METHODS AND SYSTEMS FOR ASSOCIATING PHYSICAL AND GENETIC PROPERTIES OF BIOLOGICAL PARTICLES

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/587,634, filed November 17, 2017, which is entirely incorporated herein by reference.

BACKGROUND

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

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

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

[0005] Biological samples may be processed wherein the sample information (e.g., phenotypic information) may be lost using existing methods. Such sample processing may not be useful for analyzing cell-to-cell variations in the sample. Further, existing methods can suffer from inefficient sample preparation methods, such as time-consuming procedures that may include multiple steps.

SUMMARY

[0006] Recognized herein is a need for the improved methods for sample preparation for analyzing cell-to-cell variation (e.g., phenotypic variation and sequence variation) in a biological sample.

[0006a] In one aspect, provided herein is a method for analyzing a nucleic acid molecule of a cell, comprising: (a) partitioning into a physical partition of a plurality of physical partitions:

(i) a bead comprising (I) an optically detectable moiety and (II) a plurality of nucleic acid barcode molecules, wherein a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules comprises a barcode sequence, and

(ii) said cell comprising said nucleic acid molecule,

wherein said bead comprises said optically detectable moiety prior to (a);

(b) within said physical partition, using said nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules and said nucleic acid molecule of said cell to conduct a barcoding reaction on said nucleic acid molecule to generate a barcoded nucleic acid molecule;

(c) generating (i) a first data set comprising sequence data corresponding to a nucleic acid sequence of said barcoded nucleic acid molecule or a derivative thereof and (ii) a second data set comprising data corresponding to said optically detectable moiety of said bead; and

(d) associating (i) said sequence data of said first data set with (ii) said data of said second data set.

[0007] In an example, the present disclosure provides a method for processing a nucleic acid molecule of a biological particle, comprising: (a) providing (1) a plurality of partitions, wherein a partition of said plurality of partitions comprises (i) a particle comprising a plurality of nucleic

acid barcode molecules, wherein a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules comprises a barcode sequence, and (ii) a biological particle comprising said nucleic acid molecule, and (2) a first data set comprising data indicative of one or more physical properties of said biological particle stored in computer memory, which data is generated upon sensing said biological particle; (b) using said nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules and said nucleic acid molecule of said biological particle to generate a barcoded nucleic acid molecule; (c) generating a second data set comprising data identifying a nucleic acid sequence of said barcoded nucleic acid molecule or a derivative thereof; and (d) using said first data set and said second data set to associate said one or more physical properties of said biological particle with said nucleic acid sequence.

[0008] In some examples, said one or more physical properties of said biological particle includes a size of said biological particle, a shape of said biological particle, a surface marker on said biological particle, an inclusion in said biological particle, a structure of an organelle in said biological particle, a number of organelles in said biological particle, a secretion or excretion with respect to said biological particle, or a localization of an organelle in said biological particle.

[0009] In some examples, said biological particle comprises a plurality of nucleic acid molecules. In some examples, said plurality of nucleic acid molecules comprises a plurality of ribonucleic acid molecules. In some examples, said plurality of nucleic acid molecules comprises a plurality of deoxyribonucleic acid molecules.

[0010] In some examples, said nucleic acid molecule is a ribonucleic acid molecule. In some examples, said nucleic acid molecule is a deoxyribonucleic acid molecule.

[0011] In some examples, said one or more physical properties comprises phenotypic information of said biological particle.

[0012] In some examples, the method further comprises optically detecting said particle to generate a third data set comprising data indicative of one or more physical properties of said particle or said plurality of nucleic acid barcode molecules. In some examples, said one or more physical properties of said particle comprises an optical property of said particle. In some examples, said physical property of said particle comprises a size, a shape, a circularity, a hardness, or a symmetry of said particle or a component thereof. In some examples, said optical property of said particle comprises an absorbance, a birefringence, a color, a fluorescence characteristic, a luminosity, a photosensitivity, a reflectivity, a refractive index, a scattering, or a transmittance of said particle or a component thereof.

[0013] In some examples, nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules comprise barcode sequences, which barcode sequences are identical.

[0014] In some examples, said plurality of partitions is a plurality of droplets. In some examples, said plurality of partitions is a plurality of wells.

[0015] In some examples, said particle comprises one or more optical barcodes. In some examples, said plurality of nucleic acid barcode molecules of said particle comprise said one or more optical barcodes. In some examples, said one or more optical barcodes comprises a fluorescent dye, a nanoparticle, a microparticle, or any combination thereof. In some examples, said one or more optical barcodes has an associated optical intensity or frequency that is distinct with respect to other optical barcodes of said plurality of partitions. In some examples, (a)-(d) are repeated for an additional partition among said plurality of partitions, wherein said additional partition comprises (i) an additional particle comprising an additional plurality of nucleic acid barcode molecules, wherein a nucleic acid barcode molecule of said additional plurality of nucleic acid barcode molecules comprises an additional barcode sequence different than said barcode sequence, and (ii) an additional biological particle comprising an additional nucleic acid molecule, wherein said partition and said additional partition yield optical signals at different intensities or frequencies upon sensing. In some examples, said nanoparticle comprises a quantum dot. In some examples, said nanoparticle comprises a Janus particle.

[0016] In some examples, said plurality of nucleic acid barcode molecules is configured in a geometric structure. In some examples, said geometric structure is a nucleic acid origami.

[0017] In some examples, said one or more physical properties of said biological particle are identified by imaging said partition using (i) bright field microscopy, (ii) fluorescence microscopy, (iii) phase contrast microscopy, (iv) multispectral microscopy, or (v) polarization microscopy.

[0018] In some examples, said particle is a bead. In some examples, said bead is a gel bead. In some examples, said plurality of nucleic acid barcode molecules is releasably attached to said gel bead. In some examples, said gel bead comprises a polyacrylamide polymer.

[0019] In some examples, said one or more physical properties are one or more optical properties. In some examples, the method further comprises, subsequent to (a), imaging said partition to optically detect said biological particle, thereby identifying said one or more optical

properties of said biological particle. In some examples, the method further comprises, prior to (a), imaging said biological particle to identify said one or more optical properties of said biological particle. In some examples, said biological particle is imaged prior to providing said biological particle in said partition.

[0020] In some examples, said particle is coupled to another particle comprising one or more optical barcodes.

[0021] In some examples, (a)-(d) are repeated for additional partitions among said plurality of partitions, wherein said additional partitions each comprise (i) an additional plurality of nucleic acid barcode molecules, wherein a nucleic acid barcode molecule of said additional plurality of nucleic acid barcode molecules comprises an additional barcode sequence different than said barcode sequence, and (ii) an additional biological particle comprising an additional nucleic acid molecule, and wherein said partition and said additional partitions comprise a combination of particles comprising molecular barcodes and particles comprising optical barcodes, which combination is different across said partition and said additional partitions.

[0022] In some examples, (a)-(d) are repeated for additional partitions among said plurality of partitions, wherein said additional partitions each comprise (i) an additional plurality of nucleic acid barcode molecules, wherein a nucleic acid barcode molecule of said additional plurality of nucleic acid barcode molecules comprises an additional barcode sequence different than said barcode sequence, and (ii) an additional biological particle comprising an additional nucleic acid molecule, which additional partitions comprises at least 1,000 partitions. In some examples, said additional partitions comprise at least 10,000 partitions. In some examples, said additional partitions comprise at least 100,000 partitions. In some examples, said additional partitions comprise a plurality of particles comprising nucleic acid barcode molecules comprising at least 1,000 barcode sequences, which at least 1,000 barcode sequences are different across said partition and said additional partitions. In some examples, said additional partitions comprises a plurality of particles comprising nucleic acid barcode molecules comprising at least 10,000 barcode sequences, which at least 10,000 barcode sequences are different across said partition and said additional partitions. In some examples, said additional partitions comprises a plurality of particles comprising nucleic acid barcode molecules comprising at least 100,000 barcode sequences, which at least 100,000 barcode sequences are different across said partition and said additional partitions.

[0023] In some examples, said biological particle is a cell.

[0024] In some examples, said biological particle comprises a cell, or one or more components thereof, in a matrix. In some examples, said matrix is a polymeric matrix. In some examples, said matrix is a gel matrix.

[0025] In some examples, the method further comprises associating a protein of said biological particle with said one or more physical properties of said biological particle with said nucleic acid sequence. In some examples, said protein is a cell surface protein.

[0026] In some examples, the method further comprises associating one or more ribonucleic acid sequences or one or more deoxyribonucleic acid (DNA) sequences of said biological particle with said one or more physical properties. In some embodiments, said one or more DNA sequences comprise epigenetic information. In some embodiments, said one or more DNA sequences comprise chromatin information.

[0027] In another example, the present disclosures provides a kit comprising a plurality of particles, which plurality of particles comprise (i) a plurality of nucleic acid barcode molecules coupled thereto, wherein said plurality of barcode molecules comprise a plurality of barcode sequences, and (ii) a plurality of optical barcodes, wherein said plurality of optical barcodes comprise a plurality of optical codes, wherein each particle of said plurality of particles comprises (i) a subset of said plurality of nucleic acid barcode molecules coupled thereto and (ii) a subset of said plurality of optical barcodes, wherein barcode sequences of subsets of said plurality of nucleic acid barcode molecules differ across particles of said plurality of particles, and wherein optical codes of subsets of said plurality of optical barcodes differ across said particles.

[0028] In some examples, each subset of said subsets of said plurality of optical barcodes comprise a single optical barcode.

[0029] In some examples, each subset of said subsets of said plurality of optical barcodes comprise two or more optical barcodes.

[0030] In some examples, said plurality of optical barcodes confers optical properties to said plurality of particles. In some examples, said optical properties are selected from the group consisting of an absorbance, a birefringence, a color, a fluorescence characteristic, a luminosity, a photosensitivity, a reflectivity, a refractive index, a scattering, or a transmittance of said particle or a component thereof.

[0031] In some examples, said plurality of optical barcodes comprises one or more fluorescent dyes, nanoparticles, microparticles, or any combination thereof. In some examples, said plurality of optical barcodes comprises a plurality of nanoparticles. In some

examples, said plurality of optical barcodes comprises a plurality of quantum dots. In some examples, said plurality of optical barcodes comprises a plurality of Janus particles. In some examples, said plurality of optical barcodes comprises a plurality of fluorescent dyes. In some examples, said plurality of fluorescent dyes comprises between 2-10 fluorescent dyes. In some examples, said plurality of fluorescent dyes comprises a plurality of fluorescent dyes having the same emission wavelength and different intensities. In some examples, said plurality of fluorescent dyes comprises at least 20 different intensities.

[0032] In some examples, each subset of said subsets of said plurality of optical barcodes comprises different optical codes.

[0033] In some examples, each subset of said subsets of said plurality of optical barcodes comprises a different combination of optical codes.

[0034] In some examples, said plurality of optical barcodes has associated optical intensities or frequencies that are distinct with respect to one another.

[0035] In some examples, said plurality of optical barcodes comprises at least 1,000 different optical codes. In some examples, said plurality of optical barcodes comprises at least 10,000 different optical codes. In some examples, said plurality of optical barcodes comprises at least 100,000 different optical codes.

[0036] In some examples, nucleic acid barcode molecules within a given subset of said subsets of said plurality of nucleic acid barcode molecules comprise identical barcode sequences.

[0037] In some examples, said plurality of barcode sequences comprises at least 1,000 different barcode sequences. In some examples, said plurality of barcode sequences comprises at least 10,000 different barcode sequences. In some examples, said plurality of barcode sequences comprises at least 100,000 different barcode sequences.

[0038] In some examples, said plurality of particles comprises at least 10,000 particles. In some examples, said plurality of partitions comprises at least 100,000 particles.

[0039] In some examples, said plurality of nucleic acid barcode molecules comprise a plurality of functional sequences, which plurality of functional sequences are configured to interact with a plurality of target molecules. In some examples, said plurality of target molecules comprises a plurality of deoxyribonucleic acid molecules. In some examples, said plurality of target molecules comprises a plurality of ribonucleic acid molecules. In some examples, said plurality of functional sequences is configured to capture said plurality of target molecules.

