

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, 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 biological sample to the cell-tagging agent such that a cell in the biological sample is tagged with the spatially barcoded-oligonucleotide; (d) ~~hybridizing~~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 hybridization comprises the first hybridization domain of the spatially-barcoded oligonucleotide hybridizing to the second hybridization domain of the first bead-bound oligonucleotide; (e) ~~hybridizing the~~allowing an analyte from the cell to ~~interact with~~ the capture domain of the second bead-bound oligonucleotide; and (f) determining (i) a sequence corresponding to both the analyte 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 analyte in the biological sample~~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 example 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

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 ~~example~~^{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,

In another example 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 example 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

WHAT IS ~~ITEMIZED~~CLAIMED IS:

1. 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-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.
2. The method of ~~claimitem~~ 1, comprising dissociating the cell from the biological sample.
3. The method of ~~claimitem~~ 1 or 2, wherein 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.

4. The method of [claimitem](#) 3, wherein the priming oligonucleotide coupled to the cell-tagging agent is substantially complementary to the priming domain of the spatially barcoded-oligonucleotide.
5. The method of [claimitem](#) 3, 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.
6. The method of any one of [claimitems](#) 1 to 5, comprising determining the sequence of at least a portion of the spatially-barcoded oligonucleotide.
7. The method of [claimitem](#) 6, wherein determining the sequence comprises in situ sequencing.
8. The method of [claimitem](#) 7, 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).
9. The method of any one of [claimitems](#) 1-8, wherein providing the cell comprising the spatially barcoded-oligonucleotide to the bead comprises hybridization.
10. The method of [claimitem](#) 9, wherein the hybridization comprises the first hybridization domain hybridizing to the second hybridization domain.
11. The method of any one of [claimitems](#) 1 to 10, wherein the spatially-barcoded oligonucleotide comprises one or more of a unique molecular identifier, an attachment sequence, a cleavage domain, and a functional domain.
12. The method of [claimitem](#) 11, wherein the attachment sequence comprises one or more of a flow cell attachment sequence and a substrate attachment sequence.
13. The method of any one of [claimitems](#) 1-12, wherein allowing the analyte from the cell to interact with the capture domain comprises releasing the analyte from the cell.
14. The method of [claimitem](#) 13, wherein releasing comprises permeabilization of the cell.
15. The method of any one of [claimitems](#) 1-14, wherein associating the analyte bound to the capture domain with the cellular domain comprises identifying the analyte.

16. The method of any one of [claimitem](#)s 1-15, wherein associating the cellular barcode with the spatial barcode comprises extending the hybridized first bead-bound oligonucleotide and spatially barcoded-oligonucleotide.
17. The method of [claimitem](#) 16, comprising determining the sequence of the extended bead-bound oligonucleotide and spatially barcoded-oligonucleotide.
18. 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 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.
19. The method of [claimitem](#) 18, wherein 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 oligonucleotide.
20. The method of any one of [claimitem](#)s 18 to 19, wherein 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.
21. The method of any one of [claimitem](#)s 18 to 20, wherein the cell-tagging agent is coupled to the spatially-barcoded oligonucleotide with a linker.
22. The method of any one of [claimitem](#)s 18 to 21, wherein the spatially-barcoded oligonucleotide comprises one or more of a unique molecular identifier, an attachment sequence, a restriction enzyme sequence, and a functional domain.
23. The method of [claimitem](#) 22, wherein the attachment sequence comprises one or more of a flow cell attachment sequence and a substrate attachment sequence.
24. The method of any one of [claimitem](#)s 18 to 23, comprising:

