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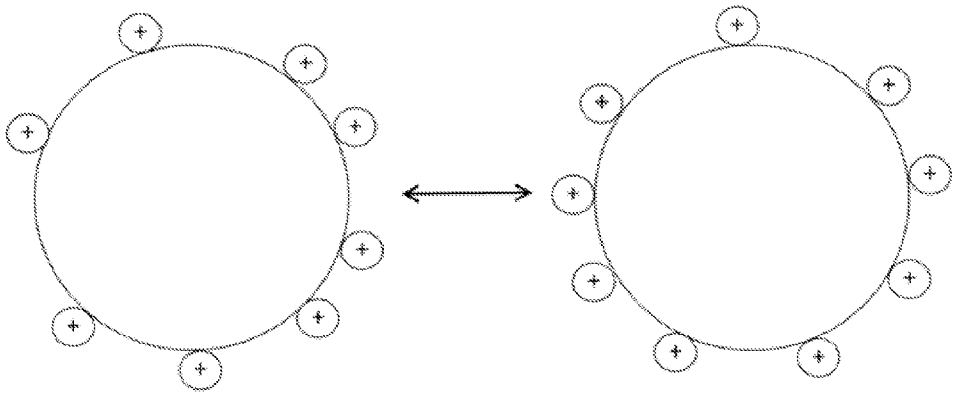

FIG. 7

(57) Abstract: Particles can bind to the surface of cells, wherein the particles comprise a property that can prevent the cells from adhering to one another.

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METHODS FOR SAMPLE PREPARATION

CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Application No. 63/272,911, filed on October 28, 2021, which is herein incorporated by reference in its entirety.

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.

SUMMARY

[0005] In one aspect, the present disclosure provides methods for preventing cell to cell adhesion in a sample preparation process. In one embodiment, the method comprises providing a mixture of particles and cells. The mixture of particles and cells may include a plurality of particles and a plurality of cells. In another embodiment, a particle of the plurality of particles is coupled to a cell of the plurality of cells. In one other embodiment, the particle coupled to the cell prevents the cell from adhering to another cell of the plurality of cells. In other embodiments, the plurality of particles comprises at least 10 particles, at least 1,000 particles, or at least 100,000 particles. In another embodiment, the number of particles of the plurality of particles is at least 2 times a number of cells of the plurality of cells, at least 10 times a number of cells of the plurality of cells, or at least 100 times a number of cells of the plurality of cells.

[0006] In other embodiments, the plurality of particles comprises a plurality of beads. In another embodiment, the plurality of particles is homogenous or heterogeneous. In other embodiments, the plurality of particles comprises particles having different sizes, particles having different weights, and/or particles having different functional groups. In one embodiment, the particle comprises a microsphere or the particle is a magnetic particle. In other embodiments, the

particle is a charged particle, e.g., such as a positively charged particle. In one additional embodiment, the particle comprises more than one particle.

[0007] In other embodiments, the particle is a bead. The particle may comprise a dimension of less than 10 μm or a dimension of less than 50 μm. The particle may comprise a diameter no more than 1/2 of a diameter of the cell, a diameter no more than 1/3 of a diameter of the cell, a diameter no more than 1/10 of a diameter of the cell or a diameter no more than 1/100 of a diameter of the cell.

[0008] In some embodiments, the particle further comprises a protein. The protein may be an enzyme, such as a DNAase. In one embodiment, the method of the present disclosure further comprises removing the DNAase by removing the particle.

[0009] In other embodiments, the particle further comprises an oligonucleotide.

[0010] In one aspect, the particles are coupled to single cells. In one embodiment, the particle of the plurality of particles is coupled to one cell or a single cell of the plurality of cells. In one other embodiment, at least two particles of the plurality of particles are coupled to the one cell or the single cell. In one embodiment, the at least two particles surround the one cell or the single cell. In another embodiment, the particle coupled to the one cell or the single cell prevents the one cell or the single cell from adhering to another cell of the plurality of cells. In one other embodiment, the plurality of cells may comprise single cells, cultured cells or harvested cells. In an additional embodiment, the plurality of cells comprises hepatocytes, such as for example HepG2 cells. In other embodiments, the plurality of cells comprises at least 10 cells, at least 100 cells, at least 1,000 cells, at least 10,000 cells, or at least 100,000 cells.

[0011] In other embodiments, the method further comprises freezing the cell. The method may further comprise processing the plurality of cells. Processing may include fluorescence-activated cell sorting (FACS) and/or performing an assay.

[0012] In some embodiments, the method further comprises partitioning the particle coupled to the cell to a partition. In one embodiment, the method further comprises co-partitioning the particle coupled to the cell to a same partition as a nucleic acid barcode molecule. In other embodiments, the partition comprises no more than one cell. In one embodiment, a nucleic acid barcode molecule is coupled to a nucleic acid molecule of the cell. In some embodiments, the partition is a well or a droplet. In another embodiment, the method further comprises pooling the partition with a partition comprising a second cell from another plurality of cells. The particle may be coupled to a capture moiety. In one embodiment, the method further comprises lysing the cell to provide access an intracellular molecule of the cell. The intracellular molecule of the cell may be bound or captured using the capture moiety.

[0013] In another aspect, the present disclosure provides methods for isolating a positively charged particle coupled to a cell based at least on a density of the positively charged particle. In one embodiment, the method comprises providing a mixture comprising a plurality of positively charged particles and a plurality of cells. In another embodiment, a particle of the plurality of positively charged particles is coupled to a cell of the plurality of cells. In a further embodiment, the method further comprises isolating the particle coupled to the cell based at least in part on a density of the particle coupled to the cell. In other embodiments, the plurality of positively charged particles comprises at least 10 positively charged particles, at least 1,000 positively charged particles, or at least 100,000 positively charged particles. In another embodiment, a number of positively charged particles of the plurality of positively charged particles is at least 2 times a number of cells of the plurality of cells, a number of positively charged particles of the plurality of positively charged particles is at least 10 times a number of cells of the plurality of cells, or a number of positively charged particles of the plurality of positively charged particles is at least 100 times a number of cells of the plurality of cells. In additional embodiments, the plurality of positively charged particles comprises a plurality of beads. The plurality of positively charged particles is a plurality of homogenous particles or a plurality of heterogeneous particles. In another embodiment, the plurality of positively charged particles comprises positively charged particles having different sizes, the plurality of positively particles comprises positively charged particles having different weights, or the plurality of positively charged particles comprises positively charged particles having different functional groups. In one embodiment, the positively charged particle comprises a microsphere. In one other embodiment, the positively charged particle comprises more than one positively charged particle. In other embodiments, a dimension of the positively charged particle is less than about 10 μm or a dimension of the positively charged particle is less than about 50 μm. In additional embodiments, the positively charged particle comprises a diameter no more than 1/2 of a diameter of the cell, a diameter no more than 1/3 of a diameter of the cell, a diameter no more than 1/10 of a diameter of the cell, or a diameter no more than 1/100 of a diameter of the cell. In one other embodiment, positively charged particle comprises more than one positively charged particle.

[0014] In other embodiments, the positively charged particle further comprises a protein or an oligonucleotide.

[0015] In one aspect, the positively charged particles are coupled to single cells. In one embodiment, the positively charged particle of the plurality of particles is coupled to one cell or a single cell of the plurality of cells. In one other embodiment, at least two positively charged

particles of the plurality of positively charged particles are coupled to the one cell or the single cell. In one embodiment, the at least two positively charged particles surround the one cell or the single cell. In another embodiment, the positively charged particle coupled to the one cell or the single cell prevents the one cell or the single cell from adhering to another cell of the plurality of cells. In one other embodiment, the plurality of cells may comprise single cells, cultured cells, oocytes, or harvested cells. In other embodiments, the plurality of cells comprises at least 10 cells, at least 100 cells, at least 1,000 cells, at least 10,000 cells, or at least 100,000 cells.

[0016] In one other aspect, the present disclosure provides methods for isolating a positively charged particle coupled to a cell based at least on a density of the positively charged particle, wherein the isolating comprises sedimentation. In one embodiment, the sedimentation comprises differential sedimentation. In other embodiments, the isolating comprises collecting a layer comprising cells. The layer comprising cells may be a liquid layer.

[0017] In additional embodiments, the method further comprises partitioning the positively charged particle coupled to the cell to a partition. In one embodiment, the method further comprises co-partitioning the positively charged particle coupled to the cell to a same partition as a nucleic acid barcode molecule. In other embodiments, the partition comprises no more than one cell. In one embodiment, a nucleic acid barcode molecule is coupled to a nucleic acid molecule of the cell. In some embodiments, the partition is a well or a droplet. In another embodiment, the method further comprises pooling the partition with a partition comprising a second cell from another plurality of cells. The positively charged particle may be coupled to a capture moiety. In one embodiment, the method further comprises lysing the cell to provide access an intracellular molecule of the cell. The intracellular molecule of the cell may be bound or captured using the capture moiety.

[0018] 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.

[0019] 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.

[0020] 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

[0021] 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

[0022] 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:

[0023] FIG. 1 shows an example of a microfluidic channel structure for partitioning individual biological particles.

[0024] FIG. 2 shows an example of a microfluidic channel structure for delivering barcode carrying beads to droplets.

[0025] FIG. 3 shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets.

[0026] FIG. 4 illustrates an example of a barcode carrying bead.

[0027] FIG. 5 illustrates another example of a barcode carrying bead.

[0028] FIG. 6 shows a computer system that is programmed or otherwise configured to implement methods provided herein.

[0029] FIG. 7 shows two biological particles, e.g., cells or nuclei, each coupled to a plurality of charged particles, such that the charged particles prevent the biological particles, e.g., cells or nuclei from adhering to one another.

[0030] FIG. 8 shows a workflow for using particles to prevent biological particles, e.g., cells or nuclei, from adhering to one another.

[0031] FIG. 9 shows an exemplary microwell array schematic.

[0032] FIG. 10 shows an exemplary microwell array workflow for processing nucleic acid molecules.

DETAILED DESCRIPTION

[0033] 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.

[0034] 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.

[0035] The terms "a," "an," and "the," as used herein, generally refers to singular and plural references unless the context clearly dictates otherwise.

[0036] 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.

[0037] 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.

[0038] The term "about" as used herein referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary between 1% and 15% of the stated number or numerical range.

[0039] 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.

[0040] 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.

[0041] 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).

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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 or nucleus 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.

[0047] 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 biological particle sample, e.g., a cell or nuclei sample. The sample may be a cell line or cell culture sample. The sample can include one or more cells or nuclei. 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.

[0048] The term "biological particle," as used herein, generally refers to a discrete biological system derived from a biological sample. The biological particle may be a macromolecule. The biological particle may be a small molecule. The biological particle may be a virus. The biological particle may be a cell or derivative of a cell. The biological particle may be an organelle. Examples of an organelle from a cell include, without limitation, a nucleus, endoplasmic reticulum, a ribosome, a Golgi apparatus, an endoplasmic reticulum, a chloroplast, an endocytic vesicle, an exocytic vesicle, a vacuole, and a lysosome. The biological particle may be a rare cell from a population of cells. The biological particle 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 biological particle may be a constituent of a cell. The biological particle may be or may include DNA, RNA, organelles, proteins, or any combination thereof. The biological particle 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 biological particle may be obtained from a tissue of a subject. The

biological particle may be a hardened cell. Such hardened cell may or may not include a cell wall or cell membrane. The biological particle 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.

[0049] The term "macromolecular constituent," as used herein, generally refers to a macromolecule contained within or from a biological particle. The macromolecular constituent may comprise a nucleic acid. In some cases, the biological particle 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.

[0050] 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.

[0051] 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.

[0052] Properties of different cell types and/or experimental methods can influence the behavior of cells in applications such as fluidics-based analysis methods. For example, cell-to cell clumping and differential sedimentation properties of cells may present a problem for single-cell gene expression methods. Provided herein are methods to prevent cell clumping. In some cases, such methods can be implemented without negatively affecting an assay or process on the cells.

Methods

[0053] Provided herein are methods for preventing a biological particle, e.g., a cell or nucleus, from adhering to another biological particle (e.g., cell-cell or nucleus-nucleus clumping). Particles can be coupled to biological particles, e.g., cells or nuclei, to prevent adhering or clumping of the biological particles, e.g., cells or nuclei, to one another. In some embodiments, a property of the particles can prevent clumping, for example, by overcoming or preventing a mechanical, chemical, biochemical, or spatial association between two biological particles, e.g., two cells or two nuclei. An example workflow of such a method is provided in **FIG. 8**.

[0054] Methods can comprise providing a mixture comprising a plurality of particles and a plurality of biological particles, e.g., cells or nuclei, wherein a particle of the plurality of particles is coupled to a biological particle of the plurality of biological particle. In some embodiments, the particle coupled to the biological particle, e.g., cell or nucleus, prevents the biological particle from adhering to another of the plurality of biological particles.

[0055] A particle can be an entity smaller than a biological particle, e.g., a cell or nucleus, that can bind to a biological particle to prevent clumping. For example, in some cases, a particle can be a bead, a microbead, a microsphere, a nanoparticle, or another type of particle that can prevent a biological particle, e.g., a cell or nucleus, from adhering to another biological particle. In some embodiments, a plurality of biological particles, e.g., cells or nuclei, can comprise a plurality of beads.

[0056] A plurality of particles can be homogenous or heterogeneous. In some embodiments, a heterogeneous plurality of particles can comprise particles that can prevent biological particle adhesion, e.g., cell or nucleus adhesion by different mechanisms (e.g., size and charge or size

and magnetic property) or by different values of a same mechanism (e.g., different charges, different magnetic properties, or different sizes).

[0057] In some embodiments, the plurality of particles can comprise particles having different functional groups. A functional group can be, for example, a grouping of atoms within a molecule associated with a particle or within or on the particle itself that can have their own characteristic properties, for example regardless of other atoms present. Examples of functional groups can include an alkene, an alkyne, an arene, a haloalkane, an alcohol, an aldehyde, a ketone, a carboxylic acid, an ester, an ether, an epoxide, an amine, an amide, a nitrate, a nitrite, a nitrile, an imine, an imide, an azide, a cyanate, an isocyanate, an azo compound, a thiol, a sulfide, a sulfone, a sulfonic acid, a sulfinic acid, a sulfonate ester, a thiocyanate, an isothiocyanate, a thial, a thioketone, or another appropriate functional group.

[0058] A particle can have a property that can prevent a biological particle, e.g., a cell or a nucleus, from adhering to another biological particle, e.g., a cell or a nucleus. In some embodiments, for example, a particle can be a magnetic particle. In some such cases, coupling of such magnetic particle(s) to a biological particle, e.g., a cell or a nucleus (or a plurality of cells or nuclei) can prevent the biological particle from adhering to another biological particle by magnetic repulsion. In some cases, beads coupled to different biological particles, e.g., cells or nuclei, can repel one another, thus repelling the biological particles, e.g., cells or nuclei, they are coupled to. In some cases, a magnetic particle can display paramagnetism or superparamagnetism.

[0059] In some embodiments, a particle can be a charged particle. A charged particle can comprise a positive charge or a negative charge. In some embodiments, a positively charged particle can bind to a negative charge on the surface of a biological particle, e.g., a cell or a nucleus. A charged particle on a biological particle, e.g., a cell or nucleus, can repel a charge on the surface of another biological particle, e.g., a cell surface of another cell or a nuclear surface of another nucleus, thus physically separating the biological particles and preventing adhesion, e.g., cell adhesion or nucleus adhesion. In some embodiments, a charged particle on a biological particle, e.g., a cell or nucleus, can repel a charged particle on another biological particle, e.g., a cell or nucleus.