[0040] In some examples, said plurality of particles comprises a plurality of beads. In some examples, said plurality of particles comprises a plurality of gel beads. In some examples, said plurality of nucleic acid barcode molecules is releasably coupled to said plurality of gel beads. In some examples, said plurality of gel beads comprises a polyacrylamide polymer.

[0041] In some examples, said plurality of optical barcodes is included on surfaces of said plurality of particles.

[0042] In some examples, said plurality of optical barcodes is included within said plurality of particles.

[0043] In some examples, first particles of said plurality of particles are coupled to second particles of said plurality of particles, wherein said first particles comprise nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules and said second particles comprise optical barcodes of said plurality of optical barcodes.

[0044] Additional examples and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, 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

[0045] 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

[0046] 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:

[0047] **FIG. 1** shows an example of a microfluidic channel structure for partitioning individual biological particles.

[0048] **FIG. 2** shows an example of a microfluidic channel structure for delivering barcode carrying beads to droplets.

[0049] **FIG. 3** shows an example of a microfluidic channel structure for co-partitioning biological particles and reagents.

[0050] **FIG. 4** shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets.

[0051] **FIG. 5** shows an example of a microfluidic channel structure for increased droplet generation throughput.

[0052] **FIG. 6** shows another example of a microfluidic channel structure for increased droplet generation throughput.

[0053] **FIG. 7A** shows a cross-section view of another example of a microfluidic channel structure with a geometric feature for controlled partitioning. **FIG. 7B** shows a perspective view of the channel structure of **FIG. 7A**.

[0054] **FIG. 8** shows an example of a microfluidic channel structure for optical detection of partitions.

[0055] **FIG. 9** shows an example of a procedure for electronically associating genetic and phenotypic information of biological particles.

[0056] **FIG. 10** shows a computer system that is programmed or otherwise configured to implement methods provided herein.

DETAILED DESCRIPTION

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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. 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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). 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.

[0066] 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 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.

[0067] 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 virus. The biological particle may be a cell or derivative of a cell. The biological particle may be an organelle. 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 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.

[0068] The biological particle may be a fixed cell or a population of fixed cells, such as a tissue. The biological particle may be FFPE cells or tissues. The biological particle may be obtained from an FFPE sample by laser capturing the cells.

[0069] A cell may split into two or more daughter cells, such as by binary fission, for example. The cell can be at any stage of cell division (e.g., prophase, metaphase). One or more physical properties of the biological particle can include a number of daughter cells, stage of cell division, and/or type of cell division.

[0070] 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. 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 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 mainly 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.

[0071] 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.

[0072] 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. The partition may isolate space or volume from another space or volume. The partition may be a droplet or well, for example. 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.

[0073] The present disclosure provides methods and systems for obtaining genetic and/or proteomic information from a biological particle (e.g., a cell) and associating such information with one or more physical properties of the biological particle, such as size, shape, or density, or any other phenotypic property of the biological particle. This may permit genetic and/or

proteomic information from the biological particle to be linked to the one or more physical properties of the biological particle.

Methods and systems for associating physical and genetic properties of biological particles

[0074] In an aspect, the present disclosure provides a method for processing a nucleic acid molecule of a biological particle (e.g., a cell). The method may comprise providing a plurality of partitions (e.g., a plurality of wells or droplets). A partition of the plurality of partitions may comprise (i) a particle comprising a plurality of nucleic acid barcode molecules; and (ii) a biological particle comprising the nucleic acid molecule. A nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules may comprise a barcode sequence. The method may further comprise providing a first data set comprising data indicative of one or more physical properties of the biological particle. The first data set may be stored in computer memory. The first data set may be generated upon sensing the biological particle, for example. The one or more physical properties may include, for example, a size, shape, or density of the biological particle. The sensing of the one or more physical properties may generate optical information, such as by optically detecting the biological particle and/or the partition.

[0075] A given nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules may be used to barcode the nucleic acid molecule of the biological particle (e.g., included within or derived from the biological particle), to generate a barcoded nucleic acid molecule. A second data set comprising data identifying a nucleic acid sequence of the barcoded nucleic acid molecule or a derivative thereof may then be generated (e.g., using nucleic acid sequencing). The first data set and the second data set may then be used to associate (e.g., electronically associate) the one or more physical properties of the biological particle with the nucleic acid sequence.

[0076] The biological particle can be a cell. As an alternative, the biological particle can be a gel or polymer matrix comprising the cell, a derivative of the cell, or one or more constituents of the cell.

[0077] In some examples, the biological particle is a cell. The cell may split into two or more daughter cells, such as by binary fission, for example. The cell can be at any stage of cell division (e.g., prophase, metaphase). The one or more physical properties of the cell can include a number of daughter cells, stage of cell division, and/or type of cell division. The biological particle may comprise a plurality of nucleic acid molecules. Some or all of the nucleic acid molecules may be disposed in the interior of the cell. Alternatively, the nucleic acid molecules

may be disposed external or partially external to the cell. The cell may be lysed or permeabilized to provide access to one or more nucleic acid molecules within the cell.

[0078] One or more nucleic acid molecules of or associated with the biological particle may be associated with the one or more physical properties of the biological particle. For example, at least 1, 10, 100, 1,000, 10,000, 100,000 or 1,000,000 nucleic acid molecules from or associated with the biological particle may be associated with the one or more physical properties of the biological particle.

[0079] One or more nucleic acid molecules may be conjugated to one or more antibodies coupled to one or more proteins coupled to a surface of the biological particle (e.g., cell). Identifying a sequence of such one or more nucleic acid molecules may permit identifying the one or more antibodies and the one or more proteins. The one or more proteins from the biological particle may subsequently be associated with the one or more physical properties of the biological particle. Such proteomic information may be used in association with genetic information from the biological particle, such as DNA sequence information, transcriptome information (i.e., sequences of transcripts), or both. For example, a cell surface protein of a cell can be identified using one or more antibodies. The cell surface protein can be associated with one or more physical properties of the cell (e.g., a shape of the cell) with a nucleic acid molecule. The one or more physical properties can be characterized by imaging the cell. The nucleic acid molecule of a derivative thereof of the cell can be sequenced to obtain a nucleic acid sequence. The nucleic acid sequence can be associated with the cell surface protein, in turn, with the one or more physical properties of the cell (e.g., a shape of the cell). In some cases, one or more ribonucleic acid (RNA) sequences and/or one or more deoxyribonucleic acid (DNA) sequences of a biological particle (e.g., cell) can be associated with one or more physical properties. In some cases, the one or more DNA sequences can comprise epigenetic information. For example, the one or more DNA sequences can include methylated DNA (e.g., 5-methylcytosine) as indicated by an epigenetic assay (e.g., bisulfite sequencing). In some cases, the one or more DNA sequences can include chromatin information, such as a lower nucleosome occupancy resulting in a highly accessible chromatin or "open" chromatin. The open chromatin can be as assessed by subjecting the one or more DNA sequences to an enzymatic treatment (e.g., DNase I, transposase), releasing a segment of nucleic acid molecules between two nucleosomes in an open chromatin.

[0080] A partition (e.g., droplet) can comprise a particle (e.g., bead) and a biological particle (e.g., cell). The partition can be distinguished from other partitions in order to uniquely identify

the partition. The partition can be characterized by, for example, imaging the partition to obtain optical information of the particle, a plurality of nucleic acid barcode molecules attached to the particle and/or the biological particle. In an example, the partition may be distinguished from other partitions by the particle included in the partition. For example, the particle may include one or more features such as one or more optical barcodes or other characteristics that may be used to distinguish the partition from other partitions. A lookup table (LUT) can be used to associate the plurality of nucleic acid barcode molecules to the particle. The optical information of the partition can permit associating the particle (e.g., gel bead) with the biological particle (e.g., cell) in the partition (e.g., droplet). The association of the particle with the biological particle can further permit associating a nucleic acid sequence of a nucleic acid molecule of the biological particle to one or more physical properties (e.g., a color of a cell) of the biological particle. For example, the partition (e.g., droplet) can include a particle (e.g., bead) having a first color and a biological particle (e.g., cell) having a second color or other optical characteristic, such as a shape or morphology of the biological particle, which first color and second color or characteristic can be associated with one another. The particle can have a plurality of nucleic acid barcode molecules attached thereto. The plurality of nucleic acid barcode molecules can comprise barcode sequences. The plurality of nucleic acid molecules attached to a given particle can have the same barcode sequence.

[0081] In some examples, the LUT can be used to associate a feature (e.g., an optical barcode, such as a color and/or intensity) of the particle with the barcode sequence. The feature may derive from the particle or an optical tag associated with the particle. The partition (e.g., droplet) can be imaged to obtain optical information of the partition, including, for example, the feature (e.g., color and/or intensity) of the particle or the optical tag associated with the particle, and optical information of the biological particle in the partition. For example, an image can include optical information in the visible spectrum, non-visible spectrum, or both. For example, multiple images may be obtained of a partition across various optical frequencies.

[0082] The biological particle can comprise a nucleic acid molecule which can be barcoded with a barcode sequence of a nucleic acid barcode molecule of the particle in the partition to provide a barcoded nucleic acid molecule. The barcoded nucleic acid molecule can be sequenced to obtain a nucleic acid sequence. The nucleic acid sequence can comprise genetic information of the biological particle. The nucleic acid sequence may comprise the barcode sequence, or a complement thereof. The barcode sequence, or complement thereof, of the nucleic acid sequence can be electronically associated with the feature (e.g., color and/or intensity) of the

particle using the LUT to identify the particle. Alternatively, the LUT may be used to look up the barcode sequence to identify the feature (e.g., color and/or intensity), which may subsequently be used to identify an image having such feature, which may identify the partition comprising the particle. Identification of the particle can be useful in identifying the partition. This may in turn permit identification of the biological particle by using the optical information of the partition. This may permit genotypic information from a biological particle (e.g., cell) to be associated with phenotypic information of the biological particle.

[0083] In an example, an image of a partition (e.g., droplet) comprising a biological particle (e.g., cell or cell bead) and a particle (e.g., bead) comprising nucleic acid barcode molecules is captured. The nucleic acid barcode molecules include the same barcode sequence. The image identifies the particle as having a color (e.g., a green color) and also captures a shape of the biological particle (e.g., cell). The nucleic acid barcode molecules are used to barcode a nucleic acid molecule (e.g., an RNA molecule) from the biological particle (e.g., cell) to generate a sequencing library that is subsequently sequenced to yield a plurality of sequencing reads. The some or all of the plurality of sequencing reads include the barcode sequence. Next, an LUT is used to associate the color of the particle from the captured image to the barcode sequence, which permits sequencing reads generated from the sequencing library to be identified. The sequencing reads are then associated with the shape of the biological particle.

[0084] In some cases, one or more optical properties of the partition (e.g., droplet) and/or components (e.g., particle or biological particle) thereof can be used to distinguish the individual partition from other partitions. In some cases, the optical properties of the partition, such as color of a partitioning fluid (e.g., aqueous fluid) can be used to distinguish the partition from other partitions. For example, the partitions can have different colors associated therewith to be able to distinguish from the partitions from one another. In some cases, the partitions can include droplets having different colors associated therewith. The partition can be any container or a vessel, such as a well, microwell, tube, nanoarrays or other containers. In some cases, the plurality of partitions can include at least 1,000 partitions, at least 10,000 partitions or at least 100,000 partitions. In such cases, at least a portion of the plurality of partitions can have one or more unique optical properties. In an example, one or more optical properties of the partition (e.g., droplet) and/or components (e.g., particle or biological particle) thereof may be used to identify whether the partition includes a particle and/or biological particle or not (e.g., whether the partition is occupied or unoccupied).

[0085] Non-limiting examples of optical properties of a partition or a component thereof (e.g., particle or biological particle) can include absorbance, birefringence, color, fluorescence, luminosity, photosensitivity, reflectivity, refractive index, scattering, or transmittance. Such optical properties may be associated with the partition, particle, or biological particle, such as associated with a signal directly from the partition, particle, or biological particle, or from one or more moieties coupled to the partition, particle, or biological particle.

