

droplet. The droplet may contain a gel bead which may contain a tag (such as a barcode) that may be used to barcode macromolecular constituents (e.g., nucleic acid molecules) of a single biological particle.

[0005] Methods and systems of the present disclosure may be used to generate target sequence or sequencing reads (“reads”) specific to macromolecular constituents of interest at a higher rate than non-target specific reads. For instance, the methods and systems are characterized by their suppression of no template control (NTC) effects.

[0005a] In one aspect, the present disclosure refers to a method for processing or analyzing one or more components from a sample, comprising:

(a) providing a plurality of cell beads and a plurality of barcode beads, wherein (i) a cell bead of said plurality of cell beads comprises said cell, wherein said cell comprises a nucleic acid molecule, and (ii) a barcode bead of said plurality of barcode beads comprises a plurality of nucleic acid barcode molecules for barcoding said nucleic acid molecule;

(b) subjecting said plurality of cell beads to bulk denaturation, which bulk denaturation comprises denaturing said nucleic acid molecule to generate a denatured nucleic acid molecule in said cell bead; and

partitioning said plurality of cell beads and said plurality of barcode beads into a plurality of partitions, wherein upon partitioning, a partition of said plurality of partitions comprises said cell bead and said barcode bead.

[0006] In an example, the present disclosure provides a method for analysis of a single biological particle, comprising (a) providing a first liquid phase comprising a plurality of biological particles; (b) providing a second liquid phase comprising a plurality of beads each including a tag to barcode one or more macromolecular constituents of each of the plurality of biological particles; (c) bringing the plurality of biological particles from the first liquid phase and the plurality of beads from the second liquid phase in contact with a third liquid phase that is immiscible with the first or second liquid phase, to partition each of the plurality of biological particles and the plurality of beads into a plurality of partitions (e.g., droplets), wherein upon partitioning, a given partition of the plurality of partitions includes a single biological particle from the plurality of biological particles and a single bead from the plurality of beads; (d) in the given partition (e.g., droplet), using the tag from the single bead to barcode the one or more macromolecular constituents of the single biological particle, forming one or more barcoded macromolecules; and (e) subjecting the barcoded macromolecules to sequencing to generate reads characterized by a specific target read(s) to non-target specific read(s) ratio greater than 1,

which specific target read(s) of the reads is indicative of the one or more macromolecular constituents.

[0007] In some examples, the sequencing is nucleic acid sequencing. In some examples, the nucleic acid sequencing is massively parallel sequencing. In some examples, the nucleic acid sequencing is digital polymerase chain reaction (PCR).

[0008] In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 100. In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 1,000. In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 10,000. In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 100,000. In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 1,000,000. In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 10,000,000. In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 100,000,000. In some examples, the specific target read(s) to non-target specific read(s) ratio is greater than 1,000,000,000.

[0009] In some examples, the specific target read(s) correspond to one or more nucleic acid sequences from the single biological particle. In some examples, the non-target specific read(s) corresponds to one or more exogenous nucleic acid sequences.

[0010] In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells.

[0011] In some examples, a given bead of the plurality of beads includes one or more tags coupled to a surface thereof and/or enclosed within the given bead.

[0012] In some examples, the plurality of partitions is part of a population of partitions that includes one or more partitions that are unoccupied by biological particles and/or beads.

[0013] In another example, the present disclosure provides a method for analysis of a single biological particle, comprising (a) providing a first liquid phase comprising a plurality of biological particles; (b) providing a second liquid phase comprising a plurality of beads each including a tag to barcode one or more macromolecular constituents of each of the plurality of biological particles; and
(c) bringing the plurality of biological particles from the first liquid phase and the plurality of beads from the second liquid phase in contact with a third liquid phase that is immiscible with the first or second liquid phase, to partition each of the plurality of biological particles and the plurality of beads into a plurality of partitions, wherein upon partitioning, a given partition of the plurality of partitions includes a single biological particle from the plurality of biological

particles and a single bead from the plurality of beads, wherein the single biological particle includes or is enclosed within a polymer or gel matrix.