- (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,
5 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
10 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
 - 15 (f) associating the cellular barcode with the spatially barcoded-oligonucleotide.
25. The method of any one of [claimitem](#)s 1 to 24, wherein the biological sample is a tissue sample.
 26. The method of [claimitem](#) 25, wherein the tissue sample is a fresh-frozen tissue sample.
 - 20 27. The method of [claimitem](#) 25, wherein the tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.
 28. The method of [claimitem](#) 25, wherein the tissue sample comprises a tumor cell.
 29. The method of [claimitem](#) 25, wherein the tissue sample comprises a tissue section.
 - 25 30. The method of any one of [claimitem](#)s 1 to 29, comprising imaging the biological sample.
 31. The method of any one of [claimitem](#)s 1 to 30, wherein the analyte comprises at least one of RNA, DNA, protein, lipid, peptide, metabolite, small molecule, and a cell labeling agent.
 - 30 32. The method of any one of [claimitem](#)s 1 to 31, wherein the analyte comprises RNA.
 33. 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.
34. The method of [claim item](#) 33, wherein amplifying the two or more of the spatially-
barcoded oligonucleotides on the substrate comprises bridge amplification.
35. The method of any one of [claim items](#) 33 to 34, comprising determining the
identity of the two or more spatially-barcoded oligonucleotides of the array.
36. The method of [claim item](#) 35, wherein determining the identity of the two or more
spatially-barcoded oligonucleotides comprises sequencing the spatially-barcoded
oligonucleotide.
37. The method of [claim item](#) 36, wherein sequencing comprises in situ sequencing.
38. The method of [claim item](#) 37, wherein the in situ sequencing comprises
hybridizing a priming oligonucleotide to the two or more spatially-barcoded
oligonucleotides.
39. The method of [claim item](#) 38, wherein the priming oligonucleotide is coupled to a
cell-tagging agent.
40. The method of any one of [claim items](#) 37 to 39, wherein the in situ sequencing
comprises one or more of sequencing by synthesis, sequencing by ligation, and
rolling circle amplification sequencing.
41. The method of any one of [claim items](#) 37 to 39, wherein the in situ sequencing
comprises sequencing by synthesis.
42. The method of any one of [claim items](#) 40-41, wherein the sequencing by synthesis
comprises hybridizing a priming oligonucleotide with the two or more spatially-
barcoded oligonucleotides.

43. The method of [claimitem](#) 42, wherein the priming oligonucleotide is coupled to a cell-tagging agent.
44. The method of any one of [claimitems](#) 33 to 43, wherein 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.
45. The method of any one of [claimitems](#) 33 to 43, wherein the two or more of the spatially-barcoded oligonucleotides comprise a spatial barcode.
46. The method of any one of [claimitems](#) 33 to 43, wherein the two or more of the spatially-barcoded oligonucleotides comprise a cleavage domain.
47. The method of any one of [claimitems](#) 1 to 46, wherein the cell-tagging agent comprises an extracellular cell-tagging agent.
48. The method of any one of [claimitems](#) 1 to 46, wherein the cell-tagging agent comprises an intracellular cell-tagging agent.
49. The method of any one of [claimitems](#) 1 to 46, wherein the cell-tagging agent localizes to an internal component of the cell.
50. The method of [claimitem](#) 49, 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.
51. The method of [claimitem](#) any one of [claimitems](#) 1 to 46, 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.
52. The method of [claimitem](#) any one of [claimitems](#) 1 to 46, wherein the cell-tagging agent is amphiphilic.
53. The method of [claimitem](#) any one of [claimitems](#) 1 to 46, wherein the cell-tagging agent is lipophilic.
54. The method of [claimitem](#) any one of [claimitems](#) 1 to 46, wherein the cell-tagging agent is a cholesterol moiety.

55. The method of any one of claim items 1 to 46, wherein the cell-tagging agent is coupled to the priming oligonucleotide by a linker.
56. The method of any one of claim items 21 or 55, 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).

WHAT IS CLAIMED IS:

1. 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, 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 biological sample to the cell-tagging agent such that a cell in the biological sample is tagged with the spatially barcoded-oligonucleotide;
 - (d) ~~hybridizing~~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 hybridization comprises the first hybridization domain of the spatially-barcoded oligonucleotide hybridizing to the second hybridization domain of the first bead-bound oligonucleotide;
 - (e) ~~hybridizing the~~allowing an analyte from the cell to ~~interact with~~ the capture domain of the second bead-bound oligonucleotide; and
 - (f) ~~associating the analyte bound to the capture domain of the second bead bound oligonucleotide with the cellular barcode; and~~determining (i) a sequence corresponding to both the analyte 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 analyte in the biological sample.
~~(g) associating the cellular barcode with the spatial barcode, thereby determining the location of the analyte present in the biological sample.~~

2. The method of claim 1, comprising dissociating the cell from the biological sample.

~~3. — The method of claim 1 or 2, wherein 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.~~

4.3. The method of claim 1 or 23, wherein the priming oligonucleotide coupled to the cell-tagging agent is substantially complementary to the priming domain of the spatially barcoded-oligonucleotide.