[0086] The partitions and/or components thereof (e.g., particles or biological particles) can be made optically detectable by including an optical material in the partition to distinguish the partitions from one another. For example, the plurality of partitions can comprise a first partition and a second partition. The first partition and the second partition can yield optical signals at different intensities and/or frequencies. For example, the first and second partitions can include one or more optical materials such as one or more fluorescent dyes, nanoparticles, and/or microparticles that can provide a distinct optical signal. The one or more optical materials may be associated with the partition (e.g., a partitioning fluid), the particle (e.g., nucleic acid barcode molecules of the particle), or the biological particle. In some examples, the partitioning fluid (e.g., aqueous fluid) can include a fluorescent dye. In some examples, the partitioning fluid can include nanoparticles, such as quantum dots, to provide enhanced spectral resolution in order to distinguish between the partitions. In some cases, the one or more optical materials can be introduced prior to or subsequent to generating partitions. For example, a fluorescent dye can be injected into the partitioning fluid after generating partitions (e.g., droplets). In some examples, a fluorescent dye can be included in the partitioning fluid before generating partitions (e.g., droplets).

[0087] In some cases, optical properties of the particles (e.g., beads) can be used for optical detection of the particles (e.g., within the partitions). For example, the particles can have different colors to be able to distinguish from one another. In some examples, the particles can have different refractive indices based on the composition of the particles. The particles may be beads such as gel beads. Gel beads may comprise a polyacrylamide polymer. Gel beads may have different birefringence values based on degree of polymerization, chain length, or monomer chemistry. The particle can include (e.g., encapsulate or have attached thereto) a plurality of nucleic acid molecules, which can be nucleic acid barcode molecules. In some cases, optical properties of the nucleic acid barcode molecules can be used for optical detection of the particles. For example, the absorbance of light by the nucleic acid barcode molecules can be used to distinguish the particles from one another.

[0088] The particles can be made optically detectable by including optical barcodes in the particles, on surfaces of the particles, or both in and on surfaces of the particles. The optical barcodes can be on interior and/or exterior surfaces of the particles. Optical barcodes may comprise optical codes (e.g., one or more characteristic frequencies and/or intensities). Non-limiting examples of the optical barcodes can include fluorescent dyes, nanoparticles, and/or microparticles. For example, the particles can include a plurality of different fluorescent dyes. Fluorescent dyes may be attached to the surface of the particles and/or may be incorporated into the particles for use as optical barcodes or components thereof. Fluorescent dyes may be attached to a surface of particles, incorporated within particles, and/or attached to nucleic acid barcode molecules coupled to particles. Different intensities of the different fluorescent dyes may be used to increase the number of optical barcodes (e.g., optical codes) that may be used. For example, if N is the number of fluorescent dyes (e.g., between 2 and 10 fluorescent dyes, such as 4 fluorescent dyes) and M is the possible intensities for the dyes (e.g., between 2 and 50 intensities, such as 20 intensities), then M^N are the possible distinct optical barcodes. In one example, 4 fluorescent dyes with 20 possible intensities will be used to generate 160,000 distinct optical barcodes. In some examples, nanoparticles, such as quantum dots or Janus particles, can be used as optical barcodes or components thereof. In some examples, the quantum dots can be attached to nucleic acid barcode molecules of the particles. Optical barcodes (e.g., optical codes) of particles may provide enhanced spectral resolution in order to distinguish between particles with unique nucleic acid barcode molecules (e.g., particles comprising unique barcode sequences). In an example, a first particle comprises a first optical barcode and nucleic acid barcode molecules each having a first barcode sequence. A second particle comprises a second optical barcode and nucleic acid barcode molecules each having a second barcode sequence. The first optical barcode and second optical barcode may be different (e.g., provided by two different fluorescent dyes or the same fluorescent dye at two different intensities). The first and second barcode sequences may be different nucleic acid sequences. The particles may be imaged to sense the first and second optical barcodes and the first and second optical barcodes may then be used to associate the first and second optical barcodes with the first and second barcode sequences, respectively.

[0089] Optical barcodes may be included while generating the particles. For example, the particles can be beads (e.g., gel beads). Gel beads can comprise polyacrylamide polymers. Optical barcodes can be included in the polymer structure, or attached at the pre-polymer or monomer stage in bead production. In some cases, the beads may comprise moieties that can

attach to one or more optical barcodes (e.g., at a surface of a bead and/or within a bead). In some cases, optical barcodes can be loaded into the beads with one or more reagents. For example, reagents and optical barcodes can be loaded into the beads by diffusion of the reagents (e.g., a solution of reagents comprising the optical barcodes). In some cases, optical barcodes can be included while preparing nucleic acid barcode molecules. For example, nucleic acid barcode molecules can be prepared by synthesizing molecules comprising barcode sequences (e.g., using a split pool or combinatorial approach). Optical barcodes may be attached to nucleic acid barcode molecules prior to attaching the nucleic acid barcode molecules to a particle. In some cases, optical barcodes can be included after attaching nucleic acid barcode molecules to a particle. For example, optical barcodes can be attached to nucleic acid barcode molecules coupled to the particle. Nucleic acid barcode molecules or sequences thereof can be releasably attached to the particle. Optical barcodes can be releasably or non-releasably attached to the particle. In some cases, a firstparticle (e.g., a particle comprising a plurality of nucleic acid barcode molecules) can be coupled to a second particle comprising one or more optical barcodes. For example, the first particle can be covalently coupled to the second particle via a chemical bond. In some cases, the first particle can be non-covalently associated with the second particle. The first and/or second particle may comprise a plurality of nucleic acid barcode molecules. The plurality of nucleic acid barcode molecules coupled to a given particle can comprise the same barcode sequences. Where both the first and second particles comprise nucleic acid barcode molecules, the first and second particles may comprise nucleic acid barcode molecules comprising the same barcode sequences or different barcode sequences.

[0090] Optical properties of a biological particles (e.g., cell) can be used for optical detection of the biological particles. The optical detection of the biological particles can provide one or more physical properties (e.g., optical information) of the biological particle. The one or more physical properties of the biological particle can include phenotypic information. The phenotypic information of the biological particle can include one or more members selected from the group consisting of a size of the biological particle, a shape of the biological particle, a surface marker on the biological particle, an inclusion in the biological particle, a structure of an organelle in the biological particle, number of organelles in the biological particle, secretion or excretion with respect to the biological particle, a localization of an organelle in the biological particle, a circularity of the biological particle, a density of the biological particle, symmetry of the biological particle, and hardness of the biological particle. Optical properties comprising or correlated with phenotypic information can be used to characterize the biological particle. For

example, absorbance of an inclusion in the cell (e.g., pigment granules) can be used to distinguish biological particles from one another. In some cases, a biological particle can include one or more components of a cell in a matrix (e.g., polymeric matrix or a gel matrix). In such cases, optical properties of the matrix can be used for optical detection of a biological particle (e.g., cell) or components thereof included in the matrix. In some cases, phenotypic information of the biological particle (e.g., cell) can be preserved by encapsulating (e.g., in microcapsule) the biological particle prior to partitioning.

[0091] Biological particles can be made optically detectable by including optical materials (e.g., optical barcodes or labels) in the biological particles, on surfaces of the biological particles, or both in and on surfaces of the biological particles. Optical materials can be on the interior and or exterior surfaces (e.g., cell membranes) of biological particles. For example, biological particles (e.g., cells) or the components thereof (e.g., nucleic acid molecule) can be labelled with fluorescent dyes. In some examples, nucleic acid molecules, such as DNA molecules, can be fluorescently labelled using 4',6-diamidino-2-phenylindole (DAPI). The fluorescence from binding of DAPI to DNA can be imaged to optically detect the biological particle. In some cases, optical materials can be attached to antibodies. For example, a biological particle or components thereof can be optically detected using optical materials (e.g., fluorescent dyes such as Alexa Fluor) conjugated to an antibody against a protein. In some cases, biological particles can be engineered to express one or more optical materials. For example, a biological particle (e.g., cell) can be engineered to express Green Fluorescent Protein (GFP).

[0092] One or more physical properties of a partition (e.g., droplet) and/or components (e.g., particle and/or biological particle) thereof can be used to distinguish a partition from other partitions. Non-limiting examples of the physical properties can include size, shape, circularity, hardness, volume, or symmetry of the partition and/or components thereof. In some cases, physical properties of a partition can be used to distinguish the partition from other partitions. For examples, the volume of the partitioning fluid (e.g., aqueous fluid) can be used to distinguish the partition from other partitions. In other examples, the shape and/or the symmetry of the partition (which may reflect characteristics of the particle and/or biological particle included therein) can be used to distinguish the partition form other partitions. Partitions can be made physically detectable by generating partitions with unique physical properties. In some cases, the plurality of partitions can include at least 1,000 partitions, at least 10,000 partitions or at least 100,000 partitions. In such cases, at least a portion of the partitions can have one or more unique physical properties. For example, the partitions can be generated with different volumes. In some

cases, the volume of partitioning fluids (e.g., aqueous fluid) can be different in the plurality of partitions.

[0093] Physical properties of particles (e.g., beads) can be used to characterize the particles (e.g., within partitions). The particles can be beads, such as gel beads. The particles can be biological particles. The particles can include (e.g., encapsulate or have attached thereto) nucleic acid molecules, which can be nucleic acid barcode molecules. The nucleic acid barcode molecules can include barcode sequences (e.g., nucleic acid barcode sequences). The barcode sequences can be the same across a plurality of the nucleic acid barcode molecules (e.g., a plurality of nucleic acid barcode molecules coupled to a given bead). Non-limiting examples of physical properties of particles can include size, shape, circularity, density, symmetry, and hardness. For example, particles can be of different sizes. Different sizes of particles can be obtained by using microfluidic channel networks configured to provide specific sized particles (e.g., based on channel sizes, flow rates, etc). In some examples, particles can have different hardness values that can be obtained by varying the concentration of polymer used to generate the particles. In some cases, a nucleic acid barcode molecule attached to a particle (e.g., bead) can be made optically detectable using a physical property of the nucleic acid molecule. For example, a nucleic acid origami, such as a deoxyribonucleic acid (DNA) origami can be used to generate an optically detectable nucleic acid barcode molecule. For example, a nucleic acid molecule, or a plurality of nucleic acid molecules, can be folded to create two-and/or three-dimensional geometric shapes. The different geometric shapes can then be further optically detected. In some examples, special types of nanoparticles with more than one distinct physical property can be used to make the particles physically distinguishable. In an example, Janus particles with both hydrophilic and hydrophobic surfaces can be used to provide unique physical property. In some cases, physical properties of particles can be characterized prior to and/or subsequent to partitioning.

[0094] A partition can be characterized using one or more physical and/or optical properties of the partitions and/or components thereof (e.g., particles, nucleic acid barcode molecules of particles, and/or biological particles) by imaging the partition. The partition can be imaged using an imaging unit. The imaging unit can be configured to image the partition based on an expected property (e.g., optical property) of the partition to be characterized. For example, an imaging unit can be configured to excite and subsequently detect an emission wavelength of a partition comprising one or more fluorescent dyes. The imaging unit can be configured to perform bright field microscopy, fluorescence microscopy, phase contrast microscopy, multispectral

microscopy, or polarization microscopy. The imaging unit can be configured to include an optical sensor based on the microscopy performed. In some examples, the imaging unit can be configured to perform bright field microscopy to determine the physical property of the partition (e.g., shape and/or size of the partition, particle and the biological particle). In other examples, the imaging unit can be configured to perform fluorescence microscopy to image the partition (e.g., fluorescently-labelled partition, particle and/or biological particle). In some cases, microfluidic chips can include additional features for imaging the individual partitions without interference. For example, the microfluidic chip can include features, such as rails, asymmetric turns, herringbone mixers, to facilitate the imaging of the partitions without interference.

