

(e.g., RNA-seq). Such approaches, however, rely on a small set of pre-defined markers, therefore introducing selection bias that limits discovery and making it costly and laborious to localize RNA transcriptome-wide.

SUMMARY

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Provided herein are methods of manufacturing of spatial arrays and determining the location of an analyte present in a biological sample (e.g., by utilizing a cell-tagging agent).

10 In one example, a method for determining the location of an analyte present in a biological sample, comprising (a) providing a substrate comprising an arrayed plurality of spatially-barcoded oligonucleotides, wherein a spatially-barcoded oligonucleotide of the plurality of spatially-barcoded oligonucleotides comprises a spatial barcode, a priming domain, and a first hybridization domain; (b) coupling a cell-tagging agent to the spatially-barcoded oligonucleotide; (c) contacting the biological sample to the cell-
15 tagging agent such that a cell in the biological sample is tagged with the spatially barcoded-oligonucleotide; (d) providing the cell comprising the spatially barcoded-oligonucleotide to a bead, wherein the bead comprises (1) a first bead-bound oligonucleotide, wherein the first bead-bound oligonucleotide comprises a cellular barcode and a second hybridization domain, and (2) a second bead-bound
20 oligonucleotide, wherein the second bead-bound oligonucleotide comprises the cellular barcode and a capture domain; (e) allowing an analyte from the cell to interact with the capture domain of the second bead-bound oligonucleotide; (f) associating the analyte bound to the capture domain of the second bead-bound oligonucleotide with the cellular barcode; and (g) associating the cellular barcode with the spatial barcode, thereby
25 determining the location of the analyte present in the biological sample.

In one aspect, the present disclosure refers to a method for determining the location of an analyte present in a biological sample, comprising: (a) providing a substrate comprising a plurality of spatially-barcoded oligonucleotides, wherein a spatially-barcoded oligonucleotide of the plurality of spatially-barcoded oligonucleotides comprises a spatial barcode, a priming domain, and a first hybridization domain; (b) coupling a cell-tagging agent to the spatially-barcoded oligonucleotide; (c) contacting the biological sample to the cell-tagging agent such that a cell in the biological sample is tagged with the spatially barcoded-oligonucleotide; (d) providing the cell comprising the spatially barcoded-oligonucleotide to a bead, wherein the bead comprises: 1. a first bead-bound oligonucleotide, wherein the first bead-bound oligonucleotide comprises a cellular barcode and a second hybridization domain, and 2. a second bead-bound oligonucleotide, wherein the second bead-bound oligonucleotide comprises the cellular barcode and a capture domain; (e) allowing an analyte from the cell to interact with the capture domain of the second bead-bound oligonucleotide; (f) associating the analyte bound to the capture domain of the second bead-bound oligonucleotide with the cellular barcode; and (g) associating the cellular barcode with the spatial barcode, thereby determining the location of the analyte present in the biological sample.

In some examples, the method for determining the location of an analyte present in a biological sample comprises dissociating the cell from the biological sample.

In another aspect, the present disclosure refers to a method for determining the location of an analyte present in a biological sample, comprising: (a) providing a substrate comprising a plurality of spatially-barcoded oligonucleotides, wherein a spatially-barcoded oligonucleotide of the plurality of spatially-barcoded oligonucleotides comprises a spatial barcode, a first hybridization domain, and a cleavage domain; (b) coupling a cell-tagging agent with the spatially-barcoded oligonucleotide; and (c) determining the location of an analyte present in the biological sample.

In some examples, providing a cell-tagging agent comprises hybridizing a priming oligonucleotide to the priming domain, wherein the priming oligonucleotide is

coupled to the cell-tagging agent. In some examples, the priming oligonucleotide coupled to the cell-tagging agent is substantially complementary to the priming domain of the spatially barcoded-oligonucleotide. In some examples, the priming oligonucleotide coupled to the cell-tagging agent is substantially complementary to the priming domain, the spatial barcode, and the first hybridization domain of the spatially barcoded-oligonucleotide.

In some examples, the method for determining the location of an analyte present in a biological sample comprises determining the sequence of at least a portion of the spatially-barcoded oligonucleotide. In some examples, determining the sequence comprises in situ sequencing. In some examples, the in situ sequencing comprises one or more of sequencing by synthesis, sequencing by ligation, rolling circle amplification sequencing, fluorescent in situ sequencing (FISSEQ), and spatially-resolved transcript amplicon readout mapping (STARmap).

In some examples, providing the cell comprising the spatially barcoded-oligonucleotide to the bead comprises hybridization. In some examples, the hybridization comprises the first hybridization domain hybridizing to the second hybridization domain.