[0060] In some embodiments, a particle can have a particular size. A size of a particle can influence the interactions of a biological particle, e.g., a cell or nucleus, associated with the particle with its environment, including other biological particles, e.g., other cells or nuclei. In some embodiments, a particle can have a size that prevents the surface of a biological particle, e.g., a cell surface of a cell or nuclear surface of a nucleus, from having a close enough

proximity to the surface of another biological particle, e.g., another cell surface of another cell or another nuclear surface of another nucleus, to adhere, and in some cases a particle can have a size that can prevent clumping between two biological particles. A size of a particle can refer to a dimension of the particle such as the diameter, radius, circumference, width, height, length, or other dimension of the particle. In some embodiments, a particle can have a dimension of less than 5 μm, less than 10 μm, less than 20 μm, less than 30 μm, less than 40 μm, less than 50 μm, less than 60 μm, less than 70 μm, less than 80 μm, less than 90 μm, less than 100 μm, less than 200 μm, less than 300 μm, less than 400 μm, or less than 500 μm. In some embodiments, a particle can have a dimension of at least 5 μm, at least 10 μm, at least 20 μm, at least 30 μm, at least 40 μm, at least 50 μm, at least 60 μm, at least 70 μm, at least 80 μm, at least 90 μm, at least 100 μm, at least 200 μm, at least 300 μm, at least 400 μm, or at least 500 μm. In some embodiments, a particle can have a dimension of about 5 μm, about 10 μm, about 20 μm, about 30 μm, about 40 μm, about 50 μm, about 60 μm, about 70 μm, about 80 μm, about 90 μm, about 100 μm, about 200 μm, about 300 μm, about 400 μm, about 500 μm, or a range between any two foregoing values. For example, in some embodiments, a particle can comprise a dimension of less than 10 μm. As another example, a particle can comprise a dimension of less than 50 μm.

[0061] A dimension can be smaller than the diameter or other dimension of a cell, such as the cell the particle couples to. In some embodiments, the particle can comprise a dimension that is no more than 1/2, no more than 1/3, no more than 1/5, no more than 1/6, no more than 1/7, no more than 1/8, no more than 1/9, or no more than 1/10 the diameter of the cell. In some embodiments, the particle can comprise a diameter than is no more than 1/2, no more than 1/3, no more than 1/5, no more than 1/6, no more than 1/7, no more than 1/8, no more than 1/9, or no more than 1/10, no more than 1/25, no more than 1/50, no more than 1/100, no more than 1/500, or no more than 1/1000 the diameter of the cell.

[0062] In some embodiments, a particle can further comprise a protein. A protein can comprise a sequence of amino acids. In some embodiments, a protein can be a peptide, a single protein, a dimer, a trimer, a tetramer, or other configuration of a protein.

[0063] In some embodiments, a protein can aid in coupling the particle to the biological particle, e.g., a cell or a nucleus. In some such cases, a protein can have affinity to a biological particle surface feature, e.g., a cell surface feature of the cell or nuclear surface feature of the nucleus, such as a protein, a glycosylated protein, an otherwise modified protein, or a fatty acid.

[0064] In some embodiments, a protein can aid in preventing adhesion between biological particles, e.g., cell adhesion or nucleus-nucleus adhesion, or can prevent such types of adhesion.

For example, a protein can physically block a component of a biological particle, e.g., a cell or a nucleus, that can adhere to another biological particle or component thereof.

[0065] In some embodiments where a particle comprises a protein, and the protein can be removed after the particle is coupled to the biological particle, e.g., cell or nucleus. This can occur, for example, once a need to prevent biological particle adhesion has passed, or once the biological particle adhesion is successfully prevented. A protein can be removed, for example, by removing the particle from the biological particle, or by removing the protein from the particle.

[0066] In some embodiments, a particle can further comprise an oligonucleotide. An oligonucleotide can comprise a sequence of nucleic acids coupled to the particle. An oligonucleotide can comprise, for example, a barcode, a UMI sequence, a primer sequence, a binding sequence, or another sequence type such as those provided herein. An oligonucleotide can be useful, for example, for identifying a biological particle, e.g., a cell or a nucleus, for identifying a source of a biological particle, e.g., a cell or nucleus, for tracking a biological particle, e.g., a cell or nucleus, for labeling a biological particle, e.g., a cell or nucleus, for preventing adhesion as described herein, or to aid in performing an assay using the biological particle, e.g., the cell or nucleus.

[0067] In some embodiments, a particle can comprise or be coupled to a capture moiety or labeling agent. Examples of such capture moieties or labeling agents can include an oligonucleotide, an antibody or antigen binding fragment thereof, or a protein having affinity to a biological molecule (e.g., a protein or peptide), for example of the biological particle, e.g., a cell or nucleus. The capture moieties or labeling agents may be configured to couple to one or more features of a biological particles, such as cell features, including cell surface features, or nuclear features, including nuclear membrane features. Cell surface features or nuclear membrane features may include, but are not limited to, a receptor, an antigen, a surface protein, a transmembrane protein, a cluster of differentiation protein, a protein channel, a protein pump, a carrier protein, a phospholipid, a glycoprotein, a glycolipid, a cell-cell interaction protein complex, an antigen-presenting complex, a major histocompatibility complex, an engineered T-cell receptor, a T-cell receptor, a B-cell receptor, a chimeric antigen receptor, a gap junction, an adherens junction, or any combination thereof. In some instances, cell features may include intracellular analytes, such as proteins, protein modifications (e.g., phosphorylation status or other post-translational modifications), nuclear proteins, nuclear membrane proteins, or any combination thereof. A capture moiety or labelling agent may include, but is not limited to, a protein, a peptide, an antibody (or an epitope binding fragment thereof), a lipophilic moiety

(such as cholesterol), a cell surface receptor binding molecule, a receptor ligand, a small molecule, a bi-specific antibody, a bi-specific T-cell engager, a T-cell receptor engager, a B-cell receptor engager, a pro-body, an aptamer, a monobody, an affimer, a darpin, and a protein scaffold, or any combination thereof. The capture moieties or labelling agents can include (e.g., are attached to) a reporter oligonucleotide that is indicative of the biological particle feature (e.g., cell feature such as a cell surface or intracellular feature, or a nuclear feature such as a nuclear membrane or intranuclear feature) to which the labeling agent binds. For example, the reporter oligonucleotide may comprise a barcode sequence that permits identification of the capture moiety or labelling agent. For example, a capture moiety or labelling agent that is specific to one type of biological particle feature (e.g., a first cell surface feature or a first nuclear membrane feature) may have a first reporter oligonucleotide coupled thereto, while a capture moiety or labelling agent that is specific to a different biological particle feature (e.g., a second cell surface feature or a second nuclear membrane feature) may have a different reporter oligonucleotide coupled thereto. For a description of exemplary labelling agents, reporter oligonucleotides, and methods of use, see, e.g., U.S. Pat. 10,550,429; U.S. Pat. Pub. 20190177800; and U.S. Pat. Pub. 20190367969, each of which is herein entirely incorporated by reference for all purposes.

[0068] In some embodiments, the method can further comprise lysing the biological particle, e.g., a cell or nucleus. Upon lysing, the contents of the biological particle, e.g., a cell or nucleus, can be exposed to or accessed by the capture moiety or labeling agent. In some embodiments, the capture moiety or labeling agent can capture one or more components of the biological particle, e.g., cell or nucleus. For example, an oligonucleotide can capture a ribonucleic acid (RNA) transcript or an antibody or antigen binding fragment thereof can capture a protein of the cell. A captured component of the cell can be then further processed and/or analyzed.

[0069] In some embodiments, a particle can comprise more than one particle. For example, a particle can comprise two or more particles coupled to one another. Such coupling can be reversible or irreversible. Coupled particles can be the same particle or different particles. For example, a particle having a first property can be coupled to a particle having a second property. In some embodiments, a particle comprising more than one particle can comprise a particle that can couple with a biological particle, e.g., a cell or nucleus, and a particle that can prevent adhesion. In some embodiments, a particle comprising more than one particle can comprise a particle that can couple with a biological particle, e.g., a cell or a nucleus, and a particle that can be useful in identifying the biological particle. In some embodiments, a particle comprising more than one particle can comprise a particle that can couple with a biological particle, e.g., a cell or a nucleus, and a particle that can be useful in performing an assay. In some embodiments,

a particle comprising more than one particle can comprise a particle that can prevent adhesion and a particle that can be useful in identifying the biological particle. In some embodiments, a particle comprising more than one particle can comprise a particle that can prevent adhesion and a particle that can be useful in performing an assay. In some embodiments, a particle comprising more than one particle can comprise a particle that can be useful in identifying the biological particle, e.g., the cell or nucleus, and a particle that can be useful in performing an assay. In some embodiments, a particle comprising more than one particle can comprise a particle that can couple with a biological particle, e.g., a cell or nucleus, a particle that can prevent adhesion, and a particle that can be useful in identifying the biological particle. In some embodiments, a particle comprising more than one particle can comprise a particle that can couple with a biological particle, e.g., a cell or a nucleus, a particle that can prevent adhesion, and a particle that can be useful in performing an assay. In some embodiments, a particle comprising more than one particle can comprise a particle that can couple with a biological particle, e.g., a cell or a nucleus, a particle that can prevent adhesion, a particle that can be useful in identifying the biological particle, e.g., the cell or nucleus, and a particle that can be useful in performing an assay.

[0070] A plurality of particles can comprise at least 10 particles, at least 100 particles, at least 1,000 particles, at least 10,000 particles, at least 100,000 particles, or at least 1,000,000 particles. In some embodiments, a plurality of particles can comprise no more than 10 particles, no more than 100 particles, no more than 1,000 particles, no more than 10,000 particles, no more than 100,000 particles, or no more than 1,000,000 particles. In some embodiments, a plurality of particles can comprise about 10 particles, about 100 particles, about 1,000 particles, about 10,000 particles, about 100,000 particles, about 1,000,000 particles, or a range between any two foregoing values.

[0071] A plurality of positively charged particles can comprise at least 10 positively charged particles, at least 100 positively charged particles, at least 1,000 positively charged particles, at least 10,000 positively charged particles, at least 100,000 positively charged particles, or at least 1,000,000 positively charged particles. In some embodiments, a plurality of particles can comprise no more than 10 positively charged particles, no more than 100 positively charged particles, no more than 1,000 positively charged particles, no more than 10,000 positively charged particles, no more than 100,000 positively charged particles, or no more than 1,000,000 positively charged particles. In some embodiments, a plurality of particles can comprise about 10 positively charged particles, about 100 positively charged particles, about 1,000 positively charged particles, about 10,000 positively charged particles, about 100,000 positively charged

particles, about 1,000,000 positively charged particles, or a range between any two foregoing values.

[0072] In some embodiments, a plurality of positively charged particles can comprise about the same number of positively charged particles as there are biological particles, e.g., cells or nuclei, in the plurality of biological particles, e.g., cells or nuclei. In some embodiments, a plurality of positively charged particles can comprise more particles than there are cells in the plurality of biological particles. In some embodiments, for example, a biological particle, e.g., a cell or nucleus, to positively charged particle ratio can be at least 1:1, at least 1:2, at least 1:5, at least 1:10, at least 1:100, at least 1:1,000, at least 1:10,000, or at least 1:100,000. In some embodiments, a biological particle, e.g., a cell or nucleus, to positively charged particle ratio can be no more than 1:1, no more than 1:2, no more than 1:5, no more than 1:10, no more than 1:100, no more than 1:1,000, no more than 1:10,000, or no more than 1:100,000. In some embodiments, a cell to positively charged particle ratio can be about 1:1, about 1:2, about 1:5, about 1:10, about 1:100, about 1:1,000, about 1:10,000, about 1:100,000, or a range between any two foregoing values. For example, the number of particles of the plurality of positively charged particles can be at least 2 times a number of biological particles, e.g., cells or nuclei, of the plurality of biological particles, e.g., cells or nuclei, at least 10 times the number of cells of the plurality of biological particles, or at least 100 times the number of biological particles, in the plurality of biological particles, e.g., cells or nuclei.

[0073] In some embodiments, the plurality of positively charged particles comprises a plurality of beads. In some embodiments, the plurality of positively charged particles can comprise at least 2, at least 10, at least 100, at least 1,000, at least 10,000, or at least 100,000 beads. In some embodiments, the plurality of positively charged particles can comprise no more than 2, no more than 10, no more than 100, no more than 1,000, no more than 10,000, or no more than 100,000 beads. In some embodiments, the plurality of positively charged particles can comprise about 2, about 10, about 100, about 1,000, about 10,000, or about 100,000 beads, or a range between any two foregoing values.

[0074] In some embodiments, the plurality of charged particles can be homogenous. In some embodiments, the plurality of charged particles can be heterogeneous. For example, the plurality of charged particles can comprise particles having different charges or particles having additional properties that are different (e.g., size, magnetic property, oligonucleotide associated with the charged particle, or protein associated with the charged particle).

[0075] In some embodiments, a positively charged particle can comprise a property of any particle provided herein.

[0076] In some embodiments, the plurality of biological particles, e.g., cells or nuclei can comprise single biological particle, e.g., single cells or nuclei. For example, a plurality of cells can comprise cells that are not adhered to one another, or a plurality of cells to be un-adhered from one another. In some embodiments, a plurality of cells can comprise cultured cells. Cultured cells can comprise cells that do not adhere to one another, or cells that adhere to one another. Cultured cells can comprise for example hepatocytes, red blood cells, white blood cells (e.g., T cells, B cells, or lymphocytes), epithelial cells, cancer cells, or other types of cells. In some embodiments, cultured cells can comprise a HepG2 cell.

[0077] In some embodiments, a plurality of biological particles, e.g., cells or nuclei can comprise harvested biological particles, e.g., harvested cells or nuclei. Harvested biological particles, e.g., cells or nuclei, can comprise biological particles, e.g., cells or nuclei, harvested from a tissue, organ, fluid, or other biological component of a biological subject. For example, harvested biological particles, e.g., cells or nuclei, can comprise biological particle harvested from a mammal. In some embodiments, harvested biological particles, e.g., cells or nuclei, can comprise biological particles harvested from a human, a monkey, a mouse, a rat, a rabbit, a guinea pig, a dog, a cat, or another acceptable subject.

[0078] Cells, such as cultured or harvested cells, can be a heterogeneous plurality of cells or a homogenous plurality of cells. In some embodiments, a heterogeneous plurality of cells can comprise 1, 2, 3, 4, 5, or more cell types.

[0079] A plurality of cells can comprise at least 2 cells. In some embodiments, a plurality of cells can comprise at least 10, at least 100, at least 1,000, at least 10,000, or at least 100,000 cells. In some embodiments, a plurality of cells can comprise no more than 10, no more than 100, no more than 1,000, no more than 10,000, or no more than 100,000 cells. In some embodiments, a plurality of cells can comprise about 10, about 100, about 1,000, about 10,000, or about 100,000 cells, or a range between any two foregoing values.

[0080] In some embodiments, at least two particles of the plurality of particles are coupled to a biological particle, e.g., a cell or nucleus, of the plurality of biological particles, e.g., cells or nuclei. In some embodiments, more than two particles of the plurality of particles can be coupled to a biological particle, e.g., cell or nucleus, of the plurality of biological particles, e.g., cells or nuclei. In some cases, for example, the at least two particles can surround the biological particle, e.g., cell or nucleus.

[0081] In some embodiments, a plurality of particles can comprise about the same number of particles as there are biological particles, e.g., cells or nuclei in the plurality of biological particles, e.g., cells or nuclei. In some embodiments, a plurality of particles can comprise more

particles than there are biological particles, e.g., cells or nuclei in the plurality of biological particles, e.g., cells or nuclei. In some embodiments, for example, a biological particle, e.g., cell or nucleus, to particle ratio can be at least 1:1, at least 1:2, at least 1:5, at least 1:10, at least 1:100, at least 1:1,000, at least 1:10,000, or at least 1:100,000. In some embodiments, a biological particle, e.g., cell or nucleus, to particle ratio can be no more than 1:1, no more than 1:2, no more than 1:5, no more than 1:10, no more than 1:100, no more than 1:1,000, no more than 1:10,000, or no more than 1:100,000. In some embodiments, a biological particle, e.g., cell or nucleus, to particle ratio can be about 1:1, about 1:2, about 1:5, about 1:10, about 1:100, about 1:1,000, about 1:10,000, about 1:100,000, or a range between any two foregoing values. For example, the number of particles of the plurality of particles can be at least 2 times a number of biological particles, e.g., cells or nuclei, of the plurality of biological particles, e.g., cells or nuclei, at least 10 times the number of biological particles, e.g., cells or nuclei, of the plurality of biological particles, e.g., cells or nuclei, or at least 100 times the number of cells in the plurality of biological particles, e.g., cells or nuclei.