[0095] As illustrated in **FIG.8**, the microfluidic channel structure **800** can include the channel segments **801**, **802**, **804**, **806**, and **808** communicating at a channel junction **810**. In operation, the channel segment **801** can transport an aqueous fluid or a first liquid phase that includes a plurality of beads **812** (e.g., with nucleic acid barcode molecule), and reagent **814** along the channel segment **801**. The plurality of beads **812** can be releasably attached to a plurality of nucleic acid barcode molecules with barcode sequences and/or additional sequences (e.g., R1 and R2). The plurality of beads **812** may be sourced from a suspension of beads. For example, the channel segment **801** may be connected to a reservoir comprising an aqueous suspension of beads. The reagent **814** can include a lysis reagent, PCR reagents, ligase etc. The channel segment **802** may transport the aqueous fluid that includes a plurality of biological particles **816** along the channel segment **802** into junction **810**. The plurality of biological particles **816** may be sourced from a suspension of biological particles. The plurality of biological particles **816** can be cells or derivate thereof, such as macromolecular constituents. The plurality of biological particles **816** can be a sample obtained from formalin-fixed paraffin embedded tissues. A second fluid or a second liquid phase **818** that is immiscible with the aqueous fluid (e.g., oil) can be delivered to the junction **810** via channel segment **804**. Upon meeting of the aqueous fluid from each of channel segments **801** and **802** and the second fluid **818** from the channel segment **804** at the channel junction **810**, the aqueous fluid can be partitioned as discrete droplets **816** in the second fluid **814** and flow away from the junction **810** along channel segment **808**. The channel segment **808** may deliver the discrete droplets to an outlet reservoir fluidly coupled to the channel segment **808**, where they may be harvested. An individual droplet **820** may include a biological particle, a particle and reagents. The individual droplet **820** can be imaged with an imaging unit **822**. The imaging unit **822** can be communicatively coupled to the microfluidic channel structure **800**. The imaging unit **822** can be

configured to image the individual droplet as the droplet flows through the channel segment **808**. The imaging unit **822** can provide optical information of the droplet to a controller **824**. The controller **822** can be operatively coupled to the imaging unit **822**. The controller **824** can be configured to direct fluid flow in the channel segment **808**. The controller **824** can be configured to communicate the optical information to a processor **826** for further processing. As an alternative to the imaging unit **822** or in addition to the imaging unit **822**, other sensors may be used to detect one or more physical properties of the individual droplet **820** and/or constituents of the individual droplet **820**.

[0096] In some cases, more than one property of and/or from the partition can be assessed. For example, size of the particle and optical barcodes on the particle can be imaged using the imaging unit. In some cases, the partition can be imaged prior to injecting the particle in the partition. For example, the biological particle (e.g., cell, cell bead or microcapsule) can be imaged prior to injecting the particle (e.g., bead). The biological particle can be imaged prior to and subsequent to partitioning into the partition (e.g., droplet). In another example, the partition can be imaged after injecting the particle in the partition. For example, the partition comprising the biological particle and the particle can be imaged. Upon imaging the partition, optical information can be generated. The optical information can include optical information of the partition and/or particle. In some cases, the optical information can include optical information of the particle (e.g., bead). The optical information can include phenotypic information of the biological particle (e.g., cell). In some cases, the optical information can include the optical information of the particle and the phenotypic information of the biological particle.

[0097] Upon imaging, the individual partitions can carry out reactions to generate a barcoded nucleic acid molecule. The biological particle can comprise a nucleic acid molecule. The biological particle can comprise a plurality of nucleic acid molecules. The nucleic acid molecule(s) can comprise ribonucleic acid (RNA). The nucleic acid molecule(s) can comprise deoxyribonucleic acid (DNA). The biological particle can be a single biological particle, such as a cell or a polymer matrix (e.g., cell bead) as disclosed elsewhere herein. The biological particle can be a plurality of biological particles, such as a plurality of cells. The biological particle can comprise a live or fixed cell, such as FFPE cells. The biological particle can be any cellular derivative or combination thereof, such as DNA, RNA, nucleus, etc. The biological particle can be encapsulated in a gel matrix to preserve the particle.

[0098] The biological particle can be encapsulated prior to and/or subsequent to partitioning into a partition. The partition can be any container or a vessel, such as a well, microwell, tube,

nanoarrays or other containers. The partition can be flowable within fluid streams, such as a microcapsule having an inner fluid core surrounded by an outer barrier. The partition can be a droplet of aqueous fluid within a non-aqueous phase, such as an oil phase. Partitioning one or more materials (e.g., a particle and a biological particle) into a partition can be performed by any of the methods disclosed herein.

[0099] The particle can comprise a plurality of nucleic acid barcode molecules. The plurality of nucleic acid barcode molecules can comprise barcode sequences (e.g., nucleic acid barcode sequences). In some cases, the barcodes sequences can be the same across a plurality of nucleic acid barcode molecules. The plurality of partitions can comprise a plurality of particles comprising a plurality of nucleic acid barcode molecules. The plurality of nucleic acid barcode molecules can comprise at least 1,000 barcode sequences which at least 1,000 barcode sequences, at least 10,000 barcode sequences or at least 100,000 barcode sequences can be different across the plurality of partitions. In some cases, the nucleic acid barcode molecule can comprise additional sequences. The additional sequences can include a primer binding site, such as a sequencing primer site (e.g., R1 or R2), flow cell binding sequences (e.g., P5, P7). A primer binding site can comprise sequences for sequencing primers to hybridize with a nucleic acid in a sequencing reaction. A primer binding site can comprise sequences for primers to hybridize with a nucleic acid in amplification or other extension reactions. The additional sequences can be useful in downstream assays, such as a sequencing assay. The additional sequences can be selected based on the assay used.

[00100] Once released from the bead, a give nucleic acid barcode molecule or portion thereof of a plurality of the nucleic acid molecules coupled to a particle can barcode the nucleic acid molecule from the biological particle to generate a barcoded nucleic acid molecule. The given nucleic acid barcode molecule can attach to the nucleic acid molecule by annealing, extension and amplification reaction and/or ligation reactions. Extension and amplification reagents, such as DNA polymerase, nucleoside triphosphates, and buffers with co-factors (e.g. Mg^{2+}), can be co-partitioned with biological particles and beads. The nucleic acid barcode molecule can be attached at either one or both ends of the segment to yield a barcoded nucleic acid molecule. Alternatively or in addition, a nucleic acid hybridization and extension process may take place within a partition and additional extension or amplification reactions may take place outside a partition (e.g., upon pooling materials from a plurality of such partitions).

[00101] Barcoded nucleic acid molecules or derivatives thereof (e.g., barcoded nucleic acid molecules to which one or more functional sequences have been added, or from which one or

more features have been removed) of different partitions can be pooled and processed together for subsequent analysis such as sequencing on high throughput sequencers. Processing with pooling may be implemented using barcode sequences. For example, barcoded nucleic acid molecules of a given partition may have the same barcode sequence, which barcode sequence is different from barcode sequences of other partitions. Alternatively, barcoded nucleic acid molecules of different partitions can be processed separately for subsequent analysis (e.g., sequencing). Sequencing of barcoded nucleic acid molecules (e.g., in a pooled mixture or separately) can provide sequencing reads comprising nucleic acid sequences. Such nucleic acid sequences may comprise the barcode sequences of the barcoded nucleic acid molecules, or complements thereof. For example, a plurality of sequencing reads corresponding to a given partition (e.g., a given barcoded nucleic acid molecule or collection thereof) may be generated, in which a subset of the plurality of sequencing reads comprises the barcode sequence of the barcoded nucleic acid molecule or a complement thereof. The nucleic acid sequence may comprise a sequence corresponding to the nucleic acid barcode molecules of a particle in a partition and/or a sequence corresponding to the nucleic acid molecule of a biological particle in the partition.

[00102] A barcode sequence in a nucleic acid sequence can enable electronic association of physical and/or optical information of the biological particle (e.g., cell) from which a nucleic acid molecule (and thus barcoded nucleic acid molecule) was derived with the nucleic acid sequence. For example, a barcode sequence in a nucleic acid sequence can enable the identification of the particle from which the barcode sequence of the nucleic acid barcode molecule was derived. The identification of the particle can enable identification of the partition by electronically associating physical and/or optical information of the particle with the partition. The identification of the partition can enable identification of the biological particle in the partition by electronically associating the optical information of the biological particle (e.g., cell) with the partition. The identification of the biological particle can enable associating any phenotypic information (e.g., cell size, shape, etc.) associated with the biological particle with the nucleic acid sequence. In some cases, the electronic association of the phenotypic information of the biological particle with the nucleic acid sequence may be useful in assaying cell response to a therapy. For example, the response to the therapy may include changes in phenotypic information of the cell (e.g., number of organelles). The changes in phenotypic information of the cell can be associated with the nucleic acid sequence of the nucleic acid molecule of the cell.

[00103] As illustrated in **FIG. 9**, a plurality of partitions can include a first partition **902** and a second partition **904**. A first partition **902** can include a first biological particle (e.g., cell) **906** and a first particle (e.g., gel bead) **908**. A second partition **904** can include a second biological particle (e.g., cell) **910** and a second particle (e.g., gel bead) **912**. The first partition **902** and the second partition **904** can each have a unique physical and/or optical property for differentiating from other partitions. The unique physical and/or optical property may be a property of the partition itself (e.g., a volume or other feature of the partition) and/or a property of a component of the partition, such as a particle or biological particle of the partition. For example, the first particle **908** can be a round bead and the second particle **912** can be a square bead. The first and second particles can comprise a plurality of nucleic acid barcode molecules coupled thereto. The plurality of nucleic acid barcode molecules can comprise barcode sequences. The barcode sequences of nucleic acid barcode molecules coupled to a given particle (e.g., the first particle) can be the same (e.g., nucleic acid barcode molecules coupled to the first particle each comprise the same barcode sequence and nucleic acid barcode molecules coupled to the second particle each comprise the same barcode sequence), while the barcode sequences associated with the first particle may be different from the barcode sequences associated with the second particle. For example, the first particle can have a first nucleic acid barcode molecule comprising a first barcode sequence **914** and the second particle can have a second nucleic acid barcode molecule comprising a second barcode sequence **916**. The first barcode sequence of the first nucleic acid barcode molecule **914** can be coupled to the first particle **908** before and/or after partitioning the particle. The second barcode sequence of the second nucleic acid barcode molecule **916** can be coupled to the second particle **912** before and/or after partitioning the particle. A lookup table (LUT) can be used to associate the shape, color, or a combination of shape and color (or other optical properties) of the particle (e.g., round or square bead) with the barcode sequence (e.g., first or second barcode sequence). For example, the first particle **908** with the round bead can be associated with the first barcode sequence of the first nucleic acid barcode molecule **914.** The second particle **912** with the square bead can be associated with the second barcode sequence of the second nucleic acid barcode molecule **916**. Alternatively or in addition to, color may be used to differentiate the first particle **908** and the second particle **912**.

[00104] With continued reference to **FIG. 9**, the first and second biological particles can have different phenotypic properties, such as a circular shape of the cell and a square shape of the cell, respectively. The first and the second partitions can be imaged to obtain a first optical characterization and a second optical characterization of the partitions in operations **918** and **920**,

respectively. The first and second optical characterizations can include the physical characterization of the particles and the phenotypic information of the biological particles. The biological particles (e.g., first biological particle) can each comprise a nucleic acid molecule. The nucleic acid molecule of the first biological particle **906** can be barcoded using the first barcode sequence of the first nucleic acid barcode molecule **914** to generate a first barcoded nucleic acid molecule **922**. The nucleic acid molecule of the second biological particle **910** can be barcoded using the second barcode sequence of the second nucleic acid barcode molecule **916** to generate a second barcoded nucleic acid molecule **924**. The first and second barcoded nucleic acid molecules can be sequenced (e.g., together or separately) to generate a first nucleic acid sequence **926** and a second nucleic acid sequence **928**, respectively, corresponding to the nucleic acid molecules of the first and second biological particles and/or the first and second barcode sequences.