[0014] In some examples, the first liquid phase further comprises precursors that are capable of being polymerized or gelled. In some examples, the method comprises subjecting the first liquid phase to conditions sufficient to polymerize or gel the precursors so as to encapsulate the single biological particle in the polymer or gel matrix. In some examples, the polymer or gel matrix is diffusively permeable to reagents while retaining the one or more macromolecular constituents.

[0015] In some examples, the method comprises subjecting the single biological particle to conditions sufficient to lyse the single biological particle to provide a lysed single biological particle. In some examples, the method comprises subjecting the lysed single biological particle to conditions sufficient to denature the one or more macromolecular constituents released from the lysed single biological particle. In some examples, the method comprises subjecting the lysed single biological particle to conditions sufficient to release the one or more macromolecular constituents from the polymer or gel matrix.

[0016] In some examples, the method comprises using the tag from the single bead to barcode the one or more macromolecular constituents, forming one or more barcoded macromolecules. In some examples, the method comprises subjecting the barcoded macromolecules to sequencing.

[0017] In some examples, the polymer or gel matrix includes one or more of disulfide crosslinked polyacrylamide, agarose, alginate, polyvinyl alcohol, PEG-diacrylate, PEG-acrylate/thiol, PEG-azide/alkyne, other acrylates, chitosan, hyaluronic acid, collagen, fibrin, gelatin, and elastin.

[0018] In some examples, the conditions sufficient to lyse the single biological particle comprises exposure to sodium hydroxide (NaOH). In some examples, the conditions sufficient to denature the one or more macromolecular constituents comprises exposure to sodium hydroxide (NaOH). In some examples, the conditions sufficient to release the one or more macromolecular constituents comprises exposure to dithiothreitol (DTT). In some examples, the one or more macromolecular constituents released from the lysed single biological particle are denatured prior to (c).

[0019] In some examples, the sequencing is nucleic acid sequencing. In some examples, the nucleic acid sequencing is massively parallel sequencing. In some examples, the nucleic acid sequencing is digital polymerase chain reaction (PCR).

[0020] In some examples, the third liquid phase includes an oil. In some examples, the oil includes a fluorinated hydrocarbon. In some examples, the first liquid phase and the second liquid phase are the same phase.

[0021] In some examples, the first liquid phase and the second liquid phase are mixed to provide a mixed phase, and the mixed phase is brought in contact with the oil phase.

[0022] In some examples, the single biological particle comprises an organelle. In some examples, the single biological particle comprises a virus. In some examples, the single biological particle comprises a cell. In some examples, the cell comprises a rare cell from a population of cells.

[0023] In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^2 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^3 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^4 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^5 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^6 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^7 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^8 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^9 cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^{10} cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^{11} cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^{12} cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^{13} cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^{14} cells of the population of cells. In some examples, the rare cell is present in a sample at a concentration of at least about 1 in 10^{15} cells of the population of cells.

[0024] In some examples, the rare cell is a cancerous cell. In some examples, the cancer cell is a circulating tumor cell. In some examples, the rare cell is a cell obtained from an in vitro fertilization procedure. In some examples, the rare cell is a cell obtained from an individual displaying genetic mosaicism. In some examples, the rare cell is a cell obtained from an

organism produced using synthetic biology techniques. In some examples, the population of cells is a heterogeneous population of cells.

[0025] In some examples, the method comprises obtaining the plurality of biological particles. In some examples, the plurality of biological particles is obtained from a blood of a subject. In some examples, the plurality of biological particles includes cells. In some examples, the cells are cancerous cells. In some examples, the plurality of biological particles is obtained from a tissue of a subject.

[0026] In some examples, the one or more macromolecular constituents comprise deoxyribonucleic acid (DNA). In some examples, the one or more macromolecular constituents comprise ribonucleic acid (RNA). In some examples, the one or more macromolecular constituents comprise peptides or proteins.

[0027] In some examples, the tag is a primer. In some examples, (d) further comprises subjecting single biological particle to conditions sufficient for nucleic acid amplification. In some examples, the conditions sufficient for nucleic acid amplification comprise priming free amplification. In some examples, the priming free amplification comprises priming free amplification by polymerization at nick sites.

