

METHODS FOR ANALYTE DETECTION AND ANALYSIS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/914,296, filed October 11, 2019, which application is herein incorporated by reference in its entirety for all purposes.

BACKGROUND

[0002] Nucleic-acid based barcoding technology can be used to measure proteins by using antibodies conjugated to a DNA sequence that contains a primer for amplification/sequencing and a unique oligonucleotide that acts as an antibody barcode. It is a useful tool for detection and quantification of proteins that remain bound to the surface of a cell. However, there is a need for approaches that allow analysis of proteins and molecules secreted by a cell.

SUMMARY

[0002a] In one aspect, the present disclosure relates to a method of single cell analysis, comprising: a) contacting a cell with a reporter agent comprising a reporter nucleic acid molecule to provide a labelled cell, wherein the reporter nucleic acid molecule comprises a reporter sequence corresponding to the reporter agent, wherein the labelled cell comprises a complex coupled to a surface of the cell, and wherein the complex comprises (i) a capture agent, (ii) a secreted analyte, and (iii) the reporter agent, wherein the capture agent is configured to couple to both a cell surface molecule and the secreted analyte, and wherein the reporter agent is configured to couple to the secreted analyte; b) partitioning the labelled cell into a partition, wherein the partition is from a plurality of partitions and is a droplet or a microwell, wherein the partition comprises a plurality of barcode nucleic acid molecules which comprise a plurality of partition barcode sequences, wherein said reporter nucleic acid molecule further comprises a sequence configured to couple to the barcode nucleic acid molecule; c) in bulk or in the partition, generating a barcoded nucleic acid molecule from a barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules and the reporter nucleic acid molecule, wherein the barcoded nucleic acid molecule comprises the reporter sequence or a complement thereof and a partition barcode sequence or a complement thereof; and d) sequencing the barcoded nucleic acid molecules to obtain a nucleic acid sequence of the barcoded nucleic acid molecule, optionally wherein the barcoded nucleic acid molecules are amplified prior to sequencing.

[0003] Provided herein are methods and compositions for analyzing a secreted analyte, a soluble analyte, and/or an extracellular analyte, e.g., from a cell of interest.

[0004] Measuring secreted proteins and molecules is essential to fully understand functional heterogeneity and decipher the underlying mechanisms of cellular interactions and communication. Secreted proteins have previously been measured with single cell resolution using assays such as the “Cytokine Secretion Assay.” For example, the secreted protein of interest can be isolated using an antibody-antibody complex that coats the cell and can "catch" the molecules. The cell can then be detected by another fluorochrome-labelled antibody and subsequently analyzed using fluorescent-activated cell sorting (FACS). The FACS method is broadly similar to the enzyme-linked immunosorbent assay (ELISA) antibody format, except that the encapsulated cells remain intact. The Cytokine Secretion Assay can also be directly combined with measuring cell surface proteins for immunophenotyping and/or peptide-MHC tetramer staining for functional characterization of antigen-specific T cells. However, the same limitations associated with measuring surface proteins using FACS also apply to secreted proteins: limited ability to multiplex analytes beyond tens of proteins and inability to simultaneously measure nuclei acids from the same cell. Thus, improved methods and systems for multiplexing secreted analytes with large sample size and for simultaneous measurement of secreted analysts, mRNAs, cell surface proteins, paired $\alpha\beta$ T-cell receptor sequences, and antigen binding specificity, etc. are needed. The present disclosure provides methods and systems for such multiplexing and simultaneous measurement of various analytes of interest from a single cell.

[0005] In an [aspectexample](#), the present disclosure provides methods of processing a secreted molecule from a cell, the method comprises (a) coupling the secreted molecule to a capture agent (e.g., a first polypeptide) coupled to a surface of the cell to form a first conjugate; and (b) coupling a reporter agent (e.g., a second polypeptide) to the secreted molecule to form a second conjugate, wherein the reporter agent comprises a nucleic acid reporter molecule comprising a first barcode sequence (e.g., a reporter sequence comprising a reporter barcode sequence).

[0006] In some [embodimentexamples](#), prior to (a), the method further comprises incubating the cell with the capture agent under a sufficient condition such that the capture agent couples to the surface of the cell. In some [embodimentexamples](#), prior to (b), the method further comprises stimulating the cell to induce secretion of the secreted molecule.

[0007] In some [embodimentexamples](#), the cell is stimulated with an antigen. In some [embodimentexamples](#), the antigen is a Pattern recognition receptor (PRR) ligand. The PRR ligands may be based on or derived from a Pathogen Associated Molecular Pattern (PAMP) (also known as a Microbe Associated Molecular Pattern (MAMP) and can comprise one or more elements of a PAMP-based macromolecule (e.g., nucleic acid or amino acid). In some [embodimentexamples](#), the PRR ligand is a Toll-like receptors (TLR) ligand, a NOD-like receptor (NLRs) ligand, a RIG-I-like receptor (RLR) ligand, Absent-in-melanoma (AIM)-like receptor (ALR) ligand, a C-type lectin receptor (CLR) ligand, or a cytosolic dsDNA sensor (CDS) ligand. In some [embodimentexamples](#), the antigen is, comprises, or is derived from a viral protein (e.g., F protein from respiratory syncytial virus (RSV), or a glycoprotein from vesicular stomatitis virus (VSV)), a lipopolypeptide or lipopeptide (e.g., a triacyl lipopeptide, a diacyl lipopolypeptide), a peptidoglycan, phosphatidylserine, zymosan, flagellin, hemozoin, a lipopolysaccharide (LPS), a double-stranded DNA (dsDNA), a double-stranded RNA (dsRNA), a synthetic dsRNA, or CpG oligodeoxynucleotides (CpG ODN). In some [embodimentexamples](#), the synthetic dsRNA is polyinosinic-polycytidylic acid (poly I:C) or polyadenylic-polyuridylic acid (poly(A:U)).

[0008] In some [embodimentexamples](#), the method further comprises providing a plurality of antigens to the cell at a concentration that is sufficient to induce secretion of the secreted molecule. In some [embodimentexamples](#), the plurality of antigens is provided to the cell for a length of time that is sufficient to induce the secretion of the secreted molecule. In some [embodimentexamples](#), an antigen of the plurality of antigens is coupled to a major histocompatibility complex (MHC) molecule. In some [embodimentexamples](#), the MHC molecule is an MHC multimer. In some [embodimentexamples](#), the MHC multimer comprises a dextran polymer. In some [embodimentexamples](#), the MHC molecule is present in an antigen presenting cell.