[00105] With continued reference to **FIG. 9**, a barcode sequence in a nucleic acid sequence can enable identification of the partition from which the barcode sequence derives (e.g., the particle from which the barcode sequence derives) by electronically associating the nucleic acid sequence with physical and/or optical information of the partition. The electronic association can permit associating nucleic acid sequence with phenotypic information (e.g., shape of cell) of the biological particle (e.g., cell). The nucleic acid sequence can comprise genetic information of the biological particle. For example, in operation **930**, the first barcode sequence of the first barcode nucleic acid molecule **914** in the first nucleic acid sequence **926** can be used to identify the first particle **908** using a LUT. Identifying the first particle **908** can permit identifying of the first biological particle **906** by using the first optical characterization **918**. Further, the phenotypic information of the first biological particle **906** (e.g., circular cell) can be associated with the first nucleic acid sequence **926,** in turn to genetic information of the biological particle. Similarly, in operation **932**, the second barcode sequence of the second nucleic acid barcode molecule **916** in the second nucleic acid sequence can be used to identify the second particle **912** using a LUT. Identifying the second particle **912** can permit identifying of the second biological particle **910** by using the second optical characterization **920**. Further, the phenotypic information of the second biological particle **910** (e.g., square shape of cell) can be associated with the second nucleic acid sequence **928**, in turn to genetic information of the biological particle.

[00106] The present disclosure also provides a kit comprising a plurality of particles (e.g., as described herein). A kit may comprise a plurality of particles, which plurality of particles comprise (i) a plurality of nucleic acid barcode molecules coupled thereto, wherein the plurality

of barcode molecules comprise a plurality of barcode sequences. The plurality of particles may also comprise (ii) a plurality of optical barcodes, wherein the plurality of optical barcodes comprise a plurality of optical codes. Each particle of the plurality of particles may comprise (i) a subset of the plurality of nucleic acid barcode molecules coupled thereto and (ii) a subset of the plurality of optical barcodes. Barcode sequences of subsets of the plurality of nucleic acid barcode molecules differ across particles of the plurality of particles, and optical codes of subsets of the plurality of optical barcodes differ across the particles. Each subset of the subsets of the plurality of optical barcodes may comprise a single optical barcode or two or more optical barcodes. The plurality of optical barcodes may confer optical properties to the plurality of particles. Such optical properties may be selected from the group consisting of, for example, an absorbance, a birefringence, a color, a fluorescence characteristic, a luminosity, a photosensitivity, a reflectivity, a refractive index, a scattering, or a transmittance of the particle or a component thereof.

[00107] As described elsewhere herein, optical barcodes may comprise one or more fluorescent dyes, nanoparticles, microparticles, or any combination thereof. For example, optical barcodes may comprise a plurality of nanoparticles, such as a plurality of quantum dots or Janus particles. In another example, optical barcodes may comprise a plurality of fluorescent dyes, such as between 2-10 fluorescent dyes, such as 4 fluorescent dyes. A plurality of fluorescent dyes may comprise fluorescent dyes having the same emission wavelength and different intensities, such as between 2 and 50 different intensities, such as 20 different intensities. Each subset of the subsets of the plurality of optical barcodes may comprise different optical codes. A plurality of optical barcodes may have associated optical intensities or frequencies that are distinct with respect to one another. A plurality of optical barcodes may comprise at least 1,000 different optical codes, such as at least 10,000 different optical codes or at least 100,000 different optical codes.

[00108] As described elsewhere herein, nucleic acid barcode molecules within a given subset of the subsets of the plurality of nucleic acid barcode molecules may comprise identical barcode sequences. A plurality of barcode sequences may comprise at least 1,000 different barcode sequences, such as at least 10,000 different barcode sequences or at least 100,000 different barcode sequences. A plurality of nucleic acid barcode molecules may comprise a plurality of functional sequences (e.g., starter sequences, sequencing adapters, priming sequences, random N-mers, capture sequences, etc.). Functional sequences may be configured to interact with one or more target molecules, such as one or more different types of target molecules (e.g., RNA

molecules and DNA molecules). In an example, a plurality of nucleic acid barcode molecules attached to a given particle may each comprise an N-mer sequence (e.g., a random N-mer sequence), which N-mer sequences are configured to interact with a plurality of DNA molecules. In another example, a plurality of nucleic acid barcode molecules attached to a given particle may each comprise a poly(T) sequence, which poly(T) sequences are configured to interact with a plurality of RNA molecules (e.g., messenger RNA (mRNA) molecules). Functional sequences may be configured to capture target molecules. For example, functional sequences may be configured to hybridize to target molecules to facilitate nucleic acid extension reactions.

[00109] The plurality of particles of a kit may comprise at least 10,000 particles, such as at least 100,000 particles. The plurality of particles may comprise a plurality of beads, such as a plurality of gel beads. The plurality of gel beads may comprise polyacrylamide polymers. The plurality of gel beads may each comprise a plurality of nucleic acid barcode molecules releasably coupled thereto. For example, a first bead may comprise a first plurality of nucleic acid barcode molecules releasably coupled thereto, and a second bead may comprise a second plurality of nucleic acid barcode molecules releasably coupled thereto. Optical barcodes (e.g., optical materials such as fluorescent dyes) may be included on surfaces of particles and/or within particles. In an example, first particles of the plurality of particles are coupled to second particles of the plurality of particles, wherein the first particles comprise nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules and the second particles comprise optical barcodes of the plurality of optical barcodes.

Sample partitioning

[00110] Provided herein are methods and systems for processing a nucleic acid molecule from a cell. The methods can be useful for electronically associating optical information of the cell with a nucleic acid sequence of a nucleic acid molecule from the cell.

[00111] In an aspect, the systems and methods described herein provide for the compartmentalization, depositing, or partitioning of macromolecular constituent contents of individual biological particles 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.

[00112] A partition of the present disclosure may comprise 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 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 further below. 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.

[00113] 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 comprise droplets of aqueous fluid within a non-aqueous continuous phase (e.g., oil phase). The partitions can comprise droplets of a first phase within a second phase, wherein the first and second phases are immiscible. 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.

[00114] In the case of droplets in an emulsion, allocating individual biological particles to discrete partitions may in one non-limiting example be accomplished by introducing a flowing stream of biological particles in an aqueous fluid into a flowing stream of a non-aqueous fluid, such that droplets are generated at the junction of the two streams. By providing the aqueous stream at a certain concentration and/or flow rate of biological particles, the occupancy of the resulting partitions (e.g., number of biological particles per partition) can be controlled. Where single biological particle partitions are used, 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, cell or cellular material). In some embodiments, the relative flow rates of the fluids can be selected 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.

[00115] **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.

[00116] 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.

[00117] 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 biological particles, cell beads, and/or gel 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.

[00118] 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.

[00119] 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.

[00120] 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.

[00121] 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 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.

[00122] 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 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 (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), or the like, and/or a combination of the above.

[00123] 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.

[00124] 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.

[00125] 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.).

[00126] 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.

[00127] 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.

[00128] 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.

[00129] 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." 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.

[00130] 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. 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

[00131] 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.

[00132] In some cases, barcoded nucleic acid molecules can be initially associated with the microcapsule and then released from the microcapsule. Release of the barcoded nucleic acid molecules can be passive (e.g., by diffusion out of the microcapsule). In addition or alternatively, release from the microcapsule can be upon application of a stimulus which allows the barcoded nucleic acid molecules to dissociate or to be released from the microcapsule. Such stimulus may disrupt the microcapsule, an interaction that couples the barcoded nucleic acid molecules to or within the microcapsule, or both. Such stimulus can include, for example, a thermal stimulus, photo-stimulus, chemical stimulus (e.g., change in pH or use of a reducing agent(s)), a mechanical stimulus, a radiation stimulus; a biological stimulus (e.g., enzyme), or any combination thereof.

[00133] **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.

[00134] 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.

[00135] 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.

[00136] 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**.

[00137] 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).

[00138] 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.

[00139] 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.

[00140] 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.

[00141] 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.

[00142] Beads may be of uniform size or heterogeneous size. In some cases, the diameter of a bead may be at least about 1micrometers (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 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.

[00143] 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.

[00144] 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.

[00145] 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 or thioether bonds.

[00146] 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.

[00147] 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.

[00148] 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.

[00149] 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.

[00150] 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.

[00151] 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), which may include a priming sequence (e.g., a primer for amplifying target nucleic acids, random primer, primer sequence for messenger RNA) and/or a one or more barcode sequences. The one 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.

[00152] 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 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 nucleic acid molecule can comprise a barcode sequence. In some cases, the primer can further comprise a unique molecular identifier (UMI). In some cases, the primer can comprise an R1 primer sequence for Illumina sequencing. In some cases, the primer 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.

[00153] 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.

[00154] 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.

[00155] 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.

[00156] 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.

[00157] 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.

[00158] 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.

[00159] 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.

[00160] 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.

[00161] 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.

[00162] 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.

[00163] 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.

[00164] 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.

[00165] 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.

[00166] 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.

[00167] 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.

[00168] Species may be encapsulated in beads during bead generation (e.g., during polymerization of precursors). Such species may or may not participate in polymerization. Such species may be entered into polymerization reaction mixtures such that generated beads comprise the species upon bead formation. In some cases, such species may be added to the gel beads after formation. Such species may include, for example, nucleic acid molecules (e.g.,

oligonucleotides), reagents for a nucleic acid amplification reaction (e.g., primers, polymerases, dNTPs, co-factors (e.g., ionic co-factors), buffers) including those described herein, reagents for enzymatic reactions (e.g., enzymes, co-factors, substrates, buffers), reagents for nucleic acid modification reactions such as polymerization, ligation, or digestion, and/or reagents for template preparation (e.g., tagmentation) for one or more sequencing platforms (e.g., Nextera® for Illumina®). Such species may include one or more enzymes described herein, including without limitation, polymerase, reverse transcriptase, restriction enzymes (e.g., endonuclease), transposase, ligase, proteinase K, DNAse, etc. Such species may include one or more reagents described elsewhere herein (e.g., lysis agents, inhibitors, inactivating agents, chelating agents, stimulus). Trapping of such species may be controlled by the polymer network density generated during polymerization of precursors, control of ionic charge within the gel bead (e.g., via ionic species linked to polymerized species), or by the release of other species. Encapsulated species may be released from a bead upon bead degradation and/or by application of a stimulus capable of releasing the species from the bead. Alternatively or in addition, species may be partitioned in a partition (e.g., droplet) during or subsequent to partition formation. Such species may include, without limitation, the abovementioned species that may also be encapsulated in a bead.

[00169] 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.

[00170] 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.

[00171] 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.

[00172] 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.

[00173] 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.

[00174] 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.

[00175] 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.

[00176] 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.

[00177] 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.

[00178] 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.

[00179] 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).

[00180] 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.

[00181] 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.

[00182] 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

[00183] 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.

[00184] **FIG. 3** shows an example of a microfluidic channel structure **300** for co-partitioning biological particles and reagents. The channel structure **300** can include channel segments **301, 302**, **304**, **306** and **308**. Channel segments **301** and **302** communicate at a first channel junction **309**. Channel segments **302**, **304, 306**, and **308** communicate at a second channel junction **310**.

[00185] In an example operation, the channel segment **301** may transport an aqueous fluid **312** that includes a plurality of biological particles **314** along the channel segment **301** into the

second junction **310**. As an alternative or in addition to, channel segment 301 may transport beads (e.g., gel beads). The beads may comprise barcode molecules.