[0082] In some embodiments, the method can further comprise freezing the biological particle, e.g., cell or nucleus. For example, the biological particle, e.g., cell or nucleus, can be frozen at less than 0 °C, less than -10 °C, less than -20 °C, less than -40 °C, less than -60 °C, less than -80 °C, or less than -100 °C. In some embodiments, the biological particle, e.g., cell or nucleus can be flash frozen. In some embodiments, the biological particle, e.g., cell or nucleus, can be frozen for at least 1 minute, at least 5 minutes, at least 10 minutes, at least 10 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 6 hours, at least 12 hours, at least 1 day, or at least 1 week. In some embodiments, after freezing, the biological particle, e.g., cell or nucleus, can be thawed. In some embodiments, upon thawing, the biological particle, e.g., cell or nucleus, is not adhered to another biological particle, e.g., cell or nucleus, in the plurality of biological particles, e.g., cells or nuclei.

[0083] In some embodiments, the method further comprises processing the plurality of biological particles, e.g., cells or nuclei. Processing can comprise performing an assay on the biological particles, e.g., cells or nuclei, culturing the biological particles, e.g., cells, differentiating the biological particles, e.g., cells, identifying the biological particles, e.g., cells or nuclei, identifying a property of the biological particles, e.g., cells or nuclei, sorting the biological particles, e.g., cells or nuclei, or otherwise processing the biological particles, e.g., cells or nuclei. In some embodiments, processing can comprise fluorescence-activated cell sorting (FACS). In some embodiments, the processing can be improved or aided by the presence of the particle on the biological particle, e.g., cell or nucleus. For example, the processing can be

improved or aided by the prevention of cell adhesion, or by another property of the particle, such as a property that can aid in separation, isolation, or identification of the particle, the biological particle, e.g., cell or nucleus, or a component thereof.

[0084] In some embodiments, the method can further comprise partitioning the particle coupled to the biological particle, e.g., cell or nucleus, in a partition. Partitioning can be performed using any acceptable method, including those provided herein. A partition can be any acceptable partition, including those provided herein, such as a bead, a droplet, a well, or another partition type.

[0085] In some embodiments, the method can further comprise co-partitioning the particle coupled to the biological particle, e.g., the cell or nucleus, to a same partition as a nucleic acid barcode molecule. In some embodiments, for example, this can be accomplished using an oligonucleotide coupled to the biological particle, e.g., cell or nucleus, or to the particle. In some embodiments, this can be accomplished independent of the particle or a property of the biological particle, e.g., cell or nucleus.

[0086] In some embodiments, the nucleic acid barcode molecule can be coupled to a nucleic acid molecule of the biological particle, e.g., cell or nucleus. For example, the nucleic acid barcode molecule can be coupled to a ribonucleic acid (RNA) molecule or a deoxyribonucleic acid (DNA) molecule of the biological particle, e.g., cell or nucleus. In some embodiments, the nucleic acid barcode molecule can be coupled to an RNA molecule coding for a protein that is expressed in the biological particle, e.g., cell or nucleus.

[0087] In some embodiments, a partition can comprise no more than one biological particle, e.g., cell or nucleus. In some embodiments, a partition can comprise more than one biological particle, e.g., cell or nucleus. In some embodiments, a partition can comprise at least 2, at least 5, at least 10, at least 50, or at least 100 biological particles, e.g., cells or nuclei. In some embodiments, a partition can comprise not more than 2, not more than 5, not more than 10, not more than 50, or not more than 100 biological particles, e.g., cells or nuclei. In some embodiments, a partition can comprise about 1, about 2, about 5, about 5 about 50, or about 100 cells, or a range between any two foregoing values.

[0088] In some embodiments, the method can further comprise pooling the partition with a partition comprising a second biological particle, e.g., cell or nucleus, from another plurality of biological particles, e.g., cells or nuclei. In some cases, the second biological particle, e.g., cell or nucleus can be a same biological particle, e.g., cell or nucleus, type as the biological particle, e.g., cell or nucleus, in the partition. In some cases, the second biological particle, e.g., cell or

nucleus, can be a different biological particle, e.g., cell or nucleus, type as the biological particle, e.g., cell or nucleus, in the partition.

[0089] In some embodiments, such as when a method includes isolating a particle, for example based at least in part on a density of the particle coupled to the biological particle, e.g., cell or nucleus, the method can further comprise sedimentation. In some embodiments, the isolating can comprise differential sedimentation. In some embodiments, the isolating can comprise collecting a layer comprising the biological particles, e.g., cells or nuclei. Such collection can be performed, for example, after sedimentation or after differential sedimentation. In some embodiments, the layer can be a liquid layer.

Systems and methods for sample compartmentalization

[0090] In an aspect, the systems and methods described herein provide for the compartmentalization, depositing, or partitioning of one or more particles (e.g., biological particles, macromolecular constituents of biological particles, 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.

[0091] 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 biological particles 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. Alternatively, a partition may be unoccupied. For example, a partition may not comprise a bead. A cell bead can be a biological particle 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 biological particle 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. Alternative mechanisms may also be employed in the partitioning of individual biological particles, including porous membranes through which aqueous mixtures of cells are extruded into non-aqueous fluids.

[0092] 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.

[0093] In some instances, a droplet is 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, a droplet may be formed by exposing a mixture to ultrasound or sonication. For example, to partition contents into droplets, a mixture comprising a first fluid, a second fluid, optionally a surfactant, and the contents can be subject to such agitation techniques to generate a plurality of droplets (first fluid-in-second fluid or second fluid-in-first fluid) comprising the contents, or subsets thereof. In an example, a mixture comprises beads. Upon agitation, the beads in the mixture may limit droplet break-up into droplets smaller than the size of the beads, and a substantially monodisperse population of droplets comprising the beads may result.

[0094] In the case of droplets in an emulsion, allocating individual particles to discrete partitions may in one non-limiting 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 at the junction of the two streams (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 biological particles 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 biological particle partitions, the relative flow rates of the immiscible fluids can be selected such that, on average, the partitions may contain less than one biological particle 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 biological particle (e.g., bead, DNA, cell or cellular material). In some embodiments, 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 only 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.

[0095] **FIG. 1** shows an example of a microfluidic channel structure **100** for partitioning individual biological particles. 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 biological particles (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 an individual biological particle **114** (such as droplets **118**). A discrete droplet generated may include more than one individual biological particle **114** (not shown in **FIG. 1**). A discrete droplet may contain no biological particle **114** (such as droplet **120**). Each discrete partition may maintain separation of its own contents (e.g., individual biological particle **114**) from the contents of other partitions.

[0096] 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.

[0097] 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., biological particles, 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.

[0098] The generated droplets may comprise two subsets of droplets: (1) occupied droplets **118**, containing one or more biological particles **114**, and (2) unoccupied droplets **120**, not containing any biological particles **114**. Occupied droplets **118** may comprise singly occupied droplets (having one biological particle) and multiply occupied droplets (having more than one biological particle). As described elsewhere herein, in some cases, the majority of occupied partitions can include no more than one biological particle per occupied partition and some of the generated partitions can be unoccupied (of any biological particle). In some cases, though, some of the occupied partitions may include more than one biological particle. In some cases, the partitioning process may be controlled such that fewer than about 25% of the occupied partitions contain more than one biological particle, and in many cases, fewer than about 20% of the occupied partitions have more than one biological particle, while in some cases, fewer than about 10% or even fewer than about 5% of the occupied partitions include more than one biological particle per partition.

[0099] In some cases, it may be desirable 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 biological particles (e.g., biological particles **114**) at the partitioning junction **110**, such as to ensure that at least one biological particle is encapsulated in a partition, the Poissonian distribution may expectedly increase the number of partitions that include multiple biological particles. 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.

[00100] In some cases, the flow of one or more of the biological particles (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.

[00101] As will be appreciated, the above-described occupancy rates are also applicable to partitions that include both biological particles 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 a biological particle.

[00102] In another aspect, in addition to or as an alternative to droplet-based partitioning, biological particles may be encapsulated within a microcapsule that comprises an outer shell, layer or porous matrix in which is entrained one or more individual biological particles or small groups of biological particles. The microcapsule may include other reagents. Encapsulation of biological particles may be performed by a variety of processes. Such processes may combine an aqueous fluid containing the biological particles 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.

[00103] Preparation of microcapsules comprising biological particles 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 biological particles or small groups of biological particles. Likewise, membrane-based encapsulation systems may be used to generate microcapsules comprising encapsulated biological particles 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 biological particles **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 biological particles. 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.

[00104] 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.

[00105] 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.).

[00106] In some cases, encapsulated biological particles 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 biological particles (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.

[00107] The biological particle 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 biological particle. 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 biological particle. In this manner, the polymer or gel may act to allow the biological particle 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.

[00108] 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.

[00109] 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 biological particle may be surrounded by polyacrylamide strands linked together by disulfide bridges. In this manner, the biological particle may be encased inside of or comprise a gel or matrix (e.g., polymer matrix) to form a "cell bead."

[00110] A cell bead can contain biological particles (e.g., a cell) or macromolecular constituents (e.g., RNA, DNA, proteins, etc.) of biological particles. 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 biological particles and cell beads (and/or droplets or other partitions) containing macromolecular constituents of biological particles. Cell beads may be or include a cell, cell derivative, cellular material and/or material derived from the cell in, within, or encased in a matrix, such as a polymeric matrix. In some cases, a cell bead may comprise a live cell. In some instances, the live cell may be capable of being cultured when enclosed in a gel or polymer matrix, or of being cultured when comprising a gel or polymer matrix. In some instances, the polymer or gel may be diffusively permeable to certain components and diffusively impermeable to other components (e.g., macromolecular constituents).

[00111] Encapsulated biological particles can provide certain potential advantages of being more storable and more portable than droplet-based partitioned biological particles. Furthermore, in some cases, it may be desirable to allow biological particles to incubate for a select period of time before analysis, such as in order to characterize changes in such biological particles over time, either in the presence or absence of different stimuli (or reagents). In such cases, encapsulation may allow for longer incubation than partitioning in emulsion droplets, although in some cases, droplet partitioned biological particles 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 biological particles into which other reagents are co-partitioned. Alternatively, or in addition, encapsulated biological particles may be readily deposited into other partitions (e.g., droplets) as described above.

Beads

[00112] 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.

[00113] 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.

[00114] 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.

[00115] 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 biological particle. 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 biological particle 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.

[00116] 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 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.

[00117] **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 biological particles **216** along the channel segment **202** into junction **210**. The plurality of biological particles **216** may be sourced from a suspension of biological particles. For example, the channel segment **202** may be connected to a reservoir comprising an aqueous suspension of biological particles **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.

[00118] As an alternative, the channel segments **201** and **202** may meet at another junction upstream of the junction **210**. At such junction, beads and biological particles 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 biological particles in an alternating fashion, such that, for example, a droplet comprises a single bead and a single biological particle.

[00119] Beads, biological particles 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 biological particle. Such regular flow profiles may permit the droplets to have an occupancy (e.g., droplets having beads and biological particles) 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.

[00120] 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**.

[00121] A discrete droplet that is generated may include an individual biological particle **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 biological particle and a barcode carrying bead, such as droplets **220**. In some instances, a discrete droplet may include more than one individual biological particle or no biological particle. 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 biological particles).

[00122] Beneficially, a discrete droplet partitioning a biological particle and a barcode carrying bead may effectively allow the attribution of the barcode to macromolecular constituents of the biological particle within the partition. The contents of a partition may remain discrete from the contents of other partitions.

[00123] 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, electrokinetic pumping, vacuum, capillary or gravity flow, or the like.

[00124] 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.

[00125] 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.

[00126] 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), 5μm, 10μm, 20μm, 30μm, 40μm, 50μm, 60μm, 70μm, 80μm, 90μm, 100μm, 250μm, 500μm, 1mm, or greater. In some cases, a bead may have a diameter of less than about 10 nm, 100 nm, 500 nm, 1μm, 5μm, 10μm, 20μm, 30μm, 40μm, 50μm, 60μm, 70μm, 80μm, 90μm, 100μm, 250μm, 500μm, 1mm, or less. In some cases, a bead may have a diameter in the range of about 40-75μm, 30-75μm, 20-75μm, 40-85μm, 40-95μm, 20-100μm, 10-100μm, 1-100μm, 20-250μm, or 20-500μm.

[00127] In certain aspects, beads can be provided as a population or plurality of beads having a relatively monodisperse size distribution. Where it may be desirable to provide relatively consistent amounts of reagents within partitions, maintaining relatively consistent bead characteristics, such as size, can contribute 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.

[00128] 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.

[00129] 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.

[00130] 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.

[00131] 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.

[00132] 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.

[00133] In some cases, a bead may comprise an acrydite moiety, which in certain aspects 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.

[00134] 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.

[00135] 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, etc.) 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.

[00136] 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.

[00137] In some cases, the nucleic acid molecule can comprise one or more functional sequences. For example, a functional sequence can comprise a sequence for attachment to a sequencing flow cell, such as, for example, a P5 sequence 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 for attachment to a sequencing flow cell for Illumina sequencing. In some cases, the functional sequence can comprise a barcode sequence or multiple barcode sequences. In some cases, the functional sequence can comprise a unique molecular identifier (UMI). In some cases, the functional sequence can comprise a primer sequence (e.g., an R1 primer sequence for Illumina sequencing, an R2 primer sequence for Illumina sequencing, etc.). In some cases, a functional sequence can comprise a partial sequence, such as a partial barcode sequence, partial anchoring sequence, partial sequencing primer sequence (e.g., partial R1 sequence, partial R2 sequence, etc.), a partial sequence configured to attach to the flow cell of a sequencer (e.g., partial P5 sequence, partial P7 sequence, etc.), or a partial sequence of any other type of sequence described elsewhere herein. A partial sequence may contain a contiguous or

continuous portion or segment, but not all, of a full sequence, for example. In some cases, a downstream procedure may extend the partial sequence, or derivative thereof, to achieve a full sequence of the partial sequence, or derivative thereof.

[00138] 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.

[00139] **FIG. 4** illustrates an example of a barcode carrying bead. A nucleic acid molecule **402**, such as an oligonucleotide, can be coupled to a bead **404** by a releasable linkage **406**, such as, for example, a disulfide linker. The same bead **404** may be coupled (e.g., via releasable linkage) to one or more other nucleic acid molecules **418, 420**. The nucleic acid molecule **402** 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 **402** may comprise a functional sequence **408** that may be used in subsequent processing. For example, the functional sequence **408** 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), or partial sequence(s) thereof. The nucleic acid molecule **402** may comprise a barcode sequence **410** for use in barcoding the sample (e.g., DNA, RNA, protein, etc.). In some cases, the barcode sequence **410** can be bead-specific such that the barcode sequence **410** is common to all nucleic acid molecules (e.g., including nucleic acid molecule **402**) coupled to the same bead **404**. Alternatively or in addition, the barcode sequence **410** can be partition-specific such that the barcode sequence **410** is common to all nucleic acid molecules coupled to one or more beads that are partitioned into the same partition. The nucleic acid molecule **402** may comprise a specific priming sequence **412**, 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 **402** may comprise an anchoring sequence **414** to ensure that the specific priming sequence **412** hybridizes at the sequence end (e.g., of the mRNA). For example, the anchoring sequence **414** 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.

[00140] The nucleic acid molecule **402** may comprise a unique molecular identifying sequence **416** (e.g., unique molecular identifier (UMI)). In some cases, the unique molecular identifying sequence **416** may comprise from about 5 to about 8 nucleotides. Alternatively, the

unique molecular identifying sequence **416** may compress less than about 5 or more than about 8 nucleotides. The unique molecular identifying sequence **416** may be a unique sequence that varies across individual nucleic acid molecules (e.g., **402, 418, 420**, etc.) coupled to a single bead (e.g., bead **404**). In some cases, the unique molecular identifying sequence **416** 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. 4** shows three nucleic acid molecules **402, 418, 420** coupled to the surface of the bead **404**, 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., **408**, **410, 412**, etc.) and variable or unique sequence segments (e.g., **416**) between different individual nucleic acid molecules coupled to the same bead.

[00141] In operation, a biological particle (e.g., cell, DNA, RNA, etc.) can be co-partitioned along with a barcode bearing bead **404**. The barcoded nucleic acid molecules **402, 418, 420** can be released from the bead **404** in the partition. By way of example, in the context of analyzing sample RNA, the poly-T segment (e.g., **412**) of one of the released nucleic acid molecules (e.g., **402**) 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 **408, 410, 416** of the nucleic acid molecule **402**. Because the nucleic acid molecule **402** comprises an anchoring sequence **414**, 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 **410**. However, the transcripts made from the different mRNA molecules within a given partition may vary at the unique molecular identifying sequence **412** 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 biological particle (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.