[0028] In some examples, the method further comprises using the tag to identify the one or more macromolecular constituents of the single biological particle from the plurality of biological particles. In some examples, the method further comprises subjecting the barcoded macromolecules to nucleic acid sequencing to identify the one or more macromolecular constituents. In some examples, the nucleic acid sequencing is untargeted sequencing. In some examples, the nucleic acid sequencing is targeted sequencing.

[0029] In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells.

[0030] In some examples, a given bead of the plurality of beads includes one or more tags coupled to a surface thereof and/or enclosed within the given bead.

[0031] In some examples, the plurality of partitions is part of a population of partitions that includes one or more partitions that are unoccupied by biological particles and/or beads.

[0032] In another example, the present disclosure provides a method for analysis of a single biological particle, comprising (a) providing a plurality of biological particles, and a plurality of beads each including a tag to barcode one or more macromolecular constituents of each of the plurality of biological particles; and (b) partitioning the plurality of biological particles and the plurality of beads into a plurality of partitions, wherein upon partitioning, a given partition of the plurality of partitions includes a single biological particle from the plurality of biological

particles and a single bead from the plurality of beads, wherein the single biological particle includes or is enclosed within a gel or polymer matrix within the given partition.

[0033] In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells.

[0034] In some examples, a given bead of the plurality of beads includes one or more tags coupled to a surface thereof and/or enclosed within the given bead.

[0035] In some examples, the plurality of partitions is part of a population of partitions that includes one or more partitions that are unoccupied by biological particles and/or beads.

[0036] In another example, the present disclosure provides a system for analysis of a single biological particle, comprising a partition generator comprising (i) a first source of a first liquid phase comprising a plurality of biological particles, (ii) a second source of a second liquid phase comprising a plurality of beads each including a tag to barcode one or more macromolecular constituents of each of the plurality of biological particles, and (iii) a third source of a third liquid phase that is immiscible with the first or second liquid phase; and a controller operatively coupled to the partition generator, wherein the controller is programmed to (i) bring the first liquid phase from the first source and the second liquid phase from the second source in contact with the third liquid phase from the third source along a first channel to partition each of the plurality of biological particles and the plurality of beads into a plurality of partitions that flow along a second channel, wherein upon partitioning, a given partition of the plurality of partitions includes a single biological particle from the plurality of biological particles and a single bead from the plurality of beads; and (ii) in the given partition, use the tag from the single bead to barcode the one or more macromolecular constituents of the single biological particle, forming one or more barcoded macromolecules; and (iii) subject the barcoded macromolecules to sequencing to generate reads characterized by a specific target read(s) to non-target specific read(s) ratio greater than 1, which specific target read(s) of the reads is indicative of the one or more macromolecular constituents.

[0037] In another example, the present disclosure provides a system for analysis of a single biological particle, comprising a partition generator comprising (i) a first source of a first liquid phase comprising a plurality of biological particles, (ii) a second source of a second liquid phase comprising a plurality of beads each including a tag to barcode one or more macromolecular constituents of each of the plurality of biological particles, and (iii) a third source of a third liquid phase that is immiscible with the first or second liquid phase, wherein the first liquid phase further comprises precursors that are capable of being polymerized or gelled; and a controller operatively coupled to the partition generator, wherein the controller is programmed to bring the

plurality of biological particles from the first liquid phase and the plurality of beads from the second liquid phase in contact with the third liquid phase that is immiscible with the first or second liquid phase, to partition each of the plurality of biological particles and the plurality of beads into a plurality of partitions, wherein upon partitioning, a given partition of the plurality of partitions includes a single biological particle from the plurality of biological particles and a single bead from the plurality of beads, wherein the single biological particle includes or is enclosed within a polymer or gel matrix.

[0038] In some examples, the third liquid phase includes an oil. In some examples, the first liquid phase and the second liquid phase are the same phase.

[0039] In some examples, the plurality of biological particles includes cells. In some examples, the plurality of biological particles is obtained from a tissue of a subject.