[00186] For example, the channel segment **301** may be connected to a reservoir comprising an aqueous suspension of biological particles **314**. Upstream of, and immediately prior to reaching, the second junction **310**, the channel segment **301** may meet the channel segment **302** at the first junction **309**. The channel segment **302** may transport a plurality of reagents **315** (e.g., lysis agents) suspended in the aqueous fluid **312** along the channel segment **302** into the first junction **309**. For example, the channel segment **302** may be connected to a reservoir comprising the reagents **315**. After the first junction **309**, the aqueous fluid **312** in the channel segment **301** can carry both the biological particles **314** and the reagents **315** towards the second junction **310**. In some instances, the aqueous fluid **312** in the channel segment **301** can include one or more reagents, which can be the same or different reagents as the reagents **315**. A second fluid **316** that is immiscible with the aqueous fluid **312** (e.g., oil) can be delivered to the second junction **310** from each of channel segments **304** and **306**. Upon meeting of the aqueous fluid **312** from the channel segment **301** and the second fluid **316** from each of channel segments **304** and **306** at the second channel junction **310**, the aqueous fluid **312** can be partitioned as discrete droplets **318** in the second fluid **316** and flow away from the second junction **310** along channel segment **308**. The channel segment **308** may deliver the discrete droplets **318** to an outlet reservoir fluidly coupled to the channel segment **308**, where they may be harvested.

[00187] The second fluid **316** can comprise an oil, such as a fluorinated oil, that includes a fluorosurfactant for stabilizing the resulting droplets, for example, inhibiting subsequent coalescence of the resulting droplets **318**.

[00188] A discrete droplet generated may include an individual biological particle **314** and/or one or more reagents **315**. In some instances, a discrete droplet generated may include a barcode carrying bead (not shown), such as via other microfluidics structures described elsewhere herein. In some instances, a discrete droplet may be unoccupied (e.g., no reagents, no biological particles).

[00189] 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.

[00190] As will be appreciated, the channel segments described herein may be coupled to any of a variety of different fluid sources or receiving components, including reservoirs, tubing, manifolds, or fluidic components of other systems. As will be appreciated, the microfluidic

channel structure **300** may have other geometries. For example, a microfluidic channel structure can have more than two channel junctions. For example, a microfluidic channel structure can have 2, 3, 4, 5 channel segments or more each carrying the same or different types of beads, reagents, and/or 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.

[00191] 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 particles'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.

[00192] 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, DNase and RNase inactivating agents or inhibitors, such as proteinase K, chelating agents, such as EDTA, and other reagents employed in removing or otherwise reducing negative activity or impact of different cell lysate components on subsequent processing of nucleic acids. In addition, in the case of encapsulated biological particles, 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 aspects, 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.

[00193] 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, DNAse, etc. Additional reagents may also include reverse transcriptase enzymes, including enzymes with terminal transferase activity, primers and oligonucleotides, and switch oligonucleotides (also referred to herein as "switch oligos" or "template switching oligonucleotides") which can be used for template switching. In some cases, template switching can be used to increase the length of a cDNA. In some cases, template switching can be used to append a predefined nucleic acid sequence to the cDNA. In an example of template switching, cDNA can be generated from reverse transcription of a template, e.g., cellular mRNA, where a reverse transcriptase with terminal transferase activity can add additional nucleotides, e.g., polyC, to the cDNA in a template independent manner. Switch oligos can include sequences complementary to the additional nucleotides, e.g., polyG. The additional nucleotides (e.g., polyC) on the cDNA can hybridize to the additional nucleotides (e.g., polyG) on the switch oligo, whereby the switch oligo can be used by the reverse transcriptase as template to further extend the cDNA. Template switching oligonucleotides may comprise a hybridization region and a template region. The hybridization region can comprise any sequence capable of hybridizing to the target. In some cases, as previously described, the hybridization region comprises a series of G bases to complement the overhanging C bases at the 3' end of a cDNA molecule. The series of G bases may comprise 1 G base, 2 G bases, 3 G bases, 4 G bases, 5 G bases or more than 5 G bases. The template sequence can comprise any sequence to be incorporated into the cDNA. In some cases, the template region comprises at least 1 (e.g., at least 2, 3, 4, 5 or more) tag sequences and/or functional sequences. Switch oligos may comprise deoxyribonucleic acids; ribonucleic acids;

modified nucleic acids including 2-Aminopurine, 2,6-Diaminopurine (2-Amino-dA), inverted dT, 5-Methyl dC, 2'-deoxyInosine, Super T (5-hydroxybutynl-2'-deoxyuridine), Super G (8-aza-7-deazaguanosine), locked nucleic acids (LNAs), unlocked nucleic acids (UNAs, e.g., UNA-A, UNA-U, UNA-C, UNA-G), Iso-dG, Iso-dC, 2' Fluoro bases (e.g., Fluoro C, Fluoro U, Fluoro A, and Fluoro G), or any combination.

[00194] 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.

[00195] 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.

[00196] 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 particle, 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.

[00197] 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 **FIG. 2**). 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, a single nucleic acid barcode sequence may be associated with a given partition, although in some cases, two or more different barcode sequences may be present.

[00198] 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). 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.

[00199] 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 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 into partitions, e.g., droplets within microfluidic systems.

[00200] 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.

[00201] 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.

[00202] 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.

[00203] 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 form 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.

[00204] 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.

[00205] **FIG. 4** shows an example of a microfluidic channel structure for the controlled partitioning of beads into discrete droplets. A channel structure **400** can include a channel segment **402** communicating at a channel junction **406** (or intersection) with a reservoir **404**. The reservoir **404** can be a chamber. Any reference to "reservoir," as used herein, can also refer to a "chamber." In operation, an aqueous fluid **408** that includes suspended beads **412** may be transported along the channel segment **402** into the junction **406** to meet a second fluid **410** that is immiscible with the aqueous fluid **408** in the reservoir **404** to create droplets **416, 418** of the aqueous fluid **408** flowing into the reservoir **404**. At the junction **406** where the aqueous fluid **408** and the second fluid **410** meet, droplets can form based on factors such as the hydrodynamic forces at the junction **406**, flow rates of the two fluids **408, 410**, fluid properties, and certain geometric parameters (e.g., w, h_0, α, etc.) of the channel structure **400**. A plurality of droplets can be collected in the reservoir **404** by continuously injecting the aqueous fluid **408** from the channel segment **402** through the junction **406**.

[00206] A discrete droplet generated may include a bead (e.g., as in occupied droplets **416**). Alternatively, a discrete droplet generated may include more than one bead. Alternatively, a discrete droplet generated may not include any beads (e.g., as in unoccupied droplet **418**). In

some instances, a discrete droplet generated may contain one or more biological particles, as described elsewhere herein. In some instances, a discrete droplet generated may comprise one or more reagents, as described elsewhere herein.

[00207] In some instances, the aqueous fluid **408** can have a substantially uniform concentration or frequency of beads **412**. The beads **412** can be introduced into the channel segment **402** from a separate channel (not shown in **FIG. 4**). The frequency of beads **412** in the channel segment **402** may be controlled by controlling the frequency in which the beads **412** are introduced into the channel segment **402** and/or the relative flow rates of the fluids in the channel segment **402** and the separate channel. In some instances, the beads can be introduced into the channel segment **402** from a plurality of different channels, and the frequency controlled accordingly.

[00208] In some instances, the aqueous fluid **408** in the channel segment **402** can comprise biological particles (e.g., described with reference to **FIGS. 1** and **2**). In some instances, the aqueous fluid **408** can have a substantially uniform concentration or frequency of biological particles. As with the beads, the biological particles can be introduced into the channel segment **402** from a separate channel. The frequency or concentration of the biological particles in the aqueous fluid **408** in the channel segment **402** may be controlled by controlling the frequency in which the biological particles are introduced into the channel segment **402** and/or the relative flow rates of the fluids in the channel segment **402** and the separate channel. In some instances, the biological particles can be introduced into the channel segment **402** from a plurality of different channels, and the frequency controlled accordingly. In some instances, a first separate channel can introduce beads and a second separate channel can introduce biological particles into the channel segment **402**. The first separate channel introducing the beads may be upstream or downstream of the second separate channel introducing the biological particles.

[00209] The second fluid **410** can comprise an oil, such as a fluorinated oil, that includes a fluorosurfactant for stabilizing the resulting droplets, for example, inhibiting subsequent coalescence of the resulting droplets.

[00210] In some instances, the second fluid **410** may not be subjected to and/or directed to any flow in or out of the reservoir **404**. For example, the second fluid **410** may be substantially stationary in the reservoir **404**. In some instances, the second fluid **410** may be subjected to flow within the reservoir **404**, but not in or out of the reservoir **404**, such as via application of pressure to the reservoir **404** and/or as affected by the incoming flow of the aqueous fluid **408** at the junction **406**. Alternatively, the second fluid **410** may be subjected and/or directed to flow in or

out of the reservoir **404**. For example, the reservoir **404** can be a channel directing the second fluid **410** from upstream to downstream, transporting the generated droplets.

[00211] The channel structure **400** at or near the junction **406** may have certain geometric features that at least partly determine the sizes of the droplets formed by the channel structure **400**. The channel segment **402** can have a height, h_0 and width, w, at or near the junction **406**. By way of example, the channel segment **402** can comprise a rectangular cross-section that leads to a reservoir **404** having a wider cross-section (such as in width or diameter). Alternatively, the cross-section of the channel segment **402** can be other shapes, such as a circular shape, trapezoidal shape, polygonal shape, or any other shapes. The top and bottom walls of the reservoir **404** at or near the junction **406** can be inclined at an expansion angle, α. The expansion angle, α, allows the tongue (portion of the aqueous fluid **408** leaving channel segment **402** at junction **406** and entering the reservoir **404** before droplet formation) to increase in depth and facilitate decrease in curvature of the intermediately formed droplet. Droplet size may decrease with increasing expansion angle. The resulting droplet radius, R_d, may be predicted by the following equation for the aforementioned geometric parameters of h_0, w, and α:

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

[00212] By way of example, for a channel structure with w = 21 μm, h = 21 μm, and α = 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.

[00213] In some instances, the expansion angle, α, may be between a range of from about 0.5° to about 4°, from about 0.1° to about 10°, or from about 0° to about 90°. For example, the expansion angle can be at least about 0.01°, 0.1°, 0.2°, 0.3°, 0.4°, 0.5°, 0.6°, 0.7°, 0.8°, 0.9°, 1°, 2°, 3°, 4°, 5°, 6°, 7°, 8°, 9°, 10°, 15°, 20°, 25°, 30°, 35°, 40°, 45°, 50°, 55°, 60°, 65°, 70°, 75°, 80°, 85°, or higher. In some instances, the expansion angle can be at most about 89°, 88°, 87°, 86°, 85°, 84°, 83°, 82°, 81°, 80°, 75°, 70°, 65°, 60°, 55°, 50°, 45°, 40°, 35°, 30°, 25°, 20°, 15°, 10°, 9°, 8°, 7°, 6°, 5°, 4°, 3°, 2°, 1°, 0.1°, 0.01°, or less. In some instances, the width, w, can be between a range of from about 100 micrometers (μm) to about 500 μm. In some instances, the width, w, can be between a range of from about 10 μm to about 200 μm. Alternatively, the width can be less than about 10 μm. Alternatively, the width can be greater than about 500 μm. In some instances, the flow rate of the aqueous fluid **408** entering the junction **406** can be between about 0.04 microliters (μL)/minute (min) and about 40 μL/min. In some instances, the flow rate of the aqueous fluid **408** entering the junction **406** can be between about 0.01

microliters (μL)/minute (min) and about 100 μL/min. Alternatively, the flow rate of the aqueous fluid **408** entering the junction **406** can be less than about 0.01 μL/min. Alternatively, the flow rate of the aqueous fluid **408** entering the junction **406** 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 **408** entering the junction **406**.

[00214] 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.

[00215] The throughput of droplet generation can be increased by increasing the points of generation, such as increasing the number of junctions (e.g., junction **406**) between aqueous fluid **408** channel segments (e.g., channel segment **402**) and the reservoir **404**. Alternatively or in addition, the throughput of droplet generation can be increased by increasing the flow rate of the aqueous fluid **408** in the channel segment **402**.