[0009] In some [embodimentsexamples](#), the method further comprises contacting the cell with one or more co-stimulatory molecules. In some [embodimentsexamples](#), the one or more co-stimulatory molecules comprises one or more antibodies. In some [embodimentsexamples](#), the one or more antibodies are anti-CD3 or anti-CD28 antibodies. In some [embodimentsexamples](#), the one or more co-stimulatory molecules comprises one or more cytokines.

[0010] In some [embodimentsexamples](#), the one or more cytokines comprises an interleukin. In some [embodimentsexamples](#), the one or more co-stimulatory molecules are present on an antigen presenting cell. In some [embodimentsexamples](#), the method further comprises co-partitioning a plurality of nucleic acid barcode molecules and the cell (e.g., a labelled cell) comprising the first and the second conjugates (e.g., a complex coupled to a surface of the cell and comprising (i) a capture agent, (ii) a secreted analyte, and (iii) a reporter agent) into a partition, wherein each nucleic acid molecule of the plurality of nucleic acid molecules comprises a second barcode sequence, and wherein the second barcode sequence is different from the first barcode sequence. In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is attached to a support (e.g., a bead). In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is releasably attached to said support (e.g., bead). In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is releasably attached to the support (e.g., bead) via a labile bond. In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is releasable from the support (e.g., bead) upon application of a stimulus. In some [embodimentsexamples](#), the stimulus is a thermal stimulus, chemical stimulus, biological stimulus, or a photo-stimulus. In some [embodimentsexamples](#), the chemical stimulus is a reducing agent. In some [embodimentsexamples](#), the labile bond is a disulfide bond.

[0011] In some [embodimentsexamples](#), the method further comprises releasing nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules from the support, such as a bead. In some [embodimentsexamples](#), the bead is a gel bead. In some [embodimentsexamples](#), the bead is a degradable gel bead. In some [embodimentsexamples](#), the gel bead is degradable upon application of a stimulus. In some [embodimentsexamples](#), the stimulus is a thermal stimulus, chemical stimulus, biological stimulus, or a photo-stimulus. In some [embodimentsexamples](#), the chemical stimulus is a reducing agent. In some [embodimentsexamples](#), the partition is a droplet or a well.

[0012] In some [embodimentsexamples](#), the method further comprises lysing the cell. In some [embodimentsexamples](#), the method further comprises releasing nucleic acids from a cell. In one [embodimentexample](#) the nucleic acids comprise a messenger ribonucleic acid (mRNA) molecule from the cell. In some [embodimentsexamples](#), the method further comprises using a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules and the reporter molecule to generate a first

barcoded nucleic acid molecule comprising a sequence corresponding to the first barcode sequence and a sequence corresponding to the second barcode sequence. In some [embodimentsexamples](#), the nucleic acid barcode molecule comprises a sequence complementary to a sequence in the reporter molecule.

[0013] In some [embodimentsexamples](#), the method further comprises hybridizing the nucleic acid barcode molecule to the reporter molecule and performing a nucleic acid reaction to generate the first barcoded nucleic acid molecule. In some [embodimentsexamples](#), the nucleic acid reaction is a ligation reaction or a nucleic acid extension reaction. In some [embodimentsexamples](#), the method further comprises using nucleic acid molecules from the cell (e.g., the mRNA molecule(s)) and a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules to generate a second barcoded nucleic acid molecule comprising a sequence corresponding to the mRNA molecule and a sequence corresponding to the second barcode sequence.

[0014] In some [embodimentsexamples](#), the nucleic acid barcode molecule comprises a sequence complementary to a sequence in the mRNA molecule. In some [embodimentsexamples](#), the method further comprises hybridizing the nucleic acid barcode molecule to the mRNA molecule or a cDNA thereof and performing a nucleic acid reaction to generate the barcoded nucleic acid molecule. In some [embodimentsexamples](#), the nucleic acid reaction is a reverse transcription reaction, a ligation reaction, or a nucleic acid extension reaction. In some [embodimentsexamples](#), the method further comprises amplifying the reporter molecule and/or a cDNA molecule derived from the mRNA molecule.

[0015] In some [embodimentsexamples](#), the amplification comprises a polymerase chain reaction (PCR). In some [embodimentsexamples](#), wherein the amplification is isothermal. In some [embodimentsexamples](#), the method further comprises sorting the nucleic acid molecule and/or cDNA molecule according to their sizes. In some [embodimentsexamples](#), the method further comprises sequencing the first barcoded nucleic acid molecule or a derivative thereof and/or the second barcoded nucleic acid molecule or a derivative thereof.

[0016] In some [embodimentsexamples](#), the method further comprises performing one or more nucleic acid reactions to add one or more functional sequences to the first barcoded nucleic acid molecule and/or the second barcoded nucleic acid molecule. In some [embodimentsexamples](#), the one or more functional sequences are a primer sequence, a sequencing primer sequence, or a sequence configured to attach to a flow cell of a sequencer. In some [embodimentsexamples](#), the reporter molecule comprises one or more functional sequences selected from the group consisting of a primer sequence, a sequencing primer sequence, a sequence configured to attach to a flow cell of a

sequencer, and a unique molecular index (UMI). In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules each comprise one or more functional sequences selected from the group consisting of a primer sequence, a sequencing primer sequence, a partial sequencing primer sequence, a sequence configured to attach to a flow cell of a sequencer, and a unique molecular index (UMI).

[0017] In some [embodimentsexamples](#), the method further comprises identifying the first barcode sequence and the second barcode sequence and associating the secreted molecule and/or the mRNA molecule with the cell. In some [embodimentsexamples](#), the secreted molecule is a cytokine. In some [embodimentsexamples](#), the capture agent (e.g., the first polypeptide) is coupled to the cell via a cell surface protein. In some [embodimentsexamples](#), the capture agent (e.g., the first polypeptide) comprises a first antibody or antibody fragment and a second antibody or antibody fragment, wherein the first antibody or antibody fragment and a second antibody or antibody fragment are associated. In some [embodimentsexamples](#), the first antibody or antibody fragment is capable of coupling to the secreted molecule. In some [embodimentsexamples](#), the second antibody or antibody fragment is capable of coupling to the surface of the cell. In some [embodimentsexamples](#), the reporter agent (e.g., the second polypeptide) comprises or is an antibody or antibody fragment.

