some examples, the inner partition (e.g., core) may comprise a gel or bead. In some examples, the outer partition (e.g., shell) may comprise an aqueous solution comprising reagents and/or analyte carriers, or vice versa. A droplet may comprise several layers or partitions. For example, a droplet may comprise a core and multiple shells. Each droplet layer or partition may be generated using the methods described herein. A combination of droplet generation methods may be used.

[0084] The bead may comprise a bead as described elsewhere herein. In some cases, the bead may be a gel bead as described elsewhere herein. In some cases, the bead may be a cell bead as described elsewhere. The analyte carrier may comprise a biological particle as described elsewhere herein. In some cases, the analyte carrier may comprise a cell as described elsewhere. In some cases, the analyte carrier may be a cell bead.

[0085] The bead may comprise nucleic acid molecules coupled thereto. The nucleic acid molecules may be nucleic acid barcode molecules. In some cases, the bead may comprise a

plurality of nucleic acid barcode molecules. The plurality of nucleic acid barcode molecules comprises at least about 1,000 nucleic acid barcode molecules, at least about 5,000 nucleic acid barcode molecules, at least about 10,000 nucleic acid barcode molecules, at least about 50,000 nucleic acid barcode molecules, at least about 100,000 nucleic acid barcode molecules, at least about 500,000 nucleic acid barcode molecules, at least about 1,000,000 nucleic acid barcode molecules, at least about 5,000,000 nucleic acid barcode molecules, at least about 10,000,000 nucleic acid barcode molecules, at least about 100,000,000 nucleic acid barcode molecules, at least about 1,000,000,000 nucleic acid barcode molecules, or more. In some cases, the nucleic acid cell molecules comprise the same barcode sequence. As described elsewhere herein, a nucleic acid barcode sequence can be used to identify a cell for which species (e.g., cell nucleic acids, cell surface features) have been identified with nucleic acid molecules that have been barcoded with nucleic acid barcode sequences.

[0086] The reagent may be a reducing agent. For example, the reducing agent may be dithiothreitol (DTT) or a functional derivative thereof. For example, the reducing agent may be DTT, TCEP, etc. The reagent may dissolve the gel bead. The reagent may be a reducing agent and the bead may comprise a disulfide that is broken by the reducing agent. The reagent may dissolve the bead or may release at least a portion of the nucleic acid molecules from the bead.

[0087] In some cases, the reagent may be one or more of an oxidizing agent, an acid (e.g. a Lewis acid, a proton donor, etc.), a base (e.g. a Lewis base, a proton acceptor, etc.), a complexing agent, water, an enzyme, a protein, a ligand, one or more nucleic acid components, an oligonucleotide, a biochemical signaling agent, etc. Moreover, the reagent may be any small molecule or macromolecule, which may participate in a reaction with the bead, species associated with the bead and/or the analyte carrier. The reagent may comprise a salt or an ion thereof (e.g.: sodium ion (Na^+), magnesium ion (Mg^{2+}), potassium ion (K^+), ammonium ion (NH_4^+), chloride (Cl^-), bromide (Br^-), iodide (I^-), (F^-), etc). The reagent may comprise any molecule and/or solvent used in biochemical reactions (e.g. adenosine triphosphate (ATP), dinucleotidetriphosphates (dNTPs), dimethylsulfoxide (DMSO), dimethylformamide (DMF), etc.). The reagent may comprise any of the other reagents disclosed herein elsewhere.

[0088] The first droplet may comprise a reagent at a concentration of at least about 1 nanoMolar (nM), 2 nM, 5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM, 1 microMolar (μM), 2 μM , 5 μM , 10 μM , 20 μM , 50 μM , 100 μM , 200 μM , 500 μM , 1 mM (milliMolar), 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1 M (Molar), or more. The first droplet may comprise a reagent at a concentration of at most about 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM, 1 μM , 2 μM , 5 μM , 10 μM , 20 μM , 50 μM , 100

μM , 200 μM , 500 μM , 1 mM (milliMolar), 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1 M (Molar), 2 M, 3 M, 4 M, 5 M, 6 M, 7 M, 8 M, 9 M, 10 M, 50 M, 100 M or more.

[0089] The second droplet may comprise a reagent at a concentration of at least about 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM, 1 μM , 2 μM , 5 μM , 10 μM , 20 μM , 50 μM , 100 μM , 200 μM , 500 μM , 1 mM (milliMolar), 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1 M (Molar), or more. The second droplet may comprise a reagent at a concentration of at most about 1 nM, 2 nM, 5 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM, 1 μM , 2 μM , 5 μM , 10 μM , 20 μM , 50 μM , 100 μM , 200 μM , 500 μM , 1 mM (milliMolar), 2 mM, 5 mM, 10 mM, 20 mM, 50 mM, 100 mM, 200 mM, 500 mM, 1 M (Molar), 2 M, 3 M, 4 M, 5 M, 6 M, 7 M, 8 M, 9 M, 10 M, 50 M, 100 M or more. In some cases, the second droplet may not contain the reagent. A case where the second droplet does not contain the reagent the reagent may be at a concentration of less than or equal to 1 nM, 100 picoMolar (pM), 10 pM, 1 pM, 100 femtoMolar, or less.

[0090] In some cases, the first concentration of the reagent in the first droplet is greater than the second concentration of the reagent in the second droplet. However, in some cases, the first concentration and the second concentration may be substantially the same to within at least about 1%, 2%, 5%, 10%, 20%, or more. In other cases, the second concentration of the reagent in the second droplet is greater than the first concentration of the reagent in the first droplet.

[0091] A method of processing a droplet may further comprise, prior to providing a first and a second droplet, generating the first droplet or the second droplet with the aid of a microfluidic device or with the aid of agitation of a mixture of immiscible phases. For example, microfluidic channel networks (e.g., on a chip) can be utilized to generate droplets as described herein. For example, the first droplet and the second droplet may comprise droplets in an emulsion as described elsewhere herein. The first droplet and the second droplet may comprise droplets in a colloid. In some cases, the emulsion may comprise a microemulsion or a nanoemulsion.

[0092] The first droplet and the second droplet may be formed by creating an emulsion by mixing or agitating immiscible phases. Mixing or agitation may comprise various agitation techniques, such as vortexing, pipetting, tube flicking, or other agitation techniques. In some cases, mixing or agitation may be performed without using a microfluidic device. In some examples, the first droplet and the second droplet may be formed by exposing a mixture to ultrasound or sonication.

[0093] In some cases, a combination of droplet generation techniques may be used. For example, droplets may comprise an inner partition (a core) and an outer partition (a shell). The

core droplet (the inner droplet) can be generated with the aid of a microfluidic device while the shell droplet (the outer droplet) may be generated with the aid of agitation of the mixture comprising the core droplets, or vice versa. Alternatively, both the core and the shell droplets may be generated using an agitation technique, or both the core and the shell droplets may be generated using a microfluidic device.

[0094] The first and the second droplet may comprise droplets in a medium, such as an emulsion or colloid. The medium may comprise a fluid which may comprise different physical properties than a fluid within a droplet, such as solubility, miscibility, viscosity, phase, etc. In an example, the fluid inside the droplet comprises an aqueous solution and the medium comprises an oil. The medium may comprise a continuous phase of an emulsion. In cases in which the medium comprises a continuous phase of an emulsion, the continuous phase may comprise an oil. In some cases, the medium may comprise a fluid which is a continuous phase in which the first droplet is dispersed. In some cases, the medium may comprise a fluid which is a continuous phase in which the second droplet is dispersed. In some cases, the fluid can comprise an oil, such as fluorinated oil, that includes a fluorosurfactant for stabilizing droplets, for example, inhibiting subsequent coalescence of the resulting droplets. In some cases, however, droplets can be prepared such that they can be fused or merged.

[0095] The medium comprising the droplet may comprise a fractionation. For example, one fraction of the medium may comprise a greater number of droplets than another fraction. In another example, the medium may comprise an oil fraction, which comprises predominately oil but may comprise some droplets. The medium may comprise an aqueous fraction, which comprises predominately water but may comprise some droplets. The medium may comprise a plurality of fractions which may differ by, for example, phase transition, droplet concentration, miscibility of various types of fluids, viscosity, solubility, etc.

[0096] In some cases, the method further comprises subjecting the first droplet to conditions sufficient to transfer the reagent out of the first droplet and into a fluid. In some cases, the method may comprise subsequently providing conditions sufficient to transfer the reagent from the fluid into the second droplet. **FIG. 10, FIG. 11A, and FIG. 11B** show example modes of transferring a reagent from a first droplet into a fluid and, subsequently, from fluid into a second droplet. In some cases, providing conditions sufficient to transfer the reagent out of the first droplet and into the second droplet comprises a mode of transferring a reagent from a first droplet to a second droplet.

[0097] **FIG. 10** shows an example mode of transferring a reagent from a first droplet to a second droplet using a mediator. As shown, the medium **1002** may comprise a first droplet

1010, a second droplet **1020**, and a mediator **1030**. In the illustrated example, a reagent **1004** is indicated with stars. As shown, first droplet **1010** may comprise a surfactant **1012** comprising a surfactant head **1014** and a surfactant tail **1016**. The interior of first droplet **1010** may comprise a fluid **1018**. The fluid **1018** may comprise a fluid with different physical properties than the medium **1002** such as viscosity, density, composition, and/or other properties such as with respect to each other (e.g., solubility, miscibility, etc.). The fluid **1018** in the interior of the droplet (e.g., aqueous phase) may be substantially immiscible and/or substantially insoluble in the fluid medium (e.g., organic or oil phase). Alternatively, in some cases, the fluid **1018** in the interior of the droplet (e.g., aqueous phase) may be partially soluble in the fluid medium **1002**. In some cases, it may be preferred to minimize the solubility of the fluid **1028** in the fluid medium **1002**. In some cases, the fluid **1018** is an aqueous phase. Fluid **1018** may comprise a first concentration of reagent **1004**. As shown, second droplet **1020** may comprise a surfactant **1022** comprising a surfactant head **1024** and a surfactant tail **1026**, which surfactant **1022** may be the same as surfactant **1012**. The interior of second droplet **1020** may comprise a fluid **1028**. In some cases, the fluid **1028** is an aqueous phase. The fluid **1028** may comprise a fluid with different physical properties than the medium **1002**, such as density, viscosity, composition and/or other properties such as with respect to each other (e.g., solubility, miscibility, etc.). The fluid **1018** in the interior of the droplet (e.g., aqueous phase) may be substantially immiscible and/or substantially insoluble in the fluid medium **1002** (e.g., organic or oil phase). Alternatively, in some cases, the fluid **1018** in the interior of the droplet (e.g., aqueous phase) may be partially soluble in the fluid medium **1002**. In some cases, it may be preferred to minimize the solubility of the fluid **1028** in the fluid medium **1002**. Fluid **1028** may comprise a first concentration of reagent **1004**. In some cases, the reagent may be transferred from the first droplet to the second droplet using a mediator derived from the first droplet. In some cases, the mediator accepts the reagent from the first droplet, for example, via diffusion or by budding of a mediator from the first droplet. The mediator comprising the reagent may then transfer the reagent from the mediator to the second droplet. The mediator may be absorbed by the second droplet. The reagent may diffuse from the mediator to the second droplet.

[0098] In some examples, the mediator is a droplet component that is configured to transfer a reagent from the droplet containing the mediator away from the droplet. In another example, the transfer of the reagent comprises a transfer away from a first droplet to a second droplet. In one example, prior to the transfer of the reagent away from the first droplet, the reagent is absent from second droplet. The first droplet may be part of a first droplet population and the second droplet may be part of a second droplet population.

[0099] As shown in **FIG. 10**, the mediator **1030** may be a micelle. In another example, the mediator may comprise a liposome. The mediator may comprise a molecular assembly, which may aid in the transport of a reagent. The molecular assembly may comprise a “cage” of molecules around the reagent. The mediator may comprise a surfactant **1032** comprising a surfactant head **1034** and a surfactant tail **1036**, which may be the same surfactant as surfactant **1012** and/or **1022**. The surfactant may comprise a molecule with a head and a tail which comprise different solubility. The surfactant head may be more soluble in a fluid in the interior of the micelle, and the surfactant tail may be more soluble in medium **1002**. In an example, the solubility of the head may be greater in water and a tail may be more soluble in the oil. A mediator surfactant may comprise the same surfactant as the first and/or the second droplet. The mediator may be formed from the first of the second droplet. A micelle may comprise an aggregate of surfactant molecules dispersed in a fluid. The micelle or liposome may be spherical, may comprise ellipsoids, may be cylindrical, may be bilayers, etc. The size and shape of the micelle may be changed by adjusting surfactant and/or fluid conditions such as pH, temperature, surfactant concentration in the fluid, solute concentration in the fluid, reagent concentration in the fluid, ionic strength of the fluid, etc.

[00100] **FIG. 11A** and **FIG. 11B** show two additional example modes of transferring a reagent from a first droplet to a second droplet, which may underlie transfer using mediators as in **FIG. 10**. In a first example shown in **FIG. 11A**, the droplet may undergo Ostwald ripening. Ostwald ripening is a thermodynamically-driven process that energetically can favor larger droplets over smaller droplets. The molecules on the surface of a droplet may comprise a higher chemical potential for smaller droplets than for larger droplets, which may drive the fusion of droplets. In one example, as shown in **FIG. 11A**, at a time t_1 a medium **1102** may comprise droplets **1112**, **1114**, and **1116** of varying sizes. At a later time t_2 the medium **1102** may comprise droplets **1122** and **1124**. As shown, the average size of the droplet population at time t_2 may be larger than at time t_1 . A method of processing a droplet may further comprise, during or after providing conditions sufficient for transfer, the first droplet decreasing in size or the second droplet increasing in size. The first droplet may increase or decrease in size via Ostwald ripening.

[00101] In a second example shown in **FIG. 11B**, the reagents may be transferred between droplets via osmotically driven transport. In this example, the size of an individual droplet may remain substantially the same; however, the concentration of reagent within an individual droplet may change. The wall of the droplet may act as a semipermeable membrane. The wall of the droplet may, for example, comprise a surfactant as described elsewhere herein. As shown in

FIG. 11B, at a time t_1 a medium **1102** may comprise droplets **1112** and **1114** which may comprise differing concentrations of a reagent **1104**. In the illustrated example, droplets **1112** comprise a greater concentration of reagent **1104** than droplets **1114**. The chemical potential of droplets **1112** may be greater than droplets **1114** due to the higher concentration of the reagent. The reagent may be transferred via diffusion. In some cases, in order to reduce the gradient in the chemical potential, it may be thermodynamically favorable for reagent molecules to diffuse through medium **1102** from droplets **1112** to droplets **1114**. In another example, it may be thermodynamically favorable for a fluid inside of droplets **1114** to diffuse to droplets **1112** through the medium. At a later time t_2 the medium **1102** may comprise droplets **1122**. As shown, the average concentration of the reagent in the droplet population at time t_2 may be closer to equilibrium than at time t_1 . In cases where the reagent is insufficiently soluble in the medium **1102**, the reagent may diffuse from one droplet to another droplet with the aid of a mediator such as a micelle. In cases where the reagent is sufficiently soluble in the medium, the reagent may diffuse through the medium without a mediator.

[00102] **FIG. 12A** and **FIG. 12B** show example methods of processing a droplet. In both methods, a first droplet and a second droplet may be provided. The first droplet **1202** and the second droplet **1212** may comprise droplets in a first population of droplets **1204** and a second population of droplets **1214**, respectively. As shown, a first population of droplets **1204** and a second population of droplets **1214** may be provided. The first droplet population may comprise a first droplet fraction and a first fluid fraction **1206**, and the second droplet population may comprise a second droplet fraction and a second fluid fraction **1216**. The first droplet population may comprise the reagent at a first concentration. The first droplet fraction may comprise droplets which comprise the reagent at a first concentration as described herein. In some cases, the first fluid fraction comprises micelles **1208** which may comprise the reagent. The second droplet population may not comprise the reagent. In some cases, the second droplet population may comprise the reagent at a concentration less than the first droplet population. In some cases, the second droplet fraction may not comprise the reagent. In some cases, the second droplet fraction comprises droplets comprising a reagent at a second concentration less than the concentration of the first droplet fraction. The second droplet fraction may comprise droplets comprising the reagent at a second concentration as described herein. In some cases, the second fluid fraction comprises micelles **1218** which may not comprise the reagent. In another case, the second fluid fraction comprises micelles which comprise the reagent and which can be at a concentration less than the first fluid fraction.

[00103] In some cases, the first droplet population and the second droplet population are provided in a first container and a second container respectively. In another case, the first droplet population and the second droplet population may be provided via streams or channels in a microfluidic device as described elsewhere herein. The first and the second droplet populations may be provided via a variety of different fluid sources or receiving components, including reservoirs, tubing, manifolds, or fluidic components of other systems as described elsewhere herein.

[00104] **FIG. 12A** shows an example of a method for processing a droplet. As shown, a first population of droplets **1204** and a second population of droplets **1214** may be provided. The first and the second population may comprise different concentrations of reagents as described herein. Subsequently, the droplets **1202** from the first population may be added to droplets **1212** from the second population to form a mixture **1234**. The mixture may comprise a fluid fraction **1236**, which may contain micelles **1238**. The mixture may comprise droplets **1232** (e.g., droplets to which reagent is/was transferred). The reagent **1242** may be transferred from the second population to the first population, such as via a mediator. The reagent may be transferred from the first droplet to the second droplet within the mixture via diffusion, such as diffusion of the reagents from the first droplet to the second droplet. Such diffusion may be facilitated by the micelle **1208**. In some cases, alternatively or in addition to diffusion, other forms of mass transfer or transport phenomena such as convection, heat-mediated mass transfer, turbulent mass transfer (e.g., caused by mixing, agitation, vortexing, shear stress, and/or fluid flow), and/or other forms of mass transfer may also be involved.

[00105] In some cases, a method of processing a droplet may additionally comprise, generating a mixture comprising the first droplet and the second droplet, after providing a first droplet and a second droplet. The reagent may be transferred from the first droplet to the second droplet within the mixture. The reagent may be transferred from the first droplet to the second droplet by any mode described herein.

[00106] **FIG. 12B** shows another example method for processing a droplet. In the illustrated example, the fluid fraction **1206** from the first droplet population may be combined with droplets from the second population **1212** to form a mixture **1234**. The mixture may comprise a fluid fraction **1236**, which may contain micelles **1238**. The mixture may comprise droplets **1232** (e.g., droplets to which reagent is/was transferred). In another example, the fluid fraction from the second droplet population **1214** may be combined with droplets from the first population **1204** to form a mixture **1234**. The fluid from the fluid fraction may comprise the reagent, and the reagent may be transferred from the fluid fraction to the droplet population. In some cases, a

method of processing a droplet may additionally comprise providing the first droplet in a first container comprising a fluid and providing the second droplet in a second container. The method may further comprise transferring the fluid from the first container to the second container. The fluid fraction **1206** can include micelles **1208** that transfer a reagent from the first droplet population **1202**, which then transfer the reagent to the second population of droplets **1212**.

[00107] **FIG. 13** shows an example method of processing a droplet using a gel bead and a reducing agent. In the inset of **FIG. 13**, a reaction showing the interaction of DTT with a bead **1302** is shown. As shown, the bead **1302** may comprise molecules **1304** bound to the surface of the bead via a disulfide bond. The molecules may be nucleic acid molecules bound to the surface of the bead. The bead may comprise nucleic acid barcode molecules bound to the surface of the bead. The reagent may be a reducing agent, and the bead may comprise a disulfide that is broken by the reducing agent. In some examples, the reagent may also dissolve the bead (e.g., breaking disulfide bonds of the bead as described elsewhere herein) and/or may release at least a portion of the nucleic acid molecules from the bead. In some cases, the reagent may dissolve a gel bead. For example, the reducing agent may be dithiothreitol (DTT), a functional derivative thereof or any other suitable reducing agent including those described elsewhere herein.

[00108] **FIG. 13** also shows an example microfluidic device showing a plurality of beads in an aqueous phase of a channel. In some cases, the beads may be provided via a variety of different fluid sources or receiving components, including reservoirs, tubing, manifolds, or fluidic components of other systems. As shown, the beads may comprise different molecules bound to the surface of the bead. For example, a first bead **1302** may comprise a molecule of a first type (e.g., a first nucleic acid barcode molecule having a first barcode sequence) and a second bead **1306** may comprise a molecule of a second type (e.g., a second nucleic acid barcode molecule having a second barcode sequence). From a first channel **1327** of the device, a reagent, such as a reducing agent (for example, DTT, TCEP, etc.), may be added to the beads, forming an aqueous mixture. As the reagent is added to the channel **1327**, the reagent may begin reacting with the beads. In some examples, the reagent begins reducing the bonds connecting the surface molecules to the beads. At a second channel **1337** of the device, the resulting aqueous mixture is contacted with an immiscible phase, such as an oil (for example a fluorinated oil may be added to the channel), to form droplets. **FIG. 13** shows an enlarged illustration of the droplet population in the channel. The enlarged illustration shows droplets with varying compositions. The varying droplet conditions may comprise different reaction products. A droplet **1308** may

comprise a bead of a first type and an unbound surface molecule of a second type. A droplet **1310** may comprise a bead of a second type and an unbound surface molecule of a first type. A droplet **1312** may comprise a bead of a first type and an unbound surface molecule of a first type. A droplet **1314** may comprise a bead of a second type and an unbound surface molecule of a second type.

[00109] As shown in **FIG. 13**, droplet processing in this example can result in a plurality of different types of droplets after the addition of reagent, such as DTT. For example, droplets **1308** and **1310** may comprise a bead comprising bound surface molecules which are different than unbound surface molecules with the droplet. In some cases, the droplet resulting from the method of **FIG. 13** may result in droplets with a plurality of types of barcode molecules within a single droplet.

[00110] In some cases, methods described herein can be used to generate droplets that include molecules that are derived from a single bead and, thus, are of a single type (e.g., nucleic acid barcode molecules each having the same barcode sequence). Different from the example of **FIG. 13**, **FIG. 14** shows another example implementation of the method of processing a droplet using a bead and a reagent. **FIG. 14** shows an example microfluidic device transporting a plurality of beads in an aqueous phase of a channel. In some cases, the beads may be provided via a variety of different fluid sources or receiving components, including reservoirs, tubing, manifolds, or fluidic components of other systems. As shown, the beads may comprise different molecules bound to the surface of the bead. For example, a first bead **1402** may comprise a molecule of a first type (e.g., a first nucleic acid barcode molecule having a first barcode sequence) and a second bead **1404** may comprise a molecule of a second type (e.g., a second nucleic acid barcode molecule having a second barcode sequence). At a channel **1437** of the microfluidic device, the aqueous phase is contacted with an immiscible phase, such as an oil (for example a fluorinated oil may be added to the channel), to form droplets. **FIG. 14** shows an enlarged illustration of the droplet population in the channel. The enlarged illustration shows droplets with varying compositions. A droplet **1406** may comprise a bead of a first type. A droplet **1408** may comprise a bead of a second type.

[00111] **FIG. 14** also shows the methods of **FIG. 12A** and **FIG. 12B** in conjunction with the formation of droplets shown at the top of **FIG. 14**. A first droplet population **1410** may comprise a reagent, such as a reducing agent. In some examples, the reagent is DTT. A second droplet population **1412** may comprise the droplet population of the microfluidic device above. At an arrow **1414**, droplets from the first population may be added to droplets of the second population, similar to the example method shown in **FIG. 12A**. At an arrow **1416**, liquid (e.g., a

fluid fraction) from the first droplet population may be added to the second droplet population, similar to the example method shown in **FIG. 12B**. The reagent may transfer to the second droplet via a mode of transferring described herein. As the fluid and/or droplets are added, the reagent may begin reacting with the beads. In some examples, the reagent begins reducing the bonds connecting the surface molecules to the beads. **FIG. 14** shows an enlarged illustration of the droplet population. The enlarged illustration shows droplets with varying compositions. The varying droplet conditions may comprise different reaction products. A droplet **1418** may comprise a bead of a first type and an unbound surface molecule of a first type. A droplet **1420** may comprise a dissolved bead of a first type and an unbound surface molecule of a first type. A droplet **1422** may comprise a bead of a second type and an unbound surface molecule of a second type. A droplet **1424** may comprise a dissolved bead of a second type and an unbound surface molecule of a second type. The resulting droplets may comprise reaction products of a substantially similar type.

[00112] As shown in **FIG. 14** droplet processing in this example can generate droplets comprising reaction products of a substantially similar type after the addition of a reagent, such as DTT. For example, droplets **1418** and **1422** may comprise a bead comprising bound surface molecules which are of the same type as the unbound surface molecules within the droplet. In some cases, droplet processing in this example may result in droplets comprising surface molecules of the same type within the droplet, such as droplets **1418**, **1420**, **1422**, and **1424**. In some cases, droplet processing in this example can generate droplets with a single type of barcode molecule (e.g., barcode molecules having the same barcode sequence) within the droplet.

[00113] Methods for droplet processing described herein can be implemented with droplets comprising any suitable species and can also be implemented to initiate reactions of any type. For example, droplets may comprise labelling agents (e.g., antibodies) coupled to nucleic acid barcode molecules, with a barcode sequence that identifies it associated antibody, via labile bonds (e.g., disulfide bonds) as is described in U.S. Patent Publication No. 2015/0376609, U.S. Patent Application No. 15/720,085 and PCT Application No. PCT/US2017/068320, each of which documents is herein incorporated by reference for all purposes. Methods described herein can be used to transfer reagents from droplets comprising a reagent that breaks the labile bond, initiating release of the nucleic acid barcode molecules from the labelling agent. For example, reducing agents from a first population of droplets may be transferred to a second population of droplets each comprising labelling agents linked to nucleic acid barcode molecules coupled to the labelling agents. Transfer of the reducing agents to the second population of droplets initiates

release of nucleic acid barcode molecules from the labelling agents. Such released nucleic acid barcode molecules can then participate in barcoding reactions as is described elsewhere herein and in U.S. Patent Publication No. 2015/0376609, U.S. Patent Application No. 15/720,085 and PCT Application No. PCT/US2017/068320. Accordingly, the second droplet may comprise an antibody coupled to a nucleic acid molecule and, in some cases, the reagent may be capable of breaking a linkage between the antibody and the nucleic acid molecule.

[00114] The present disclosure provides a method for droplet processing. The method comprises: (a) providing a first droplet population and a second droplet population, wherein droplets from the first droplet population have a first concentration of a reagent and droplets from the second droplet population have a second concentration of the reagent, and wherein the droplets from the second droplet population comprise a bead or an analyte carrier; and (b) providing the first droplet population and the second droplet population with conditions sufficient to transfer the reagent between the first and the second droplet populations, thereby changing the first concentration in the first droplet population and the second concentration in the second droplet population.

[00115] In some examples, the method further comprises, in (a), the reagent is absent from the second droplet. In some examples, the reagent is selected from the group consisting of a reducing agent, sodium ion (Na^+), magnesium ion (Mg^{2+}), potassium ion (K^+), ammonium ion (NH_4^+), chloride (Cl^-), bromide (Br^-), iodide (I^-), fluoride (F^-), adenosine triphosphate (ATP), dinucleotide triphosphate (dNTP), dimethyl sulfoxide (DMSO), and dimethylformamide (DMF). In some examples, the reducing agent is dithiothreitol or a functional derivative thereof. In some examples, the first droplet population comprises a first droplet fraction and a first fluid fraction and wherein the second droplet population comprises a second droplet fraction and a second fluid fraction. In some examples, the first or the second fluid fraction comprises a mediator for reagent transfer.

[00116] In some examples, in (b), the reagent is transferred from the first droplet population to the second droplet population via the mediator. In some examples, the mediator is derived from the first droplet population. In some examples, the mediator comprises a surfactant. In some examples, the mediator is a micelle. In some examples, (b) comprises providing the first droplet population with conditions sufficient to transfer the reagent from the first droplet fraction to the first fluid fraction, and providing the second droplet population with conditions sufficient to transfer the reagent from the first fluid fraction to droplets from the second droplet population. In some examples, the first fluid fraction is a continuous phase in which the first droplet population is dispersed. In some examples, the continuous phase comprises an oil. In some

examples, the first droplet population is provided in a first container comprising the first fluid fraction and the second droplet population is provided in a second container, and wherein (b) further comprises transferring the first fluid fraction from the first container to the second container.

[00117] In some examples, the method further comprises, subsequent to (a), generating a mixture comprising the first droplet population and the second droplet population, wherein the reagent is transferred from the first droplet population to the second droplet population within the mixture. In some examples, the reagent is transferred from the first droplet population to the second droplet population within the mixture via diffusion. In some examples, the bead has nucleic acid molecules coupled thereto. In some examples, the nucleic acid molecules are nucleic acid barcode molecules. In some examples, the reagent dissolves the bead or releases at least a portion of the nucleic acid molecules from the bead. In some examples, the bead is a gel bead. In some examples, the reagent dissolves the gel bead. In some examples, the reagent is a reducing agent and the bead comprises a disulfide bond that is broken by the reducing agent. In some examples, the analyte carrier is a cell. In some examples, the analyte carrier is a cell bead. In some examples, the droplets from the second droplet comprise the bead and the analyte carrier.

[00118] In some examples, in (a), the first concentration is greater than the second concentration. In some examples, the droplets from the second droplet population comprise an antibody coupled to a nucleic acid molecule. In some examples, the reagent is capable of breaking a linkage between the antibody and the nucleic acid molecule. In some examples, during or subsequent to (b), the droplets from the first droplet population decrease in size or the droplets from second droplet population increase in size. In some examples, prior to (a), the droplets from the first droplet population or the droplets from the second droplet population are generated using a microfluidic device. In some examples, prior to (a), the first droplet population or the second droplet population is generated upon agitation of a mixture of immiscible phases.

[00119] Provided herein are methods for formation of a droplet population comprising a bead. For example, a droplet population may comprise droplets each comprising a single bead. The bead may be any bead described herein, such as a gel bead. In some cases, the gel bead may be a gel droplet in a continuous phase, such as an oil. The bead may be a bead from a population of beads. The population of beads may be monodisperse. For example, the population of beads may have substantially uniform size and/or shape. In some instances, the bead may be substantially spherical. In other instances, the bead may comprise other shapes and forms. In

some cases, the beads may be generated using a microfluidic device or other methods described herein. Alternatively, beads may be generated without using a microfluidic device. The method may comprise, prior to providing a first droplet population and a second droplet population, wherein the first droplet population comprises a first concentration of a reagent and the second droplet population comprises a second concentration of the reagent different from the first concentration of the reagent, as described elsewhere herein, generating the second droplet population from (i) a first fluid volume comprising a population of beads and a population of analyte carriers and (ii) a second fluid volume immiscible with the first fluid volume. In some cases, the second droplet population may be generated by applying energy to the first fluid volume, the second fluid volume, and/or both the first and the second fluid volumes. Energy may be mechanical energy. Energy may be provided in the form of agitation, such as vortexing. Energy may be applied in the form of rotation force, mixing, agitation, pulse of energy, shock, stress, shear stress, or other forms of force, stress, or energy. Energy may comprise energy from sound, light, fluid flow, heat, chemical energy, or other types of energy. For example, energy may comprise sonication and/or ultrasound.

[00120] The method may comprise generating the second droplet population in absence of using a microfluidic device. In some cases, not using a microfluidic device may simplify the method of generating droplets, and therefore may be less expensive. Generating droplets in absence of using a microfluidic device may be performed with simplified lab equipment and hardware, as well as less specialized operator skills. The method may be capable of being translated and implemented in simple laboratories.

[00121] A droplet from the second droplet population may comprise the bead from the population of beads and the analyte carrier from the population of analyte carriers. A droplet from the second droplet population may comprise a single bead from the population of beads. A droplet from the second droplet population may comprise a single analyte carrier, such as a single cell, from the population of analyte carriers.

[00122] In some cases, the method may comprise applying a stimulus, such as a form of energy to the mixture. The stimulus and/or energy applied to the first fluid volume and the second fluid volume may comprise mixing and/or agitation. For example, applying the stimulus and/or energy to a first fluid volume and a second fluid volume may comprise mixing and/or agitating the first fluid and the second fluid in a container. In some cases, the second droplet population may be generated by providing a mixture comprising the first fluid volume and the second fluid volume and agitating the mixture. The container may be a tube, a test tube, a PCR tube, a beaker, a flask, a substrate, a box, or other container of any type, size, or scale. Agitating

and/or mixing may comprise various mixing techniques, such as vortexing, tube flicking, pipetting, other mixing techniques, and combinations thereof.

[00123] The first fluid volume may comprise an aqueous fluid. The second fluid volume may comprise an oil. The first fluid volume and the second fluid volume may comprise any pair of immiscible fluids.

[00124] In some examples, the first fluid volume may not contain the reagent or have minimal or negligible concentrations of the reagent. In some examples, the first fluid volume may have any concentration of the reagent.

[00125] In some examples, the droplet may comprise a single bead and a single analyte carrier. In some cases, the analyte carrier may be a cell, a cell bead, or a cell nucleus. In some cases, the analyte carrier may be or comprise any analyte, such as a nucleic acid molecule.