[00142] In some instances, a bead may comprise a capture sequence or binding sequence configured to bind to a corresponding capture sequence or binding sequence. In some instances, a bead may comprise a plurality of different capture sequences or binding sequences configured to bind to different respective corresponding capture sequences or binding sequences. For example, a bead may comprise a first subset of one or more capture sequences each configured to bind to a first corresponding capture sequence, a second subset of one or more capture sequences each configured to bind to a second corresponding capture sequence, a third subset of one or more capture sequences each configured to bind to a third corresponding capture sequence, and etc. A bead may comprise any number of different capture sequences. In some instances, a bead may comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more different capture sequences or binding sequences configured to bind to different respective capture sequences or binding sequences, respectively. Alternatively or in addition, a bead may comprise at most about 10, 9, 8, 7, 6, 5, 4, 3, or 2 different capture sequences or binding sequences configured to bind to different respective capture sequences or binding sequences. In some instances, the different capture sequences or binding sequences may be configured to facilitate analysis of a same type of analyte. In some instances, the different capture sequences or binding sequences may be configured to facilitate analysis of different types of analytes (with the same bead). The capture sequence may be designed to attach to a corresponding capture sequence. Beneficially, such corresponding capture sequence may be introduced to, or otherwise induced in, a biological particle (e.g., cell, cell bead, etc.) for performing different assays in various formats (e.g., barcoded antibodies comprising the corresponding capture sequence, barcoded MHC dextramers comprising the corresponding capture sequence, barcoded guide RNA molecules comprising the corresponding capture sequence, etc.), such that the corresponding capture sequence may later interact with the capture sequence associated with the bead. In some instances, a capture sequence coupled to a bead (or other support) may be configured to attach to a linker molecule, such as a splint molecule, wherein the linker molecule is configured to couple the bead (or other support) to other molecules through the linker molecule, such as to one or more analytes or one or more other linker molecules.

[00143] **FIG. 5** illustrates another example of a barcode carrying bead. A nucleic acid molecule **505**, such as an oligonucleotide, can be coupled to a bead **504** by a releasable linkage **506**, such as, for example, a disulfide linker. The nucleic acid molecule **505** may comprise a first

capture sequence **560**. The same bead **504** may be coupled (e.g., via releasable linkage) to one or more other nucleic acid molecules **503, 507** comprising other capture sequences. The nucleic acid molecule **505** may be or comprise a barcode. As noted elsewhere herein, the structure of the barcode may comprise a number of sequence elements, such as a functional sequence **508** (e.g., flow cell attachment sequence, sequencing primer sequence, etc.), a barcode sequence **510** (e.g., bead-specific sequence common to bead, partition-specific sequence common to partition, etc.), and a unique molecular identifier **512** (e.g., unique sequence within different molecules attached to the bead), or partial sequences thereof. The capture sequence **560** may be configured to attach to a corresponding capture sequence **565**. In some instances, the corresponding capture sequence **565** may be coupled to another molecule that may be an analyte or an intermediary carrier. For example, as illustrated in **FIG. 5**, the corresponding capture sequence **565** is coupled to a guide RNA molecule **562** comprising a target sequence **564**, wherein the target sequence **564** is configured to attach to the analyte. Another oligonucleotide molecule **507** attached to the bead **504** comprises a second capture sequence **580** which is configured to attach to a second corresponding capture sequence **585**. As illustrated in **FIG. 5**, the second corresponding capture sequence **585** is coupled to an antibody **582**. In some cases, the antibody **582** may have binding specificity to an analyte (e.g., surface protein). Alternatively, the antibody **582** may not have binding specificity. Another oligonucleotide molecule **503** attached to the bead **504** comprises a third capture sequence **570** which is configured to attach to a second corresponding capture sequence **575**. As illustrated in **FIG. 5**, the third corresponding capture sequence **575** is coupled to a molecule **572**. The molecule **572** may or may not be configured to target an analyte. The other oligonucleotide molecules **503**, **507** may comprise the other sequences (e.g., functional sequence, barcode sequence, UMI, etc.) described with respect to oligonucleotide molecule **505**. While a single oligonucleotide molecule comprising each capture sequence is illustrated in **FIG. 5**, it will be appreciated that, for each capture sequence, the bead may comprise a set of one or more oligonucleotide molecules each comprising the capture sequence. For example, the bead may comprise any number of sets of one or more different capture sequences. Alternatively, or in addition, the bead **504** may comprise other capture sequences. Alternatively, or in addition, the bead **504** may comprise fewer types of capture sequences (e.g., two capture sequences). Alternatively, or in addition, the bead **504** may comprise oligonucleotide molecule(s) comprising a priming sequence, such as a specific priming sequence such as an mRNA specific priming sequence (e.g., poly-T sequence), a targeted priming sequence, and/or a random priming sequence, for example, to facilitate an assay for gene expression.

[00144] In operation, the barcoded oligonucleotides may be released (e.g., in a partition), as described elsewhere herein. Alternatively, the nucleic acid molecules bound to the bead (e.g., gel bead) may be used to hybridize and capture analytes (e.g., one or more types of analytes) on the solid phase of the bead.

[00145] 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.

[00146] 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-ethylmalieamide or iodoacetate.

[00147] Activation of disulfide linkages within a bead can be controlled such that only 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.

[00148] 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.

[00149] 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.

[00150] 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 non-limiting 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.

[00151] 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.

[00152] 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.

[00153] 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.

[00154] 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.

[00155] 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-swelling 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-swelling of the beads may be accomplished by various de-swelling 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.

[00156] 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.

[00157] 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.

[00158] In some cases, a species (e.g., oligonucleotide molecules comprising barcodes) that are attached to a solid support (e.g., a bead) may comprise a U-excising element that allows the species to release from the bead. In some cases, the U-excising element may comprise a single-stranded DNA (ssDNA) sequence that contains at least one uracil. The species may be attached to a solid support via the ssDNA sequence containing the at least one uracil. The species may be released by a combination of uracil-DNA glycosylase (e.g., to remove the uracil) and an endonuclease (e.g., to induce an ssDNA break). If the endonuclease generates a 5' phosphate group from the cleavage, then additional enzyme treatment may be included in downstream processing to eliminate the phosphate group, e.g., prior to ligation of additional sequencing handle elements, e.g., Illumina full P5 sequence, partial P5 sequence, full R1 sequence, and/or partial R1 sequence.

[00159] 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.

[00160] In addition to thermally cleavable bonds, disulfide bonds and UV sensitive bonds, other non-limiting 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.

[00161] 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, DNAase, 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.

[00162] 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 be broken and the bead 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.

[00163] 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 degrades and the barcode sequence is 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.

[00164] 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.

[00165] 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 will be desirable 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 desirable 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.

[00166] Numerous chemical triggers may be used to trigger the degradation of beads. Examples of these chemical changes may include, but are not 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.

[00167] In some embodiments, 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.

[00168] 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.

[00169] Any suitable agent may degrade beads. In some embodiments, changes in temperature or pH may be used to degrade thermo-sensitive or pH-sensitive bonds within beads. In some embodiments, 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 embodiments, 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.

[00170] 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.

[00171] Although **FIG. 1** and **FIG. 2** have been described in terms of providing substantially singly occupied partitions, above, in certain cases, it may be desirable 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 biological particle 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.

[00172] 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 biological particles (e.g., one biological particle and one bead per partition).

[00173] The partitions described herein may comprise small volumes, for example, less than about 10 microliters (μL), 5μL, 1μL, 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.

[00174] 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 biological particles 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.

[00175] 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

[00176] In accordance with certain aspects, biological particles may be partitioned along with lysis reagents in order to release the contents of the biological particles within the partition. In such cases, the lysis agents can be contacted with the biological particle suspension concurrently with, or immediately prior to, the introduction of the biological particles 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 accordance with other aspects, additionally or alternatively, biological particles may be partitioned along with other reagents, as will be described further below.

[00177] Beneficially, when lysis reagents and biological particles are co-partitioned, the lysis reagents can facilitate the release of the contents of the biological particles within the partition. The contents released in a partition may remain discrete from the contents of other partitions.

[00178] 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 structures may have other geometries and/or configurations. 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 biological particles 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.

[00179] 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. Other lysis agents may additionally or

alternatively be co-partitioned with the biological particles to cause the release of the biological particle's contents into the partitions. For example, in some cases, surfactant-based lysis solutions may be used to lyse cells, although these may be less desirable for emulsion based systems where the 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 biological particles 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.

[00180] Alternatively, or in addition to the lysis agents co-partitioned with the biological particles described above, other reagents can also be co-partitioned with the biological particles, including, for example, DNAase 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 biological particles (e.g., a cell or a nucleus in a polymer matrix), the biological particles may be exposed to an appropriate stimulus to release the biological particles or their contents from a co-partitioned microcapsule. For example, in some cases, a chemical stimulus may be co-partitioned along with an encapsulated biological particle 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 biological particle 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.

[00181] Additional reagents may also be co-partitioned with the biological particles, such as endonucleases to fragment a biological particle's DNA, DNA polymerase enzymes and dNTPs used to amplify the biological particle'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, DNAase, 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.

[00182] 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.

[00183] 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.

[00184] Once the contents of the cells are released into their respective partitions, the macromolecular components (e.g., macromolecular constituents of biological particles, 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 biological particles 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 biological particle or particles. The ability to attribute characteristics to individual biological particles or groups of biological particles is provided by the assignment of unique identifiers specifically to an individual biological particle or groups of biological particles. Unique identifiers, e.g., in the form of nucleic acid barcodes can be assigned or associated with individual biological particles or populations of biological particles, in order to tag or label the biological particle's macromolecular components (and as a result, its characteristics) with the unique identifiers. These unique identifiers can then be used to attribute the biological particle's components and characteristics to an individual biological particle or group of biological particles.

[00185] In some aspects, this is performed by co-partitioning the individual biological particle or groups of biological particles with the unique identifiers, such as described above

(with reference to **FIGS. 1-3**). In some aspects, 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 biological particle, or to other components of the biological particle, 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 aspects, 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.

[00186] 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.

[00187] The co-partitioned nucleic acid molecules can also comprise other functional sequences useful in the processing of the nucleic acids from the co-partitioned biological particles. These sequences include, e.g., targeted or random/universal amplification primer sequences for amplifying nucleic acids (e.g., mRNA, the genomic DNA) from the individual biological particles 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.

[00188] 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 embodiments, 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.

[00189] 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.

[00190] In some cases, it may be desirable to incorporate multiple different barcodes within a given partition, either attached to a single or 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.

[00191] The nucleic acid molecules (e.g., oligonucleotides) are releasable from the beads upon the application of a particular 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 biological particles, and may be degraded for release of the attached nucleic acid molecules through exposure to a reducing agent, such as DTT.

[00192] In some aspects, 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.

[00193] **FIG. 3** shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets. A channel structure **300** can include a channel segment **302** communicating at a channel junction **306** (or intersection) with a reservoir **304**. The reservoir **304** can be a chamber. Any reference to "reservoir," as used herein, can also refer to a "chamber." In operation, an aqueous fluid **308** that includes suspended beads **312** may be transported along the channel segment **302** into the junction **306** to meet a second fluid **310** that is immiscible with the aqueous fluid **308** in the reservoir **304** to create droplets **316, 318** of the aqueous fluid **308** flowing into the reservoir **304**. At the junction **306** where the aqueous fluid **308** and the second fluid **310** meet, droplets can form based on factors such as the hydrodynamic forces at the junction **306**, flow rates of the two fluids **308, 310**, fluid properties, and certain geometric parameters (e.g., w, h_0, α, etc.) of the channel structure **300**. A plurality of droplets can be collected in the reservoir **304** by continuously injecting the aqueous fluid **308** from the channel segment **302** through the junction **306**.

[00194] A discrete droplet generated may include a bead (e.g., as in occupied droplets **316**). 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 **318**). In some instances, a discrete droplet generated may contain one or more biological particles, as described elsewhere herein. In some instances, a discrete droplet generated may comprise one or more reagents, as described elsewhere herein.

[00195] In some instances, the aqueous fluid **308** can have a substantially uniform concentration or frequency of beads **312**. The beads **312** can be introduced into the channel segment **302** from a separate channel (not shown in **FIG. 3**). The frequency of beads **312** in the channel segment **302** may be controlled by controlling the frequency in which the beads **312** are introduced into the channel segment **302** and/or the relative flow rates of the fluids in the channel segment **302** and the separate channel. In some instances, the beads can be introduced into the channel segment **302** from a plurality of different channels, and the frequency controlled accordingly.

[00196] In some instances, the aqueous fluid **308** in the channel segment **302** can comprise biological particles (e.g., described with reference to **FIGS. 1** and **2**). In some instances, the aqueous fluid **308** can have a substantially uniform concentration or frequency of biological particles. As with the beads, the biological particles can be introduced into the channel segment **302** from a separate channel. The frequency or concentration of the biological particles in the aqueous fluid **308** in the channel segment **302** may be controlled by controlling the frequency in which the biological particles are introduced into the channel segment **302**

and/or the relative flow rates of the fluids in the channel segment **302** and the separate channel. In some instances, the biological particles can be introduced into the channel segment **302** 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 biological particles into the channel segment **302**. The first separate channel introducing the beads may be upstream or downstream of the second separate channel introducing the biological particles.

[00197] The second fluid **310** 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.

[00198] In some instances, the second fluid **310** may not be subjected to and/or directed to any flow in or out of the reservoir **304**. For example, the second fluid **310** may be substantially stationary in the reservoir **304**. In some instances, the second fluid **310** may be subjected to flow within the reservoir **304**, but not in or out of the reservoir **304**, such as via application of pressure to the reservoir **304** and/or as affected by the incoming flow of the aqueous fluid **308** at the junction **306**. Alternatively, the second fluid **310** may be subjected and/or directed to flow in or out of the reservoir **304**. For example, the reservoir **304** can be a channel directing the second fluid **310** from upstream to downstream, transporting the generated droplets.

[00199] The channel structure **300** at or near the junction **306** may have certain geometric features that at least partly determine the sizes of the droplets formed by the channel structure **300**. The channel segment **302** can have a height, h_0 and width, w, at or near the junction **306**. By way of example, the channel segment **302** can comprise a rectangular cross-section that leads to a reservoir **304** having a wider cross-section (such as in width or diameter). Alternatively, the cross-section of the channel segment **302** 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 **304** at or near the junction **306** can be inclined at an expansion angle, α. The expansion angle, α, allows the tongue (portion of the aqueous fluid **308** leaving channel segment **302** at junction **306** and entering the reservoir **304** 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:

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

[00200] By way of example, for a channel structure with $w = 21$ μm, $h = 21$ μm, and $\alpha = 3°$, the predicted droplet size is 121 μm. In another example, for a channel structure with $w = 25$

μm, h = 25 μm, and α = 5°, the predicted droplet size is 123 μm. In another example, for a channel structure with w = 28 μm, h = 28 μm, and α = 7°, the predicted droplet size is 124 μm.

[00201] 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 **308** entering the junction **306** 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 **308** entering the junction **306** can be between about 0.01 microliters (μL)/minute (min) and about 100 μL/min. Alternatively, the flow rate of the aqueous fluid **308** entering the junction **306** can be less than about 0.01 μL/min. Alternatively, the flow rate of the aqueous fluid **308** entering the junction **306** 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 **308** entering the junction **306**.

[00202] 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.

[00203] The throughput of droplet generation can be increased by increasing the points of generation, such as increasing the number of junctions (e.g., junction **306**) between aqueous fluid **308** channel segments (e.g., channel segment **302**) and the reservoir **304**. Alternatively or in addition, the throughput of droplet generation can be increased by increasing the flow rate of the aqueous fluid **308** in the channel segment **302**.

[00204] 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.

[00205] A variety of applications require the evaluation of the presence and quantification of different biological particle or organism types within a population of biological particles, including, for example, microbiome analysis and characterization, environmental testing, food safety testing, epidemiological analysis, e.g., in tracing contamination or the like.