[0040] In some examples, the one or more macromolecular constituents comprise deoxyribonucleic acid (DNA). In some examples, the one or more macromolecular constituents comprise ribonucleic acid (RNA). In some examples, the tag is a primer.

[0041] In some examples, the controller subjects the single biological particle to conditions sufficient for nucleic acid amplification. In some examples, the controller is programmed to subject the single biological particle to conditions sufficient to barcode at least one macromolecular constituent from the single biological particle with at least one tag from the single bead.

[0042] In another example, the present disclosure provides a non-transitory computer-readable medium comprising machine-executable code that, upon execution by one or more computer processors, implements a method for analysis of a single biological particle, the method comprising (a) providing a first liquid phase comprising a plurality of biological particles; (b) providing a second liquid phase comprising a plurality of beads each including a tag to barcode one or more macromolecular constituents of each of the plurality of biological particles; (c) bringing the plurality of biological particles from the first liquid phase and the plurality of beads from the second liquid phase in contact with a third liquid phase that is immiscible with the first or second liquid phase, to partition each of the plurality of biological particles and the plurality of beads into a plurality of partitions, wherein upon partitioning, a given partition of the plurality of partitions includes a single biological particle from the plurality of biological particles and a single bead from the plurality of beads, wherein the single biological particle includes or is enclosed within a polymer or gel matrix.

[0043] In another example, the present disclosure provides a method for cellular analysis, comprising

(a) partitioning a plurality of cells or derivatives thereof into a plurality of partitions, wherein upon partitioning, a given partition of the plurality of partitions includes a single cell or derivative thereof from the plurality of cells or derivatives thereof and a set of tags that are capable of barcoding one or more macromolecular constituents of the single cell or derivative thereof, wherein the single cell or derivative thereof includes or is enclosed within a gel or polymer matrix within the given partition;

(b) using the set of tags to barcode the one or more macromolecular constituents from the single cell, thereby providing one or more barcoded macromolecules; and (c) analyzing the one or more barcoded macromolecules or derivatives thereof.

[0044] In some examples, the one or more macromolecular constituents include deoxyribonucleic acid. In some examples, the one or more macromolecular constituents include ribonucleic acid.

[0045] In some examples, the plurality of partitions are a plurality of droplets. In some examples, the plurality of partitions are a plurality of wells. In some examples, the set of tags is coupled to a bead in the given partition.

[0046] In some examples, the method further comprises releasing the one or more barcoded macromolecules or derivatives thereof from the given partition prior to analyzing.

[0047] In some examples, the method further comprises processing the single cell to include or be enclosed within the gel or polymer matrix prior to partitioning the plurality of cells into the plurality of partitions. In some examples, the method further comprises processing the single cell to include or be enclosed within the gel or polymer matrix after partitioning the plurality of cells into the plurality of partitions. In some examples, the cells are live cells.

[0048] In some examples, the live cells are capable of being cultured. In some examples, the live cells are capable of being cultured upon enclosure in or when comprising a gel or polymer matrix.

[0049] Tags (e.g., barcodes) may be enclosed within the plurality of beads. As an alternative or in addition to, tags may be coupled to surfaces of the plurality of beads. A given bead may include a plurality of tags.

[0050] In another example, the disclosure provides a method for processing or analyzing one or more components from a cell, comprising: (a) providing a plurality of cell beads and a plurality of barcode beads, wherein (i) a cell bead of the plurality of cell beads comprises the one or more components of the cell, which one or more components comprise a nucleic acid molecule, and (ii) a barcode bead of the plurality of barcode beads comprises a plurality of nucleic acid barcode molecules for barcoding the nucleic acid molecule; and (b) partitioning the

plurality of cell beads and the plurality of barcode beads into a plurality of partitions, wherein upon partitioning, a partition of the plurality of partitions comprises the cell bead and the barcode bead.