[00126] In some cases, the method may comprise adding the first fluid comprising a plurality of beads and a plurality of analyte carriers to a container, such as a tube. In some instances, the container may be a centrifuge tube. In some instances, the first fluid may further comprise a first concentration of a reagent. The first concentration may be relatively low or close to zero. For example, the first fluid may have no, or negligible concentrations of, the reagent. In some cases, an excess amount of liquid (e.g., aqueous phase) in the first fluid may be present in the container. Optionally, any amount of excess liquid is removed from the container prior to adding in the second fluid to the container. The removal of the excess liquid from the container may contribute to optimizing subsequent droplet generation, such as for example, fewer resulting small and/or empty droplets. For example, excess liquid may be removed using a pipette or other device. The second fluid may be added to the container. The second fluid may comprise oil or other fluid that is immiscible with the first fluid. In some cases, the second fluid may comprise a surfactant. In some cases, the first fluid may further comprise a surfactant. The mixture of the first fluid and the second fluid may be mixed and/or agitated, or otherwise stimulated. Agitation may comprise vortexing. Agitation may be uniform or substantially uniform. Agitating the mixture may emulsify the mixture, thereby compartmentalizing the first fluid into droplets in a continuous fluid (e.g., the second fluid).

[00127] In some cases, the resulting droplet population may comprise droplets of similar size or substantially similar size. Droplets may be monodisperse or substantially monodisperse. In some cases, droplets may be generated in a variety of sizes and not be monodisperse. Generating monodisperse droplets may be favorable for downstream operations (e.g., single cell assays), such as by improving the likelihood of partitioning single analyte carriers in the droplets. The presence of the population of beads in the first fluid may contribute to generating monodisperse

droplets. In some cases, at least a portion of the droplets may each comprise a single bead. Some droplets may be empty. Empty droplets may be smaller than droplets comprising beads, and therefore may contain smaller sample amounts. Other droplets may comprise more than one bead. Beads may act as templates during droplet formation to generate monodisperse droplets. The method may comprise generating droplets without using a microfluidic device. Beads may be rigid, semi-rigid, or substantially rigid. Beads (or particles) may resist droplet breakup below the bead size.

[00128] In some cases, droplets may be generated without using beads. In such examples, the first fluid may not comprise beads. A first fluid comprising analyte carriers and, in some cases, further comprising a reagent, may be added to a container. The second fluid may be further added to the container. The mixture may be mixed and/or agitated for example by vortexing. The first fluid may be compartmentalized as a dispersed phase in the continuous second fluid. In some cases, droplet populations generated using a first fluid volume comprising the beads may be more monodisperse than droplet populations generated using fluid volumes without the beads described herein.

[00129] Mixing speed, such as vortex speed, and mixing duration may be determined and adjusted as necessary. For example, a mixing duration of at least about 30 seconds (s), 40 s, 50 s, 60 s, 70 s, 80 s, 90 s, 100 s, or more may be used. Alternatively or in addition, the mixing duration may be at most about 100 s, 90 s, 80 s, 70 s, 60 s, 50 s, 40 s, 30 s, or less. For example, a vortex speed of at least about 2000 revolutions per minute (rpm), 2100 rpm, 2200 rpm, 2300 rpm, 2400 rpm, 2500 rpm, 2600 rpm, 2700 rpm, or other vortex speeds may be used. In some cases, a vortex speed may be at most about 2000 revolutions per minute (rpm), 2100 rpm, 2200 rpm, 2300 rpm, 2400 rpm, 2500 rpm, 2600 rpm, 2700 rpm, or less. For example, the mixture may be vortexed at 2300 rpm for 30 s. The time to reach the final droplet size and the monodispersity of the emulsion may depend on fluid and/or bead properties. For example, the self-affinity and size of the beads may affect the droplet generation results. Fluid density, viscosity and interfacial tension may affect droplet generation. In some cases, the mixing duration may be independent of total mixture volume. This may be beneficial to the scalability of the process.

[00130] Beads may be introduced into the droplets by different approaches, such as the methods described elsewhere herein. In some examples, the method may further comprise generating the population of beads. The method may comprise applying a stimulus to a third fluid volume and a fourth fluid volume to generate the population of beads. The population of beads may comprise droplets in an emulsion (e.g., gel droplets in a continuous phase). In some

examples, a population of beads may be generated using similar methods for generating droplets as described herein. For example, the population of beads may be generated with the aid of a microfluidic device and/or by applying a stimulus, such as agitation, such as vortexing, to generate the beads. The population of beads may be further encapsulated in droplets using any of the methods described herein (e.g., by applying stimulus and/or energy, using a microfluidic device, etc.).

[00131] In some examples, the method may further comprise generating the population of analyte carriers. In some cases, the population of analyte carriers may comprise a plurality of transposed nuclei. The method may further comprise subjecting a population of cell nuclei to transposition in bulk to yield the plurality of transposed nuclei. The method may further comprise mixing the plurality of transposed nuclei and beads to provide the first fluid. In some instances, the beads (e.g., gel beads) may comprise one or more nucleic acid barcode molecules coupled thereto. The first fluid may comprise transposed nuclei. The first fluid may further comprise one or more biochemical reagents. The first fluid may further comprise beads. The first fluid comprising the transposed nuclei, biochemical reagent(s), and beads may be added to a container such as a tube. In some cases, excess liquid in the first fluid may be removed from the container. The mixture may be agitated by, for example, vortexing. Agitation may emulsify the mixture to generate droplets. Generated droplets may comprise transposed nuclei. Generated droplets may further comprise beads, such as barcoded beads comprising one or more nucleic acid barcode molecules coupled thereto as described elsewhere herein. Some generated droplets may comprise transposed nuclei and beads. Some generated droplets may each comprise a single transposed nucleus and a single bead.

[00132] The method may further comprise, subsequent to subjecting the first droplet population and the second droplet population to conditions sufficient to transfer the reagent between the first droplet population and the second droplet population, using a nucleic acid molecule derived from the analyte carrier and a nucleic acid barcode molecule coupled to the bead to generate a barcoded nucleic acid molecule. For example, the first fluid may comprise transposed nuclei, one or more biochemical reagents, and a plurality of barcoded beads. Agitation may emulsify the mixture to generate droplets. At least a subset of the generated droplets may comprise transposed nuclei and barcoded beads.

[00133] The method may further comprise subjecting the first droplet population and the second droplet population to conditions sufficient to transfer the reagent between the first droplet population and the second droplet population. In some cases, the reagent may be a reducing agent. In some examples, the reagent may be dithiothreitol (DTT). The reagent may be

transported between the first droplet population and the second droplet population using the methods described elsewhere herein, such as with the aid of a mediator, such as micelle. The transfer of DTT to the droplet may dissolve the beads. The transfer of a reducing agent (e.g., DTT) to the droplet may dissolve the beads and/or facilitate release of nucleic acid molecules coupled thereto in the droplets. In some cases, the reagent may comprise a lysis agent. The transfer of a lysis agent to the droplet may facilitate lysis of the analyte carrier and/or release of a nucleic acid molecule (or derivative thereof) from the analyte carrier. Droplets may comprise barcoded analyte carriers. Droplet contents may be subjected to amplification, such as for example, linear amplification. The method may further comprise removing the oil from the container. The method may further comprise sequencing the barcoded nucleic acid molecule, or derivative thereof.

[00134] The disclosure provides a method for nucleic acid processing, comprising: (a) agitating (i) a first fluid volume comprising a population of beads and a population of analyte carriers and (ii) a second fluid volume immiscible with the first fluid volume, to generate a plurality of droplets. The population of beads may comprise a plurality of nucleic acid barcode molecules. The population of analyte carriers may comprise a plurality of nucleic acid molecules. A first droplet from the plurality of droplets may comprise a bead from the population of beads and an analyte carrier from the population of analyte carriers. The bead may comprise a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules, and the analyte carrier may comprise a nucleic acid molecule of the plurality of nucleic acid molecules. The first droplet may have a first concentration of a reagent. The reagent may be configured to release the nucleic acid barcode molecule or release the nucleic acid molecule in the droplet. The method may further comprise (b) subjecting the first droplet and a second droplet having a second concentration of the reagent to conditions sufficient to transfer the reagent between the first droplet and the second droplet, thereby changing the first concentration of the reagent in the first droplet and the second concentration of the reagent in the second droplet; and (c) generating a barcoded nucleic acid molecule using the nucleic acid barcode molecule and the nucleic acid molecule in the first droplet.

[00135] In some examples, the agitating comprises mixing and/or agitating using techniques such as vortexing, tube flicking, pipetting, or other techniques.

[00136] The first fluid volume may comprise an aqueous fluid and the second fluid volume may comprise an oil.

[00137] In some examples, the first fluid volume may not include the reagent. In some cases, the first droplet comprises a single bead and a single analyte carrier. The analyte carrier may be a

cell. The reagent may be a lysis reagent. The reagent may lyse the cell. The analyte carrier may be a cell bead. The analyte carrier may be a cell nucleus.

[00138] The population of analyte carriers may comprise a plurality of transposed nuclei. The method may comprise subjecting a population of cell nuclei to transposition in bulk to yield the plurality of transposed nuclei. The method may further comprise sequencing the barcoded nucleic acid molecule, or a derivative thereof.

[00139] In some example, the reagent may comprise a reducing agent, lysis agent, sodium ion (Na^+), magnesium ion (Mg^{2+}), potassium ion (K^+), ammonium ion (NH_4^+), chloride (Cl^-), bromide (Br^-), iodide (I^-), fluoride (F^-), adenosine triphosphate (ATP), dinucleotide triphosphate (dNTP), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and n-dodecyl-p-D-maltoside (DBDM). The reducing agent is dithiothreitol or a functional derivative thereof.

[00140] The plurality of droplets may be provided in a first droplet fraction and a first fluid fraction. Providing a second droplet having a second concentration of the reagent may comprise providing a second plurality of droplets in a second droplet fraction and a second fluid fraction.

[00141] The first fluid fraction or the second fluid fraction may comprise a mediator that may aid in transfer of the reagent between the first droplet and the second droplet.

[00142] In some examples the reagent is transferred between the first droplet and the second droplet via the mediator when the first droplet and the second droplet are subjected to conditions sufficient to transfer the reagent between the first droplet and the second droplet, thereby changing the first concentration of the reagent in the first droplet and the second concentration of the reagent in the second droplet. The mediator may comprise a surfactant. In some examples, the mediator is a micelle.

[00143] In some examples, the method comprises subjecting the second plurality of droplets to conditions sufficient to transfer the reagent from the second droplet fraction to the second fluid fraction and subjecting the plurality of droplets to conditions sufficient to transfer the reagent from the first fluid fraction to the first droplet fraction. The first fluid fraction may be a continuous phase in which the plurality of droplets may be dispersed. The continuous phase may comprise an oil.

[00144] The method may further comprise generating a mixture comprising the plurality of droplets and the second plurality of droplets, wherein the reagent may be between the plurality of droplets and the second plurality of droplets within the mixture.

[00145] The reagent may be transferred between the first droplet and the second droplet within the mixture via diffusion. In other examples, other mass transfer mechanisms may be involved. Mass transfer may comprise diffusion. Mass transfer may comprise convection. Mass

transfer may be affected by various conditions, such as for example, temperature and fluid properties such as density and viscosity. Heat transfer may further contribute to mass transfer. Temperature may be modulated to alter mass transfer rates. In some cases, samples may be further agitated. Agitating the samples may further increase mass transfer.

[00146] In some examples, a bead may comprise a subset of nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules coupled thereto. The reagent may dissolve the bead or in some cases, release a portion of the subset of nucleic acid barcode molecules from the bead.

[00147] The bead may be a gel bead. The reagent may dissolve the gel bead. In some examples, the reagent may be a reducing agent and the bead may comprise a disulfide bond that may break by the reducing agent.

[00148] In some examples, prior to subjecting the first droplet and the second droplet to conditions sufficient to transfer the reagent between the first droplet and the second droplet, the first concentration may be less than the second concentration.

[00149] In some examples, during or subsequent to subjecting the first droplet and the second droplet to conditions sufficient to transfer the reagent between the first droplet and the second droplet, the second droplet may decrease in diameter and/or the first droplet may increase in diameter, and/or both.

Systems and methods for sample compartmentalization

[00150] In some examples, the systems and methods described herein provide for the compartmentalization, depositing, or partitioning of one or more particles (e.g., analyte carrier, macromolecular constituents of analyte carrier, beads, reagents, etc.) into discrete compartments or partitions (referred to interchangeably herein as partitions), where each partition maintains separation of its own contents from the contents of other partitions. The partition can be a droplet in an emulsion. A partition may comprise one or more other partitions.

[00151] A partition may include one or more particles. A partition may include one or more types of particles. For example, a partition of the present disclosure may comprise one or more analyte carrier and/or macromolecular constituents thereof. A partition may comprise one or more gel beads. A partition may comprise one or more cell beads. A partition may include a single gel bead, a single cell bead, or both a single cell bead and single gel bead. A partition may include one or more reagents. In some cases, a partition may be unoccupied. For example, a partition may not comprise a bead. A cell bead can be an analyte carrier and/or one or more of its macromolecular constituents encased inside of a gel or polymer matrix, such as via polymerization of a droplet containing the analyte carrier and precursors capable of being

polymerized or gelled. Unique identifiers, such as barcodes, may be injected into the droplets previous to, subsequent to, or concurrently with droplet generation, such as via a microcapsule (e.g., bead), as described elsewhere herein. Microfluidic channel networks (e.g., on a chip) can be utilized to generate partitions as described herein. Additional mechanisms may also be employed in the partitioning of individual analyte carrier, including porous membranes through which aqueous mixtures of cells are extruded into non-aqueous fluids.

[00152] The partitions can be flowable within fluid streams. The partitions may comprise, for example, micro-vesicles that have an outer barrier surrounding an inner fluid center or core. In some cases, the partitions may comprise a porous matrix that is capable of entraining and/or retaining materials within its matrix. The partitions can be droplets of a first phase within a second phase, wherein the first and second phases are immiscible. For example, the partitions can be droplets of aqueous fluid within a non-aqueous continuous phase (e.g., oil phase). In another example, the partitions can be droplets of a non-aqueous fluid within an aqueous phase. In some examples, the partitions may be provided in a water-in-oil emulsion or oil-in-water emulsion. A variety of different vessels are described in, for example, U.S. Patent Application Publication No. 2014/0155295, which is entirely incorporated herein by reference for all purposes. Emulsion systems for creating stable droplets in non-aqueous or oil continuous phases are described in, for example, U.S. Patent Application Publication No. 2010/0105112, which is entirely incorporated herein by reference for all purposes.

[00153] In the case of droplets in an emulsion, allocating individual particles to discrete partitions may in one example be accomplished by introducing a flowing stream of particles in an aqueous fluid into a flowing stream or reservoir of a non-aqueous fluid, such that droplets are generated (see generally, e.g., **FIGs. 1-7B**). Fluid properties (e.g., fluid flow rates, fluid viscosities, etc.), particle properties (e.g., volume fraction, particle size, particle concentration, etc.), microfluidic architectures (e.g., channel geometry, etc.), and other parameters may be adjusted to control the occupancy of the resulting partitions (e.g., number of analyte carrier per partition, number of beads per partition, etc.). For example, partition occupancy can be controlled by providing the aqueous stream at a certain concentration and/or flow rate of particles. To generate single analyte carrier partitions, the relative flow rates of the immiscible fluids can be selected such that, on average, the partitions may contain less than one analyte carrier per partition in order to ensure that those partitions that are occupied are primarily singly occupied. In some cases, partitions among a plurality of partitions may contain at most one analyte carrier (e.g., bead, DNA, cell or cellular material). In some examples, the various parameters (e.g., fluid properties, particle properties, microfluidic architectures, etc.) may be

selected or adjusted such that a majority of partitions are occupied, for example, allowing for a small percentage of unoccupied partitions. The flows and channel architectures can be controlled as to ensure a given number of singly occupied partitions, less than a certain level of unoccupied partitions and/or less than a certain level of multiply occupied partitions.

[00154] FIG. 1 shows an example of a microfluidic channel structure **100** for partitioning individual analyte carriers. The channel structure **100** can include channel segments **102**, **104**, **106** and **108** communicating at a channel junction **110**. In operation, a first aqueous fluid **112** that includes suspended analyte carriers (or cells) **114** may be transported along channel segment **102** into junction **110**, while a second fluid **116** that is immiscible with the aqueous fluid **112** is delivered to the junction **110** from each of channel segments **104** and **106** to create discrete droplets **118**, **120** of the first aqueous fluid **112** flowing into channel segment **108**, and flowing away from junction **110**. The channel segment **108** may be fluidically coupled to an outlet reservoir where the discrete droplets can be stored and/or harvested. A discrete droplet generated may include individual analyte carriers **114** (such as droplets **118**). A discrete droplet generated may include more than one individual analyte carrier **114** (not shown in FIG. 1). A discrete droplet may contain no analyte carrier **114** (such as droplet **120**). Each discrete partition may maintain separation of its own contents (e.g., individual analyte carrier **114**) from the contents of other partitions.

[00155] The second fluid **116** can comprise an oil, such as a fluorinated oil, that includes a fluorosurfactant for stabilizing the resulting droplets, for example, inhibiting subsequent coalescence of the resulting droplets **118**, **120**. Examples of particularly useful partitioning fluids and fluorosurfactants are described, for example, in U.S. Patent Application Publication No. 2010/0105112, which is entirely incorporated herein by reference for all purposes.

[00156] As will be appreciated, the channel segments described herein may be coupled to any of a variety of different fluid sources or receiving components, including reservoirs, tubing, manifolds, or fluidic components of other systems. As will be appreciated, the microfluidic channel structure **100** may have other geometries. For example, a microfluidic channel structure can have more than one channel junction. For example, a microfluidic channel structure can have 2, 3, 4, or 5 channel segments each carrying particles (e.g., analyte carrier, cell beads, and/or gel beads) that meet at a channel junction. Fluid may be directed to flow along one or more channels or reservoirs via one or more fluid flow units. A fluid flow unit can comprise compressors (e.g., providing positive pressure), pumps (e.g., providing negative pressure), actuators, and the like to control flow of the fluid. Fluid may also or otherwise be controlled via

applied pressure differentials, centrifugal force, electrokinetic pumping, vacuum, capillary or gravity flow, or the like.

[00157] The generated droplets may comprise two subsets of droplets: (1) occupied droplets **118**, containing one or more analyte carriers **114**, and (2) unoccupied droplets **120**, not containing any analyte carriers **114**. Occupied droplets **118** may comprise singly occupied droplets (having one analyte carrier) and multiply occupied droplets (having more than one analyte carrier). As described elsewhere herein, in some cases, the majority of occupied partitions can include no more than one analyte carrier per occupied partition and some of the generated partitions can be unoccupied (of any analyte carrier). In some cases, though, some of the occupied partitions may include more than one analyte carrier. In some cases, the partitioning process may be controlled such that fewer than about 25% of the occupied partitions contain more than one analyte carrier, and in many cases, fewer than about 20% of the occupied partitions have more than one analyte carrier, while in some cases, fewer than about 10% or even fewer than about 5% of the occupied partitions include more than one analyte carrier per partition.

[00158] In some cases, it may be useful to minimize the creation of excessive numbers of empty partitions, such as to reduce costs and/or increase efficiency. While this minimization may be achieved by providing a sufficient number of analyte carriers (e.g., analyte carrier **114**) at the partitioning junction **110**, such as to ensure that at least one analyte carrier is encapsulated in a partition, the Poissonian distribution may expectedly increase the number of partitions that include multiple analyte carriers. As such, where singly occupied partitions are to be obtained, at most about 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5% or less of the generated partitions can be unoccupied.

[00159] In some cases, the flow of one or more of the analyte carriers (e.g., in channel segment **102**), or other fluids directed into the partitioning junction (e.g., in channel segments **104**, **106**) can be controlled such that, in many cases, no more than about 50% of the generated partitions, no more than about 25% of the generated partitions, or no more than about 10% of the generated partitions are unoccupied. These flows can be controlled so as to present a non-Poissonian distribution of single-occupied partitions while providing lower levels of unoccupied partitions. The above noted ranges of unoccupied partitions can be achieved while still providing any of the single occupancy rates described above. For example, in many cases, the use of the systems and methods described herein can create resulting partitions that have multiple occupancy rates of less than about 25%, less than about 20%, less than about 15%, less than about 10%, and in many cases, less than about 5%, while having unoccupied partitions of

less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 5%, or less.

[00160] As will be appreciated, the above-described occupancy rates are also applicable to partitions that include both analyte carriers and additional reagents, including, but not limited to, microcapsules or beads (e.g., gel beads) carrying barcoded nucleic acid molecules (e.g., oligonucleotides) (described in relation to **FIG. 2**). The occupied partitions (e.g., at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the occupied partitions) can include both a microcapsule (e.g., bead) comprising barcoded nucleic acid molecules and an analyte carrier.

[00161] In another example, in addition to or as an alternative to droplet-based partitioning, analyte carriers may be encapsulated within a microcapsule that comprises an outer shell, layer or porous matrix in which is entrained one or more individual analyte carriers or small groups of analyte carriers. The microcapsule may include other reagents. Encapsulation of analyte carriers may be performed by a variety of processes. Such processes may combine an aqueous fluid containing the analyte carriers with a polymeric precursor material that may be capable of being formed into a gel or other solid or semi-solid matrix upon application of a particular stimulus to the polymer precursor. Such stimuli can include, for example, thermal stimuli (e.g., either heating or cooling), photo-stimuli (e.g., through photo-curing), chemical stimuli (e.g., through crosslinking, polymerization initiation of the precursor (e.g., through added initiators)), mechanical stimuli, or a combination thereof.

[00162] Preparation of microcapsules comprising analyte carriers may be performed by a variety of methods. For example, air knife droplet or aerosol generators may be used to dispense droplets of precursor fluids into gelling solutions in order to form microcapsules that include individual analyte carriers or small groups of analyte carriers. Likewise, membrane-based encapsulation systems may be used to generate microcapsules comprising encapsulated analyte carriers as described herein. Microfluidic systems of the present disclosure, such as that shown in **FIG. 1**, may be readily used in encapsulating cells as described herein. In particular, and with reference to **FIG. 1**, the aqueous fluid **112** comprising (i) the analyte carriers **114** and (ii) the polymer precursor material (not shown) is flowed into channel junction **110**, where it is partitioned into droplets **118**, **120** through the flow of non-aqueous fluid **116**. In the case of encapsulation methods, non-aqueous fluid **116** may also include an initiator (not shown) to cause polymerization and/or crosslinking of the polymer precursor to form the microcapsule that includes the entrained analyte carriers. Examples of polymer precursor/initiator pairs include

those described in U.S. Patent Application Publication No. 2014/0378345, which is entirely incorporated herein by reference for all purposes.

[00163] For example, in the case where the polymer precursor material comprises a linear polymer material, such as a linear polyacrylamide, PEG, or other linear polymeric material, the activation agent may comprise a cross-linking agent, or a chemical that activates a cross-linking agent within the formed droplets. Likewise, for polymer precursors that comprise polymerizable monomers, the activation agent may comprise a polymerization initiator. For example, in certain cases, where the polymer precursor comprises a mixture of acrylamide monomer with a N,N'-bis-(acryloyl)cystamine (BAC) comonomer, an agent such as tetraethylmethylenediamine (TEMED) may be provided within the second fluid streams **116** in channel segments **104** and **106**, which can initiate the copolymerization of the acrylamide and BAC into a cross-linked polymer network, or hydrogel.

[00164] Upon contact of the second fluid stream **116** with the first fluid stream **112** at junction **110**, during formation of droplets, the TEMED may diffuse from the second fluid **116** into the aqueous fluid **112** comprising the linear polyacrylamide, which will activate the crosslinking of the polyacrylamide within the droplets **118**, **120**, resulting in the formation of gel (e.g., hydrogel) microcapsules, as solid or semi-solid beads or particles entraining the cells **114**. Although described in terms of polyacrylamide encapsulation, other 'activatable' encapsulation compositions may also be employed in the context of the methods and compositions described herein. For example, formation of alginate droplets followed by exposure to divalent metal ions (e.g., Ca^{2+} ions), can be used as an encapsulation process using the described processes. Likewise, agarose droplets may also be transformed into capsules through temperature-based gelling (e.g., upon cooling, etc.).

[00165] In some cases, encapsulated analyte carriers can be selectively releasable from the microcapsule, such as through passage of time or upon application of a particular stimulus, that degrades the microcapsule sufficiently to allow the analyte carriers (e.g., cell), or its other contents to be released from the microcapsule, such as into a partition (e.g., droplet). For example, in the case of the polyacrylamide polymer described above, degradation of the microcapsule may be accomplished through the introduction of an appropriate reducing agent, such as DTT or the like, to cleave disulfide bonds that cross-link the polymer matrix. See, for example, U.S. Patent Application Publication No. 2014/0378345, which is entirely incorporated herein by reference for all purposes.

[00166] The analyte carriers can be subjected to other conditions sufficient to polymerize or gel the precursors. The conditions sufficient to polymerize or gel the precursors may comprise

exposure to heating, cooling, electromagnetic radiation, and/or light. The conditions sufficient to polymerize or gel the precursors may comprise any conditions sufficient to polymerize or gel the precursors. Following polymerization or gelling, a polymer or gel may be formed around the analyte carrier. The polymer or gel may be diffusively permeable to chemical or biochemical reagents. The polymer or gel may be diffusively impermeable to macromolecular constituents of the analyte carrier. In this manner, the polymer or gel may act to allow the analyte carrier to be subjected to chemical or biochemical operations while spatially confining the macromolecular constituents to a region of the droplet defined by the polymer or gel. The polymer or gel may include one or more of disulfide cross-linked polyacrylamide, agarose, alginate, polyvinyl alcohol, polyethylene glycol (PEG)-diacrylate, PEG-acrylate, PEG-thiol, PEG-azide, PEG-alkyne, other acrylates, chitosan, hyaluronic acid, collagen, fibrin, gelatin, or elastin. The polymer or gel may comprise any other polymer or gel.

[00167] The polymer or gel may be functionalized to bind to targeted analytes, such as nucleic acids, proteins, carbohydrates, lipids or other analytes. The polymer or gel may be polymerized or gelled via a passive mechanism. The polymer or gel may be stable in alkaline conditions or at elevated temperature. The polymer or gel may have mechanical properties similar to the mechanical properties of the bead. For instance, the polymer or gel may be of a similar size to the bead. The polymer or gel may have a mechanical strength (e.g. tensile strength) similar to that of the bead. The polymer or gel may be of a lower density than an oil. The polymer or gel may be of a density that is roughly similar to that of a buffer. The polymer or gel may have a tunable pore size. The pore size may be chosen to, for instance, retain denatured nucleic acids. The pore size may be chosen to maintain diffusive permeability to exogenous chemicals such as sodium hydroxide (NaOH) and/or endogenous chemicals such as inhibitors. The polymer or gel may be biocompatible. The polymer or gel may maintain or enhance cell viability. The polymer or gel may be biochemically compatible. The polymer or gel may be polymerized and/or depolymerized thermally, chemically, enzymatically, and/or optically.

[00168] The polymer may comprise poly(acrylamide-co-acrylic acid) crosslinked with disulfide linkages. The preparation of the polymer may comprise a two-step reaction. In the first activation step, poly(acrylamide-co-acrylic acid) may be exposed to an acylating agent to convert carboxylic acids to esters. For instance, the poly(acrylamide-co-acrylic acid) may be exposed to 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). The polyacrylamide-co-acrylic acid may be exposed to other salts of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium. In the second cross-linking step, the ester formed in the first step may be exposed to a disulfide crosslinking agent. For instance, the ester may be

exposed to cystamine (2,2'-dithiobis(ethylamine)). Following the two steps, the analyte carrier may be surrounded by polyacrylamide strands linked together by disulfide bridges. In this manner, the analyte carrier may be encased inside of or comprise a gel or matrix (e.g., polymer matrix) to form a "cell bead." A cell bead can contain analyte carriers (e.g., a cell) or macromolecular constituents (e.g., RNA, DNA, proteins, etc.) of analyte carriers. A cell bead may include a single cell or multiple cells, or a derivative of the single cell or multiple cells. For example, after lysing and washing the cells, inhibitory components from cell lysates can be washed away and the macromolecular constituents can be bound as cell beads. Systems and methods disclosed herein can be applicable to both cell beads (and/or droplets or other partitions) containing analyte carriers and cell beads (and/or droplets or other partitions) containing macromolecular constituents of analyte carriers.

[00169] Encapsulated analyte carriers can provide certain potential advantages of being more storable and more portable than droplet-based partitioned analyte carriers. Furthermore, in some cases, analyte carriers can be allowed to incubate for a select period of time before analysis, such as in order to characterize changes in such analyte carriers over time, either in the presence or absence of different stimuli. In such cases, encapsulation may allow for longer incubation than partitioning in emulsion droplets, although in some cases, droplet partitioned analyte carriers may also be incubated for different periods of time, e.g., at least 10 seconds, at least 30 seconds, at least 1 minute, at least 5 minutes, at least 10 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, at least 5 hours, or at least 10 hours or more. The encapsulation of biological particles may constitute the partitioning of the analyte carriers into which other reagents are co-partitioned. Alternatively or in addition, encapsulated analyte carriers may be readily deposited into other partitions (e.g., droplets) as described above.

Beads

[00170] Nucleic acid barcode molecules may be delivered to a partition (e.g., a droplet or well) via a solid support or carrier (e.g., a bead). In some cases, nucleic acid barcode molecules are initially associated with the solid support and then released from the solid support upon application of a stimulus, which allows the nucleic acid barcode molecules to dissociate or to be released from the solid support. In specific examples, nucleic acid barcode molecules are initially associated with the solid support (e.g., bead) and then released from the solid support upon application of a biological stimulus, a chemical stimulus, a thermal stimulus, an electrical stimulus, a magnetic stimulus, and/or a photo stimulus.

[00171] A nucleic acid barcode molecule may contain a barcode sequence and a functional sequence, such as a nucleic acid primer sequence or a template switch oligonucleotide (TSO) sequence.

[00172] The solid support may be a bead. A solid support, e.g., a bead, may be porous, non-porous, hollow (e.g., a microcapsule), solid, semi-solid, and/or a combination thereof. Beads may be solid, semi-solid, semi-fluidic, fluidic, and/or a combination thereof. In some instances, a solid support, e.g., a bead, may be dissolvable, disruptable, and/or degradable. In some cases, a solid support, e.g., a bead, may not be degradable. In some cases, the solid support, e.g., a bead, may be a gel bead. A gel bead may be a hydrogel bead. A gel bead may be formed from molecular precursors, such as a polymeric or monomeric species. A semi-solid support, e.g., a bead, may be a liposomal bead. Solid supports, e.g., beads, may comprise metals including iron oxide, gold, and silver. In some cases, the solid support, e.g., the bead, may be a silica bead. In some cases, the solid support, e.g., a bead, can be rigid. In other cases, the solid support, e.g., a bead, may be flexible and/or compressible.

[00173] A partition may comprise one or more unique identifiers, such as barcodes. Barcodes may be previously, subsequently or concurrently delivered to the partitions that hold the compartmentalized or partitioned analyte carriers. For example, barcodes may be injected into droplets previous to, subsequent to, or concurrently with droplet generation. The delivery of the barcodes to a particular partition allows for the later attribution of the characteristics of the individual analyte carrier to the particular partition. Barcodes may be delivered, for example on a nucleic acid molecule (e.g., an oligonucleotide), to a partition via any suitable mechanism. Barcoded nucleic acid molecules can be delivered to a partition via a microcapsule. A microcapsule, in some instances, can comprise a bead. Beads are described in further detail below.

[00174] In some cases, barcoded nucleic acid molecules can be initially associated with the microcapsule and then released from the microcapsule. Release of the barcoded nucleic acid molecules can be passive (e.g., by diffusion out of the microcapsule). In addition or alternatively, release from the microcapsule can be upon application of a stimulus which allows the barcoded nucleic acid molecules to dissociate or to be released from the microcapsule. Such stimulus may disrupt the microcapsule, an interaction that couples the barcoded nucleic acid molecules to or within the microcapsule, or both. Such stimulus can include, for example, a thermal stimulus, photo-stimulus, chemical stimulus (e.g., change in pH or use of a reducing agent(s)), a mechanical stimulus, a radiation stimulus; a biological stimulus (e.g., enzyme), or any combination thereof.

