

performing a nucleic acid sequencing assay. The present disclosure also provides methods for analyzing the cellular occupancy of partitions (e.g., droplets or wells). Such methods may comprise, for example, labeling a plurality of cells with a plurality of barcodes (e.g., nucleic acid barcode sequences, such as nucleic acid barcode sequences coupled to lipophilic or amphiphilic moieties) to provide a plurality of labeled cells. Labeled cells of the plurality of labeled cells may be labeled with different barcodes. Labeled cells may be partitioned within a plurality of partitions (e.g., droplets or wells) and may be further labeled with additional barcodes (e.g., partition nucleic acid barcode sequences). The barcodes of the labeled cells may then be used to, e.g., identify labeled cells as originating from the same partition. The methods of the present disclosure may also be useful for determining the relative sizes of cells within a cellular sample, e.g., based at least in part on the uptake of barcodes (e.g., barcodes (e.g., nucleic acid barcode sequences) coupled to lipophilic or amphiphilic moieties) by the cells. The uptake of such barcodes may be measured by, for example, directly detecting barcodes associated with the cells or by performing a nucleic acid sequencing assay and measuring an abundance of various barcode sequences identified in the sequencing assay.

[0005a] In one aspect, provided herein is a method for analyzing a cell, comprising: (a) labeling said cell with a cell nucleic acid barcode sequence to generate a labeled cell, wherein a cell nucleic acid barcode molecule comprises said cell nucleic acid barcode sequence and a lipophilic moiety; (b) generating a partition comprising said labeled cell and a plurality of partition nucleic acid barcode molecules, wherein each partition nucleic acid barcode molecule of said plurality of partition nucleic acid barcode molecules comprises a partition nucleic acid barcode sequence; (c) permeabilizing or lysing said cell to provide access to a plurality of nucleic acid molecules therein; (d) generating (i) a barcoded nucleic acid molecule comprising said cell nucleic acid barcode sequence, or a complement thereof, and said partition nucleic acid barcode sequence, or a complement thereof, and (ii) a plurality of barcoded nucleic acid products each comprising a sequence of a nucleic acid molecule of said plurality of nucleic acid molecules and said partition nucleic acid barcode sequence, or a complement thereof; and (e) identifying said plurality of nucleic acid molecules as originating from said cell.

[0006] In an example, the present disclosure provides a method for analyzing cellular occupancy of partitions, comprising: (a) providing a plurality of cell nucleic acid barcode molecules comprises a plurality of cell nucleic acid barcode sequences, each cell nucleic acid barcode molecule of the plurality of cell nucleic acid barcode molecules comprising (i) a single cell nucleic acid barcode sequence of the plurality of cell nucleic acid barcode sequences and (ii) a lipophilic moiety; (b) labeling a plurality of cells with the plurality of cell nucleic acid barcode

sequences to generate a plurality of labeled cells, wherein each labeled cell of the plurality of labeled cells comprises a different cell nucleic acid barcode sequence of the plurality of cell nucleic acid barcode sequences; (c) generating a plurality of partitions comprising the plurality of labeled cells and a plurality of partition nucleic acid barcode sequences, wherein each partition of the plurality of partitions comprises a different partition nucleic barcode sequence of the plurality of partition nucleic acid barcode sequences, and wherein at least a fraction of the plurality of partitions comprises more than one labeled cell of the plurality of labeled cells; and (d) identifying at least two labeled cells of the plurality of labeled cells as originating from a same partition using (i) cell nucleic acid barcode sequences of the plurality of cell nucleic acid barcode sequences, or complements thereof, and (ii) partition nucleic acid barcode sequences of the plurality of partition nucleic acid barcode sequences, or complements thereof.

[0007] In some examples, a given cell nucleic acid barcode sequence of the plurality of cell nucleic acid barcode sequences identifies a sample from which an associated cell of the plurality of labeled cells originates. In some examples, the sample is derived from a biological fluid. In some examples, the biological fluid comprises blood or saliva.