[0051] In some examples, the method further comprises performing one or more reactions on the nucleic acid molecule. In some examples, the one or more reactions comprise nucleic acid modification, nucleic acid amplification, nucleic acid insertion, nucleic acid cleavage, reverse transcription, or any combination thereof. In some examples, the nucleic acid modification comprises ligation, digestion, methylation, random mutagenesis, bisulfite conversion, uracil hydrolysis, nucleic acid repair, capping, decapping, or any combination thereof. In some examples, the nucleic acid amplification comprises isothermal amplification or polymerase chain reaction. In some examples, the nucleic acid insertion comprises transposon-mediated insertion, CRISPR/Cas9-mediated insertion, or any combination thereof. In some examples, the nucleic acid cleavage comprises transposon-mediated cleavage, CRISPR/Cas9-mediated cleavage, or any combination thereof. In some examples, the one or more reactions are performed in the partition. In some examples, the one or more reactions are performed outside the partition. In some examples, the one or more reactions are performed prior to (a). In some examples, the one or more reactions are performed subsequent to (a).

[0052] In some examples, the method further comprises using the plurality of nucleic acid barcode molecules to generate a barcoded nucleic acid molecule from the nucleic acid molecule. In some examples, generating the barcoded nucleic acid molecule comprises nucleic acid amplification. In some examples, generating the barcoded nucleic acid molecule comprises ligation. In some examples, the method further comprises releasing the barcoded nucleic acid molecule from the partition. In some examples, the method further comprises subjecting the barcoded nucleic acid molecule or derivative thereof to sequencing. In some examples, the method further comprises, prior to the sequencing, subjecting the barcoded nucleic acid molecule or derivative thereof to nucleic acid amplification. In some examples, the nucleic acid amplification is isothermal amplification or polymerase chain reaction. In some examples, the polymerase chain reaction is digital polymerase chain reaction.

[0053] In some examples, the cell bead comprises the cell, and the cell bead comprising the cell is subjected to conditions sufficient to lyse the cell to generate the one or more components. In some examples, the cell bead is subject to the conditions sufficient to lyse the cell in the partition. In some examples, the conditions sufficient to lyse the cell comprise exposing the cell beads to a lysis agent. In some examples, the conditions sufficient to lyse the cell comprise exposing the cell beads to sodium hydroxide, potassium hydroxide, sodium dodecyl sulfate, a

non-ionic surfactant, a saponin, a proteinase, a lytic enzyme, freeze thawing, ultraviolet light, heat, or any combination thereof. In some examples, the non-ionic surfactant is 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (Triton X-100).

[0054] In some examples, the cell bead includes or is enclosed within a gel or polymer matrix within the partition. In some examples, the barcode bead includes or is enclosed within a gel or polymer matrix within the partition. In some examples, the polymer or gel matrix includes one or more members selected from the group consisting of disulfide crosslinked polyacrylamide, agarose, alginate, polyvinyl alcohol, PEG-diacrylate, PEG-acrylate/thiol, PEG-azide/alkyne, other acrylates, chitosan, hyaluronic acid, collagen, fibrin, gelatin, and elastin.

[0055] In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells. In some examples, one or more nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules are coupled to a surface of the barcode bead and/or enclosed within the barcode bead.

[0056] In some examples, the cell bead further comprises additional reagents. In some examples, the partition further comprises additional reagents. In some examples, the additional reagents comprise primers, reverse transcriptase enzymes, polymerases, nucleotides, proteases, transposons, endonucleases, switch oligonucleotides, lysis reagents, or any combination thereof. In some examples, the nucleic acid molecule is a deoxyribonucleic acid molecule. In some examples, the deoxyribonucleic acid molecule is genomic deoxyribonucleic acid. In some examples, the deoxyribonucleic acid molecule is complementary deoxyribonucleic acid. In some examples, the nucleic acid molecule is a ribonucleic acid molecule. In some examples, the ribonucleic acid molecule is messenger ribonucleic acid. In some examples, the method further comprises recovering the nucleic acid molecule or a derivative thereof from the partition.