[0018] In some [embodimentsexamples](#), the method further comprises contacting the cell with a plurality of capture agent molecules and, prior to (b), removing capture agent molecules not bound to the surface of the cell. In some [embodimentsexamples](#), the method further comprises contacting the cell with a plurality of reporter agent molecules (e.g., second polypeptide molecules) and, subsequent to (b), removing reporter agent molecules not bound to the secreted molecule.

[0019] In another [aspectexample](#), the present disclosure provides methods of processing a molecule from a cell, the methods comprise (a) generating a cell bead, wherein the cell bead comprises a cell encapsulated by a polymer matrix, wherein the polymer matrix comprises a plurality of capture agents; and (b) coupling a molecule secreted from the cell to a first capture agent of the plurality of capture agents to form a first conjugate.

[0020] In some [embodimentsexamples](#), the polymer matrix is a hydrogel matrix. In some [embodimentsexamples](#), the polymer matrix comprises collagen, laminin, and/or fibronectin. In some [embodimentsexamples](#), the plurality of the capture agents (e.g., first polypeptides) are coupled to a polymer backbone of the polymer matrix. In some [embodimentsexamples](#), prior to (b), the method further comprises stimulating the cell bead containing the cell to induce secretion of the molecule from the cell. In some [embodimentsexamples](#), the cell bead is stimulated with an antigen. In some [embodimentsexamples](#), the antigen is a Pattern recognition receptor (PRR) ligand. In some [embodiments examples](#),

the PRR ligand is a Toll-like receptors (TLR) ligand, a NOD-like receptor (NLRs) ligand, a RIG-I-like receptor (RLR) ligand, a C-type lectin receptor (CLR) ligand, or a cytosolic dsDNA sensor (CDS) ligand. In some [embodimentsexamples](#), the antigen is a lipopolysaccharide (LPS), a double-stranded DNA (dsDNA), a double-stranded RNA (dsRNA), a synthetic dsRNA, or CpG oligodeoxynucleotides (CpG ODN). In some [embodimentsexamples](#), the synthetic dsRNA is polyinosinic-polycytidylic acid (poly I:C) or polyadenylic-polyuridylic acid (poly(A:U).

[0021] In some [embodimentsexamples](#), the method further comprises providing a plurality of antigens to the cell bead at a concentration that is sufficient to induce secretion of the secreted molecule. In some [embodimentsexamples](#), the plurality of antigens is provided to the cell bead for a length of time that is sufficient to induce the secretion of the secreted molecule. In some [embodimentsexamples](#), an antigen of the plurality of antigens is coupled to a major histocompatibility complex (MHC) molecule. In some [embodimentsexamples](#), the MHC molecule is an MHC multimer. In some [embodimentsexamples](#), the MHC multimer comprises a dextran polymer. In some [embodimentsexamples](#), the MHC molecule is present in an antigen presenting cell.

[0022] In some [embodimentsexamples](#), the method further comprises contacting the cell bead with one or more co-stimulatory molecules. In some [embodimentsexamples](#), the one or more co-stimulatory molecules comprises one or more antibodies. In some [embodimentsexamples](#), the one or more antibodies are anti-CD3 or anti-CD28 antibodies. In some [embodimentsexamples](#), the one or more co-stimulatory molecules comprises one or more cytokines. In some [embodimentsexamples](#), the one or more cytokines comprises an interleukin. In some [embodimentsexamples](#), the one or more co-stimulatory molecules are present on an antigen presenting cell.

[0023] In some [embodimentsexamples](#), the method further comprises coupling a reporter agent (e.g., second polypeptide) to the molecule secreted from the cell to form a second conjugate, wherein the reporter agent comprises a nucleic acid reporter molecule comprising a first barcode sequence (e.g., reporter barcode sequence). In some [embodimentsexamples](#), the method further comprises removing unbound reporter agent molecules from the cell bead.

[0024] In some [embodimentsexamples](#), subsequent to (b), the method further comprises (c) partially digesting the cell bead with an enzyme, thereby generating a partially digested cell bead. In some [embodimentsexamples](#), the enzyme is collagenase or dispase. In some [embodimentsexamples](#), the method further comprises contacting a plurality of reporter agents comprising a barcode to the partially digested cell bead, under a condition so that a reporter agent from the plurality of reporter agents couples to the molecule secreted from the cell. In some [embodimentsexamples](#), the method further comprises co-partitioning a plurality of nucleic acid barcode molecules and the cell bead comprising the first

and the second conjugate into a partition, wherein each nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules comprises a second barcode sequence, and wherein the second barcode sequence is different from the first barcode sequence.

[0025] In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is attached to a bead. In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is releasably attached to said bead. In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is releasably attached to the bead via a labile bond. In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules is releasable from the bead upon application of a stimulus. In some [embodimentsexamples](#), the stimulus is a thermal stimulus, chemical stimulus, biological stimulus, or a photo-stimulus. In some [embodimentsexamples](#), the chemical stimulus is a reducing agent. In some [embodimentsexamples](#), the labile bond is a disulfide bond.

[0026] In some [embodimentsexamples](#), the method further comprises releasing nucleic acid barcode molecules of the plurality of nucleic acid barcode molecules. In some [embodimentsexamples](#), wherein the bead is a gel bead. In some [embodimentsexamples](#), the bead is a degradable gel bead. In some [embodimentsexamples](#), the gel bead is degradable upon application of a stimulus. In some [embodimentsexamples](#), the stimulus is a thermal stimulus, chemical stimulus, biological stimulus, or a photo-stimulus. In some [embodimentsexamples](#), the chemical stimulus is a reducing agent. In some [embodimentsexamples](#), the partition is a droplet or a well. In some [embodimentsexamples](#), the method further comprises degrading the cell bead.