[00175] FIG. 2 shows an example of a microfluidic channel structure **200** for delivering barcode carrying beads to droplets. The channel structure **200** can include channel segments **201**, **202**, **204**, **206** and **208** communicating at a channel junction **210**. In operation, the channel segment **201** may transport an aqueous fluid **212** that includes a plurality of beads **214** (e.g., with nucleic acid molecules, oligonucleotides, molecular tags) along the channel segment **201** into junction **210**. The plurality of beads **214** may be sourced from a suspension of beads. For example, the channel segment **201** may be connected to a reservoir comprising an aqueous suspension of beads **214**. The channel segment **202** may transport the aqueous fluid **212** that includes a plurality of analyte carriers **216** along the channel segment **202** into junction **210**. The plurality of biological particles **216** may be sourced from a suspension of analyte carriers. For example, the channel segment **202** may be connected to a reservoir comprising an aqueous suspension of analyte carriers **216**. In some instances, the aqueous fluid **212** in either the first channel segment **201** or the second channel segment **202**, or in both segments, can include one or more reagents, as further described below. A second fluid **218** that is immiscible with the aqueous fluid **212** (e.g., oil) can be delivered to the junction **210** from each of channel segments **204** and **206**. Upon meeting of the aqueous fluid **212** from each of channel segments **201** and **202** and the second fluid **218** from each of channel segments **204** and **206** at the channel junction **210**, the aqueous fluid **212** can be partitioned as discrete droplets **220** in the second fluid **218** and flow away from the junction **210** along channel segment **208**. The channel segment **208** may deliver the discrete droplets to an outlet reservoir fluidly coupled to the channel segment **208**, where they may be harvested.

[00176] As an alternative, the channel segments **201** and **202** may meet at another junction upstream of the junction **210**. At such junction, beads and analyte carriers may form a mixture that is directed along another channel to the junction **210** to yield droplets **220**. The mixture may provide the beads and analyte carriers in an alternating fashion, such that, for example, a droplet comprises a single bead and a single analyte carrier.

[00177] Beads, analyte carriers and droplets may flow along channels at substantially regular flow profiles (e.g., at regular flow rates). Such regular flow profiles may permit a droplet to include a single bead and a single analyte carrier. Such regular flow profiles may permit the droplets to have an occupancy (e.g., droplets having beads and analyte carriers) greater than 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. Such regular flow profiles and devices that may be used to provide such regular flow profiles are provided in, for example, U.S. Patent Publication No. 2015/0292988, which is entirely incorporated herein by reference.

[00178] The second fluid **218** can comprise an oil, such as a fluorinated oil, that includes a fluorosurfactant for stabilizing the resulting droplets, for example, inhibiting subsequent coalescence of the resulting droplets **220**.

[00179] A discrete droplet that is generated may include an individual analyte carrier **216**. A discrete droplet that is generated may include a barcode or other reagent carrying bead **214**. A discrete droplet generated may include both an individual analyte carrier and a barcode carrying bead, such as droplets **220**. In some instances, a discrete droplet may include more than one individual analyte carrier or no analyte carrier. In some instances, a discrete droplet may include more than one bead or no bead. A discrete droplet may be unoccupied (e.g., no beads, no analyte carriers).

[00180] Beneficially, a discrete droplet partitioning an analyte carrier and a barcode carrying bead may effectively allow the attribution of the barcode to macromolecular constituents of the analyte carrier within the partition. The contents of a partition may remain discrete from the contents of other partitions.

[00181] As will be appreciated, the channel segments described herein may be coupled to any of a variety of different fluid sources or receiving components, including reservoirs, tubing, manifolds, or fluidic components of other systems. As will be appreciated, the microfluidic channel structure **200** may have other geometries. For example, a microfluidic channel structure can have more than one channel junctions. For example, a microfluidic channel structure can have 2, 3, 4, or 5 channel segments each carrying beads that meet at a channel junction. Fluid may be directed flow along one or more channels or reservoirs via one or more fluid flow units. A fluid flow unit can comprise compressors (e.g., providing positive pressure), pumps (e.g., providing negative pressure), actuators, and the like to control flow of the fluid. Fluid may also or otherwise be controlled via applied pressure differentials, centrifugal force, electro-kinetic pumping, vacuum, capillary or gravity flow, or the like.

[00182] A bead may be porous, non-porous, solid, semi-solid, semi-fluidic, fluidic, and/or a combination thereof. In some instances, a bead may be dissolvable, disruptable, and/or degradable. In some cases, a bead may not be degradable. In some cases, the bead may be a gel bead. A gel bead may be a hydrogel bead. A gel bead may be formed from molecular precursors, such as a polymeric or monomeric species. A semi-solid bead may be a liposomal bead. Solid beads may comprise metals including iron oxide, gold, and silver. In some cases, the bead may be a silica bead. In some cases, the bead can be rigid. In other cases, the bead may be flexible and/or compressible.

[00183] A bead may be of any suitable shape. Examples of bead shapes include, but are not limited to, spherical, non-spherical, oval, oblong, amorphous, circular, cylindrical, and variations thereof.

[00184] Beads may be of uniform size or heterogeneous size. In some cases, the diameter of a bead may be at least about 10 nanometers (nm), 100 nm, 500 nm, 1 micrometer (μm), 5 μm , 10 μm , 20 μm , 30 μm , 40 μm , 50 μm , 60 μm , 70 μm , 80 μm , 90 μm , 100 μm , 250 μm , 500 μm , 1mm, or greater. In some cases, a bead may have a diameter of less than about 10 nm, 100 nm, 500 nm, 1 μm , 5 μm , 10 μm , 20 μm , 30 μm , 40 μm , 50 μm , 60 μm , 70 μm , 80 μm , 90 μm , 100 μm , 250 μm , 500 μm , 1mm, or less. In some cases, a bead may have a diameter in the range of about 40-75 μm , 30-75 μm , 20-75 μm , 40-85 μm , 40-95 μm , 20-100 μm , 10-100 μm , 1-100 μm , 20-250 μm , or 20-500 μm .

[00185] In some cases, beads can be provided as a population or plurality of beads having a relatively monodisperse size distribution. Moreover, relatively consistent amounts of reagents within partitions can be provided while maintaining relatively consistent bead characteristics, such as size, contributing to the overall consistency. In particular, the beads described herein may have size distributions that have a coefficient of variation in their cross-sectional dimensions of less than 50%, less than 40%, less than 30%, less than 20%, and in some cases less than 15%, less than 10%, less than 5%, or less.

[00186] A bead may comprise natural and/or synthetic materials. For example, a bead can comprise a natural polymer, a synthetic polymer or both natural and synthetic polymers. Examples of natural polymers include proteins and sugars such as deoxyribonucleic acid, rubber, cellulose, starch (e.g., amylose, amylopectin), proteins, enzymes, polysaccharides, silks, polyhydroxyalkanoates, chitosan, dextran, collagen, carrageenan, ispaghula, acacia, agar, gelatin, shellac, sterculia gum, xanthan gum, Corn sugar gum, guar gum, gum karaya, agarose, alginic acid, alginate, or natural polymers thereof. Examples of synthetic polymers include acrylics, nylons, silicones, spandex, viscose rayon, polycarboxylic acids, polyvinyl acetate, polyacrylamide, polyacrylate, polyethylene glycol, polyurethanes, polylactic acid, silica, polystyrene, polyacrylonitrile, polybutadiene, polycarbonate, polyethylene, polyethylene terephthalate, poly(chlorotrifluoroethylene), poly(ethylene oxide), poly(ethylene terephthalate), polyethylene, polyisobutylene, poly(methyl methacrylate), poly(oxymethylene), polyformaldehyde, polypropylene, polystyrene, poly(tetrafluoroethylene), poly(vinyl acetate), poly(vinyl alcohol), poly(vinyl chloride), poly(vinylidene dichloride), poly(vinylidene difluoride), poly(vinyl fluoride) and/or combinations (e.g., co-polymers) thereof. Beads may

also be formed from materials other than polymers, including lipids, micelles, ceramics, glass-ceramics, material composites, metals, other inorganic materials, and others.

[00187] In some instances, the bead may contain molecular precursors (e.g., monomers or polymers), which may form a polymer network via polymerization of the molecular precursors. In some cases, a precursor may be an already polymerized species capable of undergoing further polymerization via, for example, a chemical cross-linkage. In some cases, a precursor can comprise one or more of an acrylamide or a methacrylamide monomer, oligomer, or polymer. In some cases, the bead may comprise prepolymers, which are oligomers capable of further polymerization. For example, polyurethane beads may be prepared using prepolymers. In some cases, the bead may contain individual polymers that may be further polymerized together. In some cases, beads may be generated via polymerization of different precursors, such that they comprise mixed polymers, co-polymers, and/or block co-polymers. In some cases, the bead may comprise covalent or ionic bonds between polymeric precursors (e.g., monomers, oligomers, linear polymers), nucleic acid molecules (e.g., oligonucleotides), primers, and other entities. In some cases, the covalent bonds can be carbon-carbon bonds, thioether bonds, or carbon-heteroatom bonds.

[00188] Cross-linking may be permanent or reversible, depending upon the particular cross-linker used. Reversible cross-linking may allow for the polymer to linearize or dissociate under appropriate conditions. In some cases, reversible cross-linking may also allow for reversible attachment of a material bound to the surface of a bead. In some cases, a cross-linker may form disulfide linkages. In some cases, the chemical cross-linker forming disulfide linkages may be cystamine or a modified cystamine.

[00189] In some cases, disulfide linkages can be formed between molecular precursor units (e.g., monomers, oligomers, or linear polymers) or precursors incorporated into a bead and nucleic acid molecules (e.g., oligonucleotides). Cystamine (including modified cystamines), for example, is an organic agent comprising a disulfide bond that may be used as a crosslinker agent between individual monomeric or polymeric precursors of a bead. Polyacrylamide may be polymerized in the presence of cystamine or a species comprising cystamine (e.g., a modified cystamine) to generate polyacrylamide gel beads comprising disulfide linkages (e.g., chemically degradable beads comprising chemically-reducible cross-linkers). The disulfide linkages may permit the bead to be degraded (or dissolved) upon exposure of the bead to a reducing agent.

[00190] In some cases, chitosan, a linear polysaccharide polymer, may be crosslinked with glutaraldehyde via hydrophilic chains to form a bead. Crosslinking of chitosan polymers may be

achieved by chemical reactions that are initiated by heat, pressure, change in pH, and/or radiation.

[00191] In some cases, a bead may comprise an acrydite moiety, which in some cases may be used to attach one or more nucleic acid molecules (e.g., barcode sequence, barcoded nucleic acid molecule, barcoded oligonucleotide, primer, or other oligonucleotide) to the bead. In some cases, an acrydite moiety can refer to an acrydite analogue generated from the reaction of acrydite with one or more species, such as, the reaction of acrydite with other monomers and cross-linkers during a polymerization reaction. Acrydite moieties may be modified to form chemical bonds with a species to be attached, such as a nucleic acid molecule (e.g., barcode sequence, barcoded nucleic acid molecule, barcoded oligonucleotide, primer, or other oligonucleotide). Acrydite moieties may be modified with thiol groups capable of forming a disulfide bond or may be modified with groups already comprising a disulfide bond. The thiol or disulfide (via disulfide exchange) may be used as an anchor point for a species to be attached or another part of the acrydite moiety may be used for attachment. In some cases, attachment can be reversible, such that when the disulfide bond is broken (e.g., in the presence of a reducing agent), the attached species is released from the bead. In other cases, an acrydite moiety can comprise a reactive hydroxyl group that may be used for attachment.

[00192] Functionalization of beads for attachment of nucleic acid molecules (e.g., oligonucleotides) may be achieved through a wide range of different approaches, including activation of chemical groups within a polymer, incorporation of active or activatable functional groups in the polymer structure, or attachment at the pre-polymer or monomer stage in bead production.

[00193] For example, precursors (e.g., monomers, cross-linkers) that are polymerized to form a bead may comprise acrydite moieties, such that when a bead is generated, the bead also comprises acrydite moieties. The acrydite moieties can be attached to a nucleic acid molecule (e.g., oligonucleotide) that comprises one or more functional sequences, such as a TSO sequence or a primer sequence (e.g., a poly T sequence, or a nucleic acid primer sequence complementary to a target nucleic acid sequence and/or for amplifying a target nucleic acid sequence, a random primer, or a primer sequence for messenger RNA) that is useful for incorporation into the bead and/or one or more barcode sequences. The one or more barcode sequences may include sequences that are the same for all nucleic acid molecules coupled to a given bead and/or sequences that are different across all nucleic acid molecules coupled to the given bead. The nucleic acid molecule may be incorporated into the bead.

[00194] In some cases, the nucleic acid molecule can comprise a functional sequence, for example, for attachment to a sequencing flow cell, such as, for example, a P5 sequence (or a portion thereof) for Illumina® sequencing. In some cases, the nucleic acid molecule or derivative thereof (e.g., oligonucleotide or polynucleotide generated from the nucleic acid molecule) can comprise another functional sequence, such as, for example, a P7 sequence (or a portion thereof) for attachment to a sequencing flow cell for Illumina sequencing. In some cases, the nucleic acid molecule can comprise a barcode sequence. In some cases, the nucleic acid molecule can further comprise a unique molecular identifier (UMI). In some cases, the nucleic acid molecule can comprise an R1 primer sequence for Illumina sequencing. In some cases, the nucleic acid molecule can comprise an R2 primer sequence for Illumina sequencing. Examples of such nucleic acid molecules (e.g., oligonucleotides, polynucleotides, etc.) and uses thereof, as may be used with compositions, devices, methods and systems of the present disclosure, are provided in U.S. Patent Pub. Nos. 2014/0378345 and 2015/0376609, each of which is entirely incorporated herein by reference.

[00195] **FIG. 8** illustrates an example of a barcode carrying bead. A nucleic acid molecule **802**, such as an oligonucleotide, can be coupled to a bead **804** by a releasable linkage **806**, such as, for example, a disulfide linker. The same bead **804** may be coupled (e.g., via releasable linkage) to one or more other nucleic acid molecules **818**, **820**. The nucleic acid molecule **802** may be or comprise a barcode. As noted elsewhere herein, the structure of the barcode may comprise a number of sequence elements. The nucleic acid molecule **802** may comprise a functional sequence **808** that may be used in subsequent processing. For example, the functional sequence **808** may include one or more of a sequencer specific flow cell attachment sequence (e.g., a P5 sequence for Illumina® sequencing systems) and a sequencing primer sequence (e.g., a R1 primer for Illumina® sequencing systems). The nucleic acid molecule **802** may comprise a barcode sequence **810** for use in barcoding the sample (e.g., DNA, RNA, protein, etc.). In some cases, the barcode sequence **810** can be bead-specific such that the barcode sequence **810** is common to all nucleic acid molecules (e.g., including nucleic acid molecule **802**) coupled to the same bead **804**. Alternatively or in addition, the barcode sequence **810** can be partition-specific such that the barcode sequence **810** is common to all nucleic acid molecules coupled to one or more beads that are partitioned into the same partition. The nucleic acid molecule **802** may comprise a specific priming sequence **812**, such as an mRNA specific priming sequence (e.g., poly-T sequence), a targeted priming sequence, and/or a random priming sequence. The nucleic acid molecule **802** may comprise an anchoring sequence **814** to ensure that the specific priming sequence **812** hybridizes at the sequence end (e.g., of the mRNA). For example, the anchoring

sequence **814** can include a random short sequence of nucleotides, such as a 1-mer, 2-mer, 3-mer or longer sequence, which can ensure that a poly-T segment is more likely to hybridize at the sequence end of the poly-A tail of the mRNA.

[00196] The nucleic acid molecule **802** may comprise a unique molecular identifying sequence **816** (e.g., unique molecular identifier (UMI)). In some cases, the unique molecular identifying sequence **816** may comprise from about 5 to about 8 nucleotides. Alternatively, the unique molecular identifying sequence **816** may compress less than about 5 or more than about 8 nucleotides. The unique molecular identifying sequence **816** may be a unique sequence that varies across individual nucleic acid molecules (e.g., **802**, **818**, **820**, etc.) coupled to a single bead (e.g., bead **804**). In some cases, the unique molecular identifying sequence **816** may be a random sequence (e.g., such as a random N-mer sequence). For example, the UMI may provide a unique identifier of the starting mRNA molecule that was captured, in order to allow quantitation of the number of original expressed RNA. As will be appreciated, although **FIG. 8** shows three nucleic acid molecules **802**, **818**, **820** coupled to the surface of the bead **804**, an individual bead may be coupled to any number of individual nucleic acid molecules, for example, from one to tens to hundreds of thousands or even millions of individual nucleic acid molecules. The respective barcodes for the individual nucleic acid molecules can comprise both common sequence segments or relatively common sequence segments (e.g., **808**, **810**, **812**, etc.) and variable or unique sequence segments (e.g., **816**) between different individual nucleic acid molecules coupled to the same bead.

[00197] In operation, an analyte carrier (e.g., cell, DNA, RNA, etc.) can be co-partitioned along with a barcode bearing bead **804**. The barcoded nucleic acid molecules **802**, **818**, **820** can be released from the bead **804** in the partition. By way of example, in the context of analyzing sample RNA, the poly-T segment (e.g., **812**) of one of the released nucleic acid molecules (e.g., **802**) can hybridize to the poly-A tail of a mRNA molecule. Reverse transcription may result in a cDNA transcript of the mRNA, but which transcript includes each of the sequence segments **808**, **810**, **816** of the nucleic acid molecule **802**. Because the nucleic acid molecule **802** comprises an anchoring sequence **814**, it will more likely hybridize to and prime reverse transcription at the sequence end of the poly-A tail of the mRNA. Within any given partition, all of the cDNA transcripts of the individual mRNA molecules may include a common barcode sequence segment **810**. However, the transcripts made from the different mRNA molecules within a given partition may vary at the unique molecular identifying sequence **812** segment (e.g., UMI segment). Beneficially, even following any subsequent amplification of the contents of a given partition, the number of different UMIs can be indicative of the quantity of mRNA originating

from a given partition, and thus from the analyte carrier (e.g., cell). As noted above, the transcripts can be amplified, cleaned up and sequenced to identify the sequence of the cDNA transcript of the mRNA, as well as to sequence the barcode segment and the UMI segment. While a poly-T primer sequence is described, other targeted or random priming sequences may also be used in priming the reverse transcription reaction. Likewise, although described as releasing the barcoded oligonucleotides into the partition, in some cases, the nucleic acid molecules bound to the bead (e.g., gel bead) may be used to hybridize and capture the mRNA on the solid phase of the bead, for example, in order to facilitate the separation of the RNA from other cell contents.

[00198] In some cases, precursors comprising a functional group that is reactive or capable of being activated such that it becomes reactive can be polymerized with other precursors to generate gel beads comprising the activated or activatable functional group. The functional group may then be used to attach additional species (e.g., disulfide linkers, primers, other oligonucleotides, etc.) to the gel beads. For example, some precursors comprising a carboxylic acid (COOH) group can co-polymerize with other precursors to form a gel bead that also comprises a COOH functional group. In some cases, acrylic acid (a species comprising free COOH groups), acrylamide, and bis(acryloyl)cystamine can be co-polymerized together to generate a gel bead comprising free COOH groups. The COOH groups of the gel bead can be activated (e.g., via 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) or 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM)) such that they are reactive (e.g., reactive to amine functional groups where EDC/NHS or DMTMM are used for activation). The activated COOH groups can then react with an appropriate species (e.g., a species comprising an amine functional group where the carboxylic acid groups are activated to be reactive with an amine functional group) comprising a moiety to be linked to the bead.

[00199] Beads comprising disulfide linkages in their polymeric network may be functionalized with additional species via reduction of some of the disulfide linkages to free thiols. The disulfide linkages may be reduced via, for example, the action of a reducing agent (e.g., DTT, TCEP, etc.) to generate free thiol groups, without dissolution of the bead. Free thiols of the beads can then react with free thiols of a species or a species comprising another disulfide bond (e.g., via thiol-disulfide exchange) such that the species can be linked to the beads (e.g., via a generated disulfide bond). In some cases, free thiols of the beads may react with any other suitable group. For example, free thiols of the beads may react with species comprising an acrydite moiety. The free thiol groups of the beads can react with the acrydite via Michael

addition chemistry, such that the species comprising the acrydite is linked to the bead. In some cases, uncontrolled reactions can be prevented by inclusion of a thiol capping agent such as N-ethylmaleimide or iodoacetate.

[00200] Activation of disulfide linkages within a bead can be controlled such that a small number of disulfide linkages are activated. Control may be exerted, for example, by controlling the concentration of a reducing agent used to generate free thiol groups and/or concentration of reagents used to form disulfide bonds in bead polymerization. In some cases, a low concentration (e.g., molecules of reducing agent:gel bead ratios of less than or equal to about 1:100,000,000,000, less than or equal to about 1:10,000,000,000, less than or equal to about 1:1,000,000,000, less than or equal to about 1:100,000,000, less than or equal to about 1:10,000,000, less than or equal to about 1:1,000,000, less than or equal to about 1:100,000, less than or equal to about 1:10,000) of reducing agent may be used for reduction. Controlling the number of disulfide linkages that are reduced to free thiols may be useful in ensuring bead structural integrity during functionalization. In some cases, optically-active agents, such as fluorescent dyes may be coupled to beads via free thiol groups of the beads and used to quantify the number of free thiols present in a bead and/or track a bead.

[00201] In some cases, addition of moieties to a gel bead after gel bead formation may be advantageous. For example, addition of an oligonucleotide (e.g., barcoded oligonucleotide) after gel bead formation may avoid loss of the species during chain transfer termination that can occur during polymerization. Moreover, smaller precursors (e.g., monomers or cross linkers that do not comprise side chain groups and linked moieties) may be used for polymerization and can be minimally hindered from growing chain ends due to viscous effects. In some cases, functionalization after gel bead synthesis can minimize exposure of species (e.g., oligonucleotides) to be loaded with potentially damaging agents (e.g., free radicals) and/or chemical environments. In some cases, the generated gel may possess an upper critical solution temperature (UCST) that can permit temperature driven swelling and collapse of a bead. Such functionality may aid in oligonucleotide (e.g., a primer) infiltration into the bead during subsequent functionalization of the bead with the oligonucleotide. Post-production functionalization may also be useful in controlling loading ratios of species in beads, such that, for example, the variability in loading ratio is minimized. Species loading may also be performed in a batch process such that a plurality of beads can be functionalized with the species in a single batch.

[00202] A bead can be injected or otherwise introduced into a partition. In some examples, the bead may be generated in a spherical or semi-spherical shape, such as for example, in form of a

droplet. A bead can comprise an inner partition in a droplet, such as a droplet core described herein. In some cases, droplet generation methods, such as with the aid of a microfluidic device may be used to generate a plurality of beads. Such generated population of beads may be monodisperse. The plurality of beads may be subjected to conditions sufficient to solidify the beads. Such beads may be further partitioned and/or compartmentalized into droplets using droplet generation techniques. For example, the beads may be compartmentalized in the second droplet population described herein. In some examples, the beads may comprise the inner partition (the core) of the second droplet population. The second droplet population may be generated by applying a stimulus, such as agitation, such as vortexing to compartmentalize the beads into the droplets.

[00203] A bead injected or otherwise introduced into a partition may comprise releasably, cleavably, or reversibly attached barcodes. A bead injected or otherwise introduced into a partition may comprise activatable barcodes. A bead injected or otherwise introduced into a partition may be degradable, disruptable, or dissolvable beads.

[00204] Barcodes can be releasably, cleavably or reversibly attached to the beads such that barcodes can be released or be releasable through cleavage of a linkage between the barcode molecule and the bead, or released through degradation of the underlying bead itself, allowing the barcodes to be accessed or be accessible by other reagents, or both. In examples, cleavage may be achieved through reduction of di-sulfide bonds, use of restriction enzymes, photo-activated cleavage, or cleavage via other types of stimuli (e.g., chemical, thermal, pH, enzymatic, etc.) and/or reactions, such as described elsewhere herein. Releasable barcodes may sometimes be referred to as being activatable, in that they are available for reaction once released. Thus, for example, an activatable barcode may be activated by releasing the barcode from a bead (or other suitable type of partition described herein). Other activatable configurations are also envisioned in the context of the described methods and systems.

[00205] In addition to, or as an alternative to the cleavable linkages between the beads and the associated molecules, such as barcode containing nucleic acid molecules (e.g., barcoded oligonucleotides), the beads may be degradable, disruptable, or dissolvable spontaneously or upon exposure to one or more stimuli (e.g., temperature changes, pH changes, exposure to particular chemical species or phase, exposure to light, reducing agent, etc.). In some cases, a bead may be dissolvable, such that material components of the beads are solubilized when exposed to a particular chemical species or an environmental change, such as a change temperature or a change in pH. In some cases, a gel bead can be degraded or dissolved at elevated temperature and/or in basic conditions. In some cases, a bead may be thermally

degradable such that when the bead is exposed to an appropriate change in temperature (e.g., heat), the bead degrades. Degradation or dissolution of a bead bound to a species (e.g., a nucleic acid molecule, e.g., barcoded oligonucleotide) may result in release of the species from the bead.

[00206] As will be appreciated from the above disclosure, the degradation of a bead may refer to the disassociation of a bound or entrained species from a bead, both with and without structurally degrading the physical bead itself. For example, the degradation of the bead may involve cleavage of a cleavable linkage via one or more species and/or methods described elsewhere herein. In another example, entrained species may be released from beads through osmotic pressure differences due to, for example, changing chemical environments. By way of example, alteration of bead pore sizes due to osmotic pressure differences can generally occur without structural degradation of the bead itself. In some cases, an increase in pore size due to osmotic swelling of a bead can permit the release of entrained species within the bead. In other cases, osmotic shrinking of a bead may cause a bead to better retain an entrained species due to pore size contraction.

[00207] A degradable bead may be introduced into a partition, such as a droplet of an emulsion or a well, such that the bead degrades within the partition and any associated species (e.g., oligonucleotides) are released within the droplet when the appropriate stimulus is applied. The free species (e.g., oligonucleotides, nucleic acid molecules) may interact with other reagents contained in the partition. For example, a polyacrylamide bead comprising cystamine and linked, via a disulfide bond, to a barcode sequence, may be combined with a reducing agent within a droplet of a water-in-oil emulsion. Within the droplet, the reducing agent can break the various disulfide bonds, resulting in bead degradation and release of the barcode sequence into the aqueous, inner environment of the droplet. In another example, heating of a droplet comprising a bead-bound barcode sequence in basic solution may also result in bead degradation and release of the attached barcode sequence into the aqueous, inner environment of the droplet.

[00208] Any suitable number of molecular tag molecules (e.g., primer, barcoded oligonucleotide) can be associated with a bead such that, upon release from the bead, the molecular tag molecules (e.g., primer, e.g., barcoded oligonucleotide) are present in the partition at a pre-defined concentration. Such pre-defined concentration may be selected to facilitate certain reactions for generating a sequencing library, e.g., amplification, within the partition. In some cases, the pre-defined concentration of the primer can be limited by the process of producing nucleic acid molecule (e.g., oligonucleotide) bearing beads.

[00209] In some cases, beads can be non-covalently loaded with one or more reagents. The beads can be non-covalently loaded by, for instance, subjecting the beads to conditions sufficient

to swell the beads, allowing sufficient time for the reagents to diffuse into the interiors of the beads, and subjecting the beads to conditions sufficient to de-swell the beads. The swelling of the beads may be accomplished, for instance, by placing the beads in a thermodynamically favorable solvent, subjecting the beads to a higher or lower temperature, subjecting the beads to a higher or lower ion concentration, and/or subjecting the beads to an electric field. The swelling of the beads may be accomplished by various swelling methods. The de-swell of the beads may be accomplished, for instance, by transferring the beads in a thermodynamically unfavorable solvent, subjecting the beads to lower or high temperatures, subjecting the beads to a lower or higher ion concentration, and/or removing an electric field. The de-swell of the beads may be accomplished by various de-swell methods. Transferring the beads may cause pores in the bead to shrink. The shrinking may then hinder reagents within the beads from diffusing out of the interiors of the beads. The hindrance may be due to steric interactions between the reagents and the interiors of the beads. The transfer may be accomplished microfluidically. For instance, the transfer may be achieved by moving the beads from one co-flowing solvent stream to a different co-flowing solvent stream. The swellability and/or pore size of the beads may be adjusted by changing the polymer composition of the bead.

[00210] In some cases, an acrydite moiety linked to a precursor, another species linked to a precursor, or a precursor itself can comprise a labile bond, such as chemically, thermally, or photo-sensitive bond e.g., disulfide bond, UV sensitive bond, or the like. Once acrydite moieties or other moieties comprising a labile bond are incorporated into a bead, the bead may also comprise the labile bond. The labile bond may be, for example, useful in reversibly linking (e.g., covalently linking) species (e.g., barcodes, primers, etc.) to a bead. In some cases, a thermally labile bond may include a nucleic acid hybridization-based attachment, e.g., where an oligonucleotide is hybridized to a complementary sequence that is attached to the bead, such that thermal melting of the hybrid releases the oligonucleotide, e.g., a barcode containing sequence, from the bead or microcapsule.

[00211] The addition of multiple types of labile bonds to a gel bead may result in the generation of a bead capable of responding to varied stimuli. Each type of labile bond may be sensitive to an associated stimulus (e.g., chemical stimulus, light, temperature, enzymatic, etc.) such that release of species attached to a bead via each labile bond may be controlled by the application of the appropriate stimulus. Such functionality may be useful in controlled release of species from a gel bead. In some cases, another species comprising a labile bond may be linked to a gel bead after gel bead formation via, for example, an activated functional group of the gel bead as described above. As will be appreciated, barcodes that are releasably, cleavably or

reversibly attached to the beads described herein include barcodes that are released or releasable through cleavage of a linkage between the barcode molecule and the bead, or that are released through degradation of the underlying bead itself, allowing the barcodes to be accessed or accessible by other reagents, or both.

[00212] The barcodes that are releasable as described herein may sometimes be referred to as being activatable, in that they are available for reaction once released. Thus, for example, an activatable barcode may be activated by releasing the barcode from a bead (or other suitable type of partition described herein). Other activatable configurations are also envisioned in the context of the described methods and systems.

[00213] In addition to thermally cleavable bonds, disulfide bonds and UV sensitive bonds, other examples of labile bonds that may be coupled to a precursor or bead include an ester linkage (e.g., cleavable with an acid, a base, or hydroxylamine), a vicinal diol linkage (e.g., cleavable via sodium periodate), a Diels-Alder linkage (e.g., cleavable via heat), a sulfone linkage (e.g., cleavable via a base), a silyl ether linkage (e.g., cleavable via an acid), a glycosidic linkage (e.g., cleavable via an amylase), a peptide linkage (e.g., cleavable via a protease), or a phosphodiester linkage (e.g., cleavable via a nuclease (e.g., DNAase)). A bond may be cleavable via other nucleic acid molecule targeting enzymes, such as restriction enzymes (e.g., restriction endonucleases), as described further below.

[00214] Species may be encapsulated in beads during bead generation (e.g., during polymerization of precursors). Such species may or may not participate in polymerization. Such species may be entered into polymerization reaction mixtures such that generated beads comprise the species upon bead formation. In some cases, such species may be added to the gel beads after formation. Such species may include, for example, nucleic acid molecules (e.g., oligonucleotides), reagents for a nucleic acid amplification reaction (e.g., primers, polymerases, dNTPs, co-factors (e.g., ionic co-factors), buffers) including those described herein, reagents for enzymatic reactions (e.g., enzymes, co-factors, substrates, buffers), reagents for nucleic acid modification reactions such as polymerization, ligation, or digestion, and/or reagents for template preparation (e.g., tagmentation) for one or more sequencing platforms (e.g., Nextera® for Illumina®). Such species may include one or more enzymes described herein, including without limitation, polymerase, reverse transcriptase, restriction enzymes (e.g., endonuclease), transposase, ligase, proteinase K, DNase, etc. Such species may include one or more reagents described elsewhere herein (e.g., lysis agents, inhibitors, inactivating agents, chelating agents, stimulus). Trapping of such species may be controlled by the polymer network density generated during polymerization of precursors, control of ionic charge within the gel bead (e.g.,

via ionic species linked to polymerized species), or by the release of other species. Encapsulated species may be released from a bead upon bead degradation and/or by application of a stimulus capable of releasing the species from the bead. Alternatively or in addition, species may be partitioned in a partition (e.g., droplet) during or subsequent to partition formation. Such species may include, without limitation, the abovementioned species that may also be encapsulated in a bead.

[00215] A degradable bead may comprise one or more species with a labile bond such that, when the bead/species is exposed to the appropriate stimuli, the bond is broken and the bead degrades. The labile bond may be a chemical bond (e.g., covalent bond, ionic bond) or may be another type of physical interaction (e.g., van der Waals interactions, dipole-dipole interactions, etc.). In some cases, a crosslinker used to generate a bead may comprise a labile bond. Upon exposure to the appropriate conditions, the labile bond can break and the bead can get degraded. For example, upon exposure of a polyacrylamide gel bead comprising cystamine crosslinkers to a reducing agent, the disulfide bonds of the cystamine can be broken and the bead degraded.

[00216] A degradable bead may be useful in more quickly releasing an attached species (e.g., a nucleic acid molecule, a barcode sequence, a primer, etc) from the bead when the appropriate stimulus is applied to the bead as compared to a bead that does not degrade. For example, for a species bound to an inner surface of a porous bead or in the case of an encapsulated species, the species may have greater mobility and accessibility to other species in solution upon degradation of the bead. In some cases, a species may also be attached to a degradable bead via a degradable linker (e.g., disulfide linker). The degradable linker may respond to the same stimuli as the degradable bead or the two degradable species may respond to different stimuli. For example, a barcode sequence may be attached, via a disulfide bond, to a polyacrylamide bead comprising cystamine. Upon exposure of the barcoded-bead to a reducing agent, the bead may degrade, and the barcode sequence may be released upon breakage of both the disulfide linkage between the barcode sequence and the bead and the disulfide linkages of the cystamine in the bead.