[00216] **FIG. 5** shows an example of a microfluidic channel structure for increased droplet generation throughput. A microfluidic channel structure **500** can comprise a plurality of channel segments **502** and a reservoir **504**. Each of the plurality of channel segments **502** may be in fluid communication with the reservoir **504**. The channel structure **500** can comprise a plurality of channel junctions **506** between the plurality of channel segments **502** and the reservoir **504**. Each channel junction can be a point of droplet generation. The channel segment **402** from the channel structure **400** in **FIG. 4** and any description to the components thereof may correspond to a given channel segment of the plurality of channel segments **502** in channel structure **500** and any description to the corresponding components thereof. The reservoir **404** from the channel structure **400** and any description to the components thereof may correspond to the reservoir **504** from the channel structure **500** and any description to the corresponding components thereof.

[00217] Each channel segment of the plurality of channel segments **502** may comprise an aqueous fluid **508** that includes suspended beads **512**. The reservoir **504** may comprise a second fluid **510** that is immiscible with the aqueous fluid **508**. In some instances, the second fluid **510** may not be subjected to and/or directed to any flow in or out of the reservoir **504**. For example, the second fluid **510** may be substantially stationary in the reservoir **504**. In some instances, the

second fluid **510** may be subjected to flow within the reservoir **504**, but not in or out of the reservoir **504**, such as via application of pressure to the reservoir **504** and/or as affected by the incoming flow of the aqueous fluid **508** at the junctions. Alternatively, the second fluid **510** may be subjected and/or directed to flow in or out of the reservoir **504**. For example, the reservoir **504** can be a channel directing the second fluid **510** from upstream to downstream, transporting the generated droplets.

[00218] In operation, the aqueous fluid **508** that includes suspended beads **512** may be transported along the plurality of channel segments **502** into the plurality of junctions **506** to meet the second fluid **510** in the reservoir **504** to create droplets **516, 518**. A droplet may form from each channel segment at each corresponding junction with the reservoir **504**. At the junction where the aqueous fluid **508** and the second fluid **510** meet, droplets can form based on factors such as the hydrodynamic forces at the junction, flow rates of the two fluids **508, 510**, fluid properties, and certain geometric parameters (e.g., w, h_0, α, etc.) of the channel structure **500**, as described elsewhere herein. A plurality of droplets can be collected in the reservoir **504** by continuously injecting the aqueous fluid **508** from the plurality of channel segments **502** through the plurality of junctions **506**. Throughput may significantly increase with the parallel channel configuration of channel structure **500**. For example, a channel structure having five inlet channel segments comprising the aqueous fluid **508** may generate droplets five times as frequently than a channel structure having one inlet channel segment, provided that the fluid flow rate in the channel segments are substantially the same. The fluid flow rate in the different inlet channel segments may or may not be substantially the same. A channel structure may have as many parallel channel segments as is practical and allowed for the size of the reservoir. For example, the channel structure may have at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 500, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1500, 5000 or more parallel or substantially parallel channel segments.

[00219] The geometric parameters, w, h_0, and α, may or may not be uniform for each of the channel segments in the plurality of channel segments **502**. For example, each channel segment may have the same or different widths at or near its respective channel junction with the reservoir **504**. For example, each channel segment may have the same or different height at or near its respective channel junction with the reservoir **504**. In another example, the reservoir **504** may have the same or different expansion angle at the different channel junctions with the plurality of channel segments **502**. When the geometric parameters are uniform, beneficially, droplet size may also be controlled to be uniform even with the increased throughput. In some

instances, when it is desirable to have a different distribution of droplet sizes, the geometric parameters for the plurality of channel segments **502** may be varied accordingly.

[00220] 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.

[00221] **FIG. 6** shows another example of a microfluidic channel structure for increased droplet generation throughput. A microfluidic channel structure **600** can comprise a plurality of channel segments **602** arranged generally circularly around the perimeter of a reservoir **604**. Each of the plurality of channel segments **602** may be in fluid communication with the reservoir **604**. The channel structure **600** can comprise a plurality of channel junctions **606** between the plurality of channel segments **602** and the reservoir **604**. Each channel junction can be a point of droplet generation. The channel segment **402** from the channel structure **400** in **FIG. 2** and any description to the components thereof may correspond to a given channel segment of the plurality of channel segments **602** in channel structure **600** and any description to the corresponding components thereof. The reservoir **404** from the channel structure **400** and any description to the components thereof may correspond to the reservoir **604** from the channel structure **600** and any description to the corresponding components thereof.

[00222] Each channel segment of the plurality of channel segments **602** may comprise an aqueous fluid **608** that includes suspended beads **612**. The reservoir **604** may comprise a second fluid **610** that is immiscible with the aqueous fluid **608**. In some instances, the second fluid **610** may not be subjected to and/or directed to any flow in or out of the reservoir **604**. For example, the second fluid **610** may be substantially stationary in the reservoir **604**. In some instances, the second fluid **610** may be subjected to flow within the reservoir **604**, but not in or out of the reservoir **604**, such as via application of pressure to the reservoir **604** and/or as affected by the incoming flow of the aqueous fluid **608** at the junctions. Alternatively, the second fluid **610** may be subjected and/or directed to flow in or out of the reservoir **604**. For example, the reservoir **604** can be a channel directing the second fluid **610** from upstream to downstream, transporting the generated droplets.

[00223] In operation, the aqueous fluid **608** that includes suspended beads **612** may be transported along the plurality of channel segments **602** into the plurality of junctions **606** to meet the second fluid **610** in the reservoir **604** to create a plurality of droplets **616**. A droplet may form from each channel segment at each corresponding junction with the reservoir **604**. At

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

[00224] The reservoir **604** may have an expansion angle, α (not shown in **FIG. 6**) at or near each channel junction. Each channel segment of the plurality of channel segments **602** may have a width, w, and a height, h_0, at or near the channel junction. The geometric parameters, w, h_0, and α, may or may not be uniform for each of the channel segments in the plurality of channel segments **602**. For example, each channel segment may have the same or different widths at or near its respective channel junction with the reservoir **604**. For example, each channel segment may have the same or different height at or near its respective channel junction with the reservoir **604**.

[00225] The reservoir **604** may have the same or different expansion angle at the different channel junctions with the plurality of channel segments **602**. For example, a circular reservoir (as shown in **FIG. 6**) may have a conical, dome-like, or hemispherical ceiling (e.g., top wall) to provide the same or substantially same expansion angle for each channel segments **602** at or near the plurality of channel junctions **606**. When the geometric parameters are uniform, beneficially, resulting droplet size may be controlled to be uniform even with the increased throughput. In some instances, when it is desirable to have a different distribution of droplet sizes, the geometric parameters for the plurality of channel segments **602** may be varied accordingly.

[00226] In some instances, at least about 50% of the droplets generated can have uniform size. In some instances, at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater of the droplets generated can have uniform size. Alternatively, less

than about 50% of the droplets generated can have uniform size. The beads and/or biological particle injected into the droplets may or may not have uniform size.

[00227] **FIG. 7A** shows a cross-section view of another example of a microfluidic channel structure with a geometric feature for controlled partitioning. A channel structure **700** can include a channel segment **702** communicating at a channel junction **706** (or intersection) with a reservoir **704**. In some instances, the channel structure **700** and one or more of its components can correspond to the channel structure **100** and one or more of its components. **FIG. 7B** shows a perspective view of the channel structure **700** of **FIG. 7A**.

[00228] An aqueous fluid **712** comprising a plurality of particles **716** may be transported along the channel segment **702** into the junction **706** to meet a second fluid **714** (e.g., oil, etc.) that is immiscible with the aqueous fluid **712** in the reservoir **704** to create droplets **720** of the aqueous fluid **712** flowing into the reservoir **704**. At the junction **706** where the aqueous fluid **712** and the second fluid **714** meet, droplets can form based on factors such as the hydrodynamic forces at the junction **706**, relative flow rates of the two fluids **712, 714**, fluid properties, and certain geometric parameters (e.g., Δh, etc.) of the channel structure **700**. A plurality of droplets can be collected in the reservoir **704** by continuously injecting the aqueous fluid **712** from the channel segment **702** at the junction **706**.

[00229] A discrete droplet generated may comprise one or more particles of the plurality of particles **716**. As described elsewhere herein, a particle may be any particle, such as a bead, cell bead, gel bead, biological particle, macromolecular constituents of biological particle, or other particles. Alternatively, a discrete droplet generated may not include any particles.

[00230] In some instances, the aqueous fluid **712** can have a substantially uniform concentration or frequency of particles **716**. As described elsewhere herein (e.g., with reference to **FIG. 4**), the particles **716** (e.g., beads) can be introduced into the channel segment **702** from a separate channel (not shown in **FIG. 7**). The frequency of particles **716** in the channel segment **702** may be controlled by controlling the frequency in which the particles **716** are introduced into the channel segment **702** and/or the relative flow rates of the fluids in the channel segment **702** and the separate channel. In some instances, the particles **716** can be introduced into the channel segment **702** from a plurality of different channels, and the frequency controlled accordingly. In some instances, different particles may be introduced via separate channels. For example, a first separate channel can introduce beads and a second separate channel can introduce biological particles into the channel segment **702**. The first separate channel

introducing the beads may be upstream or downstream of the second separate channel introducing the biological particles.

[00231] In some instances, the second fluid **714** may not be subjected to and/or directed to any flow in or out of the reservoir **704**. For example, the second fluid **714** may be substantially stationary in the reservoir **704**. In some instances, the second fluid **714** may be subjected to flow within the reservoir **704**, but not in or out of the reservoir **704**, such as via application of pressure to the reservoir **704** and/or as affected by the incoming flow of the aqueous fluid **712** at the junction **706**. Alternatively, the second fluid **714** may be subjected and/or directed to flow in or out of the reservoir **704**. For example, the reservoir **704** can be a channel directing the second fluid **714** from upstream to downstream, transporting the generated droplets.

[00232] The channel structure **700** at or near the junction **706** may have certain geometric features that at least partly determine the sizes and/or shapes of the droplets formed by the channel structure **700**. The channel segment **702** can have a first cross-section height, h_1, and the reservoir **704** can have a second cross-section height, h_2. The first cross-section height, h_1, and the second cross-section height, h_2, may be different, such that at the junction **706**, there is a height difference of Δh. The second cross-section height, h_2, may be greater than the first cross-section height, h_1. In some instances, the reservoir may thereafter gradually increase in cross-section height, for example, the more distant it is from the junction **706**. In some instances, the cross-section height of the reservoir may increase in accordance with expansion angle, β, at or near the junction **706**. The height difference, Δh, and/or expansion angle, β, can allow the tongue (portion of the aqueous fluid **712** leaving channel segment **702** at junction **706** and entering the reservoir **704** before droplet formation) to increase in depth and facilitate decrease in curvature of the intermediately formed droplet. For example, droplet size may decrease with increasing height difference and/or increasing expansion angle.

[00233] The height difference, Δh, can be at least about 1 μm. Alternatively, the height difference can be at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 μm or more. Alternatively, the height difference can be at most about 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 45, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 μm or less. In some instances, the expansion angle, β, may be between a range of from about 0.5° to about 4°, from about 0.1° to about 10°, or from about 0° to about 90°. For example, the expansion angle can be at least about 0.01°, 0.1°, 0.2°, 0.3°, 0.4°, 0.5°, 0.6°, 0.7°, 0.8°, 0.9°, 1°, 2°, 3°, 4°, 5°, 6°, 7°, 8°, 9°, 10°, 15°, 20°, 25°, 30°, 35°, 40°, 45°, 50°, 55°, 60°, 65°, 70°, 75°, 80°, 85°, or higher. In

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

[00234] In some instances, the flow rate of the aqueous fluid **712** entering the junction **706** can be between about 0.04 microliters (μL)/minute (min) and about 40 μL/min. In some instances, the flow rate of the aqueous fluid **712** entering the junction **706** can be between about 0.01 microliters (μL)/minute (min) and about 100 μL/min. Alternatively, the flow rate of the aqueous fluid **712** entering the junction **706** can be less than about 0.01 μL/min. Alternatively, the flow rate of the aqueous fluid **712** entering the junction **706** can be greater than about 40 μL/min, such as 45 μL/min, 50 μL/min, 55 μL/min, 60 μL/min, 65 μL/min, 70 μL/min, 75 μL/min, 80 μL/min, 85 μL/min, 90 μL/min, 95 μL/min, 100 μL/min, 110 μL/min , 120 μL/min , 130 μL/min , 140 μL/min , 150 μL/min, or greater. At lower flow rates, such as flow rates of about less than or equal to 10 microliters/minute, the droplet radius may not be dependent on the flow rate of the aqueous fluid **712** entering the junction **706**. The second fluid **714** may be stationary, or substantially stationary, in the reservoir **704**. Alternatively, the second fluid **714** may be flowing, such as at the above flow rates described for the aqueous fluid **712**.