Well-based Analysis

[00206] As described herein, one or more processes can be performed in a partition, which can be a well. The well can be a well of a plurality of wells of a substrate, such as a microwell of a microwell array or plate, or the well can be a microwell or microchamber of a device (e.g., microfluidic device) comprising a substrate. The well can be a well of a well array or plate, or the well can be a well or chamber of a device (e.g., fluidic device). Accordingly, the wells or microwells can assume an "open" configuration, in which the wells or microwells are exposed to the environment (e.g., contain an open surface) and are accessible on one planar face of the substrate, or the wells or microwells can assume a "closed" or "sealed" configuration, in which the microwells are not accessible on a planar face of the substrate. In some instances, the wells or microwells can be configured to toggle between "open" and "closed" configurations. For instance, an "open" microwell or set of microwells can be "closed" or "sealed" using a membrane (e.g., semi-permeable membrane), an oil (e.g., fluorinated oil to cover an aqueous solution), or a lid, as described elsewhere herein. The wells or microwells can be initially provided in a "closed" or "sealed" configuration, wherein they are not accessible on a planar

surface of the substrate without an external force. For instance, the "closed" or "sealed" configuration can include a substrate such as a sealing film or foil that is puncturable or pierceable by pipette tip(s). Suitable materials for the substrate include, without limitation, polyester, polypropylene, polyethylene, vinyl, and aluminum foil.

[00207] In some embodiments, the well can have a volume of less than 1 milliliter (mL). For example, the well can be configured to hold a volume of at most 1000 microliters (μL), at most 100 μL, at most 10 μL, at most 1 μL, at most 100 nanoliters (nL), at most 10 nL, at most 1 nL, at most 100 picoliters (pL), at most 10 (pL), or less. The well can be configured to hold a volume of about 1000 μL, about 100 μL, about 10 μL, about 1 μL, about 100 nL, about 10 nL, about 1 nL, about 100 pL, about 10 pL, etc. The well can be configured to hold a volume of at least 10 pL, at least 100 pL, at least 1 nL, at least 10 nL, at least 100 nL, at least 1 μL, at least 10 μL, at least 100 μL, at least 1000 μL, or more. The well can be configured to hold a volume in a range of volumes listed herein, for example, from about 5 nL to about 20 nL, from about 1 nL to about 100 nL, from about 500 pL to about 100 μL, etc. The well can be of a plurality of wells that have varying volumes and can be configured to hold a volume appropriate to accommodate any of the partition volumes described herein.

[00208] In some instances, a microwell array or plate includes a single variety of microwells. In some instances, a microwell array or plate includes a variety of microwells. For instance, the microwell array or plate can include one or more types of microwells within a single microwell array or plate. The types of microwells can have different dimensions (e.g., length, width, diameter, depth, cross-sectional area, etc.), shapes (e.g., circular, triangular, square, rectangular, pentagonal, hexagonal, heptagonal, octagonal, nonagonal, decagonal, etc.), aspect ratios, or other physical characteristics. The microwell array or plate can include any number of different types of microwells. For example, the microwell array or plate can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more different types of microwells. A well can have any dimension (e.g., length, width, diameter, depth, cross-sectional area, volume, etc.), shape (e.g., circular, triangular, square, rectangular, pentagonal, hexagonal, heptagonal, octagonal, nonagonal, decagonal, other polygonal, etc.), aspect ratios, or other physical characteristics described herein with respect to any well.

[00209] In certain instances, the microwell array or plate includes different types of microwells that are located adjacent to one another within the array or plate. For example, a microwell with one set of dimensions can be located adjacent to and in contact with another microwell with a different set of dimensions. Similarly, microwells of different geometries can

be placed adjacent to or in contact with one another. The adjacent microwells can be configured to hold different articles; for example, one microwell can be used to contain a biological particle, such as a cell, a nucleus, or other sample (e.g., cellular components, nucleic acid molecules, etc.) while the adjacent microwell can be used to contain a support (e.g., a particle, including a bead such as a gel bead), droplet, or other reagent. In some cases, the adjacent microwells can be configured to merge the contents held within, e.g., upon application of a stimulus, or spontaneously, upon contact of the articles in each microwell.

[00210] As is described elsewhere herein, a plurality of partitions can be used in the systems, compositions, and methods described herein. For example, any suitable number of partitions (e.g., wells or droplets) can be generated or otherwise provided. For example, in the case when wells are used, at least about 1,000 wells, at least about 5,000 wells, at least about 10,000 wells, at least about 50,000 wells, at least about 100,000 wells, at least about 500,000 wells, at least about 1,000,000 wells, at least about 5,000,000 wells at least about 10,000,000 wells, at least about 50,000,000 wells, at least about 100,000,000 wells, at least about 500,000,000 wells, at least about 1,000,000,000 wells, or more wells can be generated or otherwise provided. Moreover, the plurality of wells can include both unoccupied wells (e.g., empty wells) and occupied wells.

[00211] A well can include any of the reagents described herein, or combinations thereof. These reagents can include, for example, barcode molecules, enzymes, adapters, and combinations thereof. The reagents can be physically separated from a biological particle (for example, a cell, a nucleus, or cellular components, e.g., proteins, nucleic acid molecules, etc.) that is placed in the well. This physical separation can be accomplished by containing the reagents within, or coupling to, a support (e.g., a bead such as a gel bead) that is placed within a well. The physical separation can also be accomplished by dispensing the reagents in the well and overlaying the reagents with a layer that is, for example, dissolvable, meltable, or permeable prior to introducing the polynucleotide sample into the well. This layer can be, for example, an oil, wax, membrane (e.g., semi-permeable membrane), or the like. The well can be sealed at any point, for example, after addition of the support or bead, after addition of the reagents, or after addition of either of these components. The sealing of the well can be useful for a variety of purposes, including preventing escape of beads or loaded reagents from the well, permitting select delivery of certain reagents (e.g., via the use of a semi-permeable membrane), for storage of the well prior to or following further processing, etc.

[00212] Once sealed, the well may be subjected to conditions for further processing of a cell (or cells) in the well. For instance, reagents in the well may allow further processing of the

cell, e.g., cell lysis, as further described herein. Alternatively, the well (or wells such as those of a well-based array) comprising the cell (or cells) may be subjected to freeze-thaw cycling to process the cell (or cells), e.g., cell lysis. The well containing the cell may be subjected to freezing temperatures (e.g., 0°C, below 0°C, -5°C, -10°C, -15°C, -20°C, -25°C, -30°C, -35°C, -40°C, -45°C, -50°C, -55°C, -60°C, -65°C, -70°C, -80°C, or -85°C). Freezing may be performed in a suitable manner, e.g., sub-zero freezer or a dry ice/ethanol bath. Following an initial freezing, the well (or wells) comprising the cell (or cells) may be subjected to freeze-thaw cycles to lyse the cell (or cells). In one embodiment, the initially frozen well (or wells) are thawed to a temperature above freezing (e.g., 4°C or above, 8°C or above, 12°C or above, 16°C or above, 20°C or above, room temperature, or 25°C or above). In another embodiment, the freezing is performed for less than 10 minutes (e.g., 5 minutes or 7 minutes) followed by thawing at room temperature for less than 10 minutes (e.g., 5 minutes or 7 minutes). This freeze-thaw cycle may be repeated a number of times, e.g., 2, 3, 4 or more times, to obtain lysis of the cell (or cells) in the well (or wells). In one embodiment, the freezing, thawing and/or freeze/thaw cycling is performed in the absence of a lysis buffer. Additional disclosure related to freeze-thaw cycling is provided in WO2019165181A1, which is incorporated herein by reference in its entirety.

[00213] A well can include free reagents and/or reagents encapsulated in, or otherwise coupled to or associated with, supports (e.g., beads), or droplets. In some embodiments, any of the reagents described in this disclosure can be encapsulated in, or otherwise coupled to, a support (e.g., a bead) or a droplet, with any chemicals, particles, and elements suitable for sample processing reactions involving biomolecules, such as, but not limited to, nucleic acid molecules and proteins. For example, a bead or droplet used in a sample preparation reaction for DNA sequencing can include one or more of the following reagents: enzymes, restriction enzymes (e.g., multiple cutters), ligase, polymerase, fluorophores, oligonucleotide barcodes, adapters, buffers, nucleotides (e.g., dNTPs, ddNTPs) and the like.

[00214] Additional examples of reagents include, but are not limited to: buffers, acidic solution, basic solution, temperature-sensitive enzymes, pH-sensitive enzymes, light-sensitive enzymes, metals, metal ions, magnesium chloride, sodium chloride, manganese, aqueous buffer, mild buffer, ionic buffer, inhibitor, enzyme, protein, polynucleotide, antibodies, saccharides, lipid, oil, salt, ion, detergents, ionic detergents, non-ionic detergents, oligonucleotides, nucleotides, deoxyribonucleotide triphosphates (dNTPs), dideoxyribonucleotide triphosphates (ddNTPs), DNA, RNA, peptide polynucleotides, complementary DNA (cDNA), double stranded DNA (dsDNA), single stranded DNA (ssDNA), plasmid DNA, cosmid DNA, chromosomal DNA, genomic DNA, viral DNA, bacterial DNA, mtDNA (mitochondrial DNA), mRNA, rRNA,

tRNA, nRNA, siRNA, snRNA, snoRNA, scaRNA, microRNA, dsRNA, ribozyme, riboswitch and viral RNA, polymerase, ligase, restriction enzymes, proteases, nucleases, protease inhibitors, nuclease inhibitors, chelating agents, reducing agents, oxidizing agents, fluorophores, probes, chromophores, dyes, organics, emulsifiers, surfactants, stabilizers, polymers, water, small molecules, pharmaceuticals, radioactive molecules, preservatives, antibiotics, aptamers, and pharmaceutical drug compounds. As described herein, one or more reagents in the well can be used to perform one or more reactions, including but not limited to: biological particle (e.g., a cell or a nucleus) processing such as lysis, fixation, permeabilization, nucleic acid reactions, e.g., nucleic acid extension reactions, amplification, reverse transcription, reactions, etc.

[00215] The wells disclosed herein can be provided as a part of a kit. For example, a kit can include instructions for use, a microwell array or device, and reagents (e.g., beads). The kit can include any useful reagents for performing the processes described herein, e.g., nucleic acid reactions, barcoding of nucleic acid molecules, sample processing (e.g., for biological particle lysis, fixation, and/or permeabilization).

[00216] In some cases, a well includes a support (e.g., a bead) or droplet that includes a set of reagents that has a similar attribute, for example, a set of enzymes, a set of minerals, a set of oligonucleotides, a mixture of different barcode molecules, or a mixture of identical barcode molecules. In other cases, a support (e.g., a bead) or droplet includes a heterogeneous mixture of reagents. In some cases, the heterogeneous mixture of reagents can include all components necessary to perform a reaction. In some cases, such mixture can include all components necessary to perform a reaction, except for 1, 2, 3, 4, 5, or more components necessary to perform a reaction. In some cases, such additional components are contained within, or otherwise coupled to, a different support (e.g., a bead) or droplet, or within a solution within a partition (e.g., microwell) of the system.

[00217] **FIG. 9** schematically illustrates an example of a microwell array. The array can be contained within a substrate **900**. The substrate **900** comprises a plurality of wells **902**. The wells **902** may be of any size or shape, and the spacing between the wells, the number of wells per substrate, as well as the density of the wells on the substrate **900** can be modified, depending on the application. In one such example application, a sample molecule **906**, which may comprise a cell or nucleus or cellular components (e.g., nucleic acid molecules) is co-partitioned with a bead **904**, which may comprise a nucleic acid barcode molecule coupled thereto. The wells **902** may be loaded using gravity or other loading technique (e.g., centrifugation, liquid handler, acoustic loading, optoelectronic, etc.). In some instances, at least one of the wells **902** contains a single sample molecule **906** (e.g., cell or nucleus) and a single bead **904.**

[00218] Reagents may be loaded into a well either sequentially or concurrently. In some cases, reagents are introduced to the device either before or after a particular operation. In some cases, reagents (which may be provided, in certain instances, in droplets or beads) are introduced sequentially such that different reactions or operations occur at different steps. The reagents (or droplets or beads) may also be loaded at operations interspersed with a reaction or operation step. For example, droplets or beads comprising reagents for fragmenting polynucleotides (e.g., restriction enzymes) and/or other enzymes (e.g., transposases, ligases, polymerases, etc.) may be loaded into the well or plurality of wells, followed by loading of droplets or beads comprising reagents for attaching nucleic acid barcode molecules to a sample nucleic acid molecule. Reagents may be provided concurrently or sequentially with a sample, such as a cell or nucleus or cellular components (e.g., organelles, proteins, nucleic acid molecules, carbohydrates, lipids, etc.). Accordingly, use of wells may be useful in performing multi-step operations or reactions.

[00219] As described elsewhere herein, the nucleic acid barcode molecules and other reagents may be contained within a bead or droplet. These beads or droplets may be loaded into a partition (e.g., a microwell) before, after, or concurrently with the loading of a cell or nucleus, such that each cell or nucleus is contacted with a different bead or droplet. This technique may be used to attach a unique nucleic acid barcode molecule to nucleic acid molecules obtained from each cell or nucleus. Alternatively, or in addition to, the sample nucleic acid molecules may be attached to a support. For instance, the partition (e.g., microwell) may comprise a bead which has coupled thereto a plurality of nucleic acid barcode molecules. The sample nucleic acid molecules, or derivatives thereof, may couple or attach to the nucleic acid barcode molecules on the support. The resulting barcoded nucleic acid molecules may then be removed from the partition, and in some instances, pooled and sequenced. In such cases, the nucleic acid barcode sequences may be used to trace the origin of the sample nucleic acid molecule. For example, polynucleotides with identical barcodes may be determined to originate from the same cell/nucleus or partition, while polynucleotides with different barcodes may be determined to originate from different cells/nuclei or partitions.

[00220] The samples or reagents may be loaded in the wells or microwells using a variety of approaches. The samples (e.g., a cell, a nucleus or cellular component) or reagents (as described herein) may be loaded into the well or microwell using an external force, e.g., gravitational force, electrical force, magnetic force, or using mechanisms to drive the sample or reagents into the well, e.g., via pressure-driven flow, centrifugation, optoelectronics, acoustic loading, electrokinetic pumping, vacuum, capillary flow, etc. In certain cases, a fluid handling system may be used to load the samples or reagents into the well. The loading of the samples or

reagents may follow a Poissonian distribution or a non-Poissonian distribution, e.g., super Poisson or sub-Poisson. The geometry, spacing between wells, density, and size of the microwells may be modified to accommodate a useful sample or reagent distribution; for instance, the size and spacing of the microwells may be adjusted such that the sample or reagents may be distributed in a super-Poissonian fashion.

[00221] In one particular non-limiting example, the microwell array or plate comprises pairs of microwells, in which each pair of microwells is configured to hold a droplet (e.g., comprising a single cell or nucleus) and a single bead (such as those described herein, which may, in some instances, also be provided or encapsulated in a droplet). The droplet and the bead (or droplet containing the bead) may be loaded simultaneously or sequentially, and the droplet and the bead may be merged, e.g., upon contact of the droplet and the bead, or upon application of a stimulus (e.g., external force, agitation, heat, light, magnetic or electric force, etc.). In some cases, the loading of the droplet and the bead is super-Poissonian. In other examples of pairs of microwells, the wells are configured to hold two droplets comprising different reagents and/or samples, which are merged upon contact or upon application of a stimulus. In such instances, the droplet of one microwell of the pair can comprise reagents that may react with an agent in the droplet of the other microwell of the pair. For instance, one droplet can comprise reagents that are configured to release the nucleic acid barcode molecules of a bead contained in another droplet, located in the adjacent microwell. Upon merging of the droplets, the nucleic acid barcode molecules may be released from the bead into the partition (e.g., the microwell or microwell pair that are in contact), and further processing may be performed (e.g., barcoding, nucleic acid reactions, etc.). In cases where cells or nuclei are loaded in the microwells, one of the droplets may comprise reagents for further processing, e.g., lysis reagents for lysing the cell or nucleus, upon droplet merging.

[00222] A droplet may be partitioned into a well. The droplets may be selected or subjected to pre-processing prior to loading into a well. For instance, the droplets may comprise cells or nuclei and only certain droplets, such as those containing a single cell or nucleus (or at least one cell/nucleus), may be selected for use in loading of the wells. Such a pre-selection process may be useful in efficient loading of single cells or nuclei, such as to obtain a non-Poissonian distribution, or to pre-filter cells or nuclei for a selected characteristic prior to further partitioning in the wells. Additionally, the technique may be useful in obtaining or preventing cell/nucleus doublet or multiplet formation prior to or during loading of the microwell.