[00217] As will be appreciated from the above disclosure, while referred to as degradation of a bead, in many instances as noted above, that degradation may refer to the disassociation of a bound or entrained species from a bead, both with and without structurally degrading the physical bead itself. For example, entrained species may be released from beads through osmotic pressure differences due to, for example, changing chemical environments. By way of example, alteration of bead pore sizes due to osmotic pressure differences can generally occur without structural degradation of the bead itself. In some cases, an increase in pore size due to osmotic swelling of a bead can permit the release of entrained species within the bead. In other

cases, osmotic shrinking of a bead may cause a bead to better retain an entrained species due to pore size contraction.

[00218] Where degradable beads are provided, it may be beneficial to avoid exposing such beads to the stimulus or stimuli that cause such degradation prior to a given time, in order to, for example, avoid premature bead degradation and issues that arise from such degradation, including for example poor flow characteristics and aggregation. By way of example, where beads comprise reducible cross-linking groups, such as disulfide groups, it may be useful to avoid contacting such beads with reducing agents, e.g., DTT or other disulfide cleaving reagents. In such cases, treatment to the beads described herein will, in some cases be provided free of reducing agents, such as DTT. Because reducing agents are often provided in commercial enzyme preparations, it may be useful to provide reducing agent free (or DTT free) enzyme preparations in treating the beads described herein. Examples of such enzymes include, e.g., polymerase enzyme preparations, reverse transcriptase enzyme preparations, ligase enzyme preparations, as well as many other enzyme preparations that may be used to treat the beads described herein. The terms “reducing agent free” or “DTT free” preparations can refer to a preparation having less than about 1/10th, less than about 1/50th, or even less than about 1/100th of the lower ranges for such materials used in degrading the beads. For example, for DTT, the reducing agent free preparation can have less than about 0.01 millimolar (mM), 0.005 mM, 0.001 mM DTT, 0.0005 mM DTT, or even less than about 0.0001 mM DTT. In many cases, the amount of DTT can be undetectable.

[00219] Numerous chemical triggers may be used to trigger the degradation of beads. Examples of these chemical changes may include, but not be limited to pH-mediated changes to the integrity of a component within the bead, degradation of a component of a bead via cleavage of cross-linked bonds, and depolymerization of a component of a bead.

[00220] In some examples, a bead may be formed from materials that comprise degradable chemical crosslinkers, such as BAC or cystamine. Degradation of such degradable crosslinkers may be accomplished through a number of mechanisms. In some examples, a bead may be contacted with a chemical degrading agent that may induce oxidation, reduction or other chemical changes. For example, a chemical degrading agent may be a reducing agent, such as dithiothreitol (DTT). Additional examples of reducing agents may include β -mercaptoethanol, (2S)-2-amino-1,4-dimercaptobutane (dithiobutylamine or DTBA), tris(2-carboxyethyl) phosphine (TCEP), or combinations thereof. A reducing agent may degrade the disulfide bonds formed between gel precursors forming the bead, and thus, degrade the bead. In other cases, a change in pH of a solution, such as an increase in pH, may trigger degradation of a bead. In

other cases, exposure to an aqueous solution, such as water, may trigger hydrolytic degradation, and thus degradation of the bead. In some cases, any combination of stimuli may trigger degradation of a bead. For example, a change in pH may enable a chemical agent (e.g., DTT) to become an effective reducing agent.

[00221] Beads may also be induced to release their contents upon the application of a thermal stimulus. A change in temperature can cause a variety of changes to a bead. For example, heat can cause a solid bead to liquefy. A change in heat may cause melting of a bead such that a portion of the bead degrades. In other cases, heat may increase the internal pressure of the bead components such that the bead ruptures or explodes. Heat may also act upon heat-sensitive polymers used as materials to construct beads.

[00222] Any suitable agent may degrade beads. In some examples, changes in temperature or pH may be used to degrade thermo-sensitive or pH-sensitive bonds within beads. In some examples, chemical degrading agents may be used to degrade chemical bonds within beads by oxidation, reduction or other chemical changes. For example, a chemical degrading agent may be a reducing agent, such as DTT, wherein DTT may degrade the disulfide bonds formed between a crosslinker and gel precursors, thus degrading the bead. In some examples, a reducing agent may be added to degrade the bead, which may or may not cause the bead to release its contents. Examples of reducing agents may include dithiothreitol (DTT), β -mercaptoethanol, (2S)-2-amino-1,4-dimercaptobutane (dithiobutylamine or DTBA), tris(2-carboxyethyl) phosphine (TCEP), or combinations thereof. The reducing agent may be present at a concentration of about 0.1mM, 0.5mM, 1mM, 5mM, 10mM. The reducing agent may be present at a concentration of at least about 0.1mM, 0.5mM, 1mM, 5mM, 10mM, or greater than 10 mM. The reducing agent may be present at concentration of at most about 10mM, 5mM, 1mM, 0.5mM, 0.1mM, or less.

[00223] Any suitable number of molecular tag molecules (e.g., primer, barcoded oligonucleotide) can be associated with a bead such that, upon release from the bead, the molecular tag molecules (e.g., primer, e.g., barcoded oligonucleotide) are present in the partition at a pre-defined concentration. Such pre-defined concentration may be selected to facilitate certain reactions for generating a sequencing library, e.g., amplification, within the partition. In some cases, the pre-defined concentration of the primer can be limited by the process of producing oligonucleotide bearing beads.

[00224] Although **FIG. 1** and **FIG. 2** have been described in terms of providing substantially singly occupied partitions, above, in certain cases, it may be useful to provide multiply occupied partitions, e.g., containing two, three, four or more cells and/or microcapsules (e.g., beads)

comprising barcoded nucleic acid molecules (e.g., oligonucleotides) within a single partition. Accordingly, as noted above, the flow characteristics of the analyte carrier and/or bead containing fluids and partitioning fluids may be controlled to provide for such multiply occupied partitions. In particular, the flow parameters may be controlled to provide a given occupancy rate at greater than about 50% of the partitions, greater than about 75%, and in some cases greater than about 80%, 90%, 95%, or higher.

[00225] In some cases, additional microcapsules can be used to deliver additional reagents to a partition. In such cases, it may be advantageous to introduce different beads into a common channel or droplet generation junction, from different bead sources (e.g., containing different associated reagents) through different channel inlets into such common channel or droplet generation junction (e.g., junction **210**). In such cases, the flow and frequency of the different beads into the channel or junction may be controlled to provide for a certain ratio of microcapsules from each source, while ensuring a given pairing or combination of such beads into a partition with a given number of analyte carriers (e.g., one analyte carrier and one bead per partition).

[00226] The partitions described herein may comprise small volumes, for example, less than about 10 microliters (μL), 5 μL , 1 μL , 900 picoliters (pL), 800 pL, 700 pL, 600 pL, 500 pL, 400pL, 300 pL, 200 pL, 100pL, 50 pL, 20 pL, 10 pL, 1 pL, 500 nanoliters (nL), 100 nL, 50 nL, or less.

[00227] For example, in the case of droplet-based partitions, the droplets may have overall volumes that are less than about 1000 pL, 900 pL, 800 pL, 700 pL, 600 pL, 500 pL, 400pL, 300 pL, 200 pL, 100pL, 50 pL, 20 pL, 10 pL, 1 pL, or less. Where co-partitioned with microcapsules, it will be appreciated that the sample fluid volume, e.g., including co-partitioned analyte carriers and/or beads, within the partitions may be less than about 90% of the above described volumes, less than about 80%, less than about 70%, less than about 60%, less than about 50%, less than about 40%, less than about 30%, less than about 20%, or less than about 10% of the above described volumes.

[00228] As is described elsewhere herein, partitioning species may generate a population or plurality of partitions. In such cases, any suitable number of partitions can be generated or otherwise provided. For example, at least about 1,000 partitions, at least about 5,000 partitions, at least about 10,000 partitions, at least about 50,000 partitions, at least about 100,000 partitions, at least about 500,000 partitions, at least about 1,000,000 partitions, at least about 5,000,000 partitions at least about 10,000,000 partitions, at least about 50,000,000 partitions, at least about 100,000,000 partitions, at least about 500,000,000 partitions, at least about 1,000,000,000

partitions, or more partitions can be generated or otherwise provided. Moreover, the plurality of partitions may comprise both unoccupied partitions (e.g., empty partitions) and occupied partitions.

Reagents

[00229] In some cases, analyte carriers may be partitioned along with lysis reagents in order to release the contents of the analyte carriers within the partition. In such cases, the lysis agents can be contacted with the analyte carrier suspension concurrently with, or immediately prior to, the introduction of the analyte carriers into the partitioning junction/droplet generation zone (e.g., junction **210**), such as through an additional channel or channels upstream of the channel junction. In other examples, additionally or alternatively, analyte carriers may be partitioned along with other reagents, as will be described further below.

[00230] **FIG. 3** shows an example of a microfluidic channel structure **300** for co-partitioning analyte carriers and reagents. The channel structure **300** can include channel segments **301**, **302**, **304**, **306** and **308**. Channel segments **301** and **302** communicate at a first channel junction **309**. Channel segments **302**, **304**, **306**, and **308** communicate at a second channel junction **310**.

[00231] In an example operation, the channel segment **301** may transport an aqueous fluid **312** that includes a plurality of analyte carriers **314** along the channel segment **301** into the second junction **310**. As an alternative or in addition to, channel segment **301** may transport beads (e.g., gel beads). The beads may comprise barcode molecules.

[00232] For example, the channel segment **301** may be connected to a reservoir comprising an aqueous suspension of analyte carriers **314**. Upstream of, and immediately prior to reaching, the second junction **310**, the channel segment **301** may meet the channel segment **302** at the first junction **309**. The channel segment **302** may transport a plurality of reagents **315** (e.g., lysis agents) suspended in the aqueous fluid **312** along the channel segment **302** into the first junction **309**. For example, the channel segment **302** may be connected to a reservoir comprising the reagents **315**. After the first junction **309**, the aqueous fluid **312** in the channel segment **301** can carry both the analyte carriers **314** and the reagents **315** towards the second junction **310**. In some instances, the aqueous fluid **312** in the channel segment **301** can include one or more reagents, which can be the same or different reagents as the reagents **315**. A second fluid **316** that is immiscible with the aqueous fluid **312** (e.g., oil) can be delivered to the second junction **310** from each of channel segments **304** and **306**. Upon meeting of the aqueous fluid **312** from the channel segment **301** and the second fluid **316** from each of channel segments **304** and **306** at the second channel junction **310**, the aqueous fluid **312** can be partitioned as discrete droplets **318** in the second fluid **316** and flow away from the second junction **310** along channel segment

308. The channel segment **308** may deliver the discrete droplets **318** to an outlet reservoir fluidly coupled to the channel segment **308**, where they may be harvested.

[00233] The second fluid **316** can comprise an oil, such as a fluorinated oil, that includes a fluorosurfactant for stabilizing the resulting droplets, for example, inhibiting subsequent coalescence of the resulting droplets **318**.

[00234] A discrete droplet generated may include an individual analyte carrier **314** and/or one or more reagents **315**. In some instances, a discrete droplet generated may include a barcode carrying bead (not shown), such as via other microfluidics structures described elsewhere herein. In some instances, a discrete droplet may be unoccupied (e.g., no reagents, no analyte carriers).

[00235] Beneficially, when lysis reagents and analyte carriers are co-partitioned, the lysis reagents can facilitate the release of the contents of the analyte carriers within the partition. The contents released in a partition may remain discrete from the contents of other partitions.

[00236] As will be appreciated, the channel segments described herein may be coupled to any of a variety of different fluid sources or receiving components, including reservoirs, tubing, manifolds, or fluidic components of other systems. As will be appreciated, the microfluidic channel structure **300** may have other geometries. For example, a microfluidic channel structure can have more than two channel junctions. For example, a microfluidic channel structure can have 2, 3, 4, 5 channel segments or more each carrying the same or different types of beads, reagents, and/or analyte carriers that meet at a channel junction. Fluid flow in each channel segment may be controlled to control the partitioning of the different elements into droplets. Fluid may be directed flow along one or more channels or reservoirs via one or more fluid flow units. A fluid flow unit can comprise compressors (e.g., providing positive pressure), pumps (e.g., providing negative pressure), actuators, and the like to control flow of the fluid. Fluid may also or otherwise be controlled via applied pressure differentials, centrifugal force, electrokinetic pumping, vacuum, capillary or gravity flow, or the like.

[00237] Examples of lysis agents include bioactive reagents, such as lysis enzymes that are used for lysis of different cell types, e.g., gram positive or negative bacteria, plants, yeast, mammalian, etc., such as lysozymes, achromopeptidase, lysostaphin, labiase, kitalase, lyticase, and a variety of other lysis enzymes available from, e.g., Sigma-Aldrich, Inc. (St Louis, MO), as well as other commercially available lysis enzymes. Lysis agents may comprise Mammalian Protein Extraction Reagent (MPER). Other lysis agents may additionally or alternatively be co-partitioned with the analyte carriers to cause the release of the analyte carriers' contents into the partitions. For example, in some cases, surfactant-based lysis solutions may be used to lyse cells, although surfactants can interfere with stable emulsions. In some cases, lysis solutions may

include non-ionic surfactants such as, for example, TritonX-100 and Tween 20. In some cases, lysis solutions may include ionic surfactants such as, for example, sarcosyl and sodium dodecyl sulfate (SDS). Electroporation, thermal, acoustic or mechanical cellular disruption may also be used in certain cases, e.g., non-emulsion-based partitioning such as encapsulation of analyte carriers that may be in addition to or in place of droplet partitioning, where any pore size of the encapsulate is sufficiently small to retain nucleic acid fragments of a given size, following cellular disruption.

[00238] Alternatively or in addition to the lysis agents co-partitioned with the analyte carriers described above, other reagents can also be co-partitioned with the analyte carriers, including, for example, DNase and RNase inactivating agents or inhibitors, such as proteinase K, chelating agents, such as EDTA, and other reagents employed in removing or otherwise reducing negative activity or impact of different cell lysate components on subsequent processing of nucleic acids. In addition, in the case of encapsulated analyte carriers (e.g., a cell or a nucleus in a polymer matrix), the analyte carriers may be exposed to an appropriate stimulus to release the analyte carriers or their contents from a co-partitioned microcapsule. For example, in some cases, a chemical stimulus may be co-partitioned along with an encapsulated analyte carrier to allow for the degradation of the microcapsule and release of the cell or its contents into the larger partition. In some cases, this stimulus may be the same as the stimulus described elsewhere herein for release of nucleic acid molecules (e.g., oligonucleotides) from their respective microcapsule (e.g., bead). In alternative examples, this may be a different and non-overlapping stimulus, in order to allow an encapsulated analyte carrier to be released into a partition at a different time from the release of nucleic acid molecules into the same partition. For a description of methods, compositions, and systems for encapsulating cells (also referred to as a “cell bead”), see, e.g., U.S. Pat. 10,428,326 and U.S. Pat. Pub. 20190100632, which are each incorporated by reference in their entirety.

[00239] Additional reagents may also be co-partitioned with the analyte carrier, such as endonucleases to fragment an analyte carrier’s DNA, DNA polymerase enzymes and dNTPs used to amplify the analyte carrier’s nucleic acid fragments and to attach the barcode molecular tags to the amplified fragments. Other enzymes may be co-partitioned, including without limitation, polymerase, transposase, ligase, proteinase K, DNase, etc. Additional reagents may also include reverse transcriptase enzymes, including enzymes with terminal transferase activity, primers and oligonucleotides, and switch oligonucleotides (also referred to herein as “switch oligos” or “template switching oligonucleotides”) which can be used for template switching. In some cases, template switching can be used to increase the length of a cDNA. In some cases,

template switching can be used to append a predefined nucleic acid sequence to the cDNA. In an example of template switching, cDNA can be generated from reverse transcription of a template, e.g., cellular mRNA, where a reverse transcriptase with terminal transferase activity can add additional nucleotides, e.g., polyC, to the cDNA in a template independent manner. Switch oligos can include sequences complementary to the additional nucleotides, e.g., polyG. The additional nucleotides (e.g., polyC) on the cDNA can hybridize to the additional nucleotides (e.g., polyG) on the switch oligo, whereby the switch oligo can be used by the reverse transcriptase as template to further extend the cDNA. Template switching oligonucleotides may comprise a hybridization region and a template region. The hybridization region can comprise any sequence capable of hybridizing to the target. In some cases, as previously described, the hybridization region comprises a series of G bases to complement the overhanging C bases at the 3' end of a cDNA molecule. The series of G bases may comprise 1 G base, 2 G bases, 3 G bases, 4 G bases, 5 G bases or more than 5 G bases. The template sequence can comprise any sequence to be incorporated into the cDNA. In some cases, the template region comprises at least 1 (e.g., at least 2, 3, 4, 5 or more) tag sequences and/or functional sequences. Switch oligos may comprise deoxyribonucleic acids; ribonucleic acids; modified nucleic acids including 2-Aminopurine, 2,6-Diaminopurine (2-Amino-dA), inverted dT, 5-Methyl dC, 2'-deoxyInosine, Super T (5-hydroxybutynl-2'-deoxyuridine), Super G (8-aza-7-deazaguanosine), locked nucleic acids (LNAs), unlocked nucleic acids (UNAs, e.g., UNA-A, UNA-U, UNA-C, UNA-G), Iso-dG, Iso-dC, 2' Fluoro bases (e.g., Fluoro C, Fluoro U, Fluoro A, and Fluoro G), or any combination.

[00240] In some cases, the length of a switch oligo may be at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249 or 250 nucleotides or longer.

[00241] In some cases, the length of a switch oligo may be at most about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249 or 250 nucleotides.

[00242] Once the contents of the cells are released into their respective partitions, the macromolecular components (e.g., macromolecular constituents of analyte carriers, such as RNA, DNA, or proteins) contained therein may be further processed within the partitions. In accordance with the methods and systems described herein, the macromolecular component contents of individual analyte carriers can be provided with unique identifiers such that, upon characterization of those macromolecular components they may be attributed as having been derived from the same analyte carrier or carriers. The ability to attribute characteristics to individual analyte carrier or groups of analyte carriers is provided by the assignment of unique identifiers specifically to an individual analyte carrier or groups of analyte carriers. Unique identifiers, e.g., in the form of nucleic acid barcodes can be assigned or associated with individual analyte carriers or populations of analyte carriers, in order to tag or label the analyte carrier's macromolecular components (and as a result, its characteristics) with the unique identifiers. These unique identifiers can then be used to attribute the analyte carrier's components and characteristics to an individual analyte carrier or group of analyte carriers.

[00243] In some cases, this is performed by co-partitioning the individual analyte carrier or groups of analyte carriers with the unique identifiers, such as described above (with reference to **FIG. 2**). In some cases, the unique identifiers are provided in the form of nucleic acid molecules (e.g., oligonucleotides) that comprise nucleic acid barcode sequences that may be attached to or otherwise associated with the nucleic acid contents of individual analyte carrier, or to other components of the analyte carrier, and particularly to fragments of those nucleic acids. The nucleic acid molecules are partitioned such that as between nucleic acid molecules in a given

partition, the nucleic acid barcode sequences contained therein are the same, but as between different partitions, the nucleic acid molecule can, and do have differing barcode sequences, or at least represent a large number of different barcode sequences across all of the partitions in a given analysis. In some cases, only one nucleic acid barcode sequence can be associated with a given partition, although in some cases, two or more different barcode sequences may be present.

[00244] The nucleic acid barcode sequences can include from about 6 to about 20 or more nucleotides within the sequence of the nucleic acid molecules (e.g., oligonucleotides). The nucleic acid barcode sequences can include from about 6 to about 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides. In some cases, the length of a barcode sequence may be about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides or longer. In some cases, the length of a barcode sequence may be at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides or longer. In some cases, the length of a barcode sequence may be at most about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides or shorter. These nucleotides may be completely contiguous, i.e., in a single stretch of adjacent nucleotides, or they may be separated into two or more separate subsequences that are separated by 1 or more nucleotides. In some cases, separated barcode subsequences can be from about 4 to about 16 nucleotides in length. In some cases, the barcode subsequence may be about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 nucleotides or longer. In some cases, the barcode subsequence may be at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 nucleotides or longer. In some cases, the barcode subsequence may be at most about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 nucleotides or shorter.

[00245] The co-partitioned nucleic acid molecules can also comprise other functional sequences useful in the processing of the nucleic acids from the co-partitioned analyte carriers. These sequences include, e.g., targeted or random/universal amplification primer sequences for amplifying nucleic acids (e.g., mRNA, genomic DNA) from the individual analyte carriers within the partitions while attaching the associated barcode sequences, sequencing primers or primer recognition sites, hybridization or probing sequences, e.g., for identification of presence of the sequences or for pulling down barcoded nucleic acids, or any of a number of other potential functional sequences. Other mechanisms of co-partitioning oligonucleotides may also be employed, including, e.g., coalescence of two or more droplets, where one droplet contains oligonucleotides, or microdispensing of oligonucleotides (e.g., attached to a bead) into partitions, e.g., droplets within microfluidic systems.

[00246] In an example, microcapsules, such as beads, are provided that each include large numbers of the above described barcoded nucleic acid molecules (e.g., barcoded oligonucleotides) releasably attached to the beads, where all of the nucleic acid molecules

attached to a particular bead will include the same nucleic acid barcode sequence, but where a large number of diverse barcode sequences are represented across the population of beads used. In some examples, hydrogel beads, e.g., comprising polyacrylamide polymer matrices, are used as a solid support and delivery vehicle for the nucleic acid molecules into the partitions, as they are capable of carrying large numbers of nucleic acid molecules, and may be configured to release those nucleic acid molecules upon exposure to a particular stimulus, as described elsewhere herein. In some cases, the population of beads provides a diverse barcode sequence library that includes at least about 1,000 different barcode sequences, at least about 5,000 different barcode sequences, at least about 10,000 different barcode sequences, at least about 50,000 different barcode sequences, at least about 100,000 different barcode sequences, at least about 1,000,000 different barcode sequences, at least about 5,000,000 different barcode sequences, or at least about 10,000,000 different barcode sequences, or more. Additionally, each bead can be provided with large numbers of nucleic acid (e.g., oligonucleotide) molecules attached. In particular, the number of molecules of nucleic acid molecules including the barcode sequence on an individual bead can be at least about 1,000 nucleic acid molecules, at least about 5,000 nucleic acid molecules, at least about 10,000 nucleic acid molecules, at least about 50,000 nucleic acid molecules, at least about 100,000 nucleic acid molecules, at least about 500,000 nucleic acids, at least about 1,000,000 nucleic acid molecules, at least about 5,000,000 nucleic acid molecules, at least about 10,000,000 nucleic acid molecules, at least about 50,000,000 nucleic acid molecules, at least about 100,000,000 nucleic acid molecules, at least about 250,000,000 nucleic acid molecules and in some cases at least about 1 billion nucleic acid molecules, or more. Nucleic acid molecules of a given bead can include identical (or common) barcode sequences, different barcode sequences, or a combination of both. Nucleic acid molecules of a given bead can include multiple sets of nucleic acid molecules. Nucleic acid molecules of a given set can include identical barcode sequences. The identical barcode sequences can be different from barcode sequences of nucleic acid molecules of another set.

[00247] Moreover, when the population of beads is partitioned, the resulting population of partitions can also include a diverse barcode library that includes at least about 1,000 different barcode sequences, at least about 5,000 different barcode sequences, at least about 10,000 different barcode sequences, at least at least about 50,000 different barcode sequences, at least about 100,000 different barcode sequences, at least about 1,000,000 different barcode sequences, at least about 5,000,000 different barcode sequences, or at least about 10,000,000 different barcode sequences. Additionally, each partition of the population can include at least about 1,000 nucleic acid molecules, at least about 5,000 nucleic acid molecules, at least about 10,000 nucleic

acid molecules, at least about 50,000 nucleic acid molecules, at least about 100,000 nucleic acid molecules, at least about 500,000 nucleic acids, at least about 1,000,000 nucleic acid molecules, at least about 5,000,000 nucleic acid molecules, at least about 10,000,000 nucleic acid molecules, at least about 50,000,000 nucleic acid molecules, at least about 100,000,000 nucleic acid molecules, at least about 250,000,000 nucleic acid molecules and in some cases at least about 1 billion nucleic acid molecules.

[00248] In some cases, multiple different nucleic acid barcode molecules can be incorporated within a given partition and be either attached to a single bead or to multiple beads within the partition. For example, in some cases, a mixed, but known set of barcode sequences may provide greater assurance of identification in the subsequent processing, e.g., by providing a stronger address or attribution of the barcodes to a given partition, as a duplicate or independent confirmation of the output from a given partition.

[00249] The nucleic acid molecules (e.g., oligonucleotides) may be releasable from the beads upon the application of a stimulus to the beads. In some cases, the stimulus may be a photo-stimulus, e.g., through cleavage of a photo-labile linkage that releases the nucleic acid molecules. In other cases, a thermal stimulus may be used, where elevation of the temperature of the beads environment will result in cleavage of a linkage or other release of the nucleic acid molecules from the beads. In still other cases, a chemical stimulus can be used that cleaves a linkage of the nucleic acid molecules to the beads, or otherwise results in release of the nucleic acid molecules from the beads. In one case, such compositions include the polyacrylamide matrices described above for encapsulation of analyte carriers and may be degraded for release of the attached nucleic acid molecules through exposure to a reducing agent, such as DTT.

[00250] In some examples, provided are systems and methods for controlled partitioning. Droplet size may be controlled by adjusting certain geometric features in channel architecture (e.g., microfluidics channel architecture). For example, an expansion angle, width, and/or length of a channel may be adjusted to control droplet size.

[00251] **FIG. 4** shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets. A channel structure **400** can include a channel segment **402** communicating at a channel junction **406** (or intersection) with a reservoir **404**. The reservoir **404** can be a chamber. Any reference to “reservoir,” as used herein, can also refer to a “chamber.” In operation, an aqueous fluid **408** that includes suspended beads **412** may be transported along the channel segment **402** into the junction **406** to meet a second fluid **410** that is immiscible with the aqueous fluid **408** in the reservoir **404** to create droplets **416**, **418** of the aqueous fluid **408** flowing into the reservoir **404**. At the junction **406** where the aqueous fluid

408 and the second fluid **410** meet, droplets can form based on factors such as the hydrodynamic forces at the junction **406**, flow rates of the two fluids **408**, **410**, fluid properties, and certain geometric parameters (e.g., w , h_0 , α , etc.) of the channel structure **400**. A plurality of droplets can be collected in the reservoir **404** by continuously injecting the aqueous fluid **408** from the channel segment **402** through the junction **406**.

[00252] A discrete droplet generated may include a bead (e.g., as in occupied droplets **416**). Alternatively, a discrete droplet generated may include more than one bead. Alternatively, a discrete droplet generated may not include any beads (e.g., as in unoccupied droplet **418**). In some instances, a discrete droplet generated may contain one or more analyte carriers, as described elsewhere herein. In some instances, a discrete droplet generated may comprise one or more reagents, as described elsewhere herein.

[00253] In some instances, the aqueous fluid **408** can have a substantially uniform concentration or frequency of beads **412**. The beads **412** can be introduced into the channel segment **402** from a separate channel (not shown in **FIG. 4**). The frequency of beads **412** in the channel segment **402** may be controlled by controlling the frequency in which the beads **412** are introduced into the channel segment **402** and/or the relative flow rates of the fluids in the channel segment **402** and the separate channel. In some instances, the beads can be introduced into the channel segment **402** from a plurality of different channels, and the frequency controlled accordingly.

[00254] In some instances, the aqueous fluid **408** in the channel segment **402** can comprise analyte carriers (e.g., described with reference to **FIGS. 1** and **2**). In some instances, the aqueous fluid **408** can have a substantially uniform concentration or frequency of analyte carriers. As with the beads, the analyte carriers can be introduced into the channel segment **402** from a separate channel. The frequency or concentration of the analyte carriers in the aqueous fluid **408** in the channel segment **402** may be controlled by controlling the frequency in which the analyte carriers are introduced into the channel segment **402** and/or the relative flow rates of the fluids in the channel segment **402** and the separate channel. In some instances, the analyte carriers can be introduced into the channel segment **402** from a plurality of different channels, and the frequency controlled accordingly. In some instances, a first separate channel can introduce beads and a second separate channel can introduce analyte carriers into the channel segment **402**. The first separate channel introducing the beads may be upstream or downstream of the second separate channel introducing the analyte carriers.

[00255] The second fluid **410** can comprise an oil, such as a fluorinated oil, that includes a fluorosurfactant for stabilizing the resulting droplets, for example, inhibiting subsequent coalescence of the resulting droplets.

[00256] In some instances, the second fluid **410** may not be subjected to and/or directed to any flow in or out of the reservoir **404**. For example, the second fluid **410** may be substantially stationary in the reservoir **404**. In some instances, the second fluid **410** may be subjected to flow within the reservoir **404**, but not in or out of the reservoir **404**, such as via application of pressure to the reservoir **404** and/or as affected by the incoming flow of the aqueous fluid **408** at the junction **406**. Alternatively, the second fluid **410** may be subjected and/or directed to flow in or out of the reservoir **404**. For example, the reservoir **404** can be a channel directing the second fluid **410** from upstream to downstream, transporting the generated droplets.

[00257] The channel structure **400** at or near the junction **406** may have certain geometric features that at least partly determine the sizes of the droplets formed by the channel structure **400**. The channel segment **402** can have a height, h_0 and width, w , at or near the junction **406**. By way of example, the channel segment **402** can comprise a rectangular cross-section that leads to a reservoir **404** having a wider cross-section (such as in width or diameter). Alternatively, the cross-section of the channel segment **402** can be other shapes, such as a circular shape, trapezoidal shape, polygonal shape, or any other shapes. The top and bottom walls of the reservoir **404** at or near the junction **406** can be inclined at an expansion angle, α . The expansion angle, α , allows the tongue (portion of the aqueous fluid **408** leaving channel segment **402** at junction **406** and entering the reservoir **404** before droplet formation) to increase in depth and facilitate decrease in curvature of the intermediately formed droplet. Droplet size may decrease with increasing expansion angle. The resulting droplet radius, R_d , may be predicted by the following equation for the aforementioned geometric parameters of h_0 , w , and α :

$$R_d \approx 0.44 \left(1 + 2.2 \sqrt{\tan \alpha} \frac{w}{h_0} \right) \frac{h_0}{\sqrt{\tan \alpha}}$$

[00258] By way of example, for a channel structure with $w = 21 \mu\text{m}$, $h = 21 \mu\text{m}$, and $\alpha = 3^\circ$, the predicted droplet size is $121 \mu\text{m}$. In another example, for a channel structure with $w = 25 \mu\text{m}$, $h = 25 \mu\text{m}$, and $\alpha = 5^\circ$, the predicted droplet size is $123 \mu\text{m}$. In another example, for a channel structure with $w = 28 \mu\text{m}$, $h = 28 \mu\text{m}$, and $\alpha = 7^\circ$, the predicted droplet size is $124 \mu\text{m}$.

[00259] In some instances, the expansion angle, α , may be between a range of from about 0.5° to about 4° , from about 0.1° to about 10° , or from about 0° to about 90° . For example, the expansion angle can be at least about 0.01° , 0.1° , 0.2° , 0.3° , 0.4° , 0.5° , 0.6° , 0.7° , 0.8° , 0.9° , 1° , 2° , 3° , 4° , 5° , 6° , 7° , 8° , 9° , 10° , 15° , 20° , 25° , 30° , 35° , 40° , 45° , 50° , 55° , 60° , 65° , 70° , 75° , 80° , 85° , or higher. In some instances, the expansion angle can be at most about 89° , 88° , 87° ,

86°, 85°, 84°, 83°, 82°, 81°, 80°, 75°, 70°, 65°, 60°, 55°, 50°, 45°, 40°, 35°, 30°, 25°, 20°, 15°, 10°, 9°, 8°, 7°, 6°, 5°, 4°, 3°, 2°, 1°, 0.1°, 0.01°, or less. In some instances, the width, w , can be between a range of from about 100 micrometers (μm) to about 500 μm . In some instances, the width, w , can be between a range of from about 10 μm to about 200 μm . Alternatively, the width can be less than about 10 μm . Alternatively, the width can be greater than about 500 μm . In some instances, the flow rate of the aqueous fluid **408** entering the junction **406** can be between about 0.04 microliters (μL)/minute (min) and about 40 $\mu\text{L}/\text{min}$. In some instances, the flow rate of the aqueous fluid **408** entering the junction **406** can be between about 0.01 microliters (μL)/minute (min) and about 100 $\mu\text{L}/\text{min}$. Alternatively, the flow rate of the aqueous fluid **408** entering the junction **406** can be less than about 0.01 $\mu\text{L}/\text{min}$. Alternatively, the flow rate of the aqueous fluid **408** entering the junction **406** can be greater than about 40 $\mu\text{L}/\text{min}$, such as 45 $\mu\text{L}/\text{min}$, 50 $\mu\text{L}/\text{min}$, 55 $\mu\text{L}/\text{min}$, 60 $\mu\text{L}/\text{min}$, 65 $\mu\text{L}/\text{min}$, 70 $\mu\text{L}/\text{min}$, 75 $\mu\text{L}/\text{min}$, 80 $\mu\text{L}/\text{min}$, 85 $\mu\text{L}/\text{min}$, 90 $\mu\text{L}/\text{min}$, 95 $\mu\text{L}/\text{min}$, 100 $\mu\text{L}/\text{min}$, 110 $\mu\text{L}/\text{min}$, 120 $\mu\text{L}/\text{min}$, 130 $\mu\text{L}/\text{min}$, 140 $\mu\text{L}/\text{min}$, 150 $\mu\text{L}/\text{min}$, or greater. At lower flow rates, such as flow rates of about less than or equal to 10 microliters/minute, the droplet radius may not be dependent on the flow rate of the aqueous fluid **408** entering the junction **406**.