[0027] In some [embodimentsexamples](#), the method further comprises lysing the cell. In some [embodimentsexamples](#), the method further comprises releasing a messenger ribonucleic acid (mRNA) molecule from the cell. In some [embodimentsexamples](#), the method further comprises using a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules and the reporter molecule to generate a first barcoded nucleic acid molecule comprising a sequence corresponding to the first barcode sequence and a sequence corresponding to the second barcode sequence.

[0028] In some [embodimentsexamples](#), the nucleic acid barcode molecule comprises a sequence complementary to a sequence in the reporter molecule. In some [embodimentsexamples](#), the method further comprises hybridizing the nucleic acid barcode molecule to the reporter molecule and performing a nucleic acid reaction to generate the first barcoded nucleic acid molecule. In some [embodimentsexamples](#), the method further comprises the nucleic acid reaction is a ligation reaction or a nucleic acid extension reaction. In some [embodimentsexamples](#), the method further comprises using the mRNA molecule and a nucleic acid barcode molecule of the plurality of nucleic acid barcode molecules to generate a second barcoded nucleic acid molecule comprising a sequence

corresponding to the mRNA molecule and a sequence corresponding to the second barcode sequence.

[0029] In some [embodimentsexamples](#), the nucleic acid barcode molecule comprises a sequence complementary to a sequence in the mRNA molecule. In some [embodimentsexamples](#), the method further comprises hybridizing the nucleic acid barcode molecule to the mRNA molecule or a cDNA thereof and performing a nucleic acid reaction to generate the barcoded nucleic acid molecule. In some [embodimentsexamples](#), the nucleic acid reaction is a reverse transcription reaction, a ligation reaction, or a nucleic acid extension reaction. In some [embodimentsexamples](#), the method further comprises amplifying the reporter molecule and/or a cDNA molecule derived from the mRNA molecule.

[0030] In some [embodimentsexamples](#), the amplification comprises a polymerase chain reaction (PCR). In some [embodimentsexamples](#), the amplification is isothermal. In some [embodimentsexamples](#), the method further comprises sorting the nucleic acid molecule and/or cDNA molecule according to their sizes. In some [embodimentsexamples](#), the method further comprises sequencing the first barcoded nucleic acid molecule or a derivative thereof and/or the second barcoded nucleic acid molecule or a derivative thereof. In some [embodimentsexamples](#), the method further comprises performing one or more nucleic acid reactions to add one or more functional sequences to the first barcoded nucleic acid molecule and/or the second barcoded nucleic acid molecule.

[0031] In some [embodimentsexamples](#), the one or more functional sequences are a primer sequence, a sequencing primer sequence, or a sequence configured to attach to a flow cell of a sequencer. In some [embodimentsexamples](#), the reporter molecule comprises one or more functional sequences selected from the group consisting of a primer sequence, a sequencing primer sequence, a sequence configured to attach to a flow cell of a sequencer, and a unique molecular index (UMI). In some [embodimentsexamples](#), the plurality of nucleic acid barcode molecules each comprise one or more functional sequences selected from the group consisting of a primer sequence, a sequencing primer sequence, a partial sequencing primer sequence, a sequence configured to attach to a flow cell of a sequencer, and a unique molecular index (UMI).

[0032] In some [embodimentsexamples](#), the method further comprises identifying the first barcode sequence and the second barcode sequence and associating the secreted molecule and/or the mRNA molecule with the cell. In some [embodimentsexamples](#), the secreted molecule is a cytokine. In some [embodimentsexamples](#), the second polypeptide is an antibody or antibody fragment. In some [embodimentsexamples](#), the MHC molecule comprises a nucleic acid molecule comprising a third barcode

sequence. In some [embodimentsexamples](#), the third barcode sequence is different from the first barcode sequence or the second barcode sequence.

[0033] In certain [embodimentsexamples](#), methods of processing a secreted molecule from a cell comprise (a) coupling the secreted molecule to a capture agent (e.g., a first polypeptide or a first binding agent comprising a first polypeptide) coupled to a surface of the cell to form a first conjugate; and (b) coupling a reporter agent (e.g., a second polypeptide or a second binding agent comprising a second polypeptide) to the secreted molecule to form a second conjugate, wherein the reporter agent comprises a nucleic acid molecule comprising a first barcode sequence. Further, the methods comprise prior to (a), incubating the cell with the capture agent such that the capture agent couples to the surface of the cell. Moreover, the methods comprise prior to (a), providing a plurality of antigens to the cell at a concentration that is sufficient to induce secretion of the secreted molecule. In addition, the methods comprise co-partitioning a support, such as a bead (e.g., a gel bead such as an emulsion bead), and the cell coupled to the second conjugate into a partition, wherein the bead is coupled to a plurality of nucleic acid molecules comprising a second barcode sequence, wherein the second barcode sequence is different from the first barcode sequence. The methods also comprise lysing the cells to release intracellular content such as cellular mRNA molecules, reverse transcribing the mRNA molecules thereby generating corresponding cDNA molecules that comprise the second barcode sequence that the beads (e.g., emulsion beads) may be comprised of, and attaching the second barcode sequence (e.g., partition-specific barcode sequence) to the nucleic acid molecules comprising the first, analyte-specific barcode sequence. The nucleic acid molecules (e.g., cDNAs, barcode sequences, etc.) may be amplified, and sorted by size, followed by sequencing the sorted nucleic acid molecules. Sequencing may identify secreted analytes (e.g., cytokines), mRNA molecules, and other cell-specific analytes and enable association of these cellular analytes with their corresponding cell or cell type (e.g., an immune cell such as a T cell) for, e.g., immune phenotyping etc. The present disclosure provides methods that do not only allow to obtain this information on a single cell basis, but also allows the analysis of secreted analytes (e.g., cytokines or other secreted proteins, see e.g., operation **1803**) in addition and/or simultaneous to the analysis of cellular nucleic acid molecules (e.g., mRNAs for analyzing T cell receptor (TCR) sequences, see e.g., operations **1804** and **1805**), cell surface proteins (e.g., T cell receptors, B cell receptors, etc., see e.g., operation **1801**), antigen specificity (see e.g., operation **1802**) of a single cell, etc. Exemplary methods and compositions for single cell analysis of analytes are disclosed in WO2019/157529, incorporated herein by reference in its entirety for all purposes. In addition, the methods allow for multiplexing or otherwise increasing throughput of samples for analysis through the use of

labelling agents, which comprise nucleic acid reporter molecules and are capable of binding to or otherwise coupling to one or more cells or cell features, as further described herein.