[00223] In some instances, the wells can comprise nucleic acid barcode molecules attached thereto. The nucleic acid barcode molecules may be attached to a surface of the well

(e.g., a wall of the well). The nucleic acid barcode molecule (e.g., a partition barcode sequence) of one well may differ from the nucleic acid barcode molecule of another well, which can permit identification of the contents contained with a single partition or well. In some cases, the nucleic acid barcode molecule can comprise a spatial barcode sequence that can identify a spatial coordinate of a well, such as within the well array or well plate. In some cases, the nucleic acid barcode molecule can comprise a unique molecular identifier for individual molecule identification. In some instances, the nucleic acid barcode molecules may be configured to attach to or capture a nucleic acid molecule within a sample or cell or nucleus distributed in the well. For example, the nucleic acid barcode molecules may comprise a capture sequence that may be used to capture or hybridize to a nucleic acid molecule (e.g., RNA, DNA) within the sample. In some instances, the nucleic acid barcode molecules may be releasable from the microwell. For instance, the nucleic acid barcode molecules may comprise a chemical cross-linker which may be cleaved upon application of a stimulus (e.g., photo-, magnetic, chemical, biological, stimulus). The released nucleic acid barcode molecules, which may be hybridized or configured to hybridize to a sample nucleic acid molecule, may be collected and pooled for further processing, which can include nucleic acid processing (e.g., amplification, extension, reverse transcription, etc.) and/or characterization (e.g., sequencing). In such cases, the unique partition barcode sequences may be used to identify the cell/nucleus or partition from which a nucleic acid molecule originated.

[00224] Characterization of samples within a well may be performed. Such characterization can include, in non-limiting examples, imaging of the sample (e.g., cell/nucleus or cellular components) or derivatives thereof. Characterization techniques such as microscopy or imaging may be useful in measuring sample profiles in fixed spatial locations. For instance, when cells or nuclei are partitioned, optionally with beads, imaging of each microwell and the contents contained therein may provide useful information on cell/nucleus doublet formation (e.g., frequency, spatial locations, etc.), cell-bead pair efficiency, cell viability, cell size, cell morphology, expression level of a biomarker (e.g., a surface marker, a fluorescently labeled molecule therein, etc.), cell/nucleus or bead loading rate, number of cell-bead pairs, cell-cell interactions (when two or more cells/nuclei are co-partitioned). Alternatively, or in addition to, imaging may be used to characterize a quantity of amplification products in the well.

[00225] In operation, a well may be loaded with a sample and reagents, simultaneously or sequentially. When cells or nuclei are loaded, the well may be subjected to washing, e.g., to remove excess cells from the well, microwell array, or plate. Similarly, washing may be performed to remove excess beads or other reagents from the well, microwell array, or plate. In

addition, the cells or nuclei may be lysed in the individual partitions to release the intracellular components or cellular analytes. Alternatively, the cells or nuclei may be fixed or permeabilized in the individual partitions. The intracellular components or cellular analytes may couple to a support, e.g., on a surface of the microwell, on a solid support (e.g., bead), or they may be collected for further downstream processing. For instance, after cell lysis, the intracellular components or cellular analytes may be transferred to individual droplets or other partitions for barcoding. Alternatively, or in addition to, the intracellular components or cellular analytes (e.g., nucleic acid molecules) may couple to a bead comprising a nucleic acid barcode molecule; subsequently, the bead may be collected and further processed, e.g., subjected to nucleic acid reaction such as reverse transcription, amplification, or extension, and the nucleic acid molecules thereon may be further characterized, e.g., via sequencing. Alternatively, or in addition to, the intracellular components or cellular analytes may be barcoded in the well (e.g., using a bead comprising nucleic acid barcode molecules that are releasable or on a surface of the microwell comprising nucleic acid barcode molecules). The barcoded nucleic acid molecules or analytes may be further processed in the well, or the barcoded nucleic acid molecules or analytes may be collected from the individual partitions and subjected to further processing outside the partition. Further processing can include nucleic acid processing (e.g., performing an amplification, extension) or characterization (e.g., fluorescence monitoring of amplified molecules, sequencing). At any convenient or useful step, the well (or microwell array or plate) may be sealed (e.g., using an oil, membrane, wax, etc.), which enables storage of the assay or selective introduction of additional reagents.

[00226] **Fig. 10** schematically shows an example workflow for processing nucleic acid molecules within a sample. A substrate **1000** comprising a plurality of microwells **1002** may be provided. A sample **1006** which may comprise a cell or nucleus, cellular components or analytes (e.g., proteins and/or nucleic acid molecules) can be co-partitioned, in a plurality of microwells **1002**, with a plurality of beads **1004** comprising nucleic acid barcode molecules. During process **1010**, the sample **1006** may be processed within the partition. For instance, the cell or nucleus may be subjected to conditions sufficient to lyse the cells or nuclei and release the analytes contained therein. In process **1020**, the bead **1004** may be further processed. By way of example, processes **1020a** and **1020b** schematically illustrate different workflows, depending on the properties of the bead **1004.**

[00227] In **1020a**, the bead comprises nucleic acid barcode molecules that are attached thereto, and sample nucleic acid molecules (e.g., RNA, DNA) may attach, e.g., via hybridization of ligation, to the nucleic acid barcode molecules. Such attachment may occur on the bead. In

process **1030**, the beads **1004** from multiple wells **1002** may be collected and pooled. Further processing may be performed in process **1040**. For example, one or more nucleic acid reactions may be performed, such as reverse transcription, nucleic acid extension, amplification, ligation, transposition, etc. In some embodiments, one or more reactions occur on the bead using the sample nucleic acid molecules captured on the bead via the nucleic acid barcode molecules on the bead, e.g., sample nucleic acid molecules hybridized to complementary sequences of the nucleic acid barcode molecules on the bead. In some instances, adapter sequences are ligated to the nucleic acid molecules, or derivatives thereof, as described elsewhere herein. For instance, sequencing primer sequences may be appended to each end of the nucleic acid molecule. In process **1050**, further characterization, such as sequencing may be performed to generate sequencing reads. The sequencing reads may yield information on individual cells or populations of cells or nuclei, which may be represented visually or graphically, e.g., in a plot **1055**.

[00228] In **1020b**, the bead comprises nucleic acid barcode molecules that are releasably attached thereto, as described below. The bead may degrade or otherwise release the nucleic acid barcode molecules into the well **1002**; the nucleic acid barcode molecules may then be used to barcode nucleic acid molecules within the well **1002**. Further processing may be performed either inside the partition or outside the partition. For example, one or more nucleic acid reactions may be performed, such as reverse transcription, nucleic acid extension, amplification, ligation, transposition, etc. In some instances, adapter sequences are ligated to the nucleic acid molecules, or derivatives thereof, as described elsewhere herein. For instance, sequencing primer sequences may be appended to each end of the nucleic acid molecule. In process **1050**, further characterization, such as sequencing may be performed to generate sequencing reads. The sequencing reads may yield information on individual cells or populations of cells or nuclei, which may be represented visually or graphically, e.g., in a plot **1055.**

Computer systems

[00229] The present disclosure provides computer systems that are programmed to implement methods of the disclosure. **FIG. 6** shows a computer system **601** that is programmed or otherwise configured to the computer system **601** can regulate various aspects of the present disclosure. The computer system **601** 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.

[00230] The computer system **601** includes a central processing unit (CPU, also "processor" and "computer processor" herein) **605**, which can be a single core or multi core

processor, or a plurality of processors for parallel processing. The computer system **601** also includes memory or memory location **610** (e.g., random-access memory, read-only memory, flash memory), electronic storage unit **615** (e.g., hard disk), communication interface **620** (e.g., network adapter) for communicating with one or more other systems, and peripheral devices **625**, such as cache, other memory, data storage and/or electronic display adapters. The memory **610**, storage unit **615**, interface **620** and peripheral devices **625** are in communication with the CPU **605** through a communication bus (solid lines), such as a motherboard. The storage unit **615** can be a data storage unit (or data repository) for storing data. The computer system **601** can be operatively coupled to a computer network ("network") **630** with the aid of the communication interface **620**. The network **630** can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network **630** in some cases is a telecommunication and/or data network. The network **630** can include one or more computer servers, which can enable distributed computing, such as cloud computing. The network **630**, in some cases with the aid of the computer system **601**, can implement a peer-to-peer network, which may enable devices coupled to the computer system **601** to behave as a client or a server.

[00231] The CPU **605** 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 **610**. The instructions can be directed to the CPU **605**, which can subsequently program or otherwise configure the CPU **605** to implement methods of the present disclosure. Examples of operations performed by the CPU **605** can include fetch, decode, execute, and writeback.

[00232] The CPU **605** can be part of a circuit, such as an integrated circuit. One or more other components of the system **601** can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[00233] The storage unit **615** can store files, such as drivers, libraries and saved programs. The storage unit **615** can store user data, e.g., user preferences and user programs. The computer system **601** in some cases can include one or more additional data storage units that are external to the computer system **601**, such as located on a remote server that is in communication with the computer system **601** through an intranet or the Internet.

[00234] The computer system **601** can communicate with one or more remote computer systems through the network **630**. For instance, the computer system **601** 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 **601** via the network **630**.

[00235] 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 **601**, such as, for example, on the memory **610** or electronic storage unit **615**. 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 **605**. In some cases, the code can be retrieved from the storage unit **615** and stored on the memory **610** for ready access by the processor **605**. In some situations, the electronic storage unit **615** can be precluded, and machine-executable instructions are stored on memory **610**.

[00236] 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.

[00237] Aspects of the systems and methods provided herein, such as the computer system **601**, 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.

[00238] 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.

[00239] The computer system **601** can include or be in communication with an electronic display **635** that comprises a user interface (UI) **640**. Examples of UIs include, without limitation, a graphical user interface (GUI) and web-based user interface.

[00240] 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 **605**.

[00241] 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, a biological particle (e.g., a cell or cell bead) is partitioned in a partition (e.g., droplet), and multiple analytes from the biological particle 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.

Example 1:

[00242] A plurality of positively charged particles can be introduced to a plurality of cells, such that the plurality of positively charged particles can make contact with the plurality of cells. A portion of the positively charged particles can adhere to negatively charged features of the surface of a first cell of the plurality of cells. A second portion of the positively charged particles can adhere to negatively charged features of the surface of a second cell of the plurality of cells. The positively charged particles on the first cell can repel the positively charged particles on the second cell such that the first cell and the second cell remain sufficiently far apart to prevent the first cell from adhering to the second cell. An example of a first cell coated in positively charged particles separated from a second cell coated in positively charged particles via the positively charged particles is provided in **FIG. 11**.

[00243] 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, comprising:

providing a mixture comprising a plurality of particles and a plurality of cells, wherein a particle of the plurality of particles is coupled to a cell of the plurality of cells, and wherein the particle coupled to the cell prevents the cell from adhering to another cell of the plurality of cells.

2. The method of claim 1, wherein the plurality of particles comprises at least 10 particles.

3. The method of claim 2, wherein the plurality of particles comprises at least 1,000 particles.

4. The method of claim 3, wherein the plurality of particles comprises at least 100,000 particles.

5. The method of claim 1, wherein a number of particles of the plurality of particles is at least 2 times a number of cells of the plurality of cells.

6. The method of claim 5, wherein a number of particles of the plurality of particles is at least 10 times a number of cells of the plurality of cells.

7. The method of claim 6, wherein a number of particles of the plurality of particles is at least 100 times a number of cells of the plurality of cells.

8. The method of claim 1, wherein the plurality of particles comprises a plurality of beads.

9. The method of claim 1, wherein the plurality of particles is homogenous.

10. The method of claim 1, wherein the plurality of particles is heterogeneous.

11. The method of claim 1, wherein the plurality of particles comprises particles having different sizes.

12. The method of claim 1, wherein the plurality of particles comprises particles having different weights.

13. The method of claim 1, wherein the plurality of particles comprises particles having different functional groups.

14. The method of claim 1, wherein the particle comprises a microsphere.

15. The method of claim 1, wherein the particle is a magnetic particle.

16. The method of claim 1, wherein the particle is a charged particle.

17. The method of claim 1, wherein the particle comprises more than one particle.

18. The method of claim 16, wherein the charged particle is a positively charged particle.

19. The method of claim 1, wherein the particle is a bead.

20. The method of claim 1, wherein the particle comprises a dimension of less than 50 μm.

21. The method of claim 20, wherein the particle comprises a dimension of less than 10 μm.

22. The method of claim 1, wherein the particle comprises a diameter no more than 1/2 of a diameter of the cell.

23. The method of claim 22, wherein the particle comprises a diameter no more than 1/3 of a diameter of the cell.

24. The method of claim 23, wherein the particle comprises a diameter no more than 1/10 of a diameter of the cell.

25. The method of claim 24, wherein the particle comprises a diameter no more than 1/100 of a diameter of the cell.

26. The method of claim 1, wherein the particle further comprises a protein.

27. The method of claim 26, wherein the protein is an enzyme.

28. The method of claim 27, wherein the enzyme is a DNAase.

29. The method of claim 28, further comprising removing the DNAase by removing the particle.

30. The method of claim 28, further comprising removing the DNAase by removing the DNAase from the particle.

31. The method of claim 1, wherein the particle further comprises an oligonucleotide.

32. The method of claim 1, wherein the cell comprises one cell.

33. The method of claim 1, wherein the plurality of cells comprises single cells.

34. The method of claim 1, wherein the plurality of cells comprises cultured cells.

35. The method of claim 1, wherein the plurality of cells comprises hepatocytes.

36. The method of claim 35, wherein the hepatocytes comprise a HepG2 cell.

37. The method of claim 1, wherein the plurality of cells comprises harvested cells.

38. The method of claim 1, wherein plurality of cells comprises at least 10 cells.

39. The method of claim 38, wherein plurality of cells comprises at least 100 cells.

40. The method of claim 39, wherein plurality of cells comprises at least 1,000 cells.

41. The method of claim 1, wherein at least two particles of the plurality of particles are coupled to the cell.

42. The method of claim 41, wherein the at least two particles surround the cell.

43. The method of claim 1, further comprising freezing the cell.

44. The method of claim 1, further comprising processing the plurality of cells.

45. The method of claim 44, wherein the processing comprises fluorescence-activated cell sorting (FACS).

46. The method of claim 44, wherein the processing comprises performing an assay.

47. The method of claim 1, further comprising partitioning the particle coupled to the cell to a partition.

48. The method of claim 1, further comprising co-partitioning the particle coupled to the cell to a same partition as a nucleic acid barcode molecule.

49. The method of claim 47 or claim 48, wherein the partition comprises no more than one cell.

50. The method of claim 48, wherein a nucleic acid barcode molecule is coupled to a nucleic acid molecule of the cell.

51. The method of claim 47 or claim 48, wherein the partition is a well.

52. The method of claim 47 or claim 48, wherein the partition is a droplet.

53. The method of claim 47 or claim 48, further comprising pooling the partition with a partition comprising a second cell from another plurality of cells.

54. The method of claim 1, wherein the particle is coupled to a capture moiety.

55. The method of claim 54, further comprising lysing the cell to capture an intracellular molecule of the cell using the capture moiety.

56. A method comprising:

a) providing a mixture comprising a plurality of positively charged particles and a plurality of cells, wherein a particle of the plurality of positively charged particles is coupled to a cell of the plurality of cells; and

b) isolating the particle coupled to the cell based at least in part on a density of the particle coupled to the cell.

57. The method of claim 56, wherein the plurality of positively charged particles comprises at least 10 positively charged particles.

58. The method of claim 56, wherein the plurality of positively charged particles comprises at least 1,000 positively charged particles.

59. The method of claim 58, wherein the plurality of positively charged particles comprises at least 100,000 positively charged particles.

60. The method of claim 56, wherein a number of positively charged particles of the plurality of positively charged particles is at least 2 times a number of cells of the plurality of cells.

61. The method of claim 60, wherein a number of positively charged particles of the plurality of positively charged particles is at least 10 times a number of cells of the plurality of cells.

62. The method of claim 61, wherein a number of positively charged particles of the plurality of positively charged particles is at least 100 times a number of cells of the plurality of cells.

63. The method of claim 56, wherein the plurality of positively charged particles comprises a plurality of beads.

64. The method of claim 56, wherein the plurality of positively charged particles is homogenous.

65. The method of claim 56, wherein the plurality of positively charged particles is heterogeneous.

66. The method of claim 56, wherein the plurality of positively charged particles comprises positively charged particles having different sizes.