[00235] 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.

[00236] While **FIGs. 7A** and **7B** illustrate the height difference, Δh, being abrupt at the junction **706** (e.g., a step increase), the height difference may increase gradually (e.g., from about 0 μm to a maximum height difference). Alternatively, the height difference may decrease gradually (e.g., taper) from a maximum height difference. A gradual increase or decrease in height difference, as used herein, may refer to a continuous incremental increase or decrease in height difference, wherein an angle between any one differential segment of a height profile and an immediately adjacent differential segment of the height profile is greater than 90°. For example, at the junction **706**, a bottom wall of the channel and a bottom wall of the reservoir can meet at an angle greater than 90°. Alternatively or in addition, a top wall (e.g., ceiling) of the channel and a top wall (e.g., ceiling) of the reservoir can meet an angle greater than 90°. A gradual increase or decrease may be linear or non-linear (e.g., exponential, sinusoidal, etc.). Alternatively or in addition, the height difference may variably increase and/or decrease linearly or non-linearly. While **FIGs. 7A** and **7B** illustrate the expanding reservoir cross-section height

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

[00237] The channel networks, e.g., as described above or elsewhere herein, can be fluidly coupled to appropriate fluidic components. For example, the inlet channel segments are fluidly coupled to appropriate sources of the materials they are to deliver to a channel junction. These sources may include any of a variety of different fluidic components, from simple reservoirs defined in or connected to a body structure of a microfluidic device, to fluid conduits that deliver fluids from off-device sources, manifolds, fluid flow units (e.g., actuators, pumps, compressors) or the like. Likewise, the outlet channel segment (e.g., channel segment **208,** reservoir **604**, etc.) may be fluidly coupled to a receiving vessel or conduit for the partitioned cells for subsequent processing. Again, this may be a reservoir defined in the body of a microfluidic device, or it may be a fluidic conduit for delivering the partitioned cells to a subsequent process operation, instrument or component.

[00238] 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.

[00239] 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.

Computer systems

[00240] The present disclosure provides computer systems that are programmed to implement methods of the disclosure. **FIG. 10** shows a computer system **1001** that is programmed or otherwise configured to (i) control a microfluidics system (e.g., fluid flow), (ii) sort occupied droplets from unoccupied droplets, (iii) polymerize droplets, (iv) image individual droplets to obtain optical information, (v) perform sequencing applications, (vi) generate and maintain a library of barcoded nucleic acid molecules, (vii) analyze sequencing data, (viii) mapping sequencing data to a reference genome, and (ix) electronically associate sequencing data to the individual droplet. . The computer system **1001** can regulate various aspects of the present disclosure, such as, for example, regulating polymerization application units for generating particles (e.g., beads) with different physical and/or optical properties, regulating fluid flow rate in one or more channels, regulating fluid flow to enable an imaging unit to image individual droplets flowing through the channel and storing optical information of the droplets for further processing. The computer system **1001** 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.

[00241] The computer system **1001** includes a central processing unit (CPU, also "processor" and "computer processor" herein) **1005**, which can be a single core or multi core processor, or a plurality of processors for parallel processing. The computer system **1001** also includes memory or memory location **1010** (e.g., random-access memory, read-only memory, flash memory), electronic storage unit **1015** (e.g., hard disk), communication interface **1020** (e.g., network adapter) for communicating with one or more other systems, and peripheral devices **1025**, such as cache, other memory, data storage and/or electronic display adapters. The memory **1010**, storage unit **1015**, interface **1020** and peripheral devices **1025** are in communication with the CPU **1005** through a communication bus (solid lines), such as a motherboard. The storage unit **1015** can be a data storage unit (or data repository) for storing data. The computer system **1001** can be operatively coupled to a computer network ("network") **1030** with the aid of the communication interface **1020**. The network **1030** can be the Internet, an internet and/or extranet, or an intranet and/or extranet that is in communication with the Internet. The network **1030** in some cases is a telecommunication and/or data network. The network **1030** can include one or more computer servers, which can enable distributed computing, such as cloud

computing. The network **1030**, in some cases with the aid of the computer system **1001**, can implement a peer-to-peer network, which may enable devices coupled to the computer system **1001** to behave as a client or a server.

[00242] The CPU **1005** 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 **1010**. The instructions can be directed to the CPU **1005**, which can subsequently program or otherwise configure the CPU **1005** to implement methods of the present disclosure. Examples of operations performed by the CPU **1005** can include fetch, decode, execute, and writeback.

[00243] The CPU **1005** can be part of a circuit, such as an integrated circuit. One or more other components of the system **1001** can be included in the circuit. In some cases, the circuit is an application specific integrated circuit (ASIC).

[00244] The storage unit **1015** can store files, such as drivers, libraries and saved programs. The storage unit **1015** can store user data, e.g., user preferences and user programs. The computer system **1001** in some cases can include one or more additional data storage units that are external to the computer system **1001**, such as located on a remote server that is in communication with the computer system **1001** through an intranet or the Internet.

[00245] The computer system **1001** can communicate with one or more remote computer systems through the network **1030**. For instance, the computer system **1001** 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 **1001** via the network **1030**.

[00246] 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 **1001**, such as, for example, on the memory **1010** or electronic storage unit **1015**. 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 **1005**. In some cases, the code can be retrieved from the storage unit **1015** and stored on the memory **1010** for ready access by the processor **1005**. In some situations, the electronic storage unit **1015** can be precluded, and machine-executable instructions are stored on memory **1010**.

[00247] 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.

[00248] Aspects of the systems and methods provided herein, such as the computer system **1001**, 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.

[00249] 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.

[00250] The computer system **1001** can include or be in communication with an electronic display **1035** that comprises a user interface (UI) **1040** for providing, for example, optical information of droplets, phenotypic information of biological particles (e.g., cell) results of sequencing analysis and association of sequence data to the optical information. Examples of UIs include, without limitation, a graphical user interface (GUI) and web-based user interface.

[00251] 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 **1005**. The algorithm can, for example, obtain optical information of droplets, perform sequencing, perform sequence analysis, associate sequence data to the optical information, etc.

[00252] 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) form 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.

[00253] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the invention be limited by the specific examples provided within the specification. While the invention has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions

will now occur to those skilled in the art without departing from the invention. Furthermore, it shall be understood that all aspects of the invention are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is therefore contemplated that the invention shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for analyzing a nucleic acid molecule of a cell, comprising:

(a) partitioning into a physical partition of a plurality of physical partitions:

(i) a bead comprising (I) an optically detectable moiety and (II) a plurality of nucleic acid barcode molecules, wherein a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules comprises a barcode sequence, and

(ii) said cell comprising said nucleic acid molecule,

wherein said bead comprises said optically detectable moiety prior to (a);

(b) within said physical partition, using said nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules and said nucleic acid molecule of said cell to conduct a barcoding reaction on said nucleic acid molecule to generate a barcoded nucleic acid molecule;

(c) generating (i) a first data set comprising sequence data corresponding to a nucleic acid sequence of said barcoded nucleic acid molecule or a derivative thereof and (ii) a second data set comprising data corresponding to said optically detectable moiety of said bead; and

(d) associating (i) said sequence data of said first data set with (ii) said data of said second data set.

2. The method of claim 1, wherein said cell comprises a plurality of nucleic acid molecules comprising said nucleic acid molecule.

3. The method of claim 1, wherein generating said second data set comprises optically detecting said bead.

4. The method of claim 3, wherein said second data set further comprises data corresponding to one or more physical properties of said bead, wherein said one or more physical properties comprise one or more members selected from the group consisting of: a size, a shape, a circularity, a hardness, and a symmetry of said bead.

5. The method of claim 3, wherein said second data set comprises data corresponding to one or more optical properties of said bead, wherein said one or more optical properties comprise one or more members selected from the group consisting of: an absorbance, a birefringence, a

color, a fluorescence characteristic, a luminosity, a photosensitivity, a reflectivity, a refractive index, a scattering characteristic, and a transmittance.

6. The method of claim 1, wherein additional nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules comprise said barcode sequence.

7. The method of claim 1, wherein said plurality of physical partitions is a plurality of droplets.

8. The method of claim 1, wherein said plurality of physical partitions is a plurality of wells.

9. The method of claim 1, wherein said optically detectable moiety comprises one or more optical barcodes.

10. The method of claim 9, wherein said plurality of nucleic acid barcode molecules of said bead comprise said one or more optical barcodes.

11. The method of claim 1, wherein said optically detectable moiety comprises one or more members selected from the group consisting of a fluorescent dye, a nanoparticle, and a microparticle.

12. The method of claim 9, wherein said one or more optical barcodes have an associated optical intensity or frequency that is distinct with respect to other optical barcodes of other physical partitions of said plurality of physical partitions.

13. The method of claim 1, wherein said bead is a gel bead.

14. The method of claim 1, wherein said plurality of nucleic acid barcode molecules is releasably coupled to said bead.

15. The method of claim 1, wherein said bead is coupled to another bead comprising one or more optical barcodes.

16. The method of claim 1, wherein (a)-(d) are repeated for an additional physical partition of said plurality of physical partitions, wherein said additional physical partition comprises (i) an additional bead comprising an additional optically detectable moiety and an additional plurality of nucleic acid barcode molecules, wherein an additional nucleic acid barcode molecule of said additional plurality of nucleic acid barcode molecules comprises an additional barcode sequence different than said barcode sequence and (ii) an additional cell comprising an additional nucleic acid molecule, and wherein said optically detectable moiety of said bead of said physical partition and said additional optically detectable moiety of said additional bead of said additional physical partition are different.

17. The method of claim 16, wherein said optically detectable moiety of said bead of said physical partition and said additional optically detectable moiety of said additional bead of said additional physical partition fluoresce at different wavelengths.

18. The method of claim 16, wherein said optically detectable moiety of said bead of said physical partition and said additional optically detectable moiety of said additional bead of said additional physical partition fluoresce with different intensities.

19. The method of claim 1, wherein (b) comprises performing a ligation reaction between said nucleic acid barcode molecule and said nucleic acid molecule.

20. The method of claim 1, wherein (b) comprises performing an extension reaction using said nucleic acid barcode molecule as a primer and said nucleic acid molecule as a template.

21. The method of claim 1, further comprising generating a third data set comprising data corresponding to one or more physical properties of said cell.

22. The method of claim 21, wherein generating said third data set corresponding to said one or more physical properties of said cell is performed subsequent to (a).

23. The method of claim 21, wherein generating said third data set corresponding to said one or more physical properties of said cell is performed prior to (a).

24. The method of claim 21, wherein said one or more physical properties of said cell comprise one or more members selected from the group consisting of: a size, a shape, a surface marker on said cell, an inclusion in said cell, a structure of an organelle in said cell, a number of organelles in said cell, a secretion or excretion with respect to said cell, and a localization of an organelle in said cell.

25. The method of claim 21, wherein said one or more physical properties of said cell comprise phenotypic information of said cell.

26. The method of claim 21, wherein generating said second data set or said third data set comprises imaging said physical partition using bright field microscopy, fluorescence microscopy, phase contrast microscopy, multispectral microscopy, or polarization microscopy.

27. The method of claim 21, further comprising associating (i) data corresponding to an identity of a protein of said cell with (ii) said data of said third data set corresponding to said one or more physical properties of said cell.

28. The method of claim 21, further comprising associating said sequence data of said first data set or said data of said second data set with said data of said third data set.

29. The method of claim 28, wherein said sequence data of said first data set comprises chromatin information.

30. The method of claim 1, wherein said optically detectable moiety is disposed within said bead.