[0034] Further, in certain [embodimentsexamples](#), the methods of processing a secreted molecule from a cell comprise (a) generating a cell bead, wherein the cell bead comprises a cell encapsulated by a polymer matrix (e.g., a hydrogel matrix), wherein the polymer matrix is coupled to a capture agent (e.g., a first polypeptide or a first binding agent comprising a first polypeptide), wherein the capture agent is specific for the secreted molecule; and (b) coupling the secreted molecule to the capture agent to form a first conjugate. The methods further comprise, prior to (b), providing a plurality of antigens to the cell bead at a concentration that is sufficient to induce secretion of the secreted molecule. Subsequent to forming the first conjugate, a reporter agent (e.g., a second polypeptide or a second binding agent comprising a second polypeptide) may be coupled to the secreted molecule bound to the capture agent (e.g., the first polypeptide) to form a second conjugate, wherein the reporter agent comprises a nucleic acid molecule comprising a first barcode sequence (e.g., reporter barcode sequence), thereby barcoding the secreted molecule. In addition, the methods further comprise co-partitioning the cell bead coupled to the second conjugate into a partition, wherein the partition further comprises a bead (e.g., a gel bead such as an emulsion bead), and wherein the bead comprises plurality of nucleic acid molecules comprising a second barcode sequence, wherein second the barcode sequence is different from the first barcode sequence. The polymer matrix is dissolved and the cells subsequently lysed to release intracellular content such as cellular mRNA molecules. The mRNA molecules are reverse transcribed thereby generating corresponding cDNA molecules that comprise the second barcode sequence (e.g., partition-specific barcode sequence). The nucleic acid molecules comprising the first, analyte-specific barcode sequence are also barcoded with the second barcode sequence. The nucleic acids (e.g., cDNAs, barcode sequences, etc.) may be amplified, and sorted by size, followed by sequencing the sorted nucleic acid molecules. Sequencing may identify secreted analytes (e.g., cytokines), mRNA molecules, and other cell-specific analytes and enable association of these cellular analytes with their corresponding cell or cell type (e.g., an immune cell such as a T cell) for, e.g., immune phenotyping etc. The present disclosure provides methods that do not only allow to obtain this information on a single cell basis, but also allows the analysis of secreted analytes (e.g., cytokines or other secreted proteins, see e.g., operation **1803**) in addition and/or simultaneous to the analysis of cellular nucleic acid molecules (e.g., mRNAs for analyzing T cell receptor (TCR) sequences, see e.g., operations **1804** and **1805**), cell surface proteins (e.g., T cell receptors, B cell receptors, etc., see e.g., operation **1801**), antigen specificity (see e.g., operation **1802**) of a single cell, etc. In addition, the methods allow for multiplexing or

otherwise increasing throughput of samples for analysis through the use of labelling agents, which comprise nucleic acid reporter molecules and are capable of binding to or otherwise coupling to one or more cells or cell features, as further described herein.

[0034a] In another aspect, provided herein is a method of single cell analysis, comprising: a) contacting a cell bead with a reporter agent comprising a reporter nucleic acid molecule to provide a labelled cell bead, wherein the cell bead comprises a cell in a matrix, wherein the reporter nucleic acid molecule comprises a reporter sequence corresponding to the reporter agent, wherein the labelled cell bead comprises a complex in or on the cell bead, and wherein the complex comprises (i) a capture agent, (ii) a secreted analyte, and (iii) the reporter agent, wherein said capture agent is configured to couple to both the matrix and said secreted analyte, and wherein the reporter agent is configured to couple to the secreted analyte; b) partitioning the labelled cell bead into a partition, wherein the partition is from a plurality of partitions and is a droplet or a microwell, wherein the partition comprises a plurality of barcode nucleic acid molecules which comprise a plurality of partition barcode sequences, wherein said reporter nucleic acid molecule further comprises a sequence configured to couple to the barcode nucleic acid molecule; c) in bulk or in the partition, generating a barcoded nucleic acid molecule from a barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules and the reporter nucleic acid molecule, wherein the barcoded nucleic acid molecule comprises the reporter sequence or a complement thereof and a partition barcode sequence or a complement thereof; and d) sequencing the barcoded nucleic acid molecules to obtain a nucleic acid sequence of the barcoded nucleic acid molecule, optionally wherein the barcoded nucleic acid molecules are amplified prior to sequencing, wherein the cell bead is a hydrogel, polymeric, or crosslinked material that comprises a biological particle, a virus, components of or macromolecular constituents of or derived from a cell or virus.

[0035] In some embodimentsexamples, provided herein is a method of single cell analysis, comprising: a) contacting a cell with a reporter agent comprising a reporter nucleic acid molecule to provide a labelled cell, wherein the reporter nucleic acid molecule comprises a reporter sequence corresponding to the reporter agent, wherein the labelled cell comprises a complex coupled to a surface of the cell, and wherein the complex comprises (i) a capture agent, (ii) a secreted analyte, and (iii) the reporter agent; b) partitioning the labelled cell in a partition, wherein the partition comprises a plurality of barcode nucleic acid molecules which comprise a plurality of partition barcode sequences; and c) in the partition, generating a barcoded nucleic acid molecule from a barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules and the reporter nucleic acid

molecule, wherein the barcoded nucleic acid molecule comprises the reporter sequence or a complement thereof and a partition barcode sequence or a complement thereof.

[0036] In any of the preceding [embodimentsexamples](#), the secreted analyte can comprise a secreted protein. In any of the preceding [embodimentsexamples](#), the capture agent can be configured to couple to a cell surface molecule. In any of the preceding [embodimentsexamples](#), said cell surface molecule can comprise a cell surface protein. In any of the preceding [embodimentsexamples](#), the capture agent can be configured to couple to the secreted analyte. In any of the preceding [embodimentsexamples](#), said capture agent can be configured to couple to both the cell surface protein and said secreted analyte. In any of the preceding [embodimentsexamples](#), said capture agent can be a first protein binding agent. In any of the preceding [embodimentsexamples](#), the reporter agent can be configured to couple to the secreted analyte. In any of the preceding [embodimentsexamples](#), said reporter agent can be a second protein binding agent. In any of the preceding [embodimentsexamples](#), said reporter nucleic acid molecule can further comprise a sequence configured to couple to the barcode nucleic acid molecule.