67. The method of claim 56, wherein the plurality of positively particles comprises positively charged particles having different weights.

68. The method of claim 56, wherein the plurality of positively charged particles comprises positively charged particles having different functional groups.

69. The method of claim 56, wherein the positively charged particle comprises a microsphere.

70. The method of claim 56, wherein the positively charged particle comprises more than one positively charged particle.

71. The method of claim 56, wherein a dimension of the positively charged particle is less than about 50 μm.

72. The method of claim 71, wherein a dimension of the positively charged particle is less than about 10 μm.

73. The method of claim 56, wherein the positively charged particle comprises a diameter no more than 1/2 of a diameter of the cell.

74. The method of claim 73, wherein the positively charged particle comprises a diameter no more than 1/3 of a diameter of the cell.

75. The method of claim 74, wherein the positively charged particle comprises a diameter no more than 1/10 of a diameter of the cell.

76. The method of claim 75, wherein the particle comprises a diameter no more than 1/100 of a diameter of the cell.

77. The method of claim 56, wherein the positively charged particle comprises more than one positively charged particle.

78. The method of claim 56, wherein the positively charged particle further comprises a protein.

79. The method of claim 56, wherein the positively charged particle further comprises an oligonucleotide.

80. The method of claim 56, wherein the cell comprises one cell.

81. The method of claim 56, wherein the plurality of cells comprises single cells.

82. The method of claim 56, wherein the plurality of cells comprises cultured cells.

83. The method of claim 56, wherein the plurality of cells comprises harvested cells.

84. The method of claim 83, wherein the harvested cells comprise oocytes.

85. The method of claim 56, wherein the plurality of cells comprises at least 10 cells.

86. The method of claim 85, wherein the plurality of cells comprises at least 100 cells.

87. The method of claim 86, wherein the plurality of cells comprises at least 1,000 cells.

88. The method of claim 56, wherein at least two particles of the plurality of particles are coupled to the cell.

89. The method of claim 88, wherein the at least two particles surround the cell.

90. The method of claim 56, wherein the isolating comprises sedimentation.

91. The method of claim 90, wherein the isolating comprises differential sedimentation.

92. The method of claim 56, wherein the isolating comprises collecting a layer comprising cells.

93. The method of claim 92, wherein the layer is a liquid layer.

94. The method of claim 56, further comprising partitioning the positively charged particle coupled to the cell to a partition.

95. The method of claim 56, further comprising co-partitioning the positively charged particle coupled to the cell to a partition as a nucleic acid barcode molecule.

96. The method of claim 94 or claim 95, wherein the partition comprises no more than one cell.

97. The method of claim 95, wherein a nucleic acid barcode molecule is coupled to a nucleic acid molecule of the cell.

98. The method of claim 94 or claim 95, wherein the partition is a well.

99. The method of claim 94 or claim 95, wherein the partition is a droplet.

100. The method of claim 94 or claim 95, further comprising pooling the partition with a partition comprising a second cell from another plurality of cells.

101. The method of claim 56, wherein the positively charged particle is coupled to a capture moiety.

102. The method of claim 101, further comprising lysing the cell to capture an intracellular molecule of the cell using the capture moiety.