[0008] In some examples, the method further comprises, after (c), synthesizing a plurality of barcoded nucleic acid products from the plurality of labeled cells, wherein a given barcoded nucleic acid product of the plurality of barcoded nucleic acid products comprises (i) a cell identification sequence comprising a given cell nucleic acid barcode sequence of the plurality of cell nucleic acid barcode sequences, or a complement of the given cell nucleic acid barcode sequence; and (ii) a partition identification sequence comprising a given partition nucleic acid barcode sequence of the plurality of partition nucleic acid barcode sequences, or a complement of the given partition nucleic acid barcode sequence.

[0009] In some examples, a plurality of partition nucleic acid barcode molecules comprises the plurality of partition nucleic acid barcode sequences, each partition nucleic acid barcode molecule of the plurality of partition nucleic acid barcode molecules comprising a single partition nucleic acid barcode sequence of the plurality of partition nucleic acid barcode sequences. In some examples, a given partition nucleic acid barcode molecule of the plurality of partition nucleic acid barcode molecules comprises a priming sequence that is capable of hybridizing to a sequence of a given cell nucleic acid barcode molecule of the plurality of cell nucleic acid barcode molecules. In some examples, each cell nucleic acid barcode molecule of the plurality of cell nucleic acid barcode molecules comprises the sequence. In some examples, the priming sequence is a targeted priming sequence. In some examples, the priming sequence is a random N-mer sequence. In some examples, the plurality of barcoded nucleic acid products

is synthesized via one or more primer extension reactions. In some examples, the plurality of barcoded nucleic acid products is synthesized via one or more ligation reactions. In some examples, the plurality of barcoded nucleic acid products is synthesized via one or more nucleic acid amplification reactions.

[0010] In some examples, the method further comprises sequencing the plurality of barcoded nucleic acid products or derivatives thereof to yield a plurality of sequencing reads. In some examples, the method further comprises associating each sequencing read of the plurality of sequencing reads with a labeled cell of the plurality of labeled cells via its respective cell identification sequence, and associating each sequencing read of the plurality of sequencing reads with a partition of the plurality of partitions via its respective partition identification sequence.

[0011] In some examples, the method further comprises, in (c), partitioning the plurality of labeled cells with a plurality of beads, wherein each bead of the plurality of beads comprises a partition nucleic acid barcode sequence of the plurality of partition nucleic acid barcode sequences. In some examples, each partition of the plurality of partitions comprises a single bead of the plurality of beads. In some examples, each bead of the plurality of beads comprises a plurality of partition nucleic acid barcode molecules, wherein each partition nucleic acid barcode molecule of the plurality of partition nucleic acid barcode molecules comprises a single partition nucleic acid barcode sequence of the plurality of partition nucleic acid barcode sequences. In some examples, each partition nucleic acid barcode sequence of the plurality of partition nucleic acid barcode sequences is releasably coupled to its respective bead of the plurality of beads. In some examples, each partition nucleic acid barcode sequence of the plurality of partition nucleic acid barcode sequences is releasable from its respective bead of the plurality of beads upon application of a stimulus. In some examples, the stimulus is a chemical stimulus. In some examples, the method further comprises, after (c), releasing partition nucleic acid barcode sequences of the plurality of partition nucleic acid barcode sequences from each bead of the plurality of beads. In some examples, the method further comprises degrading each bead of the plurality of beads to release the partition nucleic acid barcode sequences from each bead of the plurality of beads. In some examples, each partition of the plurality of partitions comprises an agent that is capable of degrading each bead of the plurality of beads. In some examples, the plurality of beads is a plurality of gel beads.

[0012] In some examples, the plurality of partitions is a plurality of droplets. In some examples, the plurality of partitions is a plurality of wells.

[0013] In some examples, in (b), the plurality of cells is labeled with the plurality of cell nucleic acid barcode sequences by binding cell binding moieties, each coupled to a given cell nucleic acid barcode sequence of the plurality of cell nucleic acid barcode sequences, to each cell of the plurality of cells. In some examples, the cell binding moieties are antibodies, cell surface receptor binding molecules, receptor ligands, small molecules, pro-bodies, aptamers, monobodies, affimers, darpins, or protein scaffolds. In some examples, the cell binding moieties are antibodies. In some examples, the cell binding moieties bind to a protein of cells of the plurality of cells. In some examples, the cell binding moieties bind to a cell surface species of cells of the plurality of cells. In some examples, the cell binding moieties bind to a species common to each cell of the plurality of cells.