[0057] In some examples, the barcode bead is degradable upon application of a stimulus. In some examples, the method further comprises releasing the plurality of nucleic acid barcode molecules upon application of the stimulus. In some examples, the stimulus is a chemical stimulus, a biological stimulus, a temperature change, exposure to light, a pH change, or any combination thereof. In some examples, the chemical stimulus is a reducing agent. In some examples, the reducing agent is dithiothreitol, β -mercaptoethanol, (2S)-2-amino-1,4-dimercaptobutane, tris(2-carboxyethyl) phosphine, or any combination thereof. In some examples, the stimulus is a chemical or biological stimulus, and the partition comprises the stimulus. In some examples, the cell bead is degradable upon application of a stimulus. In some examples, the stimulus is a chemical stimulus, a biological stimulus, a temperature change, exposure to light, a pH change, or any combination thereof. In some examples, the chemical

stimulus is a reducing agent. In some examples, the reducing agent is dithiothreitol, β -mercaptoethanol, (2S)-2-amino-1,4-dimercaptobutane, tris(2-carboxyethyl) phosphine, or any combination thereof. In some examples, the stimulus is a chemical or biological stimulus, and the partition comprises the stimulus.

[0058] In some examples, the plurality of partitions is part of a population of partitions that includes one or more partitions that are unoccupied by a cell bead and/or a barcode bead.

[0059] In another example, the disclosure provides a system for processing or analyzing one or more components from a cell, comprising: a first channel in fluid communication with a first source comprising a plurality of cell beads, wherein a cell bead of the plurality of cell beads comprises the one or more components of the cell, which one or more components comprise a nucleic acid molecule; a second channel in fluid communication with a second source comprising a plurality of barcode beads, wherein a barcode bead of the plurality of barcode beads comprises a plurality of nucleic acid barcode molecules for barcoding the nucleic acid molecule; and a junction that brings a first phase comprising the plurality of cell beads from the first channel and the plurality of barcode beads from the second channel in contact with a second phase that is immiscible with the first phase, to yield a plurality of droplets comprising the plurality of cell beads and the plurality of barcode beads, wherein a droplet of the plurality of droplets comprises the cell bead and the barcode bead.

[0060] In some examples, the first channel and the second channel are the same channel. In some examples, the system further comprises a third channel in fluid communication with a third source comprising additional reagents, wherein the first phase comprises the additional reagents. In some examples, the system further comprises a fourth channel in fluid communication with a fourth source comprising additional reagents, wherein the first phase comprises the additional reagents. In some examples, the additional reagents are reagents for nucleic acid amplification, reagents that can degrade or dissolve cell beads and/or barcode beads, reagents that degrade linkages between barcodes and barcode beads, or any combination thereof.

[0061] Another example of the disclosure provides a composition comprising a cell bead of a plurality of cell beads and a barcode bead of a plurality of barcode beads, wherein the cell bead comprises one or more components from a cell, which one or more components comprise a nucleic acid molecule, and wherein the barcode bead comprises a plurality of nucleic acid barcode molecules for barcoding the nucleic acid molecule. In some examples, the cell bead further comprises additional reagents. In some examples, the additional reagents comprise primers, reverse transcriptase enzymes, polymerases, nucleotides, proteases, transposons, endonucleases, switch oligonucleotides, or any combination thereof. In some examples, the

nucleic acid molecule is a deoxyribonucleic acid molecule. In some examples, the deoxyribonucleic acid molecule is genomic deoxyribonucleic acid. In some examples, the deoxyribonucleic acid molecule is complementary deoxyribonucleic acid. In some examples, the nucleic acid molecule is a ribonucleic acid molecule. In some examples, the ribonucleic acid molecule is messenger ribonucleic acid.

[0062] In another example, the disclosure provides a method for generating a cell bead, comprising: (a) providing a plurality of cells and a plurality of polymeric or gel precursors; (b) partitioning the plurality of cells and the plurality of polymeric or gel precursors into a plurality of partitions, wherein upon partitioning, a partition of the plurality of partitions comprises a cell of the plurality of cells and at least a portion of the polymeric or gel precursors; and (c) subjecting the partitions to conditions suitable for cross-linking or polymerizing the polymeric or gel precursors to generate the cell bead, wherein the cell bead encapsulates the cell. In some examples, the method further comprises, subsequent to (b), subjecting the cell bead to conditions sufficient to lyse the cell. In some examples, the conditions sufficient to lyse the cell comprise exposing the cell beads to a lysis agent. In some examples, the conditions sufficient to lyse the cell comprise exposing the cell beads to sodium hydroxide, potassium hydroxide, sodium dodecyl sulfate, a non-ionic surfactant, a saponin, a proteinase, a lytic enzyme, freeze thawing, ultraviolet light, heat, or any combination thereof. In some examples, the non-ionic surfactant is 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (Triton X-100). In some examples, in (b), the partition comprises a bead. In some examples, the bead is a magnetic bead. In some examples, the magnetic bead is a paramagnetic particle.