5.4. The method of claim 1 or 23, 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.

~~6. — The method of any one of claims 1 to 5, comprising determining the sequence of at least a portion of the spatially barcoded-oligonucleotide.~~

7.5. The method of any one of claims 1-4-6, wherein the determining step~~the sequence~~ comprises ~~in situ~~ sequencing.

8.6. The method of claim 57, 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).

~~9. — The method of any one of claims 1-8, wherein providing the cell comprising the spatially barcoded-oligonucleotide to the bead comprises hybridization.~~

~~10. — The method of claim 9, wherein the hybridization comprises the first hybridization domain hybridizing to the second hybridization domain.~~

11.7. The method of any one of claims 1 to ~~6~~10, wherein the spatially-barcoded oligonucleotide comprises one or more of a unique molecular identifier, an attachment sequence, a cleavage domain, and a functional domain.

12.8. The method of claim ~~7~~11, wherein the attachment sequence comprises one or more of a flow cell attachment sequence and a substrate attachment sequence.

13.9. The method of any one of claims 1-~~12~~8, wherein ~~hybridizing~~allowing the analyte from the cell to ~~interact with~~ the capture domain comprises releasing the analyte from the cell.

14.10. The method of claim ~~13~~9, wherein releasing comprises permeabilization of the cell.

~~15. — The method of any one of claims 1-14, wherein associating the analyte bound to the capture domain with the cellular domain comprises identifying the analyte.~~

16.11. The method of any one of claims 1-~~15~~10, wherein the determining step (f) further associating the cellular barcode with the spatial barcode comprises extending the hybridized first bead-bound oligonucleotide and spatially barcoded-oligonucleotide.

17.12. The method of claim ~~16~~11, comprising determining the sequence of the extended bead-bound oligonucleotide and spatially barcoded-oligonucleotide.

~~18. — 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.~~

~~19. — The method of claim 18, wherein 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 oligonucleotide.~~

~~20. — The method of any one of claims 18 to 19, wherein 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.~~

~~21. — The method of any one of claims 18 to 20, wherein the cell tagging agent is coupled to the spatially barcoded oligonucleotide with a linker.~~

~~22. — The method of any one of claims 18 to 21, wherein the spatially barcoded oligonucleotide comprises one or more of a unique molecular identifier, an attachment sequence, a restriction enzyme sequence, and a functional domain.~~

~~23. — The method of claim 22, wherein the attachment sequence comprises one or more of a flow cell attachment sequence and a substrate attachment sequence.~~

~~24. — The method of any one of claims 18 to 23, comprising:~~

- ~~(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 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.~~

[25.13.](#) The method of any one of claims 1 to [1224](#), wherein the biological sample is a tissue sample.

[26.14.](#) The method of claim [1325](#), wherein the tissue sample is a fresh-frozen tissue sample.

[27.15.](#) The method of claim [1325](#), wherein the tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

[28.16.](#) The method of claim [1325](#), wherein the tissue sample comprises a tumor cell.

[29.17.](#) The method of claim [1325](#), wherein the tissue sample comprises a tissue section.

[30.18.](#) The method of any one of claims 1 to [1729](#), comprising imaging the biological sample.

[31.19.](#) The method of any one of claims 1 to [1830](#), wherein the analyte comprises at least one of RNA, DNA, protein, lipid, peptide, metabolite, small molecule, and a cell labeling agent.

[32.20.](#) The method of any one of claims 1 to [1931](#), wherein the analyte comprises RNA.