[0014] In some examples, in (b), the plurality of cells is labeled with the plurality of cell nucleic acid barcode sequences by delivering nucleic acid barcode molecules each comprising an individual cell nucleic acid barcode sequence of the plurality of cell nucleic acid barcode sequences to each cell of the plurality of cells with the aid of a cell-penetrating peptide.

[0015] In some examples, in (b), the plurality of cells is labeled with the plurality of cell nucleic acid barcode sequences with the aid of liposomes, nanoparticles, electroporation, or mechanical force. In some examples, the mechanical force comprises the use of nanowires or microinjection.

[0016] In some examples, the lipophilic moiety of each nucleic acid barcode molecule of the plurality of cell nucleic acid barcode molecules is a cholesterol.

[0017] In some examples, the lipophilic moiety is linked to the plurality of cell nucleic acid barcode molecules via a linker.

[0018] In some examples, each cell of the plurality of cells comprises a plurality of nucleic acid molecules. In some examples, the plurality of nucleic acid molecules comprises a plurality of deoxyribonucleic acid molecules. In some examples, the plurality of nucleic acid molecules comprises a plurality of ribonucleic acid molecules. In some examples, the labeled cells are lysed or permeabilized to provide access to the plurality of nucleic acid molecules. In some examples, a plurality of partition nucleic acid barcode molecules comprises the plurality of partition nucleic acid barcode sequences, each partition nucleic acid barcode molecule of the plurality of partition nucleic acid barcode molecules comprising a single partition nucleic acid barcode sequence of the plurality of partition nucleic acid barcode sequences and a priming sequence that is capable of hybridizing to a sequence of at least a subset of the plurality of nucleic acid molecules. In some examples, the priming sequence is a targeted priming sequence. In some examples, the priming sequence is a random N-mer sequence.

[0019] In some examples, prior to (c), at least a subset of the cell nucleic acid barcode molecules of the plurality of cell nucleic acid barcode molecules are at least partially disposed within the plurality of labeled cells.

[0020] In another example, the present disclosure provides a method for analyzing cellular occupancy of a partition, comprising: (a) providing a first cell nucleic acid barcode molecule comprising (i) a first cell nucleic acid barcode sequence and (ii) a lipophilic moiety, and a second nucleic acid barcode molecule comprising (i) a second cell nucleic acid barcode sequence and (ii) a lipophilic moiety, wherein the first cell nucleic acid barcode sequence has a different sequence than the second cell nucleic acid barcode sequence; (b) labeling a first cell with the first cell nucleic acid barcode sequence to generate a first labeled cell and labeling a second cell with the second cell nucleic acid barcode sequence to generate a second labeled cell; (c) generating a partition comprising the first labeled cell and the second labeled cell, wherein the partition further comprises a partition nucleic acid barcode sequence; (d) generating (i) a first barcoded nucleic acid molecule comprising the first cell nucleic acid barcode sequence, or a complement thereof, and the partition nucleic acid barcode sequence, or a complement thereof, and (ii) a second barcoded nucleic acid molecule comprising the second cell nucleic acid barcode sequence, or a complement thereof, and a partition nucleic acid barcode sequence, or a complement thereof; and (e) identifying the first labeled cell and the second labeled cell as originating from the partition based on the first barcoded nucleic acid molecule and the second barcoded nucleic acid molecule having the same partition nucleic acid barcode sequence, or a complement thereof.

[0021] In some examples, the first cell nucleic acid barcode sequence and the second cell nucleic acid barcode sequence identify a sample from which the first cell and the second cell originate. In some examples, wherein the sample is derived from a biological fluid. In some examples, the biological fluid comprises blood or saliva.

[0022] In some examples, wherein the first barcoded nucleic acid molecule and the second barcoded nucleic acid molecule each comprise a priming sequence. In some examples, the priming sequence is a targeted priming sequence. In some examples, the priming sequence is a random N-mer sequence.

[0023] In some examples, the first barcode nucleic acid molecule and the second barcode nucleic acid molecule are synthesized via one or more primer extension reactions, ligation reactions, or nucleic acid amplification reactions.

