

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

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.