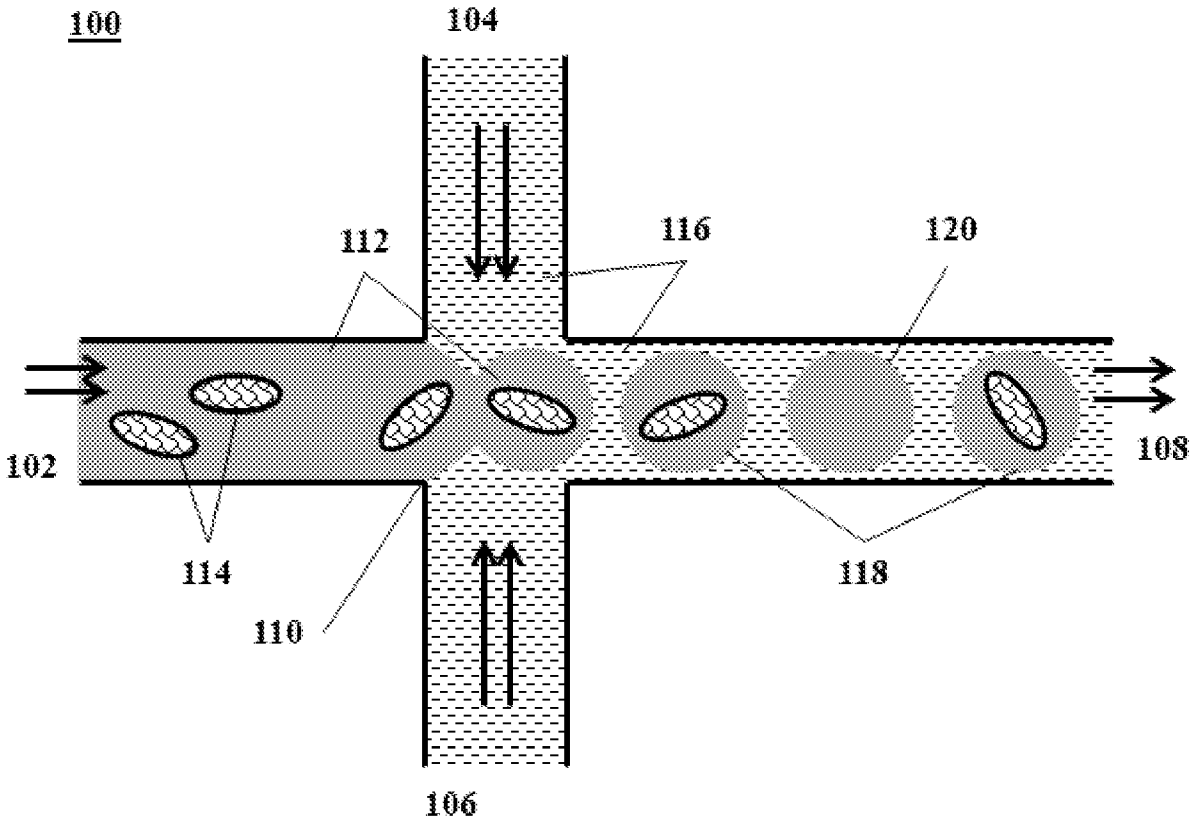

FIG. 1

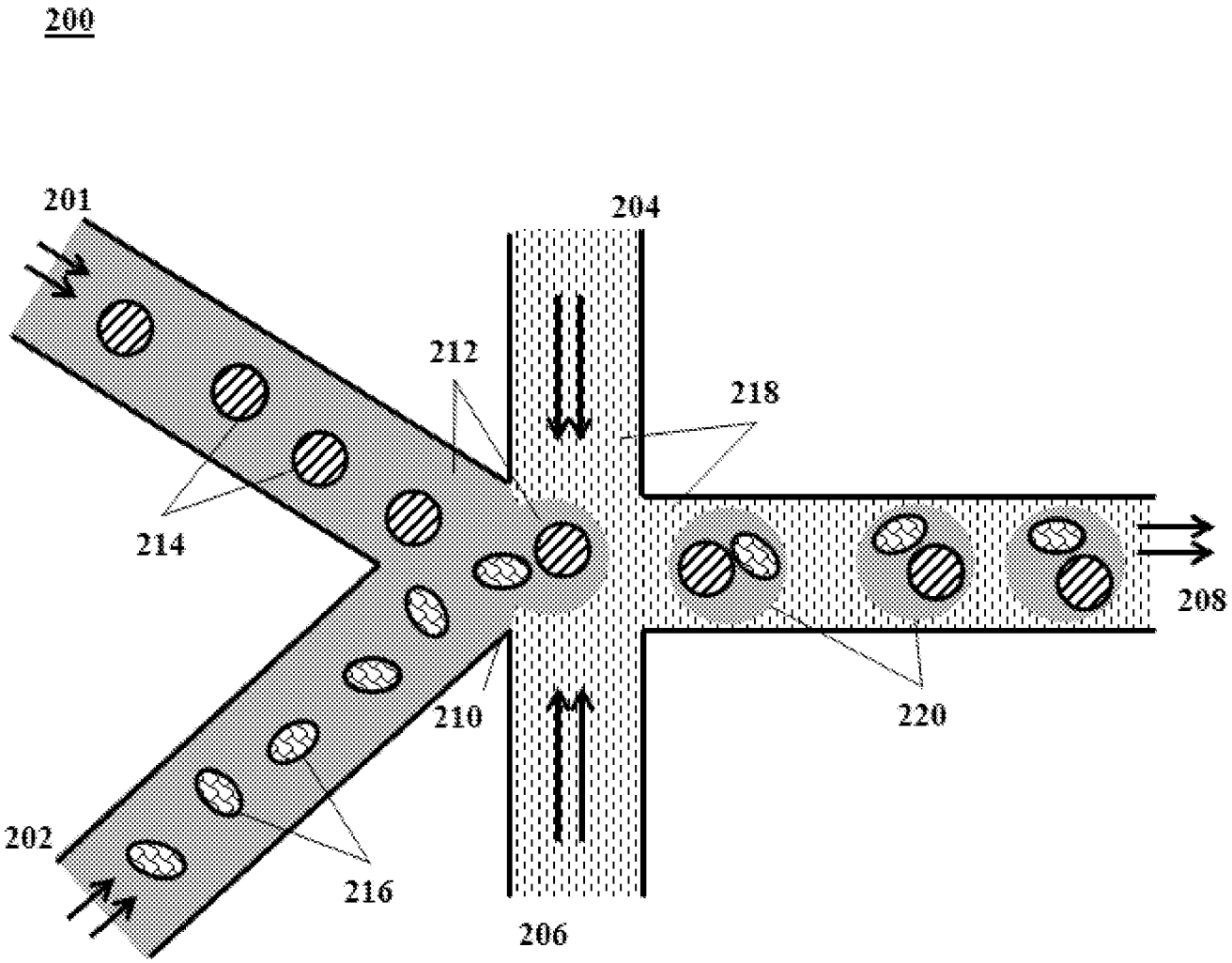

FIG. 2

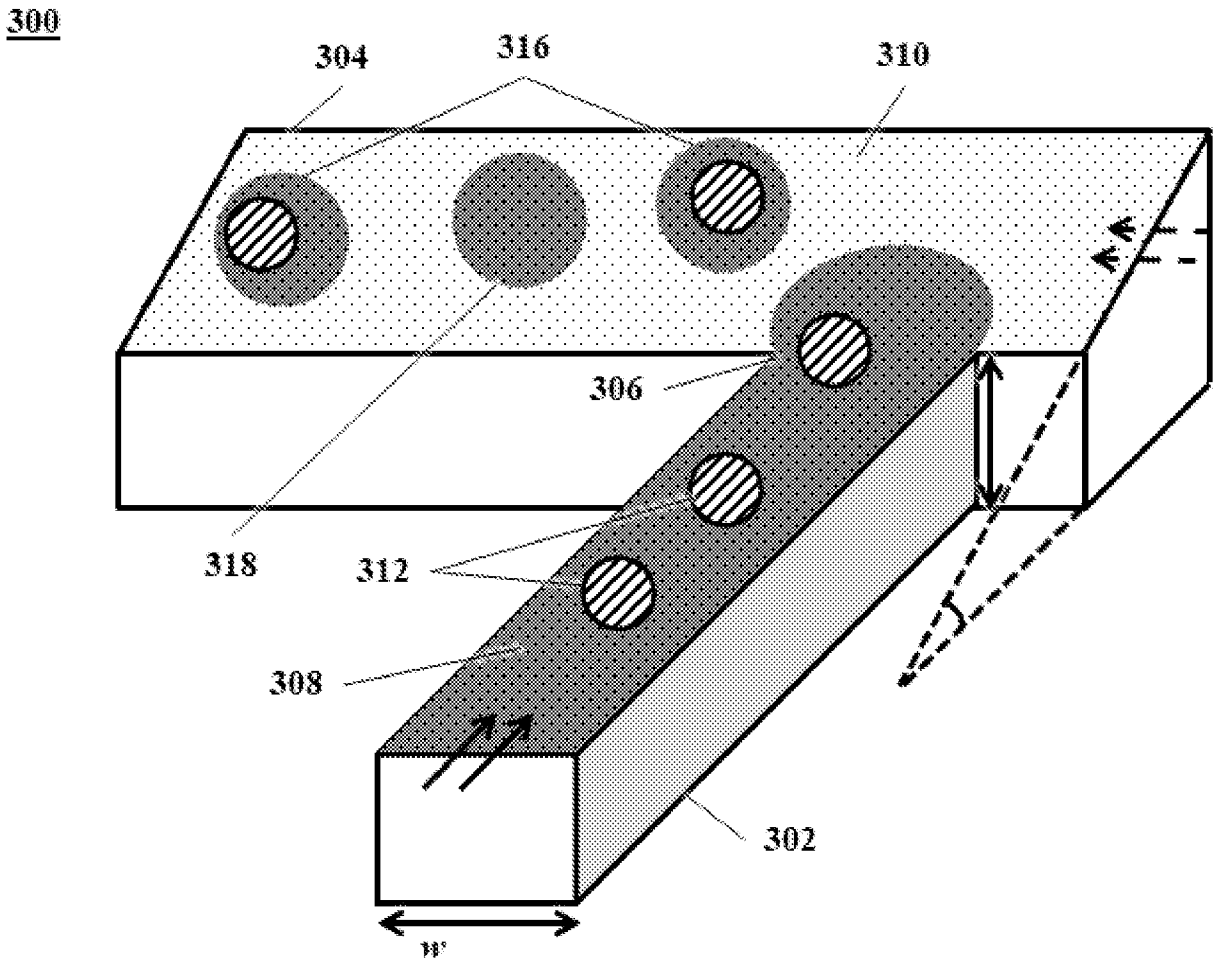

FIG. 3

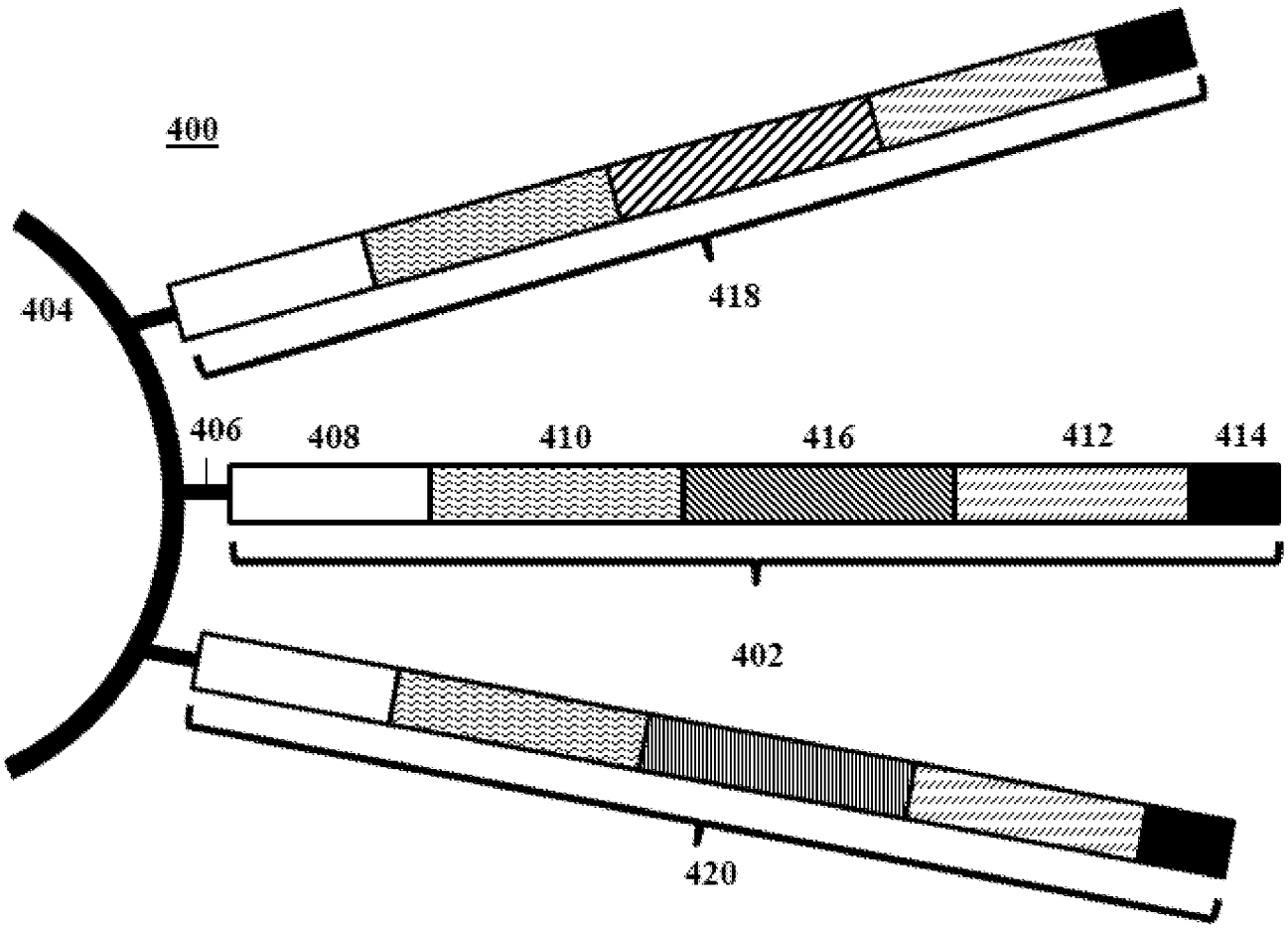

FIG. 4

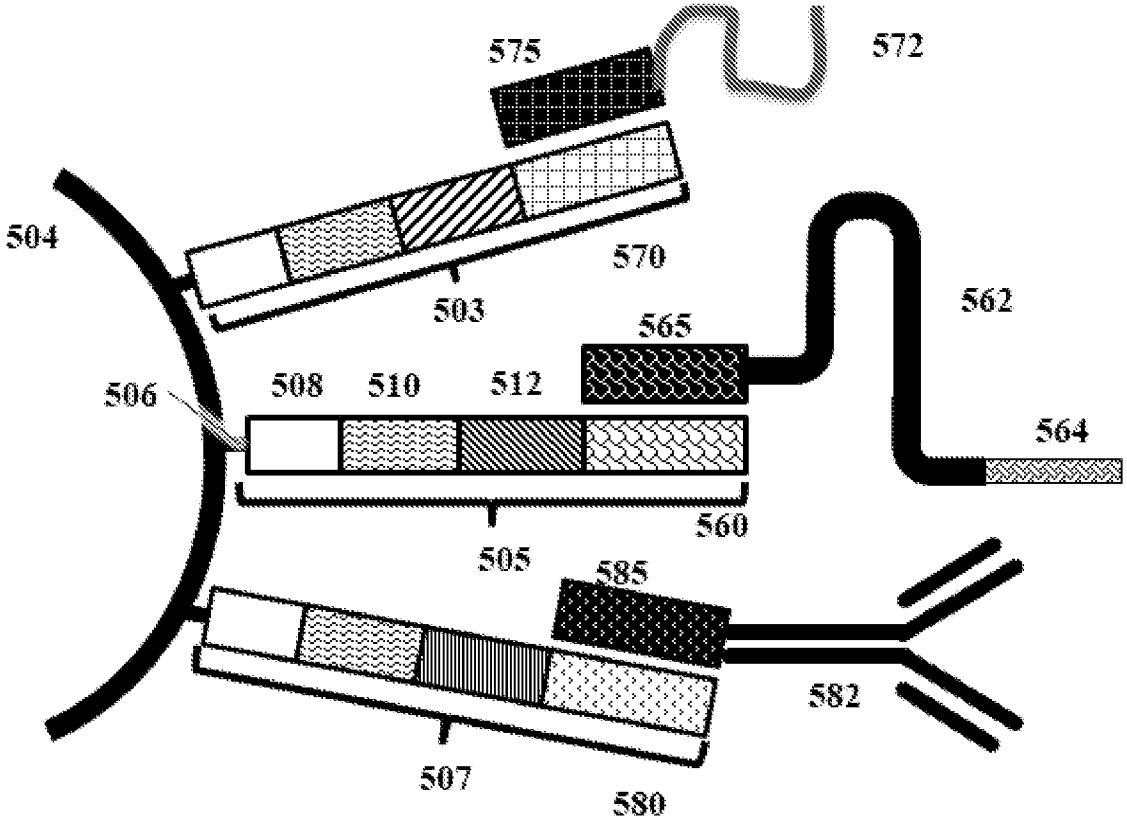
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FIG. 5

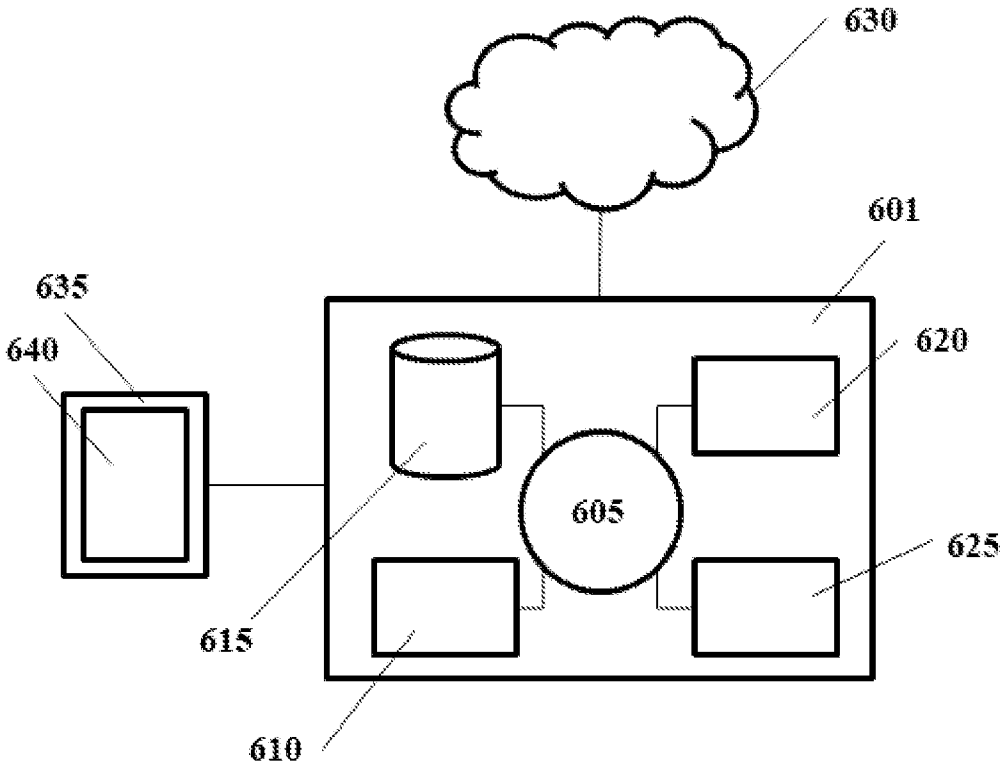

FIG. 6

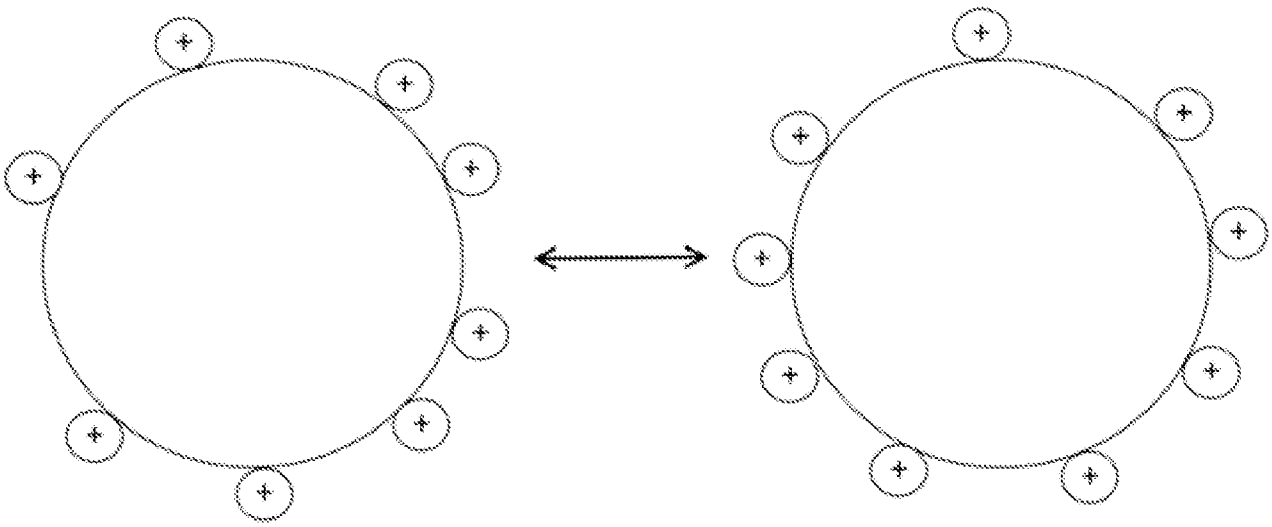

FIG. 7

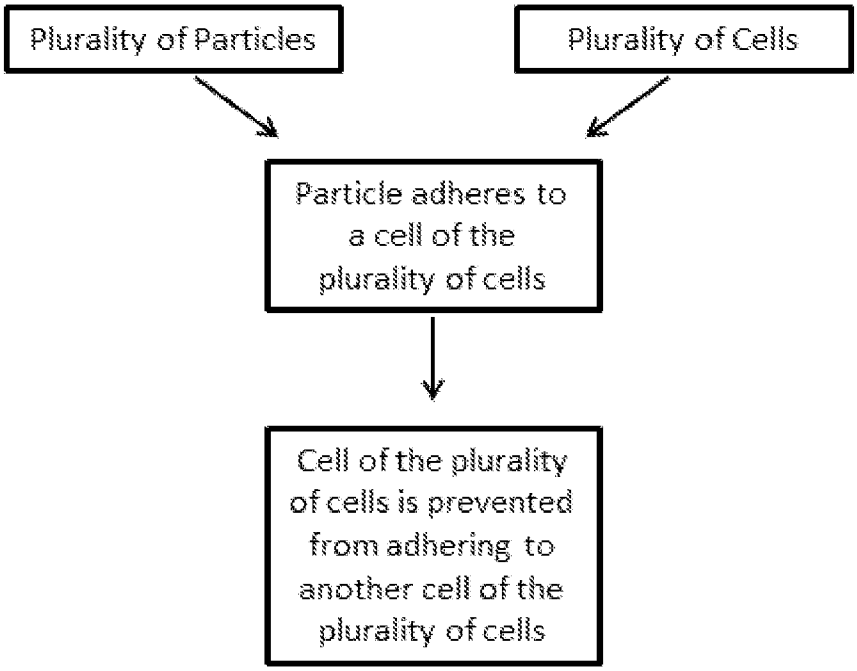
Plurality of Particles
Plurality of Cells
Particle adheres to a cell of the plurality of cells
Cell of the plurality of cells is prevented from adhering to another cell of the plurality of cells

FIG. 8

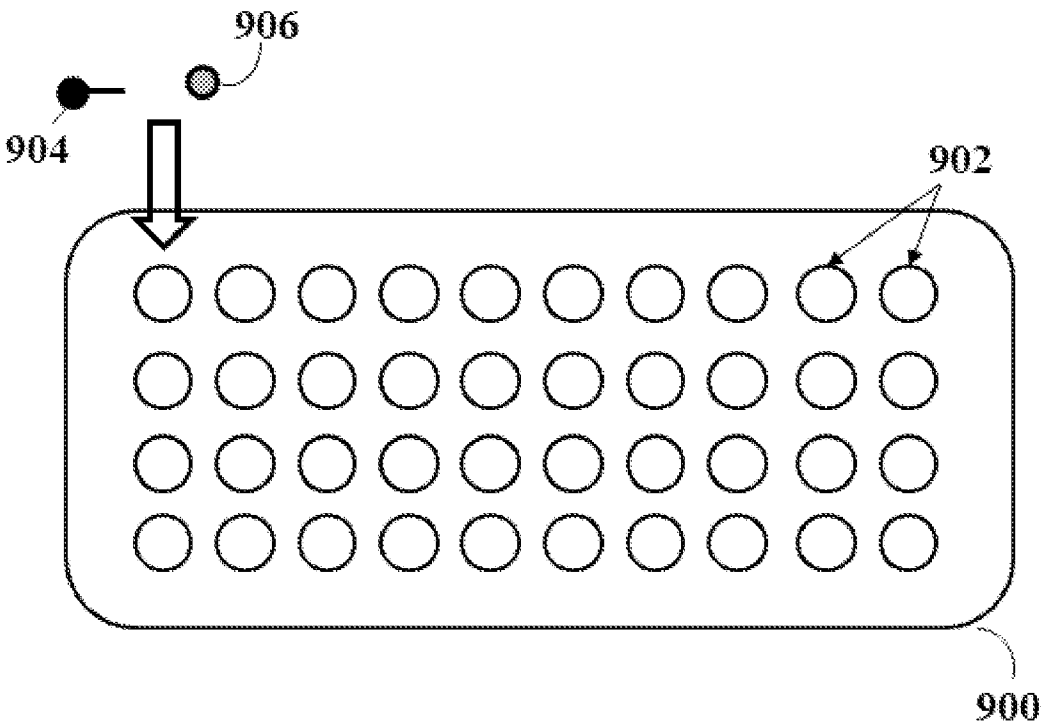

Fig. 9

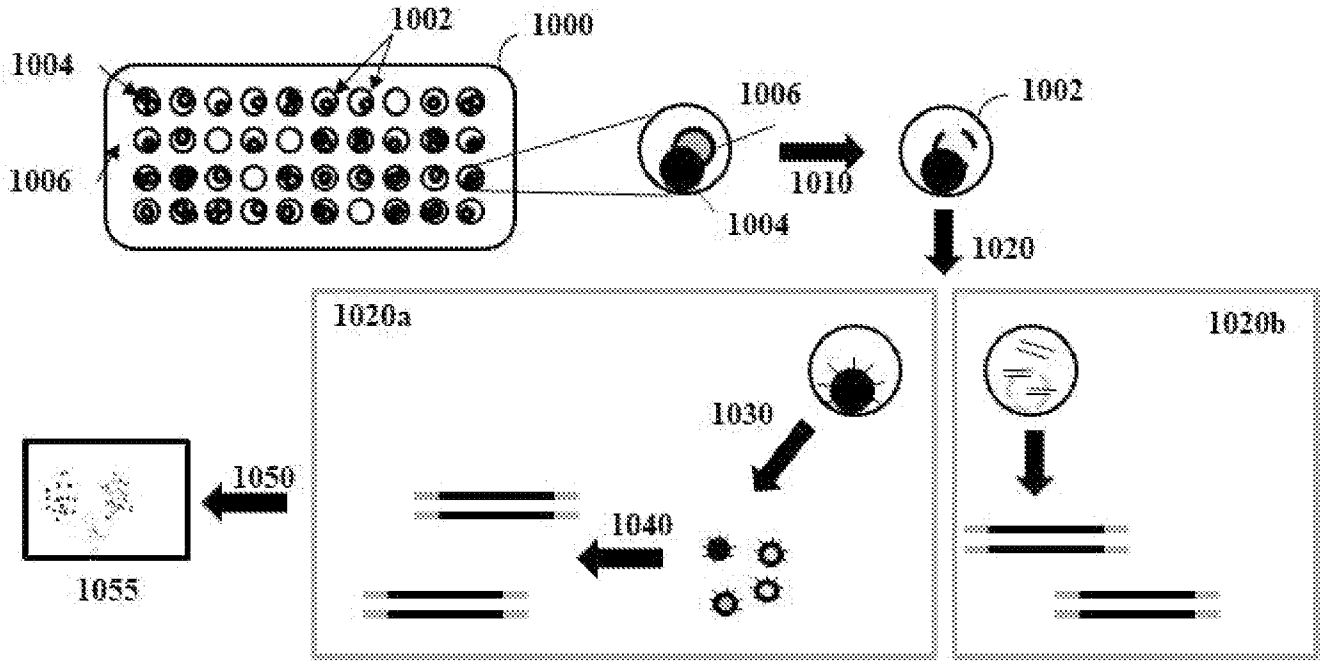
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Fig. 10