[0037] In any of the preceding [embodimentsexamples](#), said labelled cell can further comprise a second reporter agent comprising a second reporter nucleic acid molecule. In any of the preceding [embodimentsexamples](#), said second reporter agent can be configured to couple to a second cell surface protein of the cell. In any of the preceding [embodimentsexamples](#), said second reporter nucleic acid molecule can comprise a second reporter sequence corresponding to the second reporter agent. In any of the preceding [embodimentsexamples](#), the partition can further comprise a second barcode nucleic acid molecule of a plurality of second barcode nucleic acid molecules. In any of the preceding

[embodimentsexamples](#), said second reporter nucleic acid molecule can comprise a second sequence configured to couple to the second barcode nucleic acid molecule. In any of the preceding [embodimentsexamples](#), the method can further comprises generating a second barcoded nucleic acid molecule from a second barcode nucleic acid molecule from the plurality of second barcode nucleic acid molecules and the second reporter nucleic acid molecule, wherein the second barcoded nucleic acid molecule comprises sequences from the second reporter nucleic acid molecule and the second barcode nucleic acid molecule, or complements thereof.

[0038] In any of the preceding [embodimentsexamples](#), said labelled cell can further comprise a plurality of nucleic acid analytes, and a nucleic acid analyte of said plurality of nucleic acid analytes can comprise a nucleic acid analyte sequence. In any of the preceding [embodimentsexamples](#), the partition can further comprise a plurality of third barcode nucleic acid molecules. In any of the preceding [embodimentsexamples](#), a third barcode nucleic acid molecule of said plurality of third barcode nucleic acid molecules can comprise a third sequence configured to couple to the nucleic acid analyte sequence. In any of the preceding [embodimentsexamples](#), the method can further comprise generating a third barcoded nucleic acid molecule from a third barcode nucleic acid molecule from the plurality of third barcode nucleic acid molecules and the nucleic acid analyte, wherein the third barcoded nucleic acid molecule comprises sequences from the nucleic acid analyte and the third barcode nucleic acid molecule, or complements thereof.

[0039] In any of the preceding [embodimentsexamples](#), said partition can comprise a support that comprises the plurality of barcode nucleic acid molecules. In any of the preceding [embodimentsexamples](#), said support can comprise a bead. In any of the preceding [embodimentsexamples](#), said bead can comprise a gel bead. In any of the preceding [embodimentsexamples](#), said plurality of barcode nucleic acid molecules can be releasably attached to the support. In any of the preceding [embodimentsexamples](#), the partition can be from a plurality of partitions. In any of the preceding [embodimentsexamples](#), the partition can comprise a droplet or a microwell.

[0040] In some [embodimentsexamples](#), provided herein is a method of single cell analysis, comprising: a) contacting a cell bead with a reporter agent comprising a reporter nucleic acid molecule to provide a labelled cell bead, wherein the cell bead comprises a cell in a matrix, wherein the reporter nucleic acid molecule comprises a reporter sequence corresponding to the reporter agent, wherein the labelled cell bead comprises a complex in or on the cell bead, and wherein the complex comprises (i) a capture agent, (ii) a secreted analyte, and (iii) the reporter agent; b) partitioning the labelled cell bead in a partition, wherein the partition comprises a plurality of barcode nucleic acid molecules which comprise a plurality of partition barcode

sequences; and c) in the partition, generating a barcoded nucleic acid molecule from a barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules and the reporter nucleic acid molecule, wherein the barcoded nucleic acid molecule comprises the reporter sequence or a complement thereof and a partition barcode sequence or a complement thereof.

[0041] In any of the preceding [embodimentsexamples](#), said secreted analyte can comprise a secreted protein. In any of the preceding [embodimentsexamples](#), the capture agent can be configured to couple to the matrix of the cell bead. In any of the preceding [embodimentsexamples](#), said matrix can be a degradable polymer matrix. In any of the preceding [embodimentsexamples](#), the capture agent can be configured to couple to the secreted analyte. In any of the preceding [embodimentsexamples](#), said capture agent can be configured to couple to both the matrix and said secreted analyte. In any of the preceding [embodimentsexamples](#), said capture agent can be a first protein binding agent. In any of the preceding [embodimentsexamples](#), the reporter agent can be configured to couple to the secreted analyte. In any of the preceding [embodimentsexamples](#), said reporter agent can be a second protein binding agent. In any of the preceding [embodimentsexamples](#), said reporter nucleic acid molecule can further comprise a sequence configured to couple to the barcode nucleic acid molecule.

[0042] In any of the preceding [embodimentsexamples](#), said cell or said labelled cell bead can further comprise a second reporter agent comprising a second reporter nucleic acid molecule. In any of the preceding [embodimentsexamples](#), said second reporter agent can be configured to couple to a cell surface protein of the cell. In any of the preceding [embodimentsexamples](#), said second reporter nucleic acid molecule can comprise a second reporter sequence corresponding to the second reporter agent. In any of the preceding [embodimentsexamples](#), the partition can further comprise a second barcode nucleic acid molecule of a plurality of second barcode nucleic acid molecules. In any of the preceding [embodimentsexamples](#), said second reporter nucleic acid molecule can comprise a second sequence configured to couple to the second barcode nucleic acid molecule. In any of the preceding [embodimentsexamples](#), the method can further comprise generating a second barcoded nucleic acid molecule from a second barcode nucleic acid molecule from the plurality of second barcode nucleic acid molecules and the second reporter nucleic acid molecule, wherein the second barcoded nucleic acid molecule comprises sequences from the second reporter nucleic acid molecule and the second barcode nucleic acid molecule, or complements thereof.