In some examples, the spatially-barcoded oligonucleotide comprises one or more of a unique molecular identifier, an attachment sequence, a cleavage domain, and a functional domain. In some examples, the attachment sequence comprises one or more of a flow cell attachment sequence and a substrate attachment sequence.

In some examples, allowing the analyte from the cell to interact with the capture domain comprises releasing the analyte from the cell. In some examples, releasing comprises permeabilization of the cell. In some examples, associating the analyte bound to the capture domain with the cellular domain comprises identifying the analyte. In some examples, associating the cellular barcode with the spatial barcode comprises extending the hybridized first bead-bound oligonucleotide and spatially barcoded-oligonucleotide. In some examples, associating the cellular barcode with the spatial barcode comprise determining the sequence of the extended bead-bound oligonucleotide and spatially barcoded-oligonucleotide.

In another example, a method for determining the location of an analyte present in a biological sample, comprising (a) providing a substrate comprising an arrayed plurality of spatially-barcoded oligonucleotides, wherein a spatially-barcoded oligonucleotide of the plurality of spatially-barcoded oligonucleotides comprises a spatial barcode, a first
5 hybridization domain, and a cleavage domain; (b) coupling a cell-tagging agent with the spatially-barcoded oligonucleotide; and (c) determining the location of an analyte present in the biological sample. In some examples, the cell-tagging agent comprises a first associating domain that hybridizes to the spatially-barcoded oligonucleotide, and wherein the coupling comprises hybridizing the first associating domain to the spatially-barcoded
10 oligonucleotide. In some examples, the spatially-barcoded oligonucleotide comprises a second associating domain, wherein the first associating domain of the cell-tagging agent hybridizes to the second associating domain.

In some examples, the cell-tagging agent is coupled to the spatially-barcoded oligonucleotide with a linker. In some examples, the spatially-barcoded oligonucleotide
15 comprises one or more of a unique molecular identifier, an attachment sequence, a restriction enzyme sequence, and a functional domain. In some examples, the attachment sequence comprises one or more of a flow cell attachment sequence and a substrate attachment sequence.

In some examples, the method for determining the location of an analyte present
20 in a biological sample comprises (a) contacting the biological sample to the cell-tagging agent such that a cell in the biological sample is tagged with the spatially barcoded-oligonucleotide; (b) cleaving the spatially barcoded-oligonucleotide from the substrate; (c) providing the cell comprising the spatially barcoded-oligonucleotide to a bead, wherein the bead comprises (1) a first bead-bound oligonucleotide, wherein the first
25 bead-bound oligonucleotide comprises a cellular barcode and a second hybridization domain, and (2) a second bead-bound oligonucleotide, wherein the second bead-bound oligonucleotide comprises the cellular barcode and a capture domain; (d) allowing an analyte from the cell to interact with the capture domain of the second bead-bound oligonucleotide; (e) associating the analyte bound to the capture domain of the second

bead-bound oligonucleotide with the cellular barcode; and (f) associating the cellular barcode with the spatially barcoded-oligonucleotide.

In some examples, the biological sample is a tissue sample. In some examples, the tissue sample is a fresh-frozen tissue sample. In some examples, the tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample. In some examples, the tissue sample comprises a tumor cell. In some examples, the tissue sample comprises a tissue section. In some examples, the method for determining the location of an analyte present in a biological sample comprises imaging the biological sample. In some examples, the analyte comprises at least one of RNA, DNA, protein, lipid, peptide, metabolite, small molecule, and a cell labeling agent. In some examples, the analyte comprises RNA.

In another aspect, the present disclosure refers to a method for generating an array, comprising (a) providing a plurality of spatially-barcoded oligonucleotides to a substrate, wherein two or more spatially-barcoded oligonucleotides of the plurality of spatially-barcoded oligonucleotides comprise a first attachment sequence and a second attachment sequence and wherein the substrate comprises a plurality of functional domains, wherein at least two functional domains of the plurality of functional domains hybridizes to the at least two spatially-barcoded oligonucleotides, thus generating an array of spatially-barcoded oligonucleotides; and (b) amplifying the spatially-barcoded oligonucleotides on the substrate, thereby generating the array.

In some examples, amplifying the two or more of the spatially-barcoded oligonucleotides on the substrate comprises bridge amplification. In some examples, the method of generating an array comprises determining the identity of the two or more spatially-barcoded oligonucleotides of the array. In some examples, determining the identity of the two or more spatially-barcoded oligonucleotides comprises sequencing the spatially-barcoded oligonucleotide. In some examples, sequencing comprises in situ sequencing. In some examples, the in situ sequencing comprises hybridizing a priming oligonucleotide to the two or more spatially-barcoded oligonucleotides. In some examples, the priming oligonucleotide is coupled to a cell-tagging agent. In some examples, the in situ sequencing comprises one or more of sequencing by synthesis,

sequencing by ligation, and rolling circle amplification sequencing. In some examples, the in situ sequencing comprises sequencing by synthesis. In some examples, the sequencing by synthesis comprises hybridizing a priming oligonucleotide with the two or
5 more spatially-barcoded oligonucleotides. In some examples, the priming oligonucleotide is coupled to a cell-tagging agent.

