

further comprises a cleavage domain, a functional domain, and a unique molecular identifier, or combinations thereof. In some examples, the array has one or more microfluidic channels. In some examples, exposing the array to a concentration gradient comprises an optically controlled reaction. In some examples, the array comprises a plurality of features, wherein the features
5 comprise one or more pluralities of the immobilized surface probes.

In one aspect, the present disclosure refers to a method for generating a spatially barcoded array, the method comprising: (a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe of the plurality of immobilized surface probes comprises an oligonucleotide; (b) exposing the array to a concentration gradient of a first deoxyribonucleotide
10 triphosphate (dNTP), wherein the concentration gradient of the first dNTP varies along a first direction; and (c) adding one or more first nucleotides derived from the first dNTP to ~~at the~~ the 3' terminus of the oligonucleotide using a terminal transferase, wherein the number of the one or more first nucleotides that are added to the oligonucleotide correlates with the concentration of the first dNTP in the concentration gradient at the surface probe's location, thereby generating a spatially barcoded array.

In another ~~example~~ aspect, the present disclosure refers to a method for generating a spatially barcoded array, the method comprising: (a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe of the plurality of immobilized surface probes comprises an oligonucleotide; (b) exposing the array to a first dNTP and a first concentration gradient of a polymerase, wherein the first concentration gradient of the polymerase varies along a first direction; and
15 (c) adding one or more first nucleotides derived from the first dNTP to the oligonucleotide, wherein the number of the one or more first nucleotides that are added to the oligonucleotide correlates with the concentration of the polymerase in the first concentration gradient at the surface probe's location, thereby generating a spatially barcoded array. In one aspect, the present disclosure refers to a method for generating a spatially barcoded array, the method comprising: (a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe of the plurality of immobilized surface probes comprises an oligonucleotide; (b) exposing the array to a first dNTP and a first concentration gradient of a polymerase, wherein the first concentration gradient of the polymerase varies along a first direction, and wherein the polymerase is a terminal transferase; and (c) adding one or more first nucleotides derived from the first dNTP to a 3' terminus of the oligonucleotide, wherein the number of the one or more first nucleotides that are added to the oligonucleotide correlates with the concentration of the polymerase in the first concentration gradient at the surface probe's location, thereby generating a spatially barcoded array. In some examples, the oligonucleotide comprises a functional domain, a cleavage domain, a unique molecular identifier, or combinations thereof. In some examples, the method further comprises exposing the array to a second dNTP and a second concentration gradient of the polymerase, wherein the second concentration gradient of the polymerase varies along a second direction. In some examples, the first direction and the second direction forms a 90° or 180° angle. In some examples, the method further comprises exposing the array to a third dNTP and a third concentration gradient of the polymerase, wherein the third concentration gradient of the polymerase varies along a third direction. In some examples, the first direction, the second direction, and the third direction of concentration gradients are different. In some examples, the method further comprises exposing the array to a fourth dNTP and a fourth concentration gradient of the polymerase, wherein the fourth concentration gradient of the polymerase varies along a fourth direction. In some examples, the first direction, the second direction, the third direction, and the fourth direction of concentration gradients are different. In some examples, the one or more second nucleotides derived from the second dNTP are added to the oligonucleotide after the one or more first nucleotides derived from the first dNTP are added to the oligonucleotide. In some examples, the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide after the one or
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more second nucleotides derived from the second dNTP are added to the oligonucleotide. In some examples, the one or more fourth nucleotides derived from the fourth dNTP are added to the oligonucleotide after the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide. In some examples, the fourth dNTP is deoxythymidine triphosphate (dTTP). In some examples, a poly(T) oligonucleotide is added to the oligonucleotide. In some examples, the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides are added by a polymerase. In some examples, the polymerase is a terminal deoxynucleotidyl transferase. In some examples, the spatial barcode is formed by the one or more first nucleotides. In some examples, the spatial barcode is formed by the one or more first nucleotides, and the one or more second nucleotides. In some examples, the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides. In some examples, the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides. In some examples, the array comprises a plurality of features, wherein the features comprise one or more pluralities of immobilized surface probes. In some examples, exposing the array to a concentration gradient comprises an optically controlled reaction.

In another aspect, the present disclosure refers to a method for generating a spatially barcoded array, the method comprising: a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe comprises an oligonucleotide; b) exposing the array to a concentration gradient of a first deoxyribonucleotide triphosphate (dNTP), wherein the concentration gradient of the first dNTP varies along a first direction; c) exposing the array to a concentration gradient of a second dNTP, wherein the concentration gradient of the second dNTP varies along a second direction, wherein the first direction and the second direction are different; and d) adding one or more first nucleotides derived from the first dNTP and one or more second nucleotides derived from the second dNTP to the 3' terminus of the oligonucleotide using a terminal transferase, wherein the percentage of the one or more first nucleotides and the percentage of the one or more second nucleotides correlate with concentration of the first dNTP and the second dNTP at the surface probe's location, thereby generating the spatially barcoded array.

