

In one aspect, the present disclosure refers to a method for determining the location of a nucleic acid present in a tissue 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
5 comprises a spatial barcode, a priming domain, and a first hybridization domain; (b) coupling a cell-tagging agent to the spatially-barcoded oligonucleotide, wherein the coupling of the cell-tagging agent to the spatially-barcoded oligonucleotide comprises hybridizing a priming oligonucleotide coupled to the cell-tagging agent to the priming domain of the spatially-barcoded oligonucleotide; (c) contacting the tissue sample to the
10 cell-tagging agent such that a cell in the tissue sample is tagged with the spatially barcoded-oligonucleotide; (d) dissociating the cell from the tissue sample; (e) 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
15 second bead-bound oligonucleotide, wherein the second bead-bound oligonucleotide comprises the cellular barcode and a capture domain, wherein the providing comprises hybridizing the first hybridization domain of the spatially-barcoded oligonucleotide to the second hybridization domain of the first bead-bound oligonucleotide; (f) releasing the nucleic acid from the cell; (g) hybridizing the nucleic acid from the cell to the capture
20 domain of the second bead-bound oligonucleotide; and (h) determining (i) a sequence corresponding to both the nucleic acid hybridized to the capture domain or a complement thereof and the cellular barcode from the second bead-bound oligonucleotide, and (ii) a sequence corresponding to both the spatial barcode or a complement thereof and the cellular barcode from the first bead-bound oligonucleotide, and using the sequences of (i)
25 and (ii) to determine the location of the nucleic acid in the tissue 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 example, the present disclosure refers to a method for determining the location of an analyte present in a biological sample, comprising: (a) providing a
30 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.

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

WHAT IS CLAIMED IS:

1. A method for determining the location of a nucleic acid present in a tissue 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, wherein the coupling of the cell-tagging agent to the spatially-barcoded oligonucleotide comprises hybridizing a priming oligonucleotide coupled to the cell-tagging agent to the priming domain of the spatially-barcoded oligonucleotide;
 - (c) contacting the tissue sample to the cell-tagging agent such that a cell in the tissue sample is tagged with the spatially barcoded-oligonucleotide;
 - (d) dissociating the cell from the tissue sample;
 - (e) 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,wherein the providing comprises hybridizing the first hybridization domain of the spatially-barcoded oligonucleotide to the second hybridization domain of the first bead-bound oligonucleotide;
 - (f) releasing the nucleic acid from the cell;
 - (g) hybridizing the nucleic acid from the cell to the capture domain of the second bead-bound oligonucleotide; and
 - (h) determining (i) a sequence corresponding to both the nucleic acid hybridized to the capture domain or a complement thereof and the cellular barcode from the second bead-bound oligonucleotide, and (ii) a sequence corresponding to both the spatial

- barcode or a complement thereof and the cellular barcode from the first bead-bound oligonucleotide, and using the sequences of (i) and (ii) to determine the location of the nucleic acid in the tissue sample.
2. The method of claim 1, wherein the priming oligonucleotide coupled to the cell-tagging agent is substantially complementary to the priming domain of the spatially barcoded-oligonucleotide.
 3. The method of claim 1, wherein 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.
 4. The method of any one of claims 1-3, wherein the determining step comprises sequencing.
 5. The method of claim 4, wherein the sequencing comprises in situ sequencing, wherein 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).
 6. The method of any one of claims 1-5, wherein the spatially-barcoded oligonucleotide comprises one or more of a unique molecular identifier, an attachment sequence, a cleavage domain, and a functional domain.
 7. The method of claim 6, wherein the attachment sequence comprises one or more of a flow cell attachment sequence and a substrate attachment sequence.
 8. The method of claim 1, wherein releasing comprises permeabilization of the cell.

9. The method of any one of claims 1-8, wherein the determining in (h) further comprises extending the hybridized first bead-bound oligonucleotide and spatially barcoded-oligonucleotide.
10. The method of claim 9, comprising determining the sequence of the extended bead-bound oligonucleotide and spatially barcoded-oligonucleotide.
11. The method of claim 1, wherein the tissue sample is a fresh-frozen tissue sample.
12. The method of claim 1, wherein the tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.
13. The method of claim 1, wherein the tissue sample comprises a tumor cell.
14. The method of claim 1, wherein the tissue sample comprises a tissue section.
15. The method of any one of claims 1 to 14, comprising imaging the tissue sample.
16. The method of claim 1, wherein the nucleic acid comprises RNA or DNA.
17. The method of any one of claims 1-16, wherein the cell-tagging agent comprises an extracellular cell-tagging agent.
18. The method of any one of claims 1-16, wherein the cell-tagging agent comprises an intracellular cell-tagging agent.
19. The method of any one of claims 1-16, wherein the cell-tagging agent localizes to an internal component of the cell.

20. The method of claim 19, wherein the internal component of the cell comprises one or more of mitochondria, golgi apparatus, smooth endoplasmic reticulum, rough endoplasmic reticulum, nucleus, nucleolus, and lysosome.
21. The method of any one of claims 1 to 20, wherein the cell-tagging agent comprises one or more of a lipid, an antibody, a chitosan, a lectin, a streptavidin, a click-chemistry amenable moiety, a cell penetrating peptide, nanoparticles, a TIVA-tag, and liposomes/polysomes.
22. The method of any one of claims 1-16, wherein the cell-tagging agent is amphiphilic.
23. The method of any one of claims 1-16, wherein the cell-tagging agent is lipophilic.
24. The method of any one of claims 1-16, wherein the cell-tagging agent is a cholesterol moiety.
25. The method of any one of claims 1-16, wherein the cell-tagging agent is coupled to the priming oligonucleotide by a linker.
26. The method of claim 25, wherein the linker comprises one or more of a N-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).