[00260] In some instances, at least about 50% of the droplets generated can have uniform size. In some instances, at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater of the droplets generated can have uniform size. Alternatively, less than about 50% of the droplets generated can have uniform size.

[00261] The throughput of droplet generation can be increased by increasing the points of generation, such as increasing the number of junctions (e.g., junction **406**) between aqueous fluid **408** channel segments (e.g., channel segment **402**) and the reservoir **404**. Alternatively or in addition, the throughput of droplet generation can be increased by increasing the flow rate of the aqueous fluid **408** in the channel segment **402**.

[00262] **FIG. 5** shows an example of a microfluidic channel structure for increased droplet generation throughput. A microfluidic channel structure **500** can comprise a plurality of channel segments **502** and a reservoir **504**. Each of the plurality of channel segments **502** may be in fluid communication with the reservoir **504**. The channel structure **500** can comprise a plurality of channel junctions **506** between the plurality of channel segments **502** and the reservoir **504**. Each channel junction can be a point of droplet generation. The channel segment **402** from the channel structure **400** in **FIG. 4** and any description to the components thereof may correspond to a given channel segment of the plurality of channel segments **502** in channel structure **500** and any description to the corresponding components thereof. The reservoir **404** from the channel

structure **400** and any description to the components thereof may correspond to the reservoir **504** from the channel structure **500** and any description to the corresponding components thereof.

[00263] Each channel segment of the plurality of channel segments **502** may comprise an aqueous fluid **508** that includes suspended beads **512**. The reservoir **504** may comprise a second fluid **510** that is immiscible with the aqueous fluid **508**. In some instances, the second fluid **510** may not be subjected to and/or directed to any flow in or out of the reservoir **504**. For example, the second fluid **510** may be substantially stationary in the reservoir **504**. In some instances, the second fluid **510** may be subjected to flow within the reservoir **504**, but not in or out of the reservoir **504**, such as via application of pressure to the reservoir **504** and/or as affected by the incoming flow of the aqueous fluid **508** at the junctions. Alternatively, the second fluid **510** may be subjected and/or directed to flow in or out of the reservoir **504**. For example, the reservoir **504** can be a channel directing the second fluid **510** from upstream to downstream, transporting the generated droplets.

[00264] In operation, the aqueous fluid **508** that includes suspended beads **512** may be transported along the plurality of channel segments **502** into the plurality of junctions **506** to meet the second fluid **510** in the reservoir **504** to create droplets **516**, **518**. A droplet may form from each channel segment at each corresponding junction with the reservoir **504**. At the junction where the aqueous fluid **508** and the second fluid **510** meet, droplets can form based on factors such as the hydrodynamic forces at the junction, flow rates of the two fluids **508**, **510**, fluid properties, and certain geometric parameters (e.g., w , h_0 , α , etc.) of the channel structure **500**, as described elsewhere herein. A plurality of droplets can be collected in the reservoir **504** by continuously injecting the aqueous fluid **508** from the plurality of channel segments **502** through the plurality of junctions **506**. Throughput may significantly increase with the parallel channel configuration of channel structure **500**. For example, a channel structure having five inlet channel segments comprising the aqueous fluid **508** may generate droplets five times as frequently than a channel structure having one inlet channel segment, provided that the fluid flow rate in the channel segments are substantially the same. The fluid flow rate in the different inlet channel segments may or may not be substantially the same. A channel structure may have as many parallel channel segments as is practical and allowed for the size of the reservoir. For example, the channel structure may have at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 500, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1500, 5000 or more parallel or substantially parallel channel segments.

[00265] The geometric parameters, w , h_0 , and α , may or may not be uniform for each of the channel segments in the plurality of channel segments **502**. For example, each channel segment

may have the same or different widths at or near its respective channel junction with the reservoir **504**. For example, each channel segment may have the same or different height at or near its respective channel junction with the reservoir **504**. In another example, the reservoir **504** may have the same or different expansion angle at the different channel junctions with the plurality of channel segments **502**. When the geometric parameters are uniform, beneficially, droplet size may also be controlled to be uniform even with the increased throughput. In some instances, the geometric parameters for the plurality of channel segments **502** may be varied accordingly to yield different distributions of droplet sizes.

[00266] In some instances, at least about 50% of the droplets generated can have uniform size. In some instances, at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater of the droplets generated can have uniform size. Alternatively, less than about 50% of the droplets generated can have uniform size.

[00267] **FIG. 6** shows another example of a microfluidic channel structure for increased droplet generation throughput. A microfluidic channel structure **600** can comprise a plurality of channel segments **602** arranged generally circularly around the perimeter of a reservoir **604**. Each of the plurality of channel segments **602** may be in fluid communication with the reservoir **604**. The channel structure **600** can comprise a plurality of channel junctions **606** between the plurality of channel segments **602** and the reservoir **604**. Each channel junction can be a point of droplet generation. The channel segment **402** from the channel structure **400** in **FIG. 2** and any description to the components thereof may correspond to a given channel segment of the plurality of channel segments **602** in channel structure **600** and any description to the corresponding components thereof. The reservoir **404** from the channel structure **400** and any description to the components thereof may correspond to the reservoir **604** from the channel structure **600** and any description to the corresponding components thereof.

[00268] Each channel segment of the plurality of channel segments **602** may comprise an aqueous fluid **608** that includes suspended beads **612**. The reservoir **604** may comprise a second fluid **610** that is immiscible with the aqueous fluid **608**. In some instances, the second fluid **610** may not be subjected to and/or directed to any flow in or out of the reservoir **604**. For example, the second fluid **610** may be substantially stationary in the reservoir **604**. In some instances, the second fluid **610** may be subjected to flow within the reservoir **604**, but not in or out of the reservoir **604**, such as via application of pressure to the reservoir **604** and/or as affected by the incoming flow of the aqueous fluid **608** at the junctions. Alternatively, the second fluid **610** may be subjected and/or directed to flow in or out of the reservoir **604**. For example, the reservoir

604 can be a channel directing the second fluid **610** from upstream to downstream, transporting the generated droplets.

[00269] In operation, the aqueous fluid **608** that includes suspended beads **612** may be transported along the plurality of channel segments **602** into the plurality of junctions **606** to meet the second fluid **610** in the reservoir **604** to create a plurality of droplets **616**. A droplet may form from each channel segment at each corresponding junction with the reservoir **604**. At the junction where the aqueous fluid **608** and the second fluid **610** meet, droplets can form based on factors such as the hydrodynamic forces at the junction, flow rates of the two fluids **608**, **610**, fluid properties, and certain geometric parameters (e.g., widths and heights of the channel segments **602**, expansion angle of the reservoir **604**, etc.) of the channel structure **600**, as described elsewhere herein. A plurality of droplets can be collected in the reservoir **604** by continuously injecting the aqueous fluid **608** from the plurality of channel segments **602** through the plurality of junctions **606**. Throughput may significantly increase with the substantially parallel channel configuration of the channel structure **600**. A channel structure may have as many substantially parallel channel segments as is practical and allowed for by the size of the reservoir. For example, the channel structure may have at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1500, 5000 or more parallel or substantially parallel channel segments. The plurality of channel segments may be substantially evenly spaced apart, for example, around an edge or perimeter of the reservoir. Alternatively, the spacing of the plurality of channel segments may be uneven.

[00270] The reservoir **604** may have an expansion angle, α (not shown in **FIG. 6**) at or near each channel junction. Each channel segment of the plurality of channel segments **602** may have a width, w , and a height, h_0 , at or near the channel junction. The geometric parameters, w , h_0 , and α , may or may not be uniform for each of the channel segments in the plurality of channel segments **602**. For example, each channel segment may have the same or different widths at or near its respective channel junction with the reservoir **604**. For example, each channel segment may have the same or different height at or near its respective channel junction with the reservoir **604**.

[00271] The reservoir **604** may have the same or different expansion angle at the different channel junctions with the plurality of channel segments **602**. For example, a circular reservoir (as shown in **FIG. 6**) may have a conical, dome-like, or hemispherical ceiling (e.g., top wall) to provide the same or substantially same expansion angle for each channel segments **602** at or near the plurality of channel junctions **606**. When the geometric parameters are uniform, beneficially, resulting droplet size may be controlled to be uniform even with the increased throughput. In

some instances, the geometric parameters for the plurality of channel segments **602** may be varied accordingly to yield different distributions of droplet sizes.

[00272] In some instances, at least about 50% of the droplets generated can have uniform size. In some instances, at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater of the droplets generated can have uniform size. Alternatively, less than about 50% of the droplets generated can have uniform size. The beads and/or analyte carrier injected into the droplets may or may not have uniform size.

[00273] **FIG. 7A** shows a cross-section view of another example of a microfluidic channel structure with a geometric feature for controlled partitioning. A channel structure **700** can include a channel segment **702** communicating at a channel junction **706** (or intersection) with a reservoir **704**. In some instances, the channel structure **700** and one or more of its components can correspond to the channel structure **100** and one or more of its components. **FIG. 7B** shows a perspective view of the channel structure **700** of **FIG. 7A**.

[00274] An aqueous fluid **712** comprising a plurality of particles **716** may be transported along the channel segment **702** into the junction **706** to meet a second fluid **714** (e.g., oil, etc.) that is immiscible with the aqueous fluid **712** in the reservoir **704** to create droplets **720** of the aqueous fluid **712** flowing into the reservoir **704**. At the junction **706** where the aqueous fluid **712** and the second fluid **714** meet, droplets can form based on factors such as the hydrodynamic forces at the junction **706**, relative flow rates of the two fluids **712**, **714**, fluid properties, and certain geometric parameters (e.g., Δh , etc.) of the channel structure **700**. A plurality of droplets can be collected in the reservoir **704** by continuously injecting the aqueous fluid **712** from the channel segment **702** at the junction **706**.

[00275] A discrete droplet generated may comprise one or more particles of the plurality of particles **716**. As described elsewhere herein, a particle may be any particle, such as a bead, cell bead, gel bead, analyte carrier, macromolecular constituents of analyte carrier, or other particles. Alternatively, a discrete droplet generated may not include any particles.

[00276] In some instances, the aqueous fluid **712** can have a substantially uniform concentration or frequency of particles **716**. As described elsewhere herein (e.g., with reference to **FIG. 4**), the particles **716** (e.g., beads) can be introduced into the channel segment **702** from a separate channel (not shown in **FIG. 7**). The frequency of particles **716** in the channel segment **702** may be controlled by controlling the frequency in which the particles **716** are introduced into the channel segment **702** and/or the relative flow rates of the fluids in the channel segment **702** and the separate channel. In some instances, the particles **716** can be introduced into the channel segment **702** from a plurality of different channels, and the frequency controlled

accordingly. In some instances, different particles may be introduced via separate channels. For example, a first separate channel can introduce beads and a second separate channel can introduce analyte carriers into the channel segment **702**. The first separate channel introducing the beads may be upstream or downstream of the second separate channel introducing the analyte carriers.

[00277] In some instances, the second fluid **714** may not be subjected to and/or directed to any flow in or out of the reservoir **704**. For example, the second fluid **714** may be substantially stationary in the reservoir **704**. In some instances, the second fluid **714** may be subjected to flow within the reservoir **704**, but not in or out of the reservoir **704**, such as via application of pressure to the reservoir **704** and/or as affected by the incoming flow of the aqueous fluid **712** at the junction **706**. Alternatively, the second fluid **714** may be subjected and/or directed to flow in or out of the reservoir **704**. For example, the reservoir **704** can be a channel directing the second fluid **714** from upstream to downstream, transporting the generated droplets.

[00278] The channel structure **700** at or near the junction **706** may have certain geometric features that at least partly determine the sizes and/or shapes of the droplets formed by the channel structure **700**. The channel segment **702** can have a first cross-section height, h_1 , and the reservoir **704** can have a second cross-section height, h_2 . The first cross-section height, h_1 , and the second cross-section height, h_2 , may be different, such that at the junction **706**, there is a height difference of Δh . The second cross-section height, h_2 , may be greater than the first cross-section height, h_1 . In some instances, the reservoir may thereafter gradually increase in cross-section height, for example, the more distant it is from the junction **706**. In some instances, the cross-section height of the reservoir may increase in accordance with expansion angle, β , at or near the junction **706**. The height difference, Δh , and/or expansion angle, β , can allow the tongue (portion of the aqueous fluid **712** leaving channel segment **702** at junction **706** and entering the reservoir **704** before droplet formation) to increase in depth and facilitate decrease in curvature of the intermediately formed droplet. For example, droplet size may decrease with increasing height difference and/or increasing expansion angle.

[00279] The height difference, Δh , can be at least about 1 μm . Alternatively, the height difference can be at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 μm or more. Alternatively, the height difference can be at most about 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 45, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 μm or less. In some instances, the expansion angle, β , may be between a range of from about 0.5° to about 4° , from about 0.1° to about 10° , or from about 0° to about 90° . For example, the expansion angle can be

at least about 0.01°, 0.1°, 0.2°, 0.3°, 0.4°, 0.5°, 0.6°, 0.7°, 0.8°, 0.9°, 1°, 2°, 3°, 4°, 5°, 6°, 7°, 8°, 9°, 10°, 15°, 20°, 25°, 30°, 35°, 40°, 45°, 50°, 55°, 60°, 65°, 70°, 75°, 80°, 85°, or higher. In some instances, the expansion angle can be at most about 89°, 88°, 87°, 86°, 85°, 84°, 83°, 82°, 81°, 80°, 75°, 70°, 65°, 60°, 55°, 50°, 45°, 40°, 35°, 30°, 25°, 20°, 15°, 10°, 9°, 8°, 7°, 6°, 5°, 4°, 3°, 2°, 1°, 0.1°, 0.01°, or less.

[00280] In some instances, the flow rate of the aqueous fluid **712** entering the junction **706** can be between about 0.04 microliters (μL)/minute (min) and about 40 μL/min. In some instances, the flow rate of the aqueous fluid **712** entering the junction **706** can be between about 0.01 microliters (μL)/minute (min) and about 100 μL/min. Alternatively, the flow rate of the aqueous fluid **712** entering the junction **706** can be less than about 0.01 μL/min. Alternatively, the flow rate of the aqueous fluid **712** entering the junction **706** can be greater than about 40 μL/min, such as 45 μL/min, 50 μL/min, 55 μL/min, 60 μL/min, 65 μL/min, 70 μL/min, 75 μL/min, 80 μL/min, 85 μL/min, 90 μL/min, 95 μL/min, 100 μL/min, 110 μL/min, 120 μL/min, 130 μL/min, 140 μL/min, 150 μL/min, or greater. At lower flow rates, such as flow rates of about less than or equal to 10 microliters/minute, the droplet radius may not be dependent on the flow rate of the aqueous fluid **712** entering the junction **706**. The second fluid **714** may be stationary, or substantially stationary, in the reservoir **704**. Alternatively, the second fluid **714** may be flowing, such as at the above flow rates described for the aqueous fluid **712**.

[00281] In some instances, at least about 50% of the droplets generated can have uniform size. In some instances, at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater of the droplets generated can have uniform size. Alternatively, less than about 50% of the droplets generated can have uniform size.

[00282] While **FIGs. 7A** and **7B** illustrate the height difference, Δh , being abrupt at the junction **706** (e.g., a step increase), the height difference may increase gradually (e.g., from about 0 μm to a maximum height difference). Alternatively, the height difference may decrease gradually (e.g., taper) from a maximum height difference. A gradual increase or decrease in height difference, as used herein, may refer to a continuous incremental increase or decrease in height difference, wherein an angle between any one differential segment of a height profile and an immediately adjacent differential segment of the height profile is greater than 90°. For example, at the junction **706**, a bottom wall of the channel and a bottom wall of the reservoir can meet at an angle greater than 90°. Alternatively or in addition, a top wall (e.g., ceiling) of the channel and a top wall (e.g., ceiling) of the reservoir can meet an angle greater than 90°. A gradual increase or decrease may be linear or non-linear (e.g., exponential, sinusoidal, etc.). Alternatively or in addition, the height difference may variably increase and/or decrease linearly

or non-linearly. While **FIGs. 7A** and **7B** illustrate the expanding reservoir cross-section height as linear (e.g., constant expansion angle, β), the cross-section height may expand non-linearly. For example, the reservoir may be defined at least partially by a dome-like (e.g., hemispherical) shape having variable expansion angles. The cross-section height may expand in any shape.

[00283] The channel networks, e.g., as described above or elsewhere herein, can be fluidly coupled to appropriate fluidic components. For example, the inlet channel segments are fluidly coupled to appropriate sources of the materials they are to deliver to a channel junction. These sources may include any of a variety of different fluidic components, from simple reservoirs defined in or connected to a body structure of a microfluidic device, to fluid conduits that deliver fluids from off-device sources, manifolds, fluid flow units (e.g., actuators, pumps, compressors) or the like. Likewise, the outlet channel segment (e.g., channel segment **208**, reservoir **604**, etc.) may be fluidly coupled to a receiving vessel or conduit for the partitioned cells for subsequent processing. Again, this may be a reservoir defined in the body of a microfluidic device, or it may be a fluidic conduit for delivering the partitioned cells to a subsequent process operation, instrument or component.

[00284] The methods and systems described herein may be used to greatly increase the efficiency of single cell applications and/or other applications receiving droplet-based input. For example, following the sorting of occupied cells and/or appropriately-sized cells, subsequent operations that can be performed can include generation of amplification products, purification (e.g., via solid phase reversible immobilization (SPRI)), further processing (e.g., shearing, ligation of functional sequences, and subsequent amplification (e.g., via PCR)). These operations may occur in bulk (e.g., outside the partition). In the case where a partition is a droplet in an emulsion, the emulsion can be broken and the contents of the droplet pooled for additional operations. Additional reagents that may be co-partitioned along with the barcode bearing bead may include oligonucleotides to block ribosomal RNA (rRNA) and nucleases to digest genomic DNA from cells. Alternatively, rRNA removal agents may be applied during additional processing operations. The configuration of the constructs generated by such a method can help minimize (or avoid) sequencing of the poly-T sequence during sequencing and/or sequence the 5' end of a polynucleotide sequence. The amplification products, for example, first amplification products and/or second amplification products, may be subject to sequencing for sequence analysis. In some cases, amplification may be performed using the Partial Hairpin Amplification for Sequencing (PHASE) method.

[00285] A variety of applications require the evaluation of the presence and quantification of different analyte carrier or organism types within a population of analyte carriers, including, for

example, microbiome analysis and characterization, environmental testing, food safety testing, epidemiological analysis, e.g., in tracing contamination or the like.

Computer systems

[00286] The present disclosure provides computer systems that are programmed to implement methods of the disclosure. **FIG. 9** shows a computer system **901** that is programmed or otherwise configured to, for example, process and/or analyze a droplet, control addition of reagent to droplets and/or reaction mixtures, control partition generation, control of reagent addition to partitions, provide conditions sufficient for transfer of a reagent, provide conditions sufficient to conduct reactions, obtain and process sequencing data, output sequencing results to a user, provide an interface for a user to input to control devices coupled to the computer processor. The computer system **901** can regulate various aspects of the present disclosure, such as, for example, regulating fluid flow rate in one or more channels in a microfluidic structure, regulating polymerization application units, etc. The computer system **901** can be an electronic device of a user or a computer system that is remotely located with respect to the electronic device. The electronic device can be a mobile electronic device.

[00287] The computer system **901** includes a central processing unit (CPU, also “processor” and “computer processor” herein) **905**, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system **901** also includes memory or memory location **910** (e.g., random-access memory, read-only memory, flash memory), electronic storage unit **915** (e.g., hard disk), communication interface **920** (e.g., network adapter) for communicating with one or more other systems, and peripheral devices **925**, such as cache, other memory, data storage and/or electronic display adapters. The memory **910**, storage unit **915**, interface **920** and peripheral devices **925** are in communication with the CPU **905** through a communication bus (solid lines), such as a motherboard. The storage unit **915** can be a data storage unit (or data repository) for storing data. The computer system **901** can be operatively coupled to a computer network (“network”) **930** with the aid of the communication interface **920**. The network **930** can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network **930** in some cases is a telecommunication and/or data network. The network **930** can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network **930**, in some cases with the aid of the computer system **901**, can implement a peer-to-peer network, which may enable devices coupled to the computer system **901** to behave as a client or a server.

[00288] The CPU **905** can execute a sequence of machine-readable instructions, which can be embodied in a program or software. The instructions may be stored in a memory location, such

as the memory **910**. The instructions can be directed to the CPU **905**, which can subsequently program or otherwise configure the CPU **905** to implement methods of the present disclosure. Examples of operations performed by the CPU **905** can include fetch, decode, execute, and writeback.

[00289] The CPU **905** can be part of a circuit, such as an integrated circuit. One or more other components of the system **901** can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[00290] The storage unit **915** can store files, such as drivers, libraries and saved programs. The storage unit **915** can store user data, e.g., user preferences and user programs. The computer system **901** in some cases can include one or more additional data storage units that are external to the computer system **901**, such as located on a remote server that is in communication with the computer system **901** through an intranet or the Internet.

[00291] The computer system **901** can communicate with one or more remote computer systems through the network **930**. For instance, the computer system **901** can communicate with a remote computer system of a user (e.g., operator). Examples of remote computer systems include personal computers (e.g., portable PC), slate or tablet PC's (e.g., Apple® iPad, Samsung® Galaxy Tab), telephones, Smart phones (e.g., Apple® iPhone, Android-enabled device, Blackberry®), or personal digital assistants. The user can access the computer system **901** via the network **930**.

[00292] Methods as described herein can be implemented by way of machine (e.g., computer processor) executable code stored on an electronic storage location of the computer system **901**, such as, for example, on the memory **910** or electronic storage unit **915**. The machine executable or machine-readable code can be provided in the form of software. During use, the code can be executed by the processor **905**. In some cases, the code can be retrieved from the storage unit **915** and stored on the memory **910** for ready access by the processor **905**. In some situations, the electronic storage unit **915** can be precluded, and machine-executable instructions are stored on memory **910**.

[00293] The code can be pre-compiled and configured for use with a machine having a processor adapted to execute the code, or can be compiled during runtime. The code can be supplied in a programming language that can be selected to enable the code to execute in a pre-compiled or as-compiled fashion.

[00294] Aspects of the systems and methods provided herein, such as the computer system **901**, can be embodied in programming. Various aspects of the technology may be thought of as “products” or “articles of manufacture” typically in the form of machine (or processor)

executable code and/or associated data that is carried on or embodied in a type of machine readable medium. Machine-executable code can be stored on an electronic storage unit, such as memory (e.g., read-only memory, random-access memory, flash memory) or a hard disk. “Storage” type media can include any or all of the tangible memory of the computers, processors or the like, or associated modules thereof, such as various semiconductor memories, tape drives, disk drives and the like, which may provide non-transitory storage at any time for the software programming. All or portions of the software may at times be communicated through the Internet or various other telecommunication networks. Such communications, for example, may enable loading of the software from one computer or processor into another, for example, from a management server or host computer into the computer platform of an application server. Thus, another type of media that may bear the software elements includes optical, electrical and electromagnetic waves, such as used across physical interfaces between local devices, through wired and optical landline networks and over various air-links. The physical elements that carry such waves, such as wired or wireless links, optical links or the like, also may be considered as media bearing the software. As used herein, unless restricted to non-transitory, tangible “storage” media, terms such as computer or machine “readable medium” refer to any medium that participates in providing instructions to a processor for execution.

[00295] Hence, a machine readable medium, such as computer-executable code, may take many forms, including but not limited to, a tangible storage medium, a carrier wave medium or physical transmission medium. Non-volatile storage media include, for example, optical or magnetic disks, such as any of the storage devices in any computer(s) or the like, such as may be used to implement the databases, etc. shown in the drawings. Volatile storage media include dynamic memory, such as main memory of such a computer platform. Tangible transmission media include coaxial cables; copper wire and fiber optics, including the wires that comprise a bus within a computer system. Carrier-wave transmission media may take the form of electric or electromagnetic signals, or acoustic or light waves such as those generated during radio frequency (RF) and infrared (IR) data communications. Common forms of computer-readable media therefore include for example: a floppy disk, a flexible disk, hard disk, magnetic tape, any other magnetic medium, a CD-ROM, DVD or DVD-ROM, any other optical medium, punch cards paper tape, any other physical storage medium with patterns of holes, a RAM, a ROM, a PROM and EPROM, a FLASH-EPROM, any other memory chip or cartridge, a carrier wave transporting data or instructions, cables or links transporting such a carrier wave, or any other medium from which a computer may read programming code and/or data. Many of these forms

of computer readable media may be involved in carrying one or more sequences of one or more instructions to a processor for execution.

[00296] The computer system **901** can include or be in communication with an electronic display **935** that comprises a user interface (UI) **940** for providing, for example, monitoring of sample preparation, monitoring droplet preparation, monitoring reagent addition, monitoring of reactions and/or reaction conditions, monitoring sequencing, results of sequencing, and permitting user inputs for sample preparation, reaction, sequencing and/or analysis, etc.. Examples of UIs include, without limitation, a graphical user interface (GUI) and web-based user interface.

[00297] Methods and systems of the present disclosure can be implemented by way of one or more algorithms. An algorithm can be implemented by way of software upon execution by the central processing unit **905**. The algorithm can, for example, implement sample preparation protocols, implement droplet preparation protocols, implement reagent addition protocols, data analysis protocols, perform sequencing protocols, system and/or device operation protocols, etc.

[00298] Devices, systems, compositions and methods of the present disclosure may be used for various applications, such as, for example, processing a single analyte (e.g., RNA, DNA, or protein) or multiple analytes (e.g., DNA and RNA, DNA and protein, RNA and protein, or RNA, DNA and protein) from a single cell. For example, an analyte carrier (e.g., a cell or cell bead) is partitioned in a partition (e.g., droplet), and multiple analytes from the analyte carrier are processed for subsequent processing. The multiple analytes may be from the single cell. This may enable, for example, simultaneous proteomic, transcriptomic and genomic analysis of the cell.

Examples

Example 1: Transfer of Reagents Between Droplets

[00299] **FIG. 15A** shows a droplet population comprising gel beads and no DTT. **FIG. 15B** shows the results of an experiment demonstrating the method of processing a droplet. **FIG. 15A** shows a microscope image **1510** of a monodisperse water-in-fluorinated oil emulsion **1502** which was produced using a microfluidic device. In the image **1510**, each droplet **1504** may contain a single bead (e.g., a disulfide-bond crosslinked hydrogel bead **1506**) and no dithiothreitol (DTT). In some cases, some droplets may contain more than one bead. A droplet, such as droplet **1504** containing a bead **1506** may be referred to herein as a GEM. As shown in **1510**, hydrogel beads may be clearly visible inside droplets. A second disperse water-in-fluorinated oil emulsion **1522** was created by shaking to produce droplets which contained a disulfide bond reducing agent (dithiothreitol; DTT). Both droplet populations were then mixed

together in the same tube. In **FIG. 15B** in the image **1530**, the result of mixing both populations is shown. As shown, hydrogel beads were no longer visible in each droplet **1524**, indicating that DTT had been transported from one droplet population to another and subsequently caused the dissolution of hydrogel beads through reduction of their crosslinking disulfide bonds.

Example 2: Transfer of Reagents Between Droplets

[00300] Transport of a reducing agent (e.g., DTT) from one droplet population into another was used to simultaneously measure gene expression and cell surface proteins from single cells as described in U.S. Patent Publication No. 2015/0376609, U.S. Patent Application No. 15/720,085 and PCT Application No. PCT/US2017/068320. Cells were labelled with nucleic acid barcode molecule-tagged antibodies that targeted specific cell surface markers. The antibodies were linked to the nucleic acid barcode molecules through a disulfide bond. The labelled cells were then isolated and co-partitioned in aqueous droplets in an oil phase with gel beads comprising nucleic acid barcode molecules. The aqueous droplets were then contacted with a population of droplets containing DTT in a mixture, similar to the example method shown in **FIG. 12A**. Transfer of DTT from the DTT droplets to those comprising antibody and bead released nucleic acid barcode molecules from beads and antibodies, followed by barcoding of nucleic acid molecules, further processing and sequencing of barcoded constructs as is described elsewhere herein.

[00301] **Table 1** provides the various conditions used to test for the transfer of reagents between droplets. The mass transport of DTT into droplets each containing a bead (GEMs) after the generation of the GEMs was analyzed for any impact on the ability to measure gene expression profile of cells. **Tables 2A-B** provide the results for gene expression measurements/analysis. **Tables 3A-B** provide the results for cell surface protein measurements/analysis.

Table 1:

Sample	Conjugation chemistry	DTT concentration in RT Master Mix	DTT oil or emulsion volume spiked in post-GEM generation
1	Non-Disulfide (S-S)	0.5 – 500 mM	0 μ l
2		0.5 – 100 mM	0 μ l
3		0.5 – 50 mM	0 μ l
4		0.5 – 20 mM	0 μ l
5	Disulfide (S-S)	0.5 – 500 mM	0 μ l
6		0.5 – 100 mM	0 μ l
7		0.5 – 50 mM	0 μ l
8		0.5 – 20 mM	0 μ l
9	Non-Disulfide (S-S)	None	2 – 10 μ l DTT emulsion

10		None	1-10 μ l DTT emulsion
11	Disulfide (S-S)	None	1-8 μ l oil saturated with DTT
12		None	1-5 μ l DTT emulsion
13		None	1-5 μ l DTT emulsion
14		None	- 5 μ l GEM oil + 5 μ l DTT emulsion

[00302] **Table 2A** summarizes the cell expression measurements and results for Samples 1-8.

Table 2A:

Measurement	Samples 1-4	Samples 5-8
Estimated # of cells	1059 to 1257	1221 to 1305
Mean reads/cell	40257 to 55865	35061 to 60693
Valid barcodes	96.80% to 96.90%	96.80% to 97%
Reads mapped to transcriptome	47.40% to 48.20%	47.20% to 49.10%
cDNA PCR duplication (20k raw reads/cell)	66.50% to 69.00%	67.60% to 69.80%
Fraction Reads in cells	95.10% to 95.80%	95.10% to 95.90%
Median Genes/cell	1155 to 1229	1102 to 1247
Total Genes detected	16397 to 16749	16351 to 16875
Median UMI Counts per cell	2827 to 3014	2553 to 3000
Median genes per cell (20k raw reads/cell)	1053 to 1081	1019 to 1094
Median UMI counts per cell (20k raw reads/cell)	2519 to 2631	2357 to 2592

[00303] **Table 2B** summarizes the cell expression measurements and results for Samples 9-14.

Table 2B:

Measurement	Samples 9-10	Samples 11-14
Estimated # of cells	945 to 981	965 to 1063
Mean reads/cell	44253 to 72375	34001 to 39893
Valid barcodes	96.50% to 96.70%	96.50% to 96.70%
Reads mapped to transcriptome	61.60% to 62.30%	61.70% to 61.90%
cDNA PCR duplication (20k raw reads/cell)	63.60% to 64.80%	62.90% to 64.40%
Fraction Reads in cells	94.90%	94.20% to 94.70%
Median Genes/cell	1359 to 1459	1297 to 1379
Total Genes detected	15,860 to 16295	15778 to 16087

Median UMI Counts per cell	4301 to 4726	4097 to 4449
Median genes per cell (20k raw reads/cell)	1206 to 1247	1178 to 1227
Median UMI counts per cell (20k raw reads/cell)	3796 to 3956	3706 to 3923

[00304] **Table 3A** summarizes the cell surface protein measurement results for Samples 1-8.

Table 3A:

Measurement	Samples 15-18	Samples 19-22
Q30 Bases in Antibody Read	63.50% to 64.30%	62.00% to 63.90%
Valid Barcodes	98.50% to 98.50%	98.50% to 98.50%
Valid UMIs	100.00% to 100.00%	100.00% to 100.00%
Fraction Antibody Reads	78.60% to 79.00%	76.70% to 78.10%
Fraction Antibody Reads Usable	54.40% to 56.10%	15.20% to 46.90%
Fraction Antibody Reads Usable per Cell	6136 to 8277	1916 to 5881
Antibody Reads in Cells	74.20% to 75.30%	21.50% to 65.20%
Median UMIs per cell at 1k Antibody Reads Usable per cell	640 to 678	391 to 663
Median UMIs per Cell at 5k Antibody Reads Usable per Cell	1381 to 1511	1286 to 1436

[00305] **Table 3B** summarizes the cell surface protein measurement results for Samples 9-14.