[0043] In any of the preceding [embodimentsexamples](#), said cell can further comprise a plurality of nucleic acid analytes, wherein a nucleic acid analyte of said plurality of nucleic acid analytes can comprise a nucleic acid analyte sequence. In any of the preceding [embodimentsexamples](#), the partition can further comprise a plurality of third barcode nucleic acid molecules. In any of the preceding

[embodimentsexamples](#), a third barcode nucleic acid molecule of said plurality of third barcode nucleic acid molecules can comprise a third sequence configured to couple to the nucleic acid analyte sequence. In any of the preceding [embodimentsexamples](#), the method can further comprise generating a third barcoded nucleic acid molecule from a third barcode nucleic acid molecule from the plurality of third barcode nucleic acid molecules and the nucleic acid analyte, wherein the third barcoded nucleic acid molecule comprises sequences from the nucleic acid analyte and the third barcode nucleic acid molecule, or complements thereof.

[0044] In any of the preceding [embodimentsexamples](#), said partition can comprise a support that comprises the plurality of barcode nucleic acid molecules. In any of the preceding [embodimentsexamples](#), said support can comprise a bead. In any of the preceding [embodimentsexamples](#), said bead comprises a gel bead. In any of the preceding [embodimentsexamples](#), said plurality of barcode nucleic acid molecules can be releasably attached to the support. In any of the preceding [embodimentsexamples](#), the partition can be from a plurality of partitions. In any of the preceding [embodimentsexamples](#), the partition can comprise a droplet or a microwell.

[0045] Additional [aspectsexamples](#) and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative [embodimentsexamples](#) of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different [embodimentsexamples](#), 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

[0046] 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

[0047] 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

1. A method of single cell analysis, comprising:

——a) contacting a cell with a reporter agent comprising a reporter nucleic acid molecule to provide a labelled cell, wherein the reporter nucleic acid molecule comprises a reporter sequence corresponding to the reporter agent, wherein the labelled cell comprises a complex coupled to a surface of the cell, and wherein the complex comprises (i) a capture agent, (ii) a secreted analyte, and (iii) the reporter agent; wherein the capture agent is configured to couple to both a cell surface molecule and the secreted analyte, and wherein the reporter agent is configured to couple to the secreted analyte;

——b) partitioning the labelled cell ~~in~~into a partition, wherein the partition is from a plurality of partitions and is a droplet or a microwell, wherein the partition comprises a plurality of barcode nucleic acid molecules which comprise a plurality of partition barcode sequences; ~~and~~, wherein said reporter nucleic acid molecule further comprises a sequence configured to couple to the barcode nucleic acid molecule;

——c) in bulk or in the partition, generating a barcoded nucleic acid molecule from a barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules and the reporter nucleic acid molecule, wherein the barcoded nucleic acid molecule comprises the reporter sequence or a complement thereof and a partition barcode sequence or a complement thereof; and

d) sequencing the barcoded nucleic acid molecules to obtain a nucleic acid sequence of the barcoded nucleic acid molecule, optionally wherein the barcoded nucleic acid molecules are amplified prior to sequencing.

2. The method of claim 1, wherein: (i) said secreted analyte is a secreted protein;

~~3. The method of claim 1 or 2, wherein the capture agent is configured to couple to a cell surface molecule.~~

~~4. The method of claim 3, wherein; and/or (ii)~~ said cell surface molecule is a cell surface protein.

~~5. The method of any of claims 1-4, wherein the capture agent is configured to couple to the secreted analyte.~~

~~6.3. The method of claim 4, wherein said capture agent is configured to couple to both the cell surface protein and said secreted analyte;~~ 1 or claim 2, wherein:

~~7. The method of any of claims 1-6, wherein(a)~~ said capture agent is a first protein binding agent; and/or

~~8. The method of any of claims 1-7, wherein the reporter agent is configured to couple to the secreted analyte.~~

~~9. The method of claim 7 or 8, wherein (b) said reporter agent is a second protein binding agent.~~

~~10.4. The method of any of claims 1-9, wherein said reporter nucleic acid molecule further comprises a sequence configured to couple to the barcode nucleic acid molecule.~~

~~11. The method of any of claims 1-103, wherein said labelled cell further comprises a second reporter agent comprising a second reporter nucleic acid molecule, optionally wherein:~~

~~12. The method of claim 11, wherein(a) said second reporter agent is configured to couple to a second cell surface protein of the cell-~~

~~13. The method of claim 11; and/or 12, wherein~~

~~(b) said second reporter nucleic acid molecule comprises a second reporter sequence corresponding to the second reporter agent.~~

~~14.5. The method of claim any of claims 1-134, wherein the partition further comprises a second barcode nucleic acid molecule of a plurality of second barcode nucleic acid molecules, optionally wherein:~~

~~15. The method of claim 14, wherein(a) said second reporter nucleic acid molecule comprises a second sequence configured to couple to the second barcode nucleic acid molecule; and/or~~

~~16. The method of claim 14 or 15, wherein(b) step (c) further comprises generating a second barcoded nucleic acid molecule from a second barcode nucleic acid molecule from the plurality of second barcode nucleic acid molecules and the second reporter nucleic acid molecule, wherein the second barcoded nucleic acid molecule comprises sequences from the second reporter nucleic acid molecule and the second barcode nucleic acid molecule, or complements thereof.~~

~~17.6. The method of any of claims 1-165, wherein said labelled cell further comprises a plurality of nucleic acid analytes, wherein a nucleic acid analyte of said plurality of nucleic acid analytes comprises a nucleic acid analyte sequence.~~

~~18.7. The method of any of claims 1-17claim 6, wherein the partition further comprises a plurality of third barcode nucleic acid molecules-~~

~~19. The method of claim 18, optionally wherein:~~

(a) a third barcode nucleic acid molecule of said plurality of third barcode nucleic acid molecules comprises a third sequence configured to couple to the nucleic acid analyte sequence; and/or

~~20. — The method of claim 18 or 19, wherein~~(b) step (c) further comprises generating a third barcoded nucleic acid molecule from a third barcode nucleic acid molecule from the plurality of third barcode nucleic acid molecules and the nucleic acid analyte, wherein the third barcoded nucleic acid molecule comprises sequences from the nucleic acid analyte and the third barcode nucleic acid molecule, or complements thereof.