~~33.—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.~~

34. ~~—The method of claim 33, wherein amplifying the two or more of the spatially barcoded oligonucleotides on the substrate comprises bridge amplification.~~

35. ~~—The method of any one of claims 33 to 34, comprising determining the identity of the two or more spatially barcoded oligonucleotides of the array.~~

36. ~~—The method of claim 35, wherein determining the identity of the two or more spatially barcoded oligonucleotides comprises sequencing the spatially barcoded oligonucleotide.~~

37. ~~—The method of claim 36, wherein sequencing comprises in situ sequencing.~~

38. ~~—The method of claim 37, wherein the in situ sequencing comprises hybridizing a priming oligonucleotide to the two or more spatially barcoded oligonucleotides.~~

39. ~~—The method of claim 38, wherein the priming oligonucleotide is coupled to a cell tagging agent.~~

40. ~~—The method of any one of claims 37 to 39, wherein the in situ sequencing comprises one or more of sequencing by synthesis, sequencing by ligation, and rolling circle amplification sequencing.~~

~~41. — The method of any one of claims 37 to 39, wherein the in situ sequencing comprises sequencing by synthesis.~~

~~42. — The method of any one of claims 40–41, wherein the sequencing by synthesis comprises hybridizing a priming oligonucleotide with the two or more spatially barcoded oligonucleotides.~~

~~43. — The method of claim 42, wherein the priming oligonucleotide is coupled to a cell-tagging agent.~~

~~44. — The method of any one of claims 33 to 43, wherein 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.~~

~~45. — The method of any one of claims 33 to 43, wherein the two or more of the spatially barcoded oligonucleotides comprise a spatial barcode.~~

~~46. — The method of any one of claims 33 to 43, wherein the two or more of the spatially barcoded oligonucleotides comprise a cleavage domain.~~

[47.21.](#) The method of any one of claims 1 to [2046](#), wherein the cell-tagging agent comprises an extracellular cell-tagging agent.

[48.22.](#) The method of any one of claims 1 to [2046](#), wherein the cell-tagging agent comprises an intracellular cell-tagging agent.

[49.23.](#) The method of any one of claims 1 to [2046](#), wherein the cell-tagging agent localizes to an internal component of the cell.

50:24. The method of claim 2349, 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.

51:25. The method of claim any one of claims 1 to 2046, 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.

52:26. The method of claim any one of claims 1 to 2046, wherein the cell-tagging agent is amphiphilic.

53:27. The method of claim any one of claims 1 to 2046, wherein the cell-tagging agent is lipophilic.

54:28. The method of claim any one of claims 1 to 2046, wherein the cell-tagging agent is a cholesterol moiety.

55:29. The method of any one of claims 1 to 2046, wherein the cell-tagging agent is coupled to the priming oligonucleotide by a linker.

56:30. The method of ~~any one of claims 2921 or 55~~, 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).

~~57. — 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.

58. — The method of claim 57, wherein the capture probe is provided on the substrate.

59. — The method of claim 57, wherein the capture probe is provided in the well.

60. — The method of any one of claims 57–59, wherein the well is a nanowell.

61. — The method of any one of claims 57–60, wherein partitioning comprises cutting and/or stamping the biological sample.

62. — The method of any one of claims 57–60, wherein partitioning comprises laser capture microdissection of the biological sample.

63. — The method of any one of claims 57–60, wherein partitioning comprises using a hollow needle or microneedle.

64. — The method of any one of claims 57–63, wherein the method further comprises identifying a region of interest in the biological sample before partitioning.

65. — 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.

66. — The method of claim 65, wherein introducing comprises injection, particle bombardment, photoporation, magnetofection, and/or hydroporation.

67. — The method of claim 66, wherein injection comprises microinjection.

68. — The method of claim 65, wherein the capture probe further comprises a cleavage domain, and introducing comprises contacting the biological sample with a substrate comprising a plurality of capture probes and cleaving the plurality of capture probes from the substrate.

69. — The method of any one of claims 65-67, wherein the capture probe is coupled, directly or indirectly, to a lipophilic molecule.

70. — The method of claim 68, wherein the lipophilic molecule is selected from the group consisting of cholesterol, tocopherol, steryl palmitate, lignoceric acid, squalene, a fatty acid, and derivatives thereof.

71. — The method of any one of claims 65-68, wherein the capture probe is coupled, directly or indirectly, to a cell-penetrating agent.

72. — The method of claim 71, wherein the cell-penetrating agent is a cell-penetrating peptide.