Table 3B:

Measurement	Samples 23-24	Samples 25-28
Q30 Bases in Antibody Read	58.70% to 59.20%	58.70% to 59.60%
Valid Barcodes	98.80% to 98.80%	98.80% to 98.90%
Valid UMIs	100.00% to 100.00%	100.00% to 100.00%
Fraction Antibody Reads	97.10% to 97.10%	96.90% to 97.20%
Fraction Antibody Reads Usable	65.00% to 66.80%	68.20% to 69.80%
Fraction Antibody Reads Usable per Cell	12991 to 13796	13726 to 21064
Antibody Reads in Cells	67.70% to 69.60%	71.10% to 72.70%

Median UMIs per cell at 1k Antibody Reads Usable per cell	677 to 678	679 to 721
Median UMIs per Cell at 5k Antibody Reads Usable per Cell	1472 to 1540	1740 to 1796

[00306] The mass transport of DTT into GEMs after their generation had no negative impact on the ability to the measure gene expression profile of cells or to profile cell surface proteins. Samples 1-8 had disulfide conjugation chemistry between the antibody and nucleic acid barcode molecules and the DTT was provided in the RT Master Mix at four different concentrations (1 to 4 with 1 being the highest and 4 being the lowest concentration). The DTT concentrations fell within a range of 0.5 mM to 500 mM. Samples 9-14 had disulfide conjugation chemistry between the antibody and nucleic acid barcode molecules and the DTT was provided as oil or emulsion rather than in the RT Master mix.

Example 3: Sample processing in droplets formed by agitation

[00307] FIG.s 16A through 16E demonstrate an example workflow for processing analyte carriers. Referring to FIG. 16A, the method may comprise transposition of nuclei in bulk **1601**. Referring to FIG. 16B, transposed nuclei **1602**, one or more reagents, such as biochemical reagents **1603**, and beads, such as barcoded gel beads **1604**, may be added to a container, such as a tube **1605**. The contents of the container may comprise the first fluid **1606**. In some cases, excess aqueous liquid may be removed from the container, for example, using a pipette. The second fluid may be added to the container. The second fluid may comprise oil and surfactant. The container may be vortexed **1607**. Droplets **1608** may be generated in the container. Beads may limit droplet break-up into droplets smaller than the size of the beads which may contribute to the monodispersity of the generated droplets. Droplets **1608** may comprise the beads, such as barcoded gel beads **1609** or the analyte carriers, such as transposed nuclei **1610**, and/or both, such as illustrated in FIG. 16C. A droplet may comprise a single bead. A droplet may comprise a single analyte carrier. A droplet (e.g., **1613**) may comprise a single bead and a single analyte carrier. One or more reagents **1611** may be transported **1612** into the droplets (e.g., **1613**), such as illustrated in FIG. 16D. Transporting **1612** the reagent(s) into the droplets may be performed with the aid of a mediator, such as a micelle. Reagent(s) **1611** may comprise DTT. Reagent(s) may dissolve and/or degrade the beads, thereby generating a droplet **1614** comprising an analyte carrier in which the bead is dissolved. In some cases, the reagent(s) may release nucleic acid barcode molecules from the bead, such as by cleaving a bond. In some cases, the nucleic acid barcode molecules may be released into the droplet upon bead degradation. Thus, nucleic acid

barcode molecules and an analyte carrier (e.g., transposed nucleus) may be co-partitioned in the droplet **1614**. The method may further comprise processing the partitioned samples, such as generating barcoded products. For example, a nucleic acid barcode molecule and a nucleic acid molecule derived from an analyte carrier may be used to generate a barcoded product. In some cases, barcoded DNA fragments may be generated **1615** from a nucleic acid molecule derived from a transposed nucleus and a nucleic acid barcode molecule delivered on a bead. Samples may be further subjected to amplification reaction, such as PCR, such as linear amplification. Samples may be pooled. The second fluid (e.g., oil) may be removed **1616** from the container. The method may further comprise sequencing **1618**. Generating droplets with the aid of agitation such as vortexing may contribute to the scalability of the process. For example, larger sample sizes may be processed without increasing workflow time. In some cases, biochemical processes may be scaled by a few to several orders of magnitude, without increasing workflow time.

Example 4: Single cell gene expression in droplets formed by agitation

[00308] FIG.s 17A through 17D demonstrate an example workflow for single cell gene expression, wherein droplets are generated with the aid of agitation. Referring to FIG. 17A, analyte carriers, such as cells **1701**, one or more reagents, such as biochemical reagents **1702**, and beads, such as gel beads, such as barcoded gel beads **1703** may be added to a container, such as a tube. The container may comprise the first fluid **1705**. The first fluid **1705** may be an aqueous solution comprising analyte carriers (e.g., cells), reagent(s), and beads (e.g., barcoded gel beads), and in some cases, excess aqueous liquid. Excess aqueous liquid may be removed from the container. The second fluid comprising oil may be added to the container. Surfactant may be further added to the container. The container may be agitated (e.g., vortexed), such as illustrated in FIG. 17B. A droplet population may be generated by agitation. Beads may act as templates to form a monodispersed droplet population. A droplet **1707** in the droplet population may comprise a single bead **1708** and a single analyte carrier **1709**, such as a single cell. Some droplets in the droplet population may comprise a single bead. Some droplets may not comprise any beads. Some droplets may comprise more than one bead. Some droplets may comprise an analyte carrier. Some droplets may not comprise any analyte carrier. Some droplet may comprise more than one analyte carrier. The analyte carrier may be a cell. Some droplets may comprise a single cell. Some droplets (e.g., droplet **1707**) may comprise a single bead and a single cell. Droplets comprising a single cell and a single bead may be preferred for downstream operations. Referring to FIG. 17C, one or more reagents **1710** and **1711**, such as biochemical reagents may be transported to the droplets. Reagents may be transported **1712** to the droplet from other droplets with the aid of a mediator, such as micelle. In some cases, reagents may

comprise dithiothreitol (DTT) and n-dodecyl-p-D-maltoside (DBDM). DTT may dissolve the gel beads. DBDM may lyse the cells. The reagents may otherwise release a nucleic acid barcode molecule from a bead and/or release a nucleic acid molecule derived from an analyte carrier in the droplet. Gel beads may comprise barcoded beads. As such, barcode molecules may be released into the droplet. Cell contents, such as intracellular contents, nucleic acid molecules, proteins, and/or cytoplasm, may be released into the droplet. Thus, subsequent to reagent transportation, some droplets **1713** may comprise barcode molecules and cell contents (lysed cells). The method may further comprise processing the partitioned samples, such as generating barcoded products (e.g., barcoded analyte carriers or barcoded analytes derived from the analyte carriers). For example, the bead may comprise a nucleic acid barcode molecule coupled thereto. The nucleic acid barcode molecule may be used to barcode an analyte carrier in the partition, or an analyte derived from the analyte carrier. In some cases, barcoded cDNA molecules may be generated. Samples may be further subjected to amplification reactions, such as PCR, such as linear amplification. Samples may be pooled. The second fluid (e.g., oil) may be removed from the container. The method may further comprise sequencing. Generating droplets with the aid of agitation such as vortexing may contribute to the scalability of the process. For example, larger sample sizes may be processed without increasing workflow time. In some cases, biochemical processes may be scaled by few to several orders of magnitude. Scaling may be achieved without increasing workflow time.

Example 5: Addition of reagent after droplet generation

[00309] **FIG.s 18A and 18B** show the results of an experiment where transporting a lysis agent to a population of droplets comprising cells, such as single cells, can lyse the cells to release their contents into the interior of the droplets. **FIG. 18A** shows a population of droplets in a container (e.g., the second droplet population) **1801** in which cells are encapsulated. A brightfield microscopy image **1802** and a fluorescent microscopy image **1803** show the droplets, some of which comprise cells. Some droplets may be empty. Some droplets may comprise single cells. Some droplets may comprise multiple cells. In some cases, droplets may comprise one or more beads and/or one or more cells. In some cases, some droplets may comprise a single cell and a single bead. Cells may be fluorescent cells. Cells may be stained by a fluorescent dye, such as Calcein, or other fluorescent dyes. Fluorescent dyes may be visualized using an appropriate light filter on the microscope to allow visualization at a certain wavelength. Cells may be intact. Intact, unlysed cells may appear as puncta on the fluorescent image **1803**. Referring to **FIG. 18B**, one container may comprise a population of droplets **1804** (e.g., second droplet population) which may comprise cells and beads. Another container may comprise a population of droplets

1805 (e.g., the first droplet population). The first and second droplet populations **1804** and **1805** may be mixed. A droplet in the first droplet population **1805** may comprise a reagent. The reagent may comprise a lysis agent. The reagent may comprise n-dodecyl-p-D-maltoside (DBDM). Lysis agents can be transported into the second droplet population such as shown in brightfield **1806** and fluorescent **1807** microscopy images in **FIG. 18B**. The reagent can lyse the cell inside the droplet. Cell lysis inside the droplet may result in diffusion of the fluorescent dye/stain such as Calcein stain throughout the droplet rendering the cell-containing droplets fluorescent (see image **1807**). Some droplets may not be rendered fluorescent. Such droplets are suspected of not having cells in them.

[00310] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS**WHAT IS CLAIMED IS:**

1. A method for droplet processing, comprising:
 - (a) providing a first droplet population and a second droplet population, wherein droplets from said first droplet population have a first concentration of a reagent and droplets from said second droplet population have a second concentration of said reagent, and wherein said droplets from said second droplet population comprise a bead or an analyte carrier; and
 - (b) subjecting said first droplet population and said second droplet population to conditions sufficient to transfer said reagent between said first droplet population and said second droplet population, thereby changing said first concentration of said reagent in said first droplet population and said second concentration of said reagent in said second droplet population.
2. The method of claim 1, wherein in (a), said reagent is absent from said second droplet population.
3. The method of claim 1, wherein said reagent is selected from the group consisting of a reducing agent, lysis agent, sodium ion (Na⁺), magnesium ion (Mg²⁺), potassium ion (K⁺), ammonium ion (NH₄⁺), chloride (Cl⁻), bromide (Br⁻), iodide (I⁻), fluoride (F⁻), adenosine triphosphate (ATP), dinucleotide triphosphate (dNTP), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and n-dodecyl-p-D-maltoside (DBDM).
4. The method of claim 3, wherein said reducing agent is dithiothreitol or a functional derivative thereof.
5. The method of claim 1, wherein said first droplet population comprises a first droplet fraction and a first fluid fraction and wherein said second droplet population comprises a second droplet fraction and a second fluid fraction.
6. The method of claim 5, wherein said first fluid fraction or said second fluid fraction comprises a mediator that aids in transfer of said reagent between said first droplet population and said second droplet population.
7. The method of claim 6, wherein in (b), said reagent is transferred between said first droplet population and said second droplet population via the mediator.
8. The method of claim 7, wherein said mediator is derived from said first droplet population.
9. The method of claim 6, wherein said mediator comprises a surfactant.
10. The method of claim 6, wherein said mediator is a micelle.

11. The method of claim 5, wherein (b) comprises subjecting said first droplet population to conditions sufficient to transfer said reagent from said first droplet fraction to said first fluid fraction, and subjecting said second droplet population to conditions sufficient to transfer said reagent from said first fluid fraction to droplets of said second droplet population.
12. The method of claim 11, wherein said first fluid fraction is a continuous phase in which said first droplet population is dispersed.
13. The method of claim 12, wherein said continuous phase comprises an oil.
14. The method of claim 11, wherein said first droplet population is provided in a first container and said second droplet population is provided in a second container, and wherein (b) further comprises transferring said first fluid fraction of said first droplet population from said first container to said second container.
15. The method of claim 1, further comprising, subsequent to (a), generating a mixture comprising said first droplet population and said second droplet population, wherein said reagent is between said first droplet population and said second droplet population within said mixture.
16. The method of claim 15, wherein said reagent is transferred between said first droplet population and said second droplet population within said mixture via diffusion.
17. The method of claim 1, wherein said bead has nucleic acid molecules coupled thereto.
18. The method of claim 17, wherein said nucleic acid molecules are nucleic acid barcode molecules.
19. The method of claim 17, wherein said reagent dissolves said bead or releases at least a portion of said nucleic acid molecules from said bead.
20. The method of claim 1, wherein said bead is a gel bead.
21. The method of claim 20, wherein said reagent dissolves said gel bead.
22. The method of claim 1, wherein said reagent is a reducing agent and said bead comprises a disulfide bond that is broken by said reducing agent.
23. The method of claim 1, wherein said analyte carrier is a cell.
24. The method of claim 1, wherein said reagent lyses said cell.
25. The method of claim 1, wherein said analyte carrier is a cell bead.
26. The method of claim 1, wherein said droplets from said second droplet population comprise said bead and said analyte carrier.
27. The method of claim 1, wherein in (a), said first concentration is greater than said second concentration.
28. The method of claim 1, wherein said droplets from said second droplet population comprise an antibody coupled to a nucleic acid molecule.

29. The method of claim 28, wherein said reagent is capable of breaking a linkage between said antibody and said nucleic acid molecule.
30. The method of claim 1, wherein, during or subsequent to (b), said droplets from said first droplet population decrease in size or said droplets from said second droplet population increase in size.
31. The method of claim 1, wherein prior to (a), said droplets from said first droplet population or said droplets from said second droplet population are generated using a microfluidic device.
32. The method of claim 1, wherein prior to (a), said first droplet population or said second droplet population is generated upon agitation of a mixture of immiscible phases.
33. The method of claim 1, further comprising, prior to (a), generating said second droplet population from (i) a first fluid volume comprising a population of beads and a population of analyte carriers and (ii) a second fluid volume immiscible with said first fluid volume, wherein a droplet from said second droplet population comprises said bead from said population of beads and said analyte carrier from said population of analyte carriers.
34. The method of claim 33, wherein said second droplet population is generated by applying energy to said first fluid volume, said second fluid volume, or both said first fluid volume and said second fluid volume.
35. The method of claim 33, wherein said second droplet population is generated by providing a mixture comprising said first fluid volume and said second fluid volume, and agitating said mixture.
36. The method of claim 33, wherein said second droplet population is generated in absence of using a microfluidic device.
37. The method of claim 33, wherein said second droplet population is generated using a microfluidic device.
38. The method of claim 33, wherein said first fluid volume comprises an aqueous fluid.
39. The method of claim 33, wherein said second fluid volume comprises an oil.
40. The method of claim 33, wherein said first fluid volume does not include said reagent.
41. The method of claim 33, wherein said droplet comprises a single bead and a single analyte carrier.
42. The method of claim 33, wherein said analyte carrier is a cell.
43. The method of claim 33, wherein said analyte carrier is a cell bead.
44. The method of claim 33, wherein said analyte carrier is a cell nucleus.

45. The method of claim 44, wherein said population of analyte carriers comprises a plurality of transposed nuclei.
46. The method of claim 44, further comprising subjecting a population of cell nuclei to transposition in bulk to yield said plurality of transposed nuclei.
47. The method of claim 33, further comprising, subsequent to (b), using a nucleic acid molecule derived from said analyte carrier and a nucleic acid barcode molecule coupled to said bead to generate a barcoded nucleic acid molecule.
48. The method of claim 47, further comprising sequencing said barcoded nucleic acid molecule, or derivative thereof.
49. A method for nucleic acid processing, comprising:
- (a) agitating (i) a first fluid volume comprising a population of beads and a population of analyte carriers, wherein said population of beads comprises a plurality of nucleic acid barcode molecules, wherein said population of analyte carriers comprises a plurality of nucleic acid molecules, and (ii) a second fluid volume immiscible with said first fluid volume, to generate a plurality of droplets, wherein a first droplet from said plurality of droplets comprises a bead from said population of beads and an analyte carrier from said population of analyte carriers, wherein said bead comprises a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules, wherein said analyte carrier comprises a nucleic acid molecule of said plurality of nucleic acid molecules, wherein said first droplet has a first concentration of a reagent, and wherein said reagent is configured to release said nucleic acid barcode molecule or release said nucleic acid molecule in said droplet;
 - (b) subjecting said first droplet and a second droplet having a second concentration of said reagent to conditions sufficient to transfer said reagent between said first droplet and said second droplet, thereby changing said first concentration of said reagent in said first droplet and said second concentration of said reagent in said second droplet; and
 - (c) generating a barcoded nucleic acid molecule using at least said nucleic acid barcode molecule and said nucleic acid molecule in said first droplet.
50. The method of claim 49, wherein said agitating comprises vortexing.
51. The method of claim 49, wherein said first fluid volume comprises an aqueous fluid.
52. The method of claim 49, wherein said second fluid volume comprises an oil.
53. The method of claim 49, wherein said first fluid volume does not include said reagent.

54. The method of claim 49, wherein said first droplet comprises a single bead and a single analyte carrier.
55. The method of claim 49, wherein said analyte carrier is a cell.
56. The method of claim 55, wherein said reagent lyses said cell.
57. The method of claim 49, wherein said analyte carrier is a cell bead.
58. The method of claim 49, wherein said analyte carrier is a cell nucleus.
59. The method of claim 58, wherein said population of analyte carriers comprises a plurality of transposed nuclei.
60. The method of claim 58, further comprising subjecting a population of cell nuclei to transposition in bulk to yield said plurality of transposed nuclei.
61. The method of claim 49, further comprising sequencing said barcoded nucleic acid molecule, or derivative thereof.
62. The method of claim 49, wherein said reagent is selected from the group consisting of a reducing agent, lysis agent, sodium ion (Na^+), magnesium ion (Mg^{2+}), potassium ion (K^+), ammonium ion (NH_4^+), chloride (Cl^-), bromide (Br^-), iodide (I^-), fluoride (F^-), adenosine triphosphate (ATP), dinucleotide triphosphate (dNTP), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and n-dodecyl-p-D-maltoside (DBDM).
63. The method of claim 62, wherein said reducing agent is dithiothreitol or a functional derivative thereof.
64. The method of claim 49, wherein said plurality of droplets is provided in a first droplet fraction and a first fluid fraction, wherein (b) comprises providing a second plurality of droplets in a second droplet fraction and a second fluid fraction.
65. The method of claim 64, wherein said first fluid fraction or said second fluid fraction comprises a mediator that aids in transfer of said reagent between said first droplet and said second droplet.
66. The method of claim 65, wherein in (b), said reagent is transferred between said first droplet and said second droplet via said mediator.
67. The method of claim 65, wherein said mediator comprises a surfactant.
68. The method of claim 65, wherein said mediator is a micelle.
69. The method of claim 64, wherein (b) comprises subjecting said second plurality of droplets to conditions sufficient to transfer said reagent from said second droplet fraction to said second fluid fraction, and subjecting said plurality of droplets to conditions sufficient to transfer said reagent from said first fluid fraction to said first droplet fraction.

70. The method of claim 64, wherein said first fluid fraction is a continuous phase in which said plurality of droplets is dispersed.
71. The method of claim 70, wherein said continuous phase comprises an oil.
72. The method of claim 64, further comprising, in (b), generating a mixture comprising said plurality of droplets and said second plurality of droplets, wherein said reagent is between said plurality of droplets and said second plurality of droplets within said mixture.
73. The method of claim 72, wherein said reagent is transferred between said first droplet and said second droplet within said mixture via diffusion.
74. The method of claim 49, wherein said bead has a subset of nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules coupled thereto.
75. The method of claim 74, wherein said reagent dissolves said bead or releases at least a portion of said subset of nucleic acid barcode molecules from said bead.
76. The method of claim 49, wherein said bead is a gel bead.
77. The method of claim 76, wherein said reagent dissolves said gel bead.
78. The method of claim 49, wherein said reagent is a reducing agent and said bead comprises a disulfide bond that is broken by said reducing agent.
79. The method of claim 49, wherein prior to (b), said first concentration is less than said second concentration.
80. The method of claim 49, wherein, during or subsequent to (b), said second droplet decreases in diameter or said first droplet increases in diameter.