~~21.8. The method of any of claims 1-207, wherein said partition comprises a support that comprises the plurality of barcode nucleic acid molecules;~~ optionally wherein:

~~22. — The method of claim 21, wherein~~(a) said support is a bead-

~~23. — The method of claim 22,~~ optionally wherein said bead is a gel bead; and/or

~~24. — The method of any of claims 21-23, wherein~~(b) said plurality of barcode nucleic acid molecules are releasably attached to the support.

~~25. — The method of any of claims 1-24, wherein the partition is from a plurality of partitions.~~

~~26. — The method of claim 25, wherein the partition is a droplet or a microwell.~~

~~27.9.~~ A method of single cell analysis, comprising:

——— a) contacting a cell bead with a reporter agent comprising a reporter nucleic acid molecule to provide a labelled cell bead, wherein the cell bead comprises a cell in a matrix, wherein the reporter nucleic acid molecule comprises a reporter sequence corresponding to the reporter agent, wherein the labelled cell bead comprises a complex in or on the cell bead, and wherein the complex comprises (i) a capture agent, (ii) a secreted analyte, and (iii) the reporter agent; wherein said capture agent is configured to couple to both the matrix and said secreted analyte, and wherein the reporter agent is configured to couple to the secreted analyte;

——— b) partitioning the labelled cell bead ~~into~~ into a partition, wherein the partition is from a plurality of partitions and is a droplet or a microwell, wherein the partition comprises a plurality of barcode nucleic acid molecules which comprise a plurality of partition barcode sequences; ~~and,~~ wherein said reporter nucleic acid molecule further comprises a sequence configured to couple to the barcode nucleic acid molecule;

——— c) in bulk or in the partition, generating a barcoded nucleic acid molecule from a barcode nucleic acid molecule of the plurality of barcode nucleic acid molecules and the reporter nucleic acid molecule, wherein the barcoded nucleic acid molecule

comprises the reporter sequence or a complement thereof and a partition barcode sequence or a complement thereof; and

~~28. — The method d) sequencing the barcoded nucleic acid molecules to obtain a nucleic acid sequence of claim 27, the barcoded nucleic acid molecule, optionally wherein the barcoded nucleic acid molecules are amplified prior to sequencing,~~

~~wherein the cell bead is a hydrogel, polymeric, or crosslinked material that comprises a biological particle, a virus, components of or macromolecular constituents of or derived from a cell or virus.~~

~~10. The method of claim 9, wherein:~~

~~(a) said secreted analyte is a secreted protein;~~

~~29. — The method of claim 27 or 28, wherein the capture agent is configured to couple to the matrix of the cell bead.~~

~~30. — The method of claim 29, wherein (b) said matrix is a degradable polymer matrix;~~

~~31. — The method of any of claims 27–30, wherein the capture agent is configured to couple to the secreted analyte.~~

~~32. — The method of claim 30, wherein said capture agent is configured to couple to both the matrix and said secreted analyte.~~

~~33. — The method of any of claims 27–32, wherein (c) said capture agent is a first protein binding agent; and/or~~

~~34. — The method of any of claims 27–33, wherein the reporter agent is configured to couple to the secreted analyte.~~

~~35. — The method of claim 33 or 34, wherein (d) said reporter agent is a second protein binding agent.~~

~~36. — The method of any of claims 27–35, wherein said reporter nucleic acid molecule further comprises a sequence configured to couple to the barcode nucleic acid molecule.~~

~~37. — The method of any of claims 27–36~~11. The method of claim 9 or claim 10,
wherein said cell or said labelled cell bead further comprises a second reporter agent comprising a second reporter nucleic acid molecule; optionally wherein:

~~38. — The method of claim 37, wherein (a) said second reporter agent is configured to couple to a cell surface protein of the cell.~~

~~39. — The method of claim 37; and/or 38, wherein~~

~~(b) said second reporter nucleic acid molecule comprises a second reporter sequence corresponding to the second reporter agent.~~

~~40.~~

~~12.~~ The method of claim any of claims ~~27-399-11~~, wherein the partition further comprises a second barcode nucleic acid molecule of a plurality of second barcode nucleic acid molecules; optionally wherein:

~~41.—The method of claim 40, wherein(a)~~ said second reporter nucleic acid molecule comprises a second sequence configured to couple to the second barcode nucleic acid molecule; and/or

~~42.—The method of claim 40 or 41,(b)~~ wherein step (c) further comprises generating a second barcoded nucleic acid molecule from a second barcode nucleic acid molecule from the plurality of second barcode nucleic acid molecules and the second reporter nucleic acid molecule, wherein the second barcoded nucleic acid molecule comprises sequences from the second reporter nucleic acid molecule and the second barcode nucleic acid molecule, or complements thereof.

~~43.13.~~ The method of any of claims ~~27-429-12~~, wherein said cell further comprises a plurality of nucleic acid analytes, wherein a nucleic acid analyte of said plurality of nucleic acid analytes comprises a nucleic acid analyte sequence.

~~44.14.~~ The method of any of claims ~~27-439-13~~, wherein the partition further comprises a plurality of third barcode nucleic acid molecules-

~~45.—The method of claim 44, optionally~~ wherein:

(a) a third barcode nucleic acid molecule of said plurality of third barcode nucleic acid molecules comprises a third sequence configured to couple to the nucleic acid analyte sequence; and/or

~~46.—The method of claim 44 or 45, wherein(b)~~ step (c) further comprises generating a third barcoded nucleic acid molecule from a third barcode nucleic acid molecule from the plurality of third barcode nucleic acid molecules and the nucleic acid analyte, wherein the third barcoded nucleic acid molecule comprises sequences from the nucleic acid analyte and the third barcode nucleic acid molecule, or complements thereof.

~~47.15.~~ The method of any of claims ~~27-469-14~~, wherein said partition comprises a support that comprises the plurality of barcode nucleic acid molecules; optionally wherein:

~~48.—The method of claim 47, wherein(a)~~ said support is a bead-

~~49.—The method of claim 48, optionally~~ wherein said bead is a gel bead; and/or

~~50.—The method of any of claims 47-49, wherein(b)~~ said plurality of barcode nucleic acid molecules are releasably attached to the support.

51. The method of any of claims 27-50, wherein the partition is from a plurality of partitions.

52. The method of claim 51, wherein the partition is a droplet or a microwell.