[0063] Another example of the present disclosure provides a method for processing one or more nucleic acid molecules from a cell, comprising (a) providing a plurality of cells and a plurality of polymeric or gel precursors; (b) partitioning the plurality of cells and the plurality of polymeric or gel precursors into a plurality of partitions, wherein upon partitioning, a partition of the plurality of partitions comprises (i) a nucleic acid molecule, (ii) a cell of the plurality of cells and (iii) at least a portion of the polymeric or gel precursors; (c) subjecting the plurality of partitions to conditions sufficient to cross-link or polymerize the polymeric or gel precursors to form a plurality of cell beads; and (d) partitioning the plurality of cell beads and a plurality of barcode beads comprising a plurality of nucleic acid barcode molecules into an additional plurality of partitions, wherein upon partitioning, a partition of the additional plurality of partitions comprises the cell bead and the barcode bead. In some examples, the method further comprises, subsequent to (a), subjecting the plurality of partitions to conditions sufficient to lyse the plurality of cells, releasing the nucleic acid molecule from the cell into the partition. In some

examples, the nucleic acid molecule is a deoxyribonucleic acid molecule. In some examples, the nucleic acid molecule is a ribonucleic acid molecule. In some examples, in (b), the partition comprises a bead. In some examples, the bead is a magnetic bead. In some examples, the magnetic bead is a paramagnetic particle. In some examples, the method further comprises performing one or more reactions on the nucleic acid molecule. In some examples, the method further comprises barcoding the nucleic acid molecule to generate a barcoded nucleic acid molecule. In some examples, the method further comprises, subsequent to (d), releasing the barcoded nucleic acid molecule from the partition. In some examples, the method further comprises subjecting the barcoded nucleic acid molecule or derivative thereof to sequencing.

[0064] Another example of the present disclosure provides a non-transitory computer readable medium comprising machine executable code that, upon execution by one or more computer processors, implements any of the methods above or elsewhere herein.

[0065] Another example of the present disclosure provides a system comprising one or more computer processors and computer memory coupled thereto. The computer memory comprises machine executable code that, upon execution by the one or more computer processors, implements any of the methods above or elsewhere herein.

[0066] 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

[0067] 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

[0068] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be

obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings (also “Figure” and “FIG.” herein), of which:

[0069] FIG. 1A schematically illustrates an example method for generating droplets comprising a barcoded bead and a cell bead (e.g., comprising a cell or a cell component(s));

[0070] FIG. 1B photographically illustrates an example microfluidic architecture for generating cell beads;

CLAIMS

WHAT IS CLAIMED IS:

1. A method for processing or analyzing one or more components from a sample, comprising:
 - (a) providing a plurality of cell beads and a plurality of barcode beads, wherein (i) a cell bead of said plurality of cell beads comprises said cell, wherein said cell comprises a nucleic acid molecule, and (ii) a barcode bead of said plurality of barcode beads comprises a plurality of nucleic acid barcode molecules for barcoding said nucleic acid molecule;
 - (b) subjecting said plurality of cell beads to bulk denaturation, which bulk denaturation comprises denaturing said nucleic acid molecule to generate a denatured nucleic acid molecule in said cell bead; and
 - (c) partitioning said plurality of cell beads and said plurality of barcode beads into a plurality of partitions, wherein upon partitioning, a partition of said plurality of partitions comprises said cell bead and said barcode bead.
2. The method of claim 1, further comprising using a nucleic acid barcode molecule of said plurality of nucleic acid barcode molecules and said denatured nucleic acid molecule or a derivative thereof to generate a barcoded nucleic acid molecule comprising a first sequence corresponding to said denatured nucleic acid molecule and a second sequence corresponding to a barcode sequence of said nucleic acid barcode molecule.
3. The method of claim 2, further comprising subjecting said barcoded nucleic acid molecule or derivative thereof to sequencing.
4. The method of claim 1, wherein the plurality of partitions is a plurality of droplets or a plurality of wells.
5. The method of claim 1, wherein said barcode bead or said cell bead is degradable upon application of a stimulus selected from the group consisting of a chemical stimulus, a biological stimulus, a temperature change, exposure to light, and a pH change.
6. The method of claim 1, further comprising: prior to (a), partitioning a plurality of cells comprising said cell and polymer or gel precursors into a plurality of first partitions; and subjecting said plurality of first partitions to conditions sufficient to polymerize or crosslink said polymer or gel precursors in said plurality of first partitions to generate said plurality of cell beads.