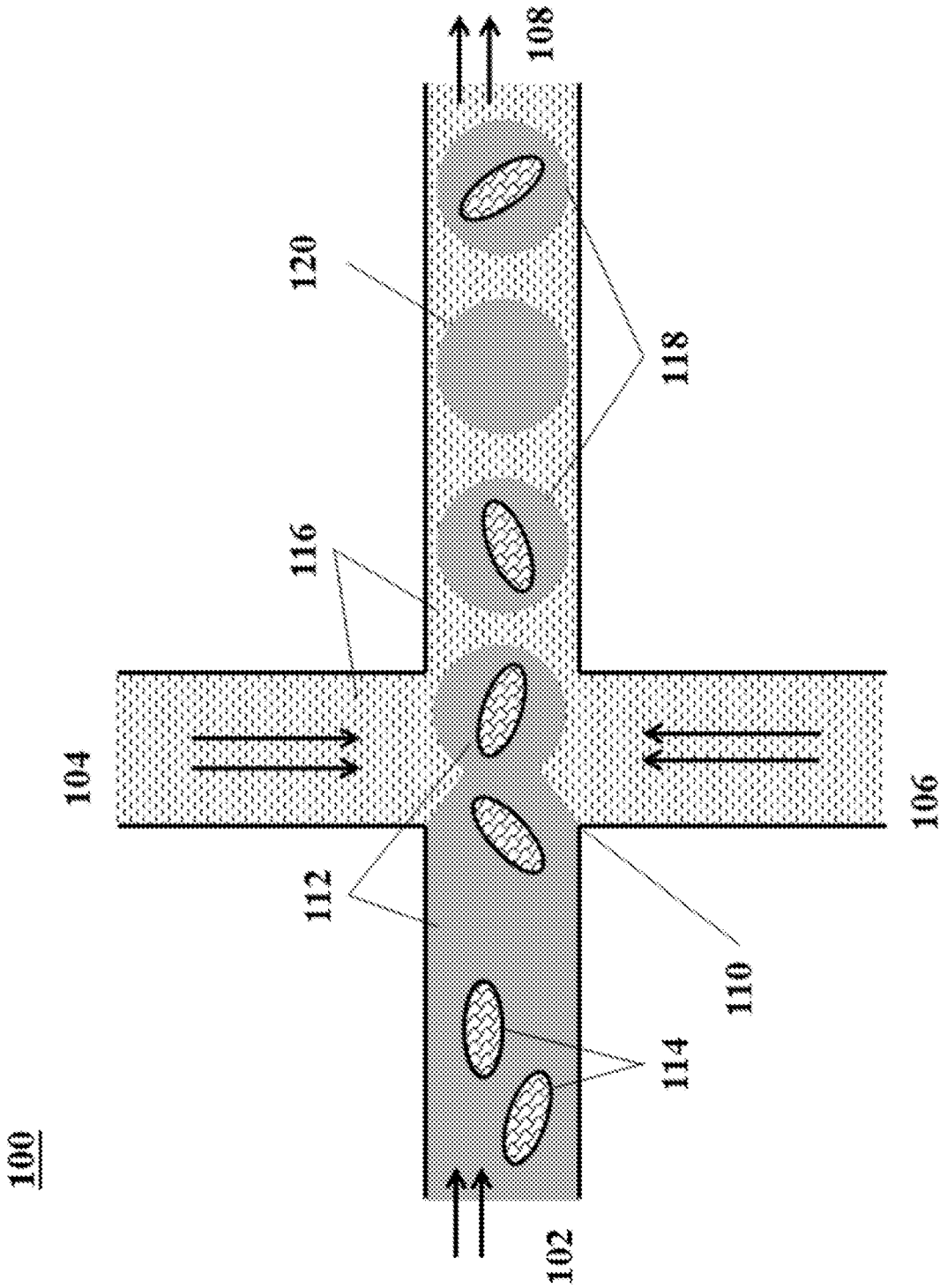


FIG. 1

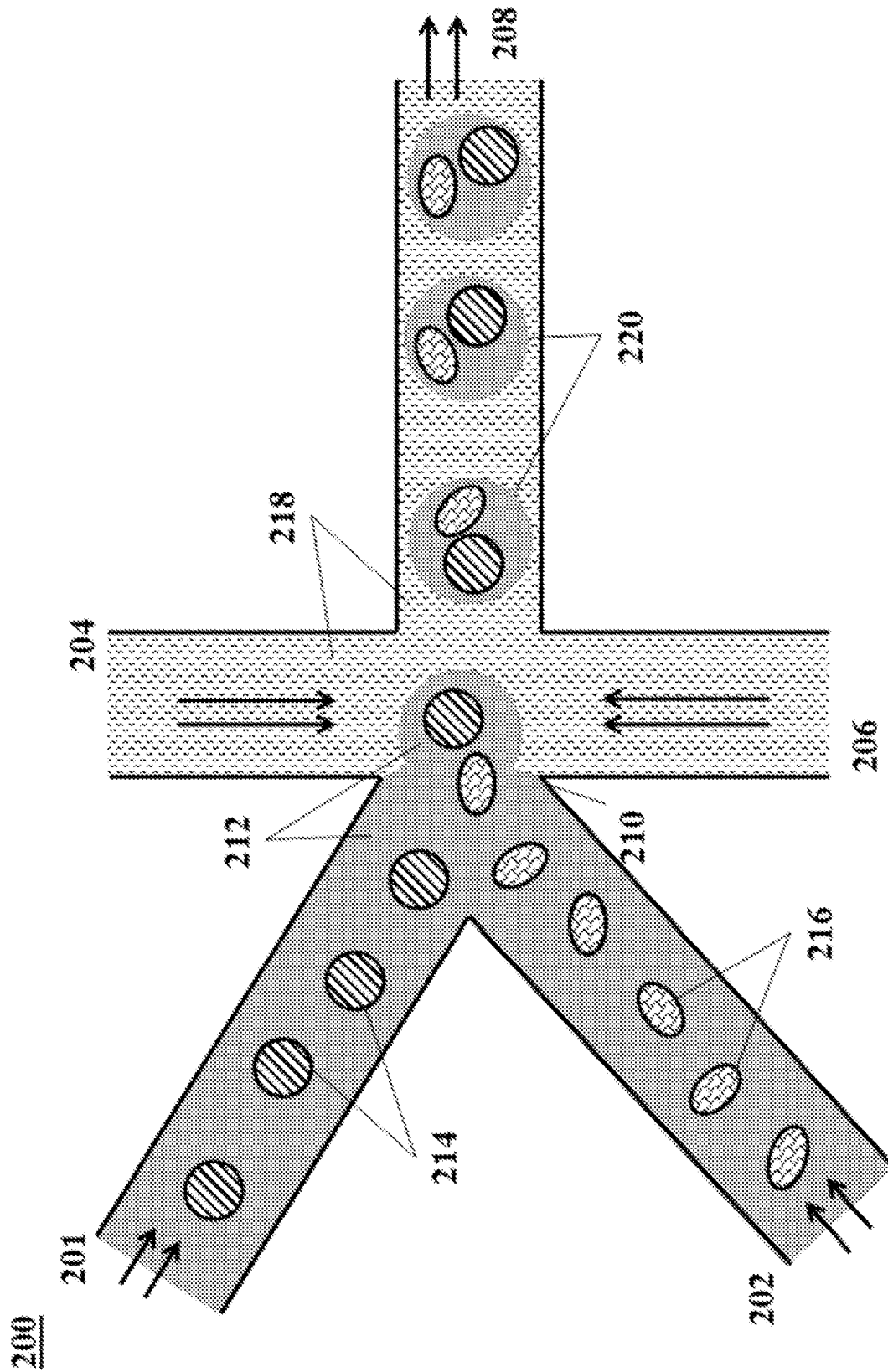


FIG. 2

300

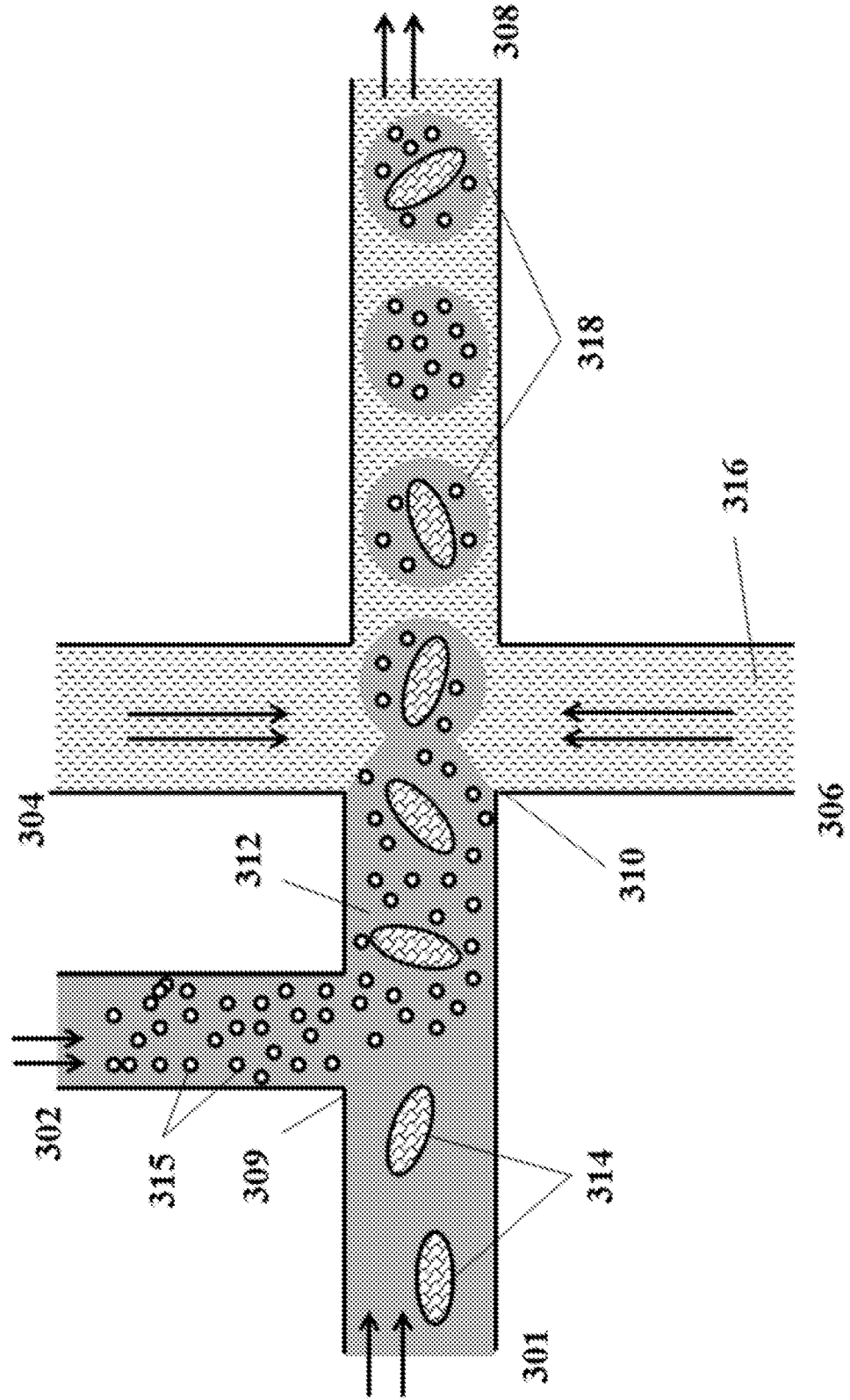


FIG. 3

400

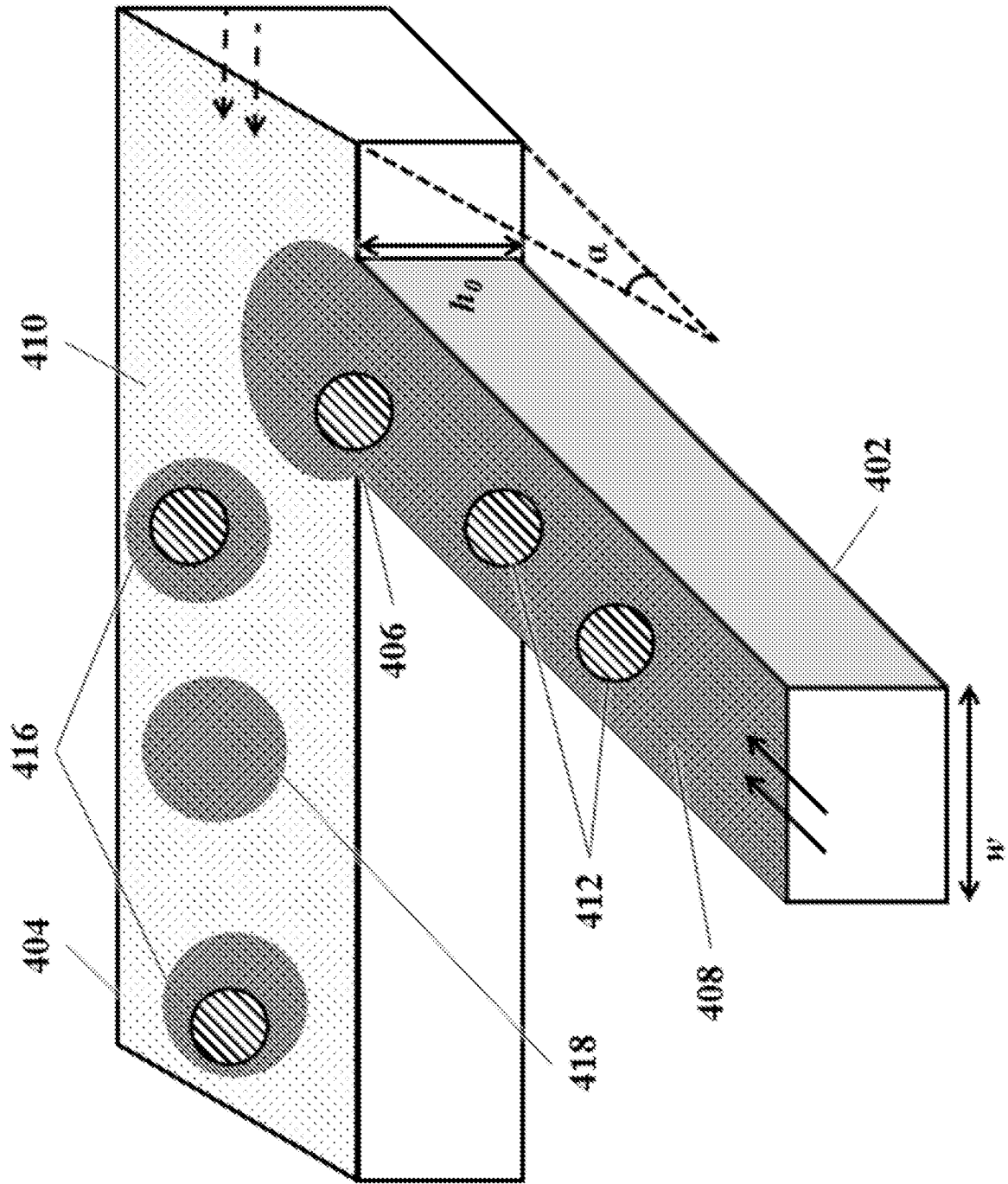


FIG. 4

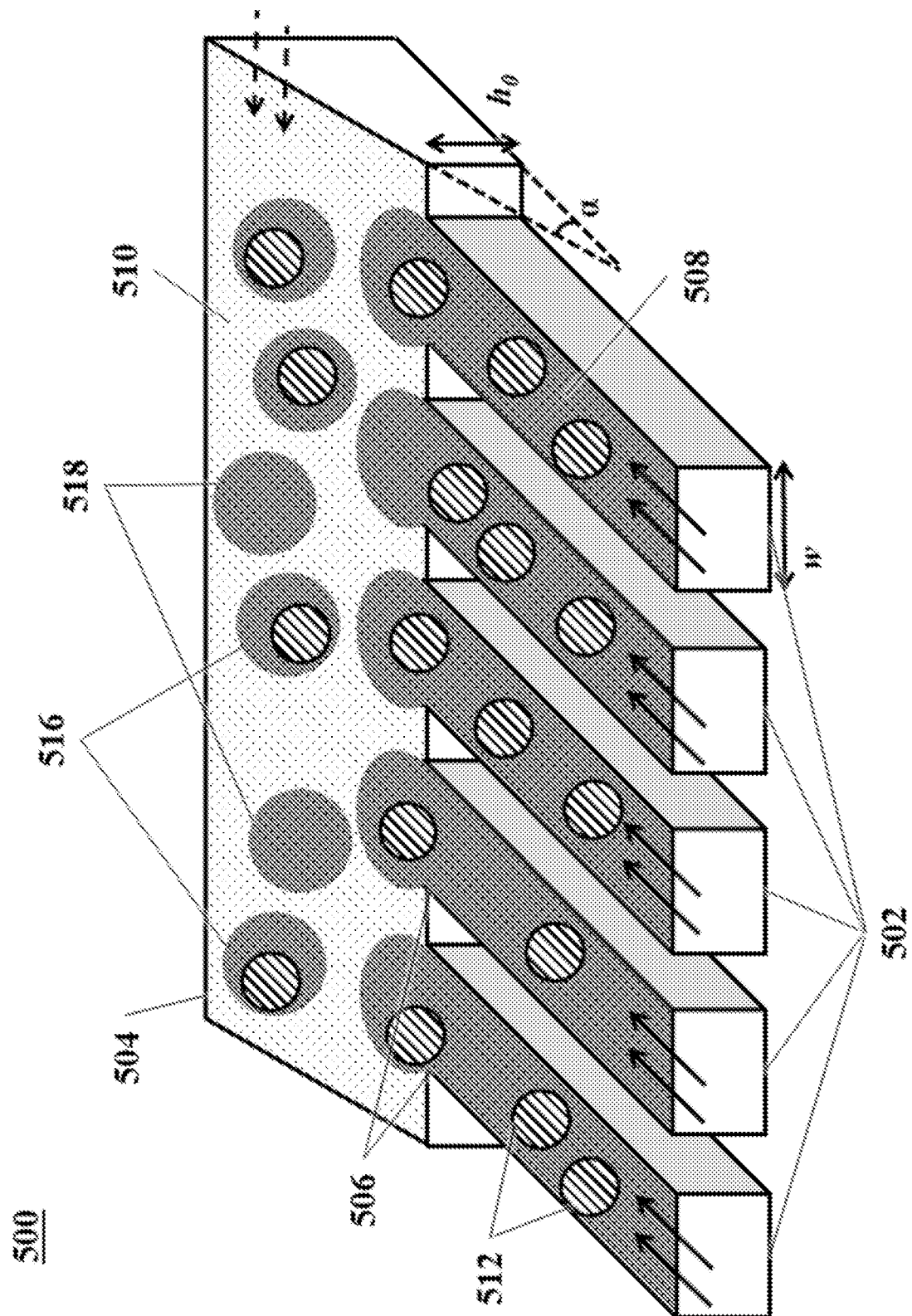


FIG. 5

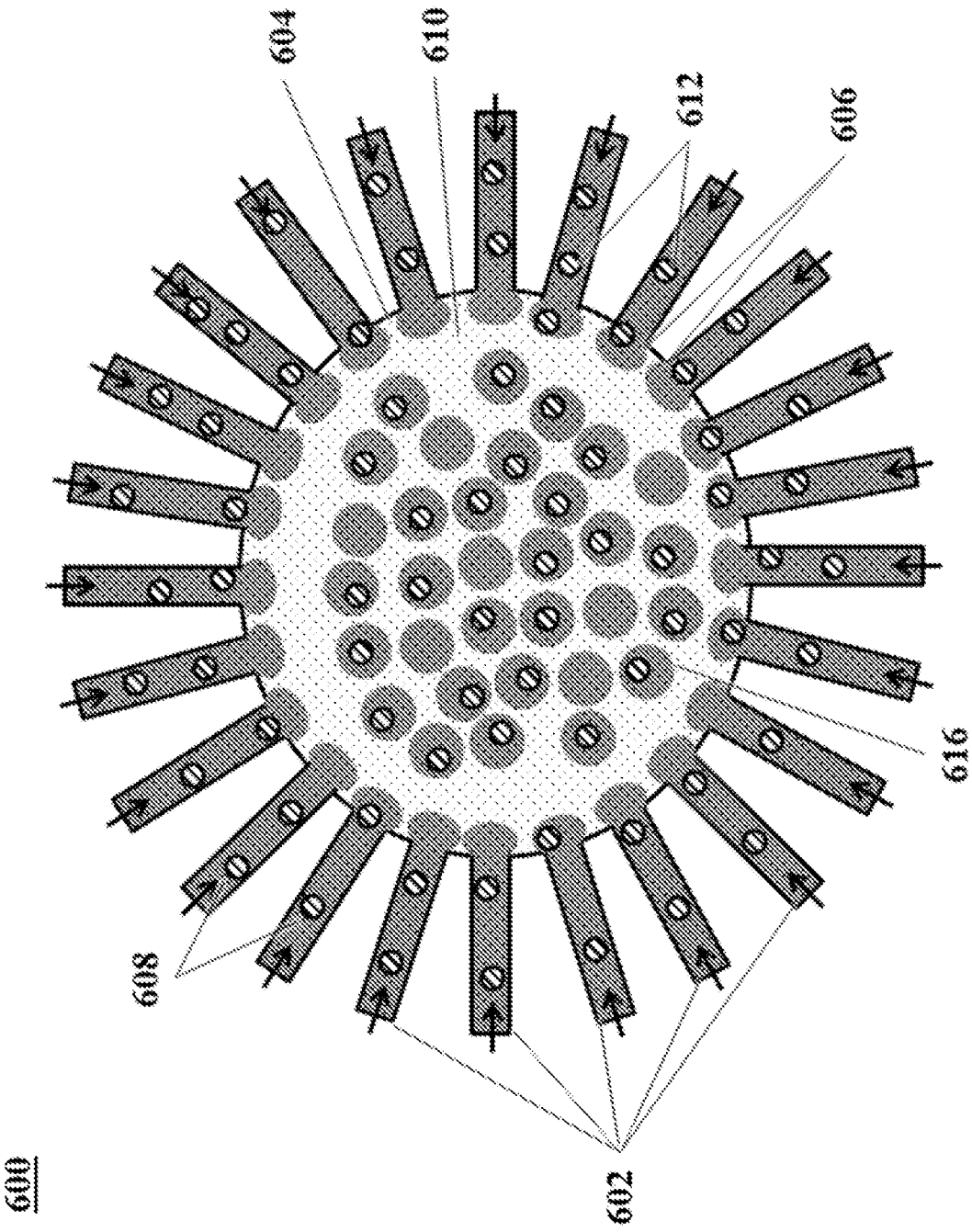


FIG. 6

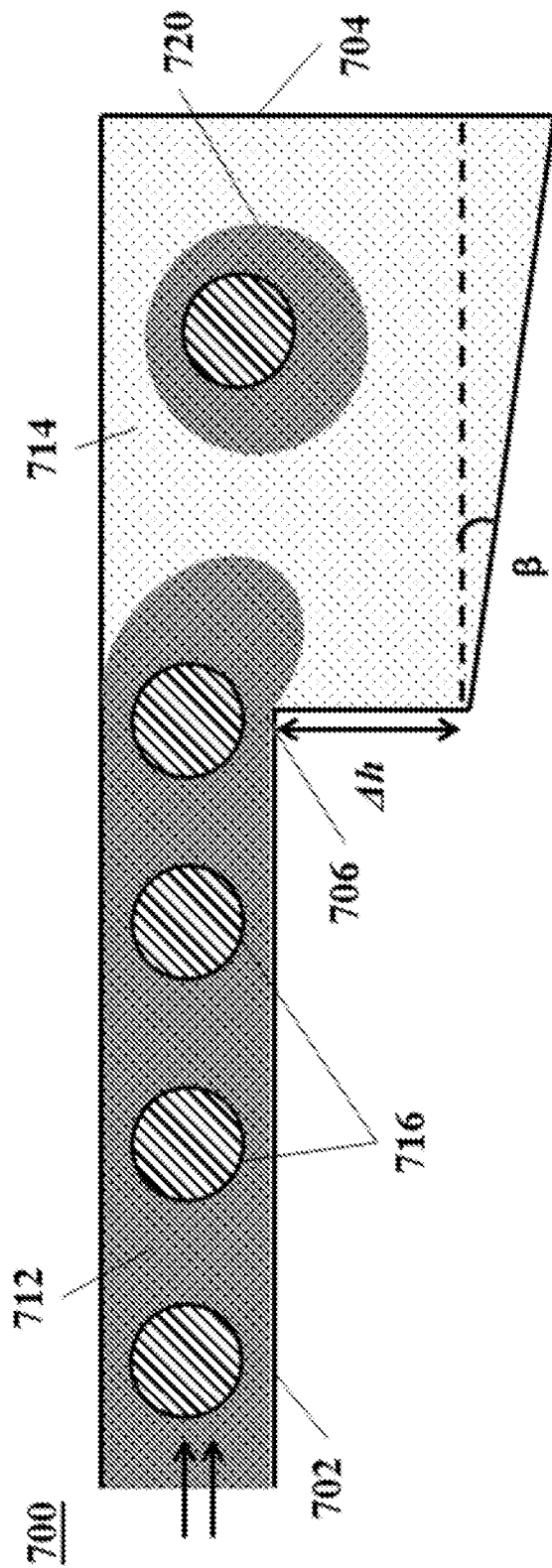


FIG. 7A

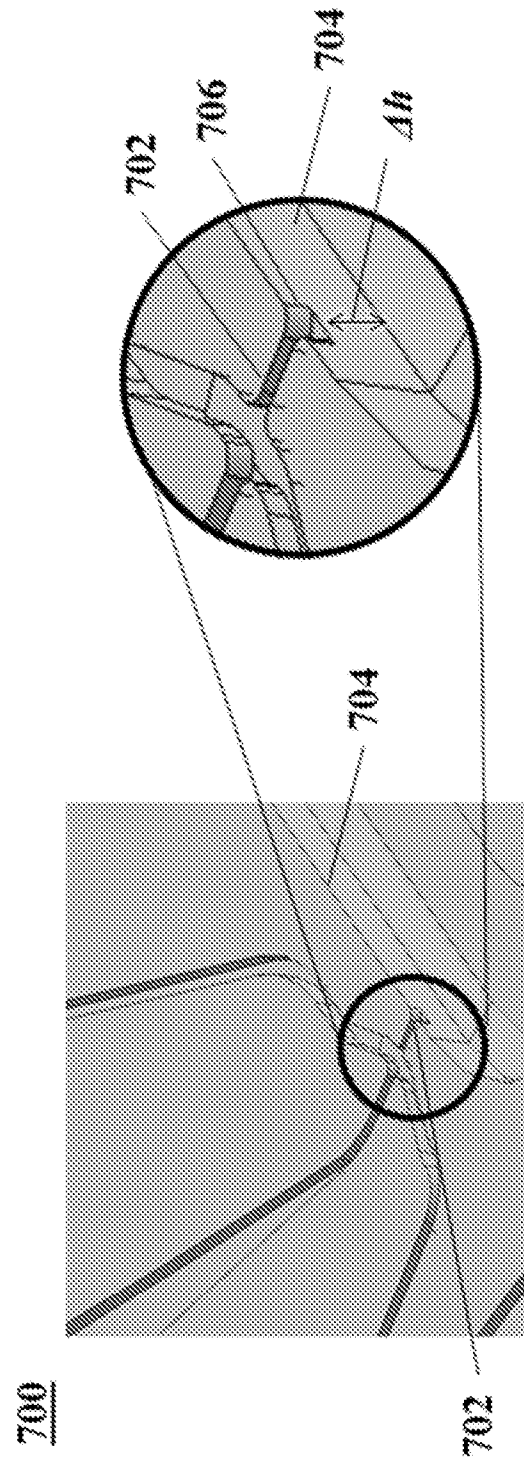


FIG. 7B

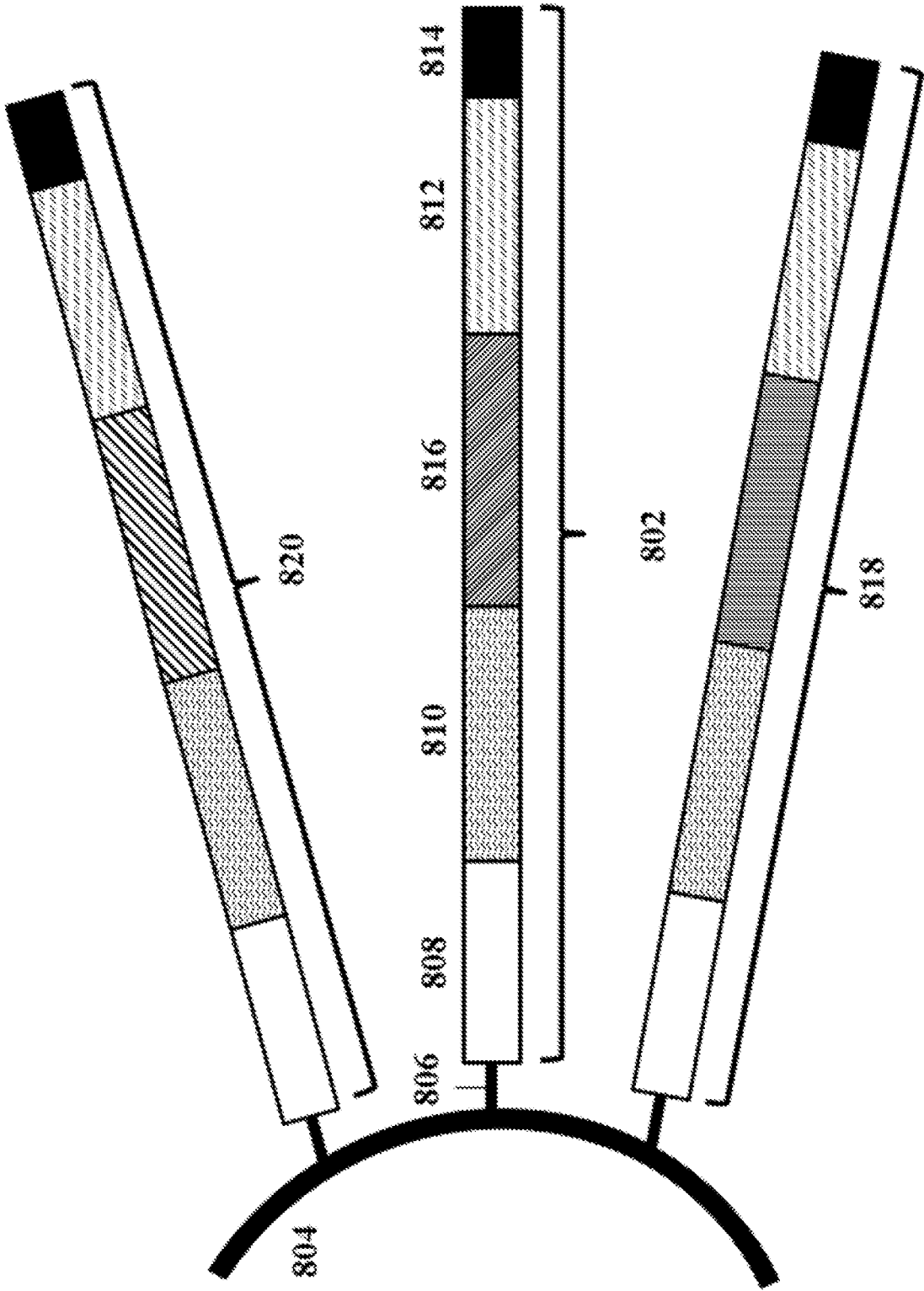


FIG. 8

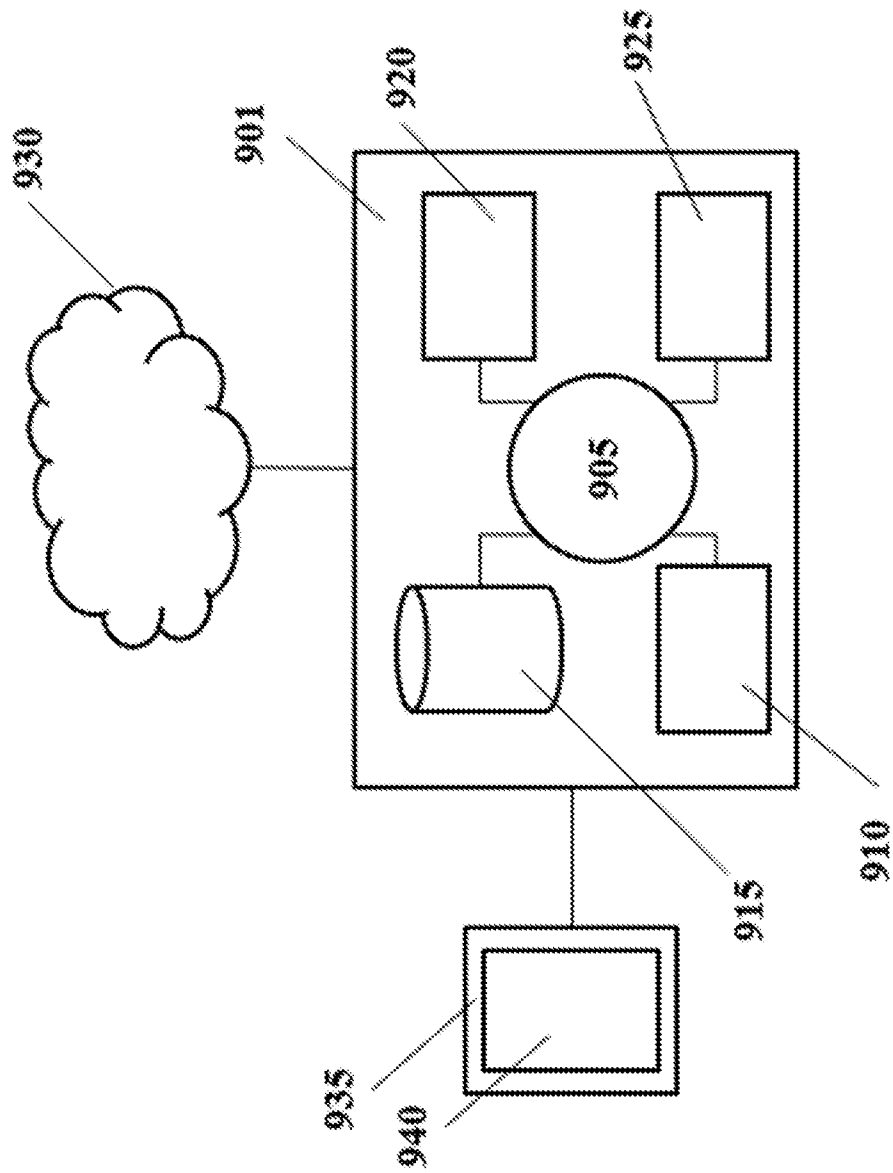


FIG. 9

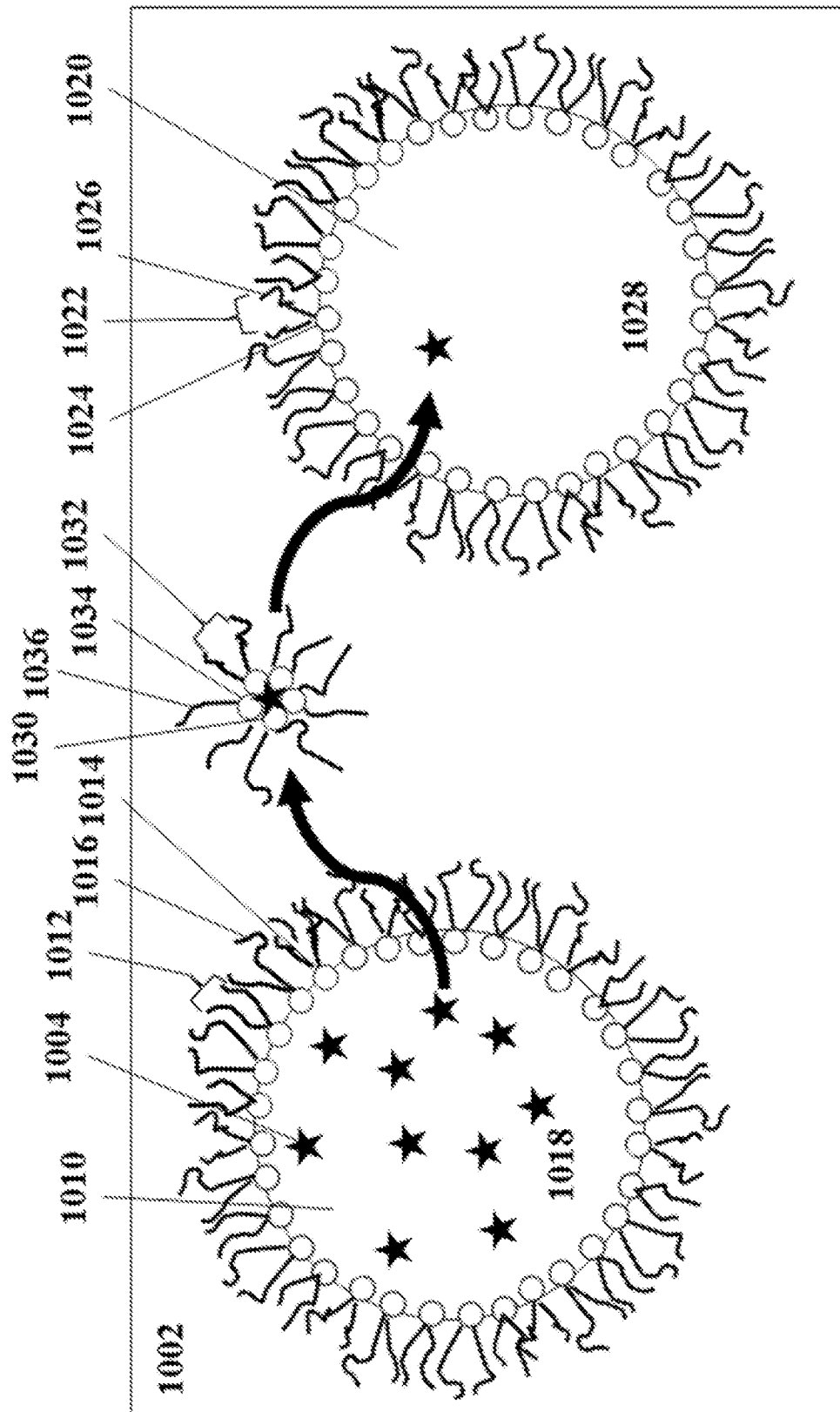


FIG. 10

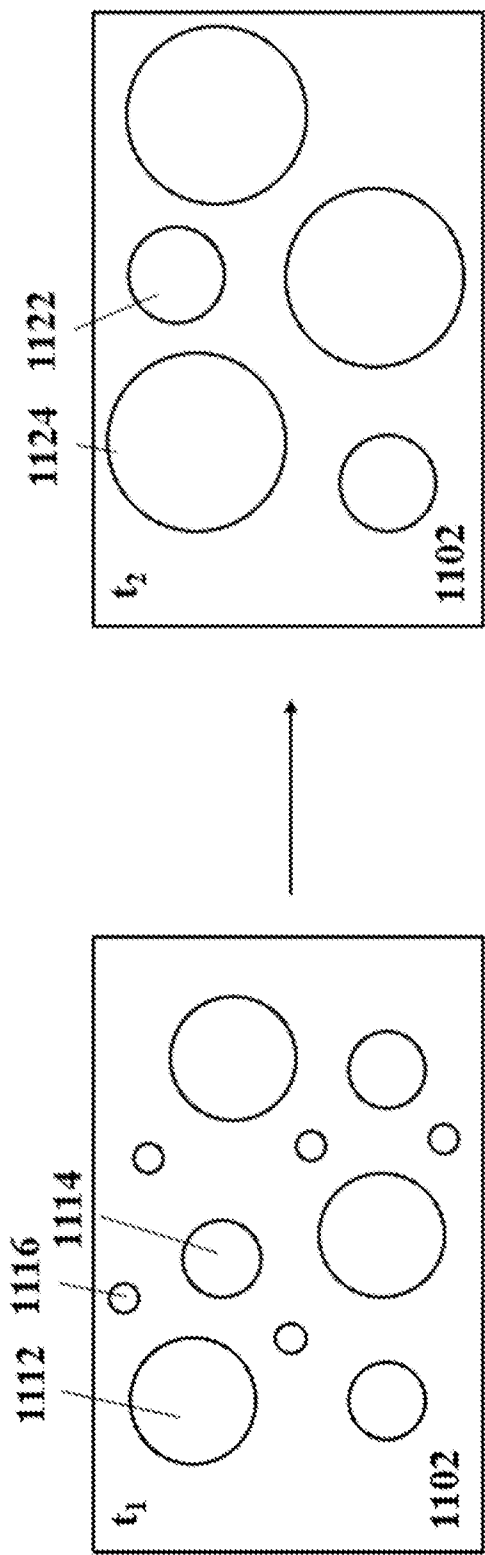


FIG. 11A

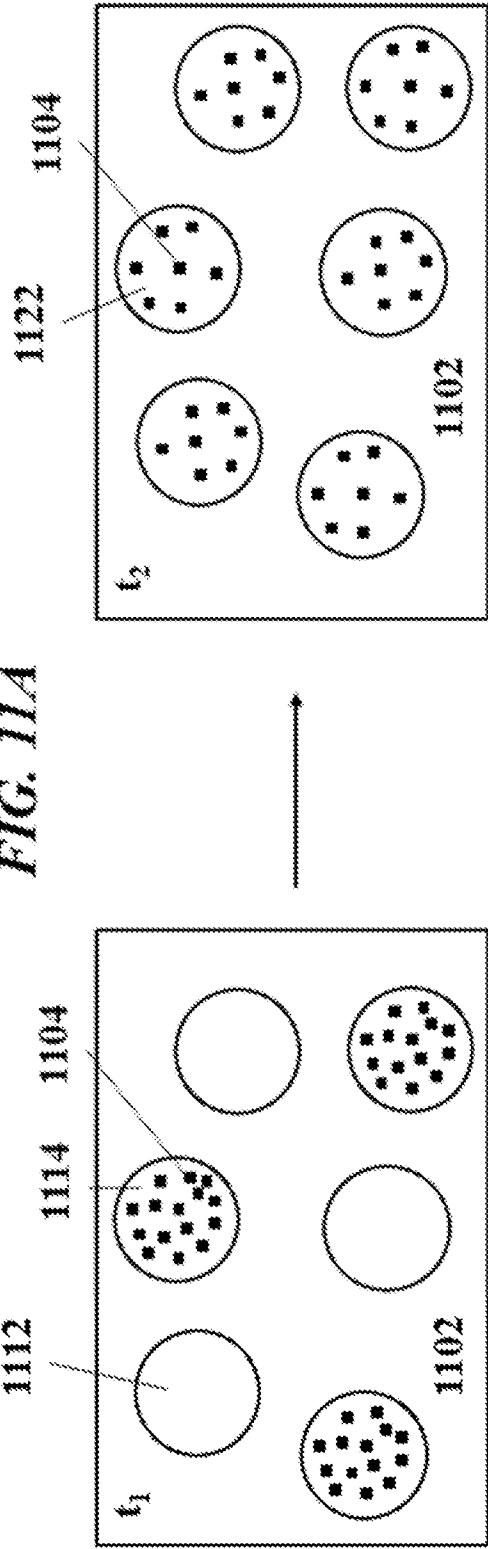


FIG. 11B

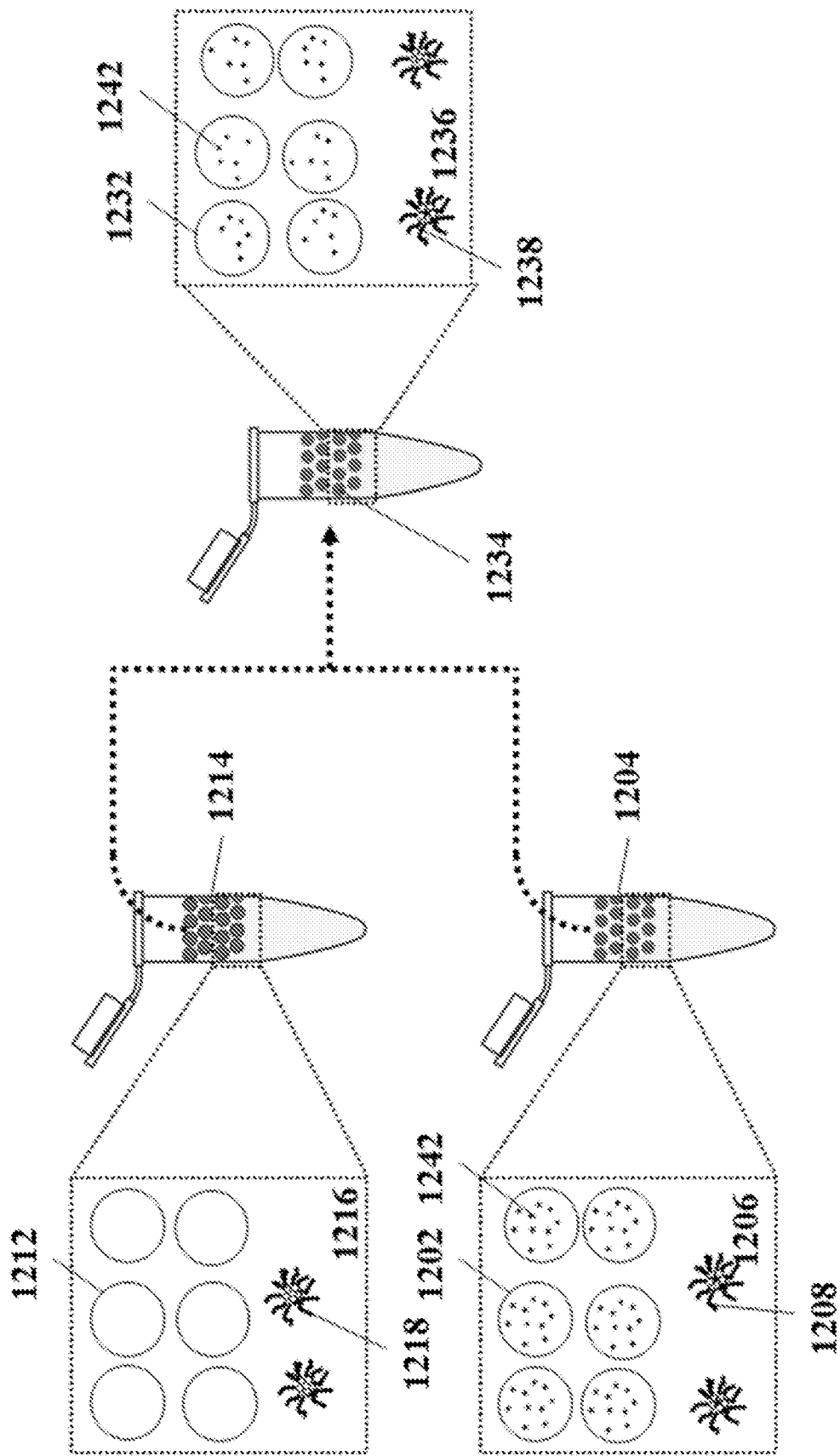


FIG. 12A

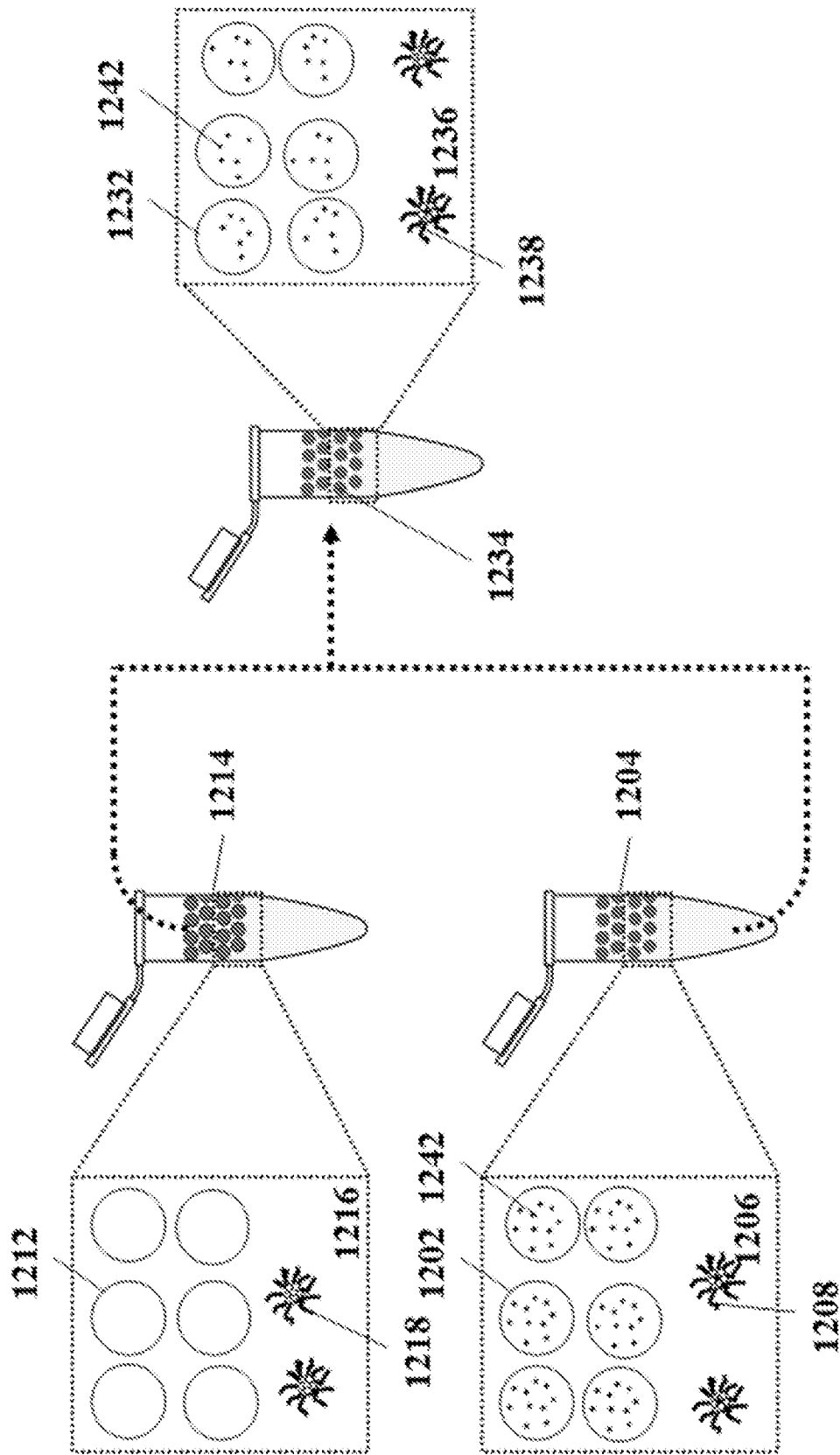


FIG. 12B

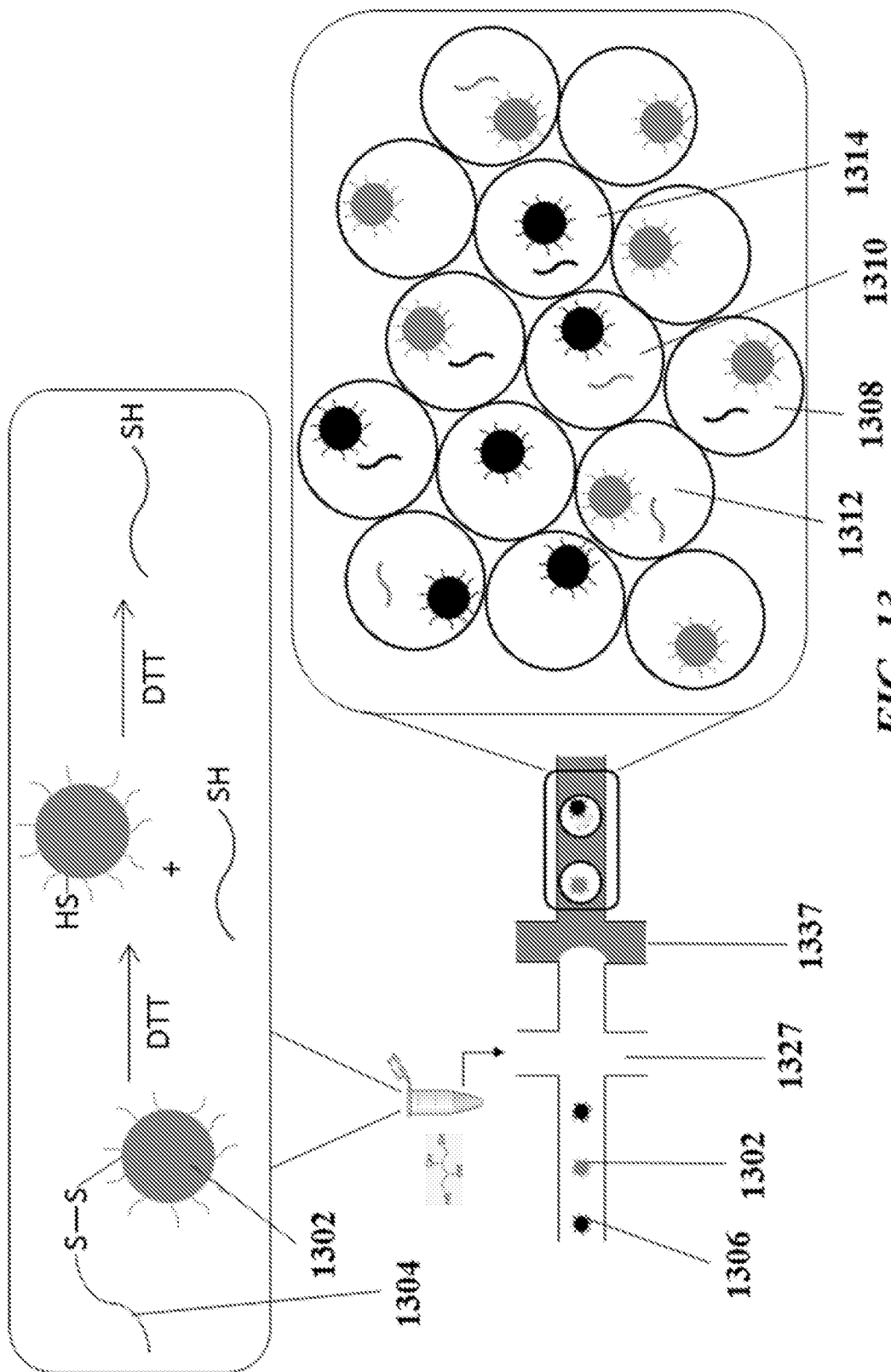


FIG. 13

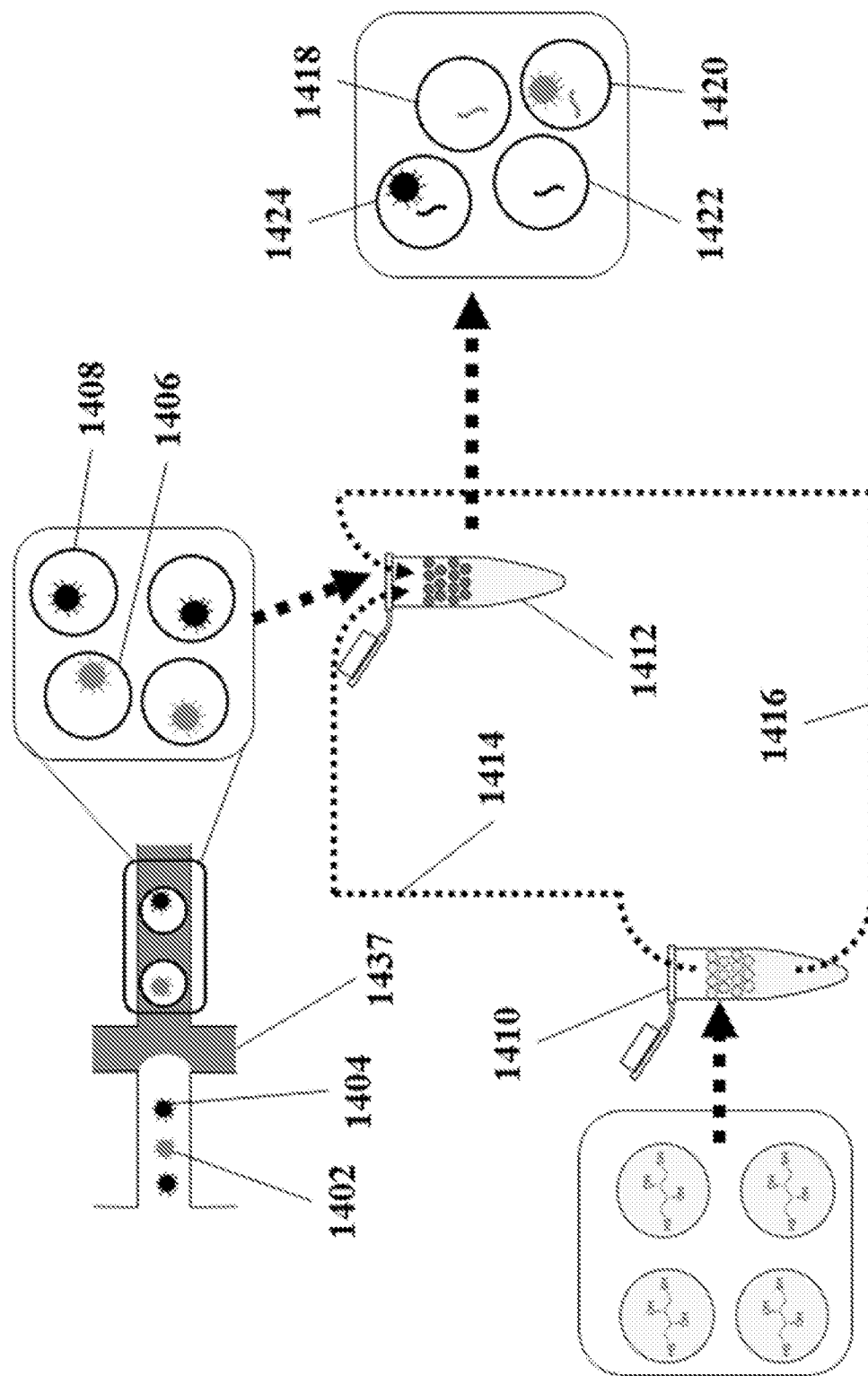


FIG. 14

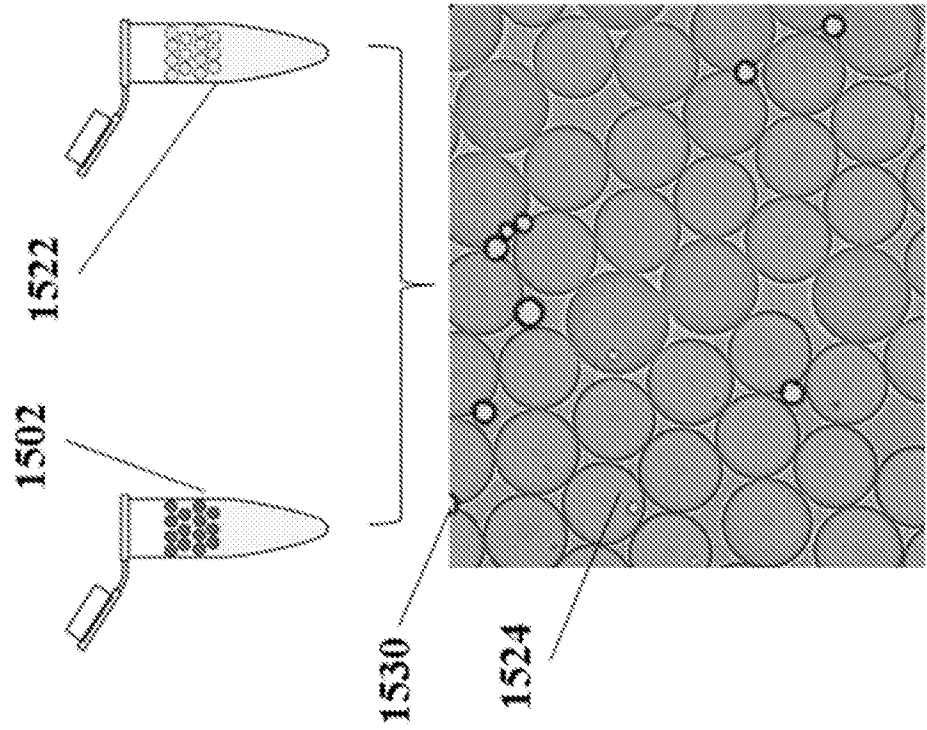


FIG. 15B

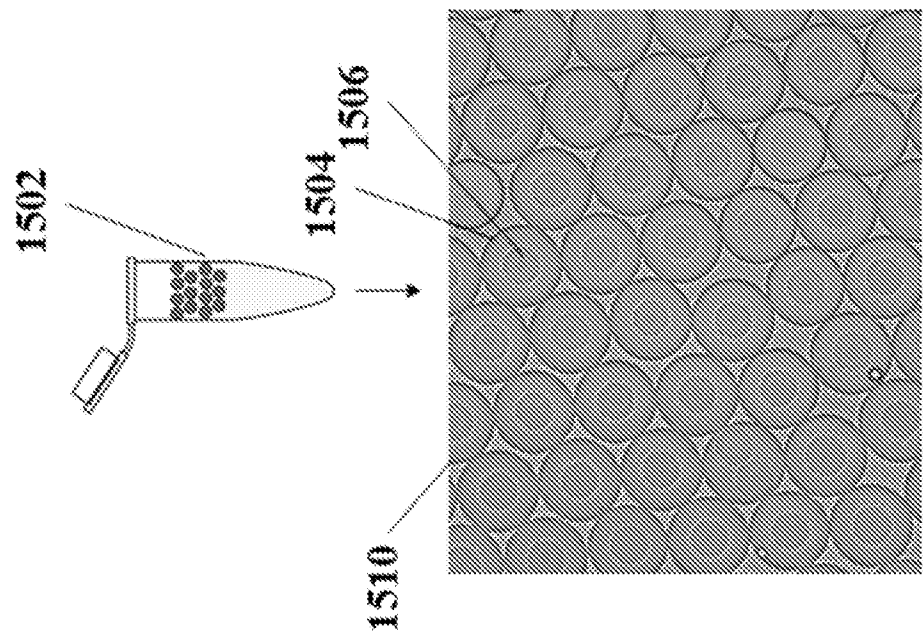


FIG. 15A

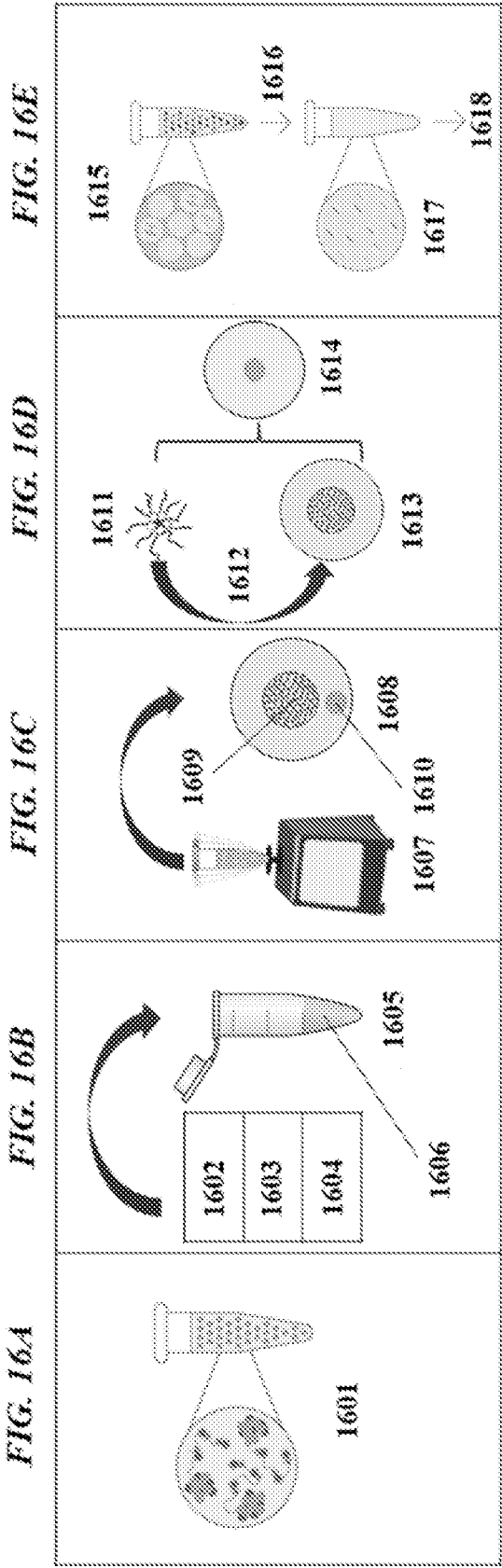


FIG. 17A

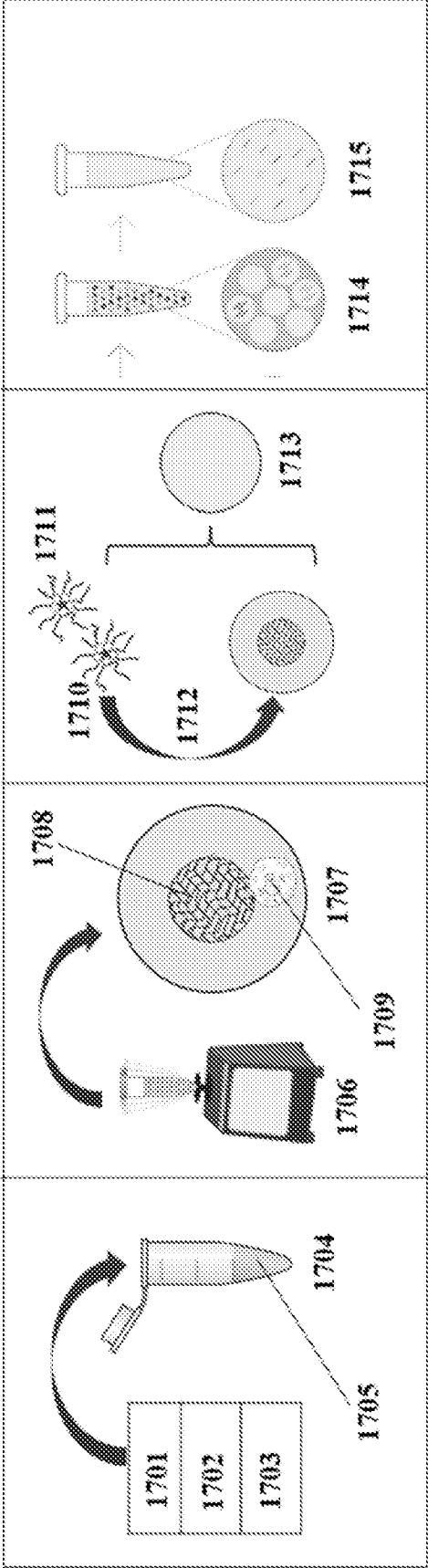


FIG. 17B

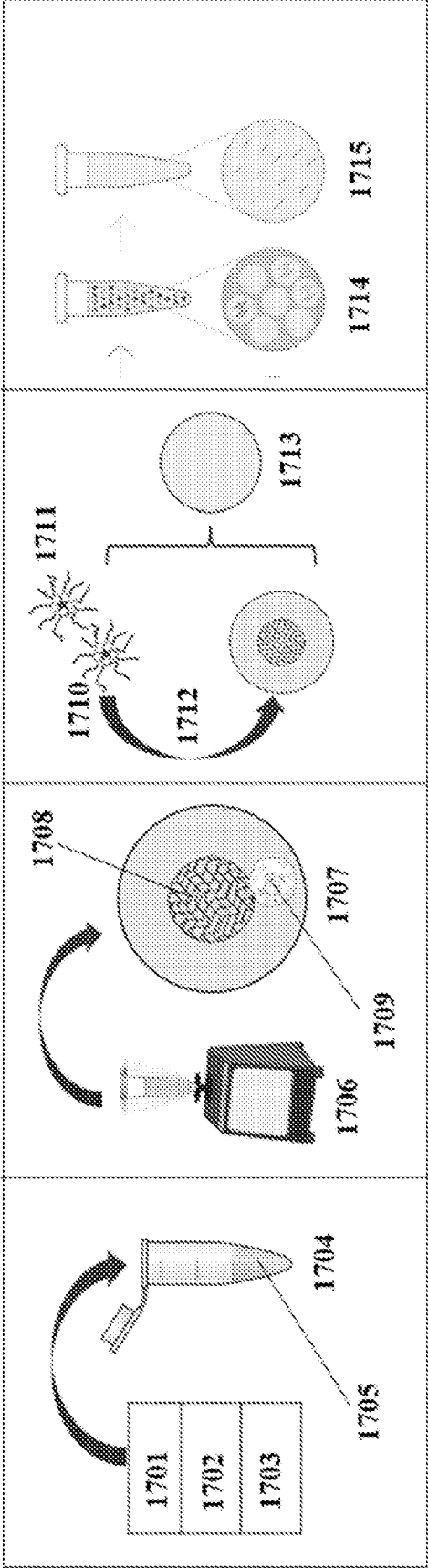


FIG. 17C

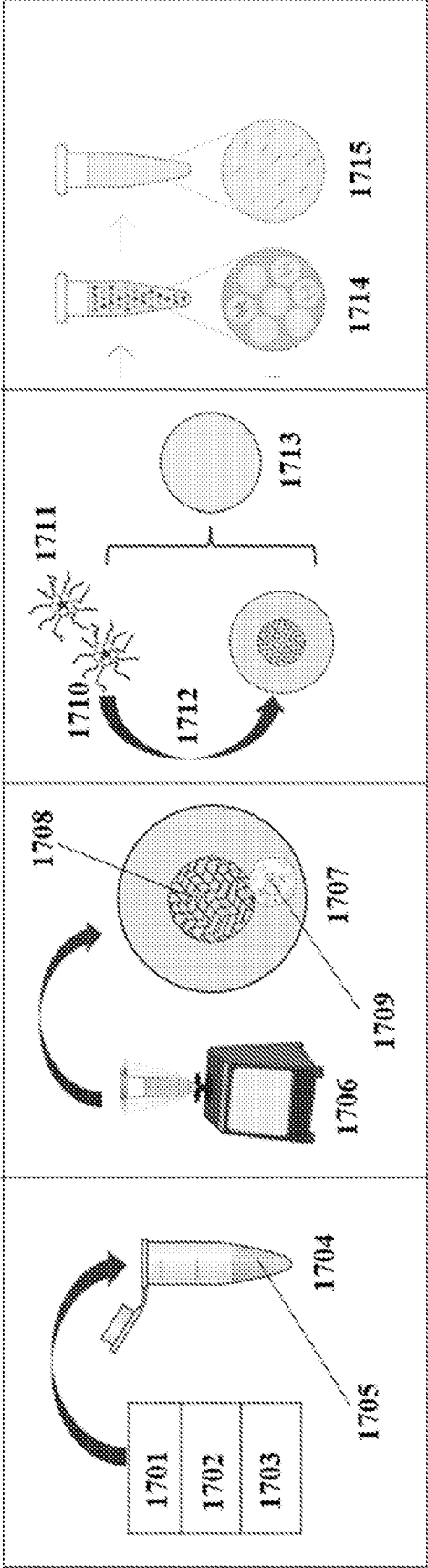


FIG. 17D

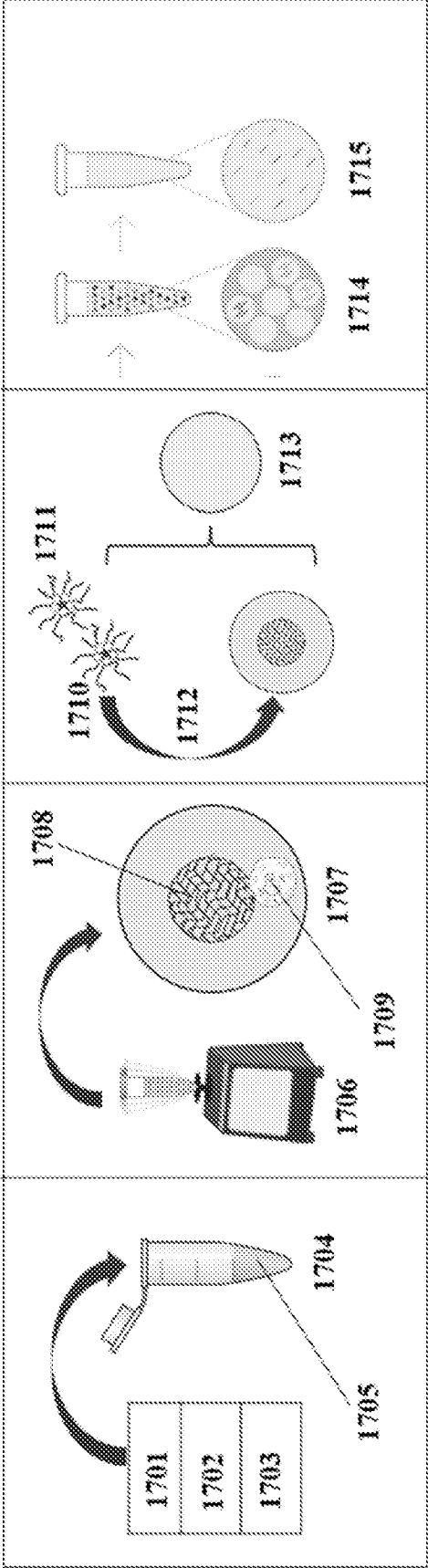


FIG. 18B

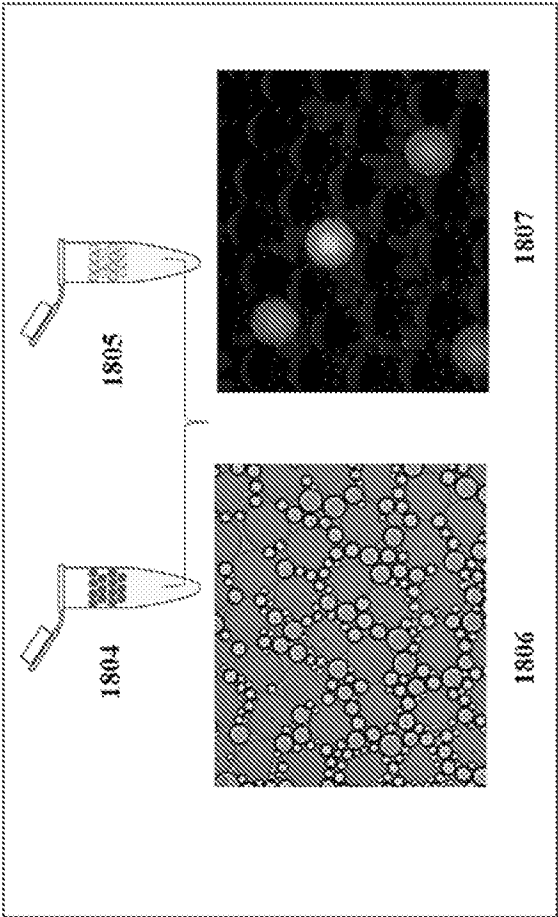
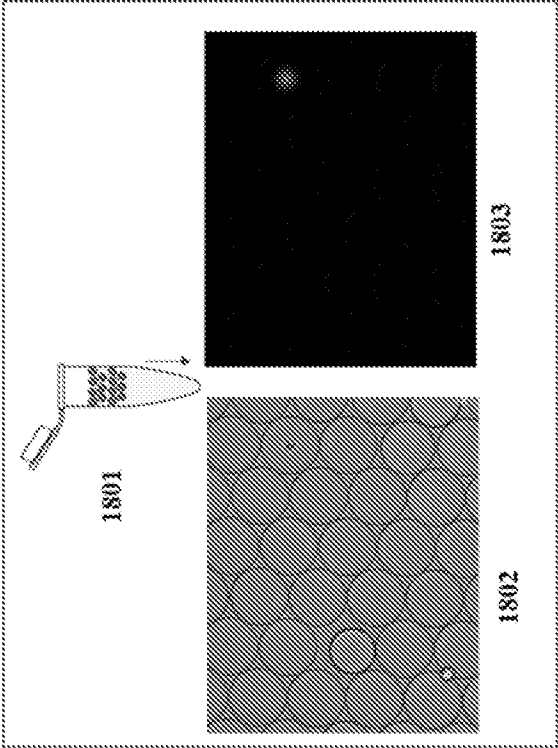


FIG. 18A



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/017785

A. CLASSIFICATION OF SUBJECT MATTER

INV. B01L3/00 C12Q1/6804 C12Q1/6806 G01N33/543
ADD.

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)

B01L C12Q G01N B01J

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)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/356430 A1 (THIAM ABDOU RACHID [FR] ET AL) 13 December 2018 (2018-12-13) paragraphs [0013], [0025], [0028], [0044], [0048], [0052], [0076], [0086], [0093], [0105], [0120] -----	1-16,27, 30
X	BARBARA SCHLICHT ET AL: "Droplet-interface-bilayer assays in microfluidic passive networks", SCIENTIFIC REPORTS, vol. 5, 24 April 2015 (2015-04-24), page 9951, XP055260973, DOI: 10.1038/srep09951 pg. 3 col. 2; paragraph bridging pg. 4 and 5.; figures 1,4 ----- -/--	1,2, 5-16,27