[0024] In some examples, the method further comprises sequencing the first barcode nucleic acid molecule and the second barcode nucleic acid molecule, or derivatives thereof, to yield a

plurality of sequencing reads. In some examples, the method further comprises associating each sequencing read of the plurality of sequencing reads with the first labeled cell or the second labeled cell via its cell nucleic acid barcode sequence, and associating each sequencing read of the plurality of sequencing reads with the partition via its respective partition nucleic acid sequence.

[0025] In some examples, the method further comprises, in (c), partitioning the first labeled cell and the second labeled cell with a bead, which bead comprises a plurality of nucleic acid barcode molecules, each of which comprises the partition nucleic acid barcode sequence. In some examples, the partition nucleic acid barcode sequence of each nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules is releasably coupled to the bead. In some examples, the method further comprises, after (c), releasing partition nucleic acid barcode sequences of the plurality of partition nucleic acid barcode molecules from the bead. In some examples, the bead is a gel bead.

[0026] In some examples, the partition is a well. In some examples, the partition is a droplet.

[0027] In some examples, the lipophilic moiety of the first cell nucleic acid barcode molecule and the second cell nucleic acid barcode molecule is a cholesterol.

[0028] In some examples, the first cell and the second cell each comprise a plurality of nucleic acid molecules. In some examples, the first labeled cell and the second labeled cell are lysed or permeabilized to provide access to the pluralities of nucleic acid molecules. In some examples, a plurality of partition nucleic acid barcode molecules each comprise the partition nucleic acid barcode sequence and a priming sequence that is capable of hybridizing to a sequence of at least a subset of the plurality of nucleic acid molecules. In some examples, the priming sequence is a targeted priming sequence. In some examples, the priming sequence is a random N-mer sequence.

[0029] In some examples, prior to (c), at least a subset of the cell nucleic acid barcode molecules of the plurality of cell nucleic acid barcode molecules are at least partially disposed within the plurality of labeled cells.

[0030] In another example, the present disclosure provides a method for analyzing a cell, comprising: (a) labeling the cell with a cell nucleic acid barcode sequence to generate a labeled cell, wherein a cell nucleic acid barcode molecule comprises the cell nucleic acid barcode sequence and a lipophilic moiety; (b) generating a partition comprising the labeled cell and a plurality of partition nucleic acid barcode molecules, wherein each partition nucleic acid barcode molecule of the plurality of partition nucleic acid barcode molecules comprises a partition

nucleic acid barcode sequence; (c) permeabilizing the cell to provide access to a plurality of nucleic acid molecules therein; (d) generating (i) a barcoded nucleic acid molecule comprising the cell nucleic acid barcode sequence, or a complement thereof, and the partition nucleic acid barcode sequence, or a complement thereof, and (ii) a plurality of barcoded nucleic acid products each comprising a sequence of a nucleic acid molecule of the plurality of nucleic acid molecules and the partition nucleic acid barcode sequence, or a complement thereof; and (e) identifying the plurality of nucleic acid molecules as originating from the cell.

[0031] In some examples, the cell nucleic acid barcode sequence identifies a sample from which the cell originates. In some examples, the sample is derived from a biological fluid. In some examples, the biological fluid comprises blood or saliva.

[0032] In some examples, the barcoded nucleic acid molecule comprises a priming sequence. In some examples, each partition nucleic acid barcode molecule of the plurality of partition nucleic acid barcode molecules comprises a priming sequence. In some examples, the priming sequence is a targeted priming sequence. In some examples, the priming sequence is a random N-mer sequence. In some examples, the priming sequence is capable of hybridizing to a sequence of at least a subset of the plurality of nucleic acid molecules. In some examples, the priming sequence is capable of hybridizing to a sequence of the cell nucleic acid barcode molecule.

[0033] In some examples, the barcoded nucleic acid molecule and the plurality of barcoded nucleic acid products are synthesized via one or more primer extension reactions, ligation reactions, or nucleic acid amplification reactions.

[0034] In some examples, the method further comprises sequencing the barcoded nucleic acid molecule and the barcoded nucleic acid products, or derivatives thereof, to yield a plurality of sequencing reads. In some examples, the method further

comprises associating each sequencing read of the plurality of sequencing reads with the partition via its partition nucleic acid barcode sequence.