7. The method of claim 1, wherein said plurality of nucleic acid barcode molecules is a plurality of double-stranded nucleic acid barcode molecules.
8. The method of claim 7, further comprising (i) performing one or more nucleic acid extension reactions on said denatured nucleic acid molecule to generate a double-stranded nucleic acid molecule comprising a sequence of said denatured nucleic acid molecule and (ii) ligating said nucleic acid barcode molecule to said double-stranded nucleic acid molecule to generate a barcoded nucleic acid molecule.
9. The method of claim 8, wherein said one or more nucleic acid extension reactions comprise:
 - (i) annealing a first primer to said denatured nucleic acid molecule and performing a first nucleic acid extension reaction in the presence of uracil to generate a first nucleic acid extension product comprising a uracil-containing moiety;
 - (ii) excising said uracil-containing moiety to generate a nick in said first nucleic acid extension product;
 - (iii) performing a second nucleic acid extension reaction on said first nucleic acid extension product comprising said nick to generate a plurality of single-stranded nucleic acid fragments;
 - (iv) annealing a second primer to a single-stranded nucleic acid fragment of said plurality of single-stranded nucleic acid fragments; and
 - (v) performing a third nucleic acid extension reaction to generate a second nucleic acid extension product.
10. The method of claim 9, wherein said first primer or said second primer comprises a random primer sequence or a targeted primer sequence.
11. The method of claim 9, wherein said first primer and said second primer comprises a random primer sequence.
12. The method of claim 9, wherein said second nucleic acid extension reaction is completed with use of a polymerase having strand displacement activity, wherein the polymerase engages the first nucleic acid extension product at the nick, and wherein said plurality of single stranded nucleic acid fragments are displaced from the first nucleic acid extension product.
13. The method of claim 1, wherein said bulk processing comprises contacting said plurality of cell beads with a chemical agent.
14. The method of claim 13, wherein said chemical agent is sodium hydroxide.

15. The method of claim 1, wherein said plurality of cell beads comprise a plurality of magnetic particles.
16. The method of claim 15, further comprising, prior to (c), using a magnetic source to purify said plurality of cell beads subsequent to said bulk processing.
17. The method of claim 1, wherein said plurality of nucleic acid barcode molecules are releasable from said barcode bead upon application of a stimulus selected from the group consisting of a chemical stimulus, a biological stimulus, a temperature change, exposure to light, and a pH change.
18. The method of claim 1, wherein said bulk processing comprises subjecting said cell bead to conditions sufficient to lyse said cell.
19. The method of claim 18, wherein said conditions sufficient to lyse said cell comprise exposing said plurality of cell beads to sodium hydroxide, potassium hydroxide, sodium dodecyl sulfate, a non-ionic surfactant, a saponin, a proteinase, a lytic enzyme, freeze thawing, ultraviolet light, heat, or any combination thereof.
20. The method of claim 1, wherein in (a), said cell bead comprises said cell enclosed within a gel or polymer matrix comprising one or more members selected from the group consisting of disulfide crosslinked polyacrylamide, agarose, alginate, polyvinyl alcohol, PEG-diacrylate, PEG-acrylate/thiol, PEG-azide/alkyne, other acrylates, chitosan, hyaluronic acid, collagen, fibrin, gelatin, and elastin.