In some examples, the two or more of the spatially-barcoded oligonucleotides comprise one or more of a spatial barcode, a priming domain, a hybridization domain, a unique molecular identifier, a functional domain, and a cleavage domain. In some
10 examples, the two or more of the spatially-barcoded oligonucleotides comprise a spatial barcode. In some examples, the two or more of the spatially-barcoded oligonucleotides comprise a cleavage domain.

In some examples, the cell-tagging agent comprises an extracellular cell-tagging agent. In some examples, the cell-tagging agent comprises an intracellular cell-tagging
15 agent. In some examples, the cell-tagging agent localizes to an internal component of the cell. In some examples, the internal component of the cell comprises one or more of mitochondria, golgi apparatus, smooth endoplasmic reticulum, rough endoplasmic reticulum, nucleus, nucleolus, and lysosome. In some examples, the cell-tagging agent comprises one or more of a lipid, an antibody, a chitosan, a lectin, a streptavidin, a click-
20 chemistry amenable moiety, a cell penetrating peptide, nanoparticles, a TIVA-tag, and liposomes/polysomes. In some examples, the cell-tagging agent is amphiphilic. In some examples, the cell-tagging agent is lipophilic. In some examples, the cell-tagging agent is a cholesterol moiety. In some examples, the cell-tagging agent is coupled to the priming oligonucleotide by a linker. In some examples, the linker comprises one or more of a N-
25 hydroxysuccinimide(NHS) linker, a bifunctional NHS linker, an azide, an alkyne, glycol chitosan, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol) (DSPE-PEG), and succinimidyl-3-(2-pyridyldithio)propionate (SPDP).

In another aspect, the present disclosure refers to a method of determining a location of an analyte in a biological sample, the method comprising: (a) contacting the biological sample with a substrate; (b) partitioning the biological sample into a plurality of pieces, a piece of the plurality of pieces being translocated into a well of a plurality of wells, wherein the piece comprises one or more analytes; (c) providing a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain, wherein the capture domain binds specifically to an analyte of the one or more analytes in the piece; and (d) determining (i) all or a portion of a sequence corresponding to the spatial barcode or a complement thereof, and (ii) all or a portion of a sequence corresponding to the analyte or a complement thereof, and using the determined sequences of (i) and (ii) to determine the location of the analyte in the biological sample.

In another aspect, the present disclosure refers to a method for determining a location of an analyte in a biological sample, the method comprising: introducing into the biological sample a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain, wherein the capture domain binds specifically to the analyte, and wherein the capture probe can pass through a cell membrane of the biological sample; and determining (i) all or a portion of a sequence corresponding to a spatial barcode or a complement thereof, and (ii) all or a portion of a sequence corresponding to the analyte or a complement thereof, and using the determined sequences of (i) and (ii) to determine the location of the analyte in the biological sample.

All publications, patents, patent applications, and information available on the internet and mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, patent application, or item of

information was specifically and individually indicated to be incorporated by reference. To the extent publications, patents, patent applications, and items of information 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.

Where values are described in terms of ranges, it should be understood that the description includes the disclosure of all possible sub-ranges within such ranges, as well as specific numerical values that fall within such ranges irrespective of whether a specific numerical value or specific sub-range is expressly stated.

The term “each,” when used in reference to a collection of items, is intended to identify an individual item in the collection but does not necessarily refer to every item in the collection, unless expressly stated otherwise, or unless the context of the usage clearly indicates otherwise.

Various examples of the features of this disclosure are described herein. However, it should be understood that such examples are provided merely by way of example, and numerous variations, changes, and substitutions can occur to those skilled in the art without departing from the scope of this disclosure. It should also be understood that various alternatives to the specific examples described herein are also within the scope of this disclosure.

BRIEF DESCRIPTION OF DRAWINGS

The following drawings illustrate certain embodiments of the features and advantages of this disclosure. These embodiments are not intended to limit the scope of the appended claims in any manner. Like reference symbols in the drawings indicate like elements.

FIG. 1 shows an exemplary spatial analysis workflow.

FIG. 2 shows an exemplary spatial analysis workflow.

FIG. 3 shows an exemplary spatial analysis workflow.

FIG. 4 shows an exemplary spatial analysis workflow.

FIG. 5 shows an exemplary spatial analysis workflow.