[0035] In some examples, the method further comprises, in (b), partitioning the labeled cell with a bead, which bead comprises the plurality of partition nucleic acid barcode molecules. In some examples, the partition nucleic acid barcode sequence of each nucleic acid barcode molecule of the plurality of partition nucleic acid barcode molecules is releasably coupled to the bead. In some examples, the method further comprises, after (b), releasing partition nucleic acid barcode sequences of the plurality of partition nucleic acid barcode molecules from the bead. In some examples, the bead is a gel bead.

[0036] In some examples, the partition is a well. In some examples, the partition is a droplet.

[0037] In some examples, the lipophilic moiety of the cell nucleic acid barcode molecule is a cholesterol.

[0038] In some examples, the plurality of nucleic acid molecules comprise a plurality of deoxyribonucleic acid molecules. In some examples, the plurality of nucleic acid molecules comprise a plurality of ribonucleic acid molecules.

[0039] In some examples, prior to (b), the cell nucleic acid barcode molecule is at least partially disposed within the labeled cells.

[0040] 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

[0041] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or

CLAIMS

WHAT IS CLAIMED IS:

1. A method for analyzing a cell, comprising:
 - (a) labeling said cell with a cell nucleic acid barcode sequence to generate a labeled cell, wherein a cell nucleic acid barcode molecule comprises said cell nucleic acid barcode sequence and a lipophilic moiety;
 - (b) generating a partition comprising said labeled cell and a plurality of partition nucleic acid barcode molecules, wherein each partition nucleic acid barcode molecule of said plurality of partition nucleic acid barcode molecules comprises a partition nucleic acid barcode sequence;
 - (c) permeabilizing or lysing said cell to provide access to a plurality of nucleic acid molecules therein;
 - (d) generating (i) a barcoded nucleic acid molecule comprising said cell nucleic acid barcode sequence, or a complement thereof, and said partition nucleic acid barcode sequence, or a complement thereof, and (ii) a plurality of barcoded nucleic acid products each comprising a sequence of a nucleic acid molecule of said plurality of nucleic acid molecules and said partition nucleic acid barcode sequence, or a complement thereof; and
 - (e) identifying said plurality of nucleic acid molecules as originating from said cell.
2. The method of claim 1, wherein said cell nucleic acid barcode sequence identifies a sample from which said cell originates.
3. The method of claim 1 or claim 2, wherein each partition nucleic acid barcode molecule of said plurality of partition nucleic acid barcode molecules comprises a priming sequence.
4. The method of claim 3, wherein said priming sequence is capable of hybridizing to a sequence of said cell nucleic acid barcode molecule or a sequence of at least a subset of said plurality of nucleic acid molecules.
5. The method of any one of claims 1 to 4, wherein said barcoded nucleic acid molecule and said plurality of barcoded nucleic acid products are synthesized via one or more primer extension reactions, ligation reactions, or nucleic acid amplification reactions.
6. The method of any one of claims 1 to 5, further comprising sequencing said barcoded nucleic acid molecule and said barcoded nucleic acid products, or derivatives thereof, to yield a plurality of sequencing reads.
7. The method of claim 6, further comprising associating each sequencing read of said plurality of sequencing reads with said partition via its partition nucleic acid barcode sequence.
8. The method of any one of claims 1 to 7, further comprising, in (b), partitioning said labeled cell with a bead, which bead comprises said plurality of partition nucleic acid barcode molecules.

9. The method of claim 8, wherein said partition nucleic acid barcode sequence of each nucleic acid barcode molecule of said plurality of partition nucleic acid barcode molecules is releasably coupled to said bead.
10. The method of claim 9, further comprising, after (b), releasing partition nucleic acid barcode sequences of said plurality of partition nucleic acid barcode molecules from said bead.
11. The method of any one of claims 8 to 10, wherein said bead is a gel bead.
12. The method of any one of claims 1 to 11, wherein said partition is a droplet.
13. The method of any one of claims 1 to 12, wherein said lipophilic moiety of said cell nucleic acid barcode molecule is a cholesterol.
14. The method of any one of claims 1 to 13, wherein, prior to (b), said cell nucleic acid barcode molecule is at least partially disposed within said labeled cells.
15. The method of any one of claims 1 to 14, wherein each partition nucleic acid barcode molecule of said plurality of partition nucleic acid barcode molecules comprises a poly-T sequence.