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

13 May 2020

Date of mailing of the international search report

16/07/2020

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Goodman, Marco

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/017785

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2011/053798 A1 (HINDSON BENJAMIN J [US] ET AL) 3 March 2011 (2011-03-03) paragraphs [0094], [0106]; figure 7 -----	1-16,27, 30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/017785

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.:
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 additional 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 an 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.:

2-16, 27, 30(completely); 1(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2-16, 27, 30(completely); 1(partially)

A method for droplet processing, further providing details of the nature of the reagent, solving the problem of how to transfer a reagent between populations of droplets

2. claims: 17-26, 28, 29(completely); 1(partially)

A method for droplet processing wherein a droplet comprises a nucleic acid, solving the problem of how to provide a method for characterizing nucleic acids.

3. claims: 31-48(completely); 1(partially)

A method for droplet processing further detailing how to generate droplet populations, solving the problem as how to provide droplet populations.

4. claims: 49-80

A method for nucleic acid processing wherein a bar coded nucleic acid molecule is generated.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/017785

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
US 2018356430	A1	13-12-2018	CN 108780096 A	09-11-2018
			EP 3377906 A1	26-09-2018
			US 2018356430 A1	13-12-2018
			WO 2017085189 A1	26-05-2017

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			EP 2473618 A1	11-07-2012
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			JP 6155418 B2	05-07-2017
			JP 2013503630 A	04-02-2013
			US 2011053798 A1	03-03-2011
			US 2016059204 A1	03-03-2016
			US 2019242795 A1	08-08-2019
			WO 2011028539 A1	10-03-2011