In another example the present disclosure generally describes a method for generating a spatially barcoded array, the method comprising: providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe comprises an oligonucleotide; exposing the array to a concentration gradient of a first deoxyribonucleotide triphosphate (dNTP), wherein the concentration gradient of the first dNTP varies along a first direction; exposing the array to a concentration gradient of a second dNTP, wherein the concentration gradient of the second dNTP varies along a second direction, wherein the first direction and the second direction are different; adding one or more first nucleotides derived from the first dNTP and one or more second nucleotides derived from the second dNTP to the oligonucleotide, wherein the percentage of the one or more first nucleotides and the percentage of the one or more second nucleotides correlate with concentration of the first dNTP and the second dNTP at the surface probe's location, thereby generating the spatially barcoded array. In some examples, the method further comprises exposing the array to a concentration gradient

of a third dNTP, wherein the concentration gradient of the third dNTP varies along a third direction, and wherein the third direction is different than the first direction and the second direction. In some examples, the method of claim 50, wherein the method further comprises exposing the array to a concentration gradient of a fourth dNTP, wherein the concentration
5 gradient of the fourth dNTP varies along a fourth direction, and wherein the fourth direction is different than the first direction, the second direction, and the third direction. In some examples, the method of claim 50 or claim 51, wherein one or more third nucleotides derived from the third dNTP are added to the oligonucleotide in about the same time when one or more first nucleotides and the one or more second nucleotides are added to the oligonucleotides. In some examples, one
10 or more fourth nucleotides derived from the fourth dNTP are added to the oligonucleotide in about the same time when the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides are added to the oligonucleotide. In some examples, the percentages of the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides correlate with the concentration
15 gradient of the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP, thereby generating a spatially barcoded array. In some examples, a poly(T) oligonucleotide is added to the oligonucleotide after the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides are added to the oligonucleotide. In some examples, the fourth dNTP is dTTP.

20 In another example~~aspect~~, the present disclosure refers to a method of generating an array, the method comprising: (a) providing a plurality of first oligonucleotides on a substrate, wherein a first oligonucleotide of the plurality comprises a first spatial barcode and is attached to the substrate; (b) delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode; and (c) ligating the
25 first oligonucleotide and the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.

In another example~~aspect~~, the present disclosure refers to a method of generating an array, the method comprising: (a) providing a plurality of first oligonucleotides on a substrate, wherein a first oligonucleotide of the plurality is attached to the substrate and comprises a first
30 spatial barcode; (b) delivering a plurality of second oligonucleotides to the

substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode, a surface linker that binds to the substrate, and a cleavage domain; (c) attaching the second oligonucleotide to the substrate; (d) ligating the first oligonucleotide and the second oligonucleotide; and (e) cleaving the cleavage domain in the second oligonucleotide, thereby
5 generating a capture probe comprising the first spatial barcode and the second spatial barcode.

In another example~~aspect~~, the present disclosure refers to a method of generating an array, the method comprising: (a) delivering a plurality of first oligonucleotides to a substrate, wherein a first oligonucleotide of the plurality comprises a first spatial barcode and a surface linker that binds to the substrate; (b) delivering a plurality of second oligonucleotides to the
10 substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode; and (c) ligating the first oligonucleotide and the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.

In another example~~aspect~~, the present disclosure refers to a method of generating an array, the method comprising: (a) delivering a plurality of first oligonucleotides to a substrate, wherein a first oligonucleotide comprises a spatial barcode and a surface linker that binds to the
15 substrate; (b) delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide comprises a spatial barcode, a surface linker that binds to the substrate, and a cleavage domain; (c) ligating the first oligonucleotide and the second oligonucleotide; and (d) cleaving the cleavage domain in the second oligonucleotide, thereby generating a capture probe
20 comprising the first spatial barcode and the second spatial barcode. In some examples, the surface linker of the first oligonucleotide is attached to the substrate prior to the delivery of the plurality of second oligonucleotide. In some examples, the first oligonucleotide is attached at a first distinct spatial position on the substrate. In some examples, the second oligonucleotide is delivered to a distinct spatial position on the substrate. In some examples, the second
25 oligonucleotide attaches to the substrate prior to ligating the first oligonucleotide and the second oligonucleotide. In some examples, the first oligonucleotide comprises an overhanging sequence. In some examples, the first oligonucleotide comprises an overhanging sequence and the second oligonucleotide comprises an overhanging sequence, wherein the overhanging sequence of the first oligonucleotide and the overhanging sequence of the second oligonucleotide hybridize to
30 each other. In some examples, the substrate is washed prior to ligating the first oligonucleotide and the second oligonucleotide. In some examples, members of the plurality

claimed array; (b) releasing an analyte from the biological sample, wherein the analyte is bound by a capture probe at a distinct spatial position of the substrate; (c) detecting the bound analyte; and (d) correlating the analyte with a barcode of the capture probe at the distinct spatial position of the substrate, thus associating presence or abundance of an analyte with a location in the biological sample.

In another example aspect, the present disclosure refers to a method of associating presence or abundance of an analyte with a location in a biological sample, comprising: (a) contacting the biological sample with an array produced as disclosed herein; (b) releasing an analyte from the biological sample, wherein the analyte is bound by a capture probe at a distinct spatial position of the substrate; (c) detecting the bound analyte; and (d) correlating the analyte with a barcode of the capture probe at the distinct spatial position of the substrate, thus associating presence or abundance of an analyte with a location in the biological sample.

In another example aspect, the present disclosure refers to a method for transferring an analyte from a biological sample to a substrate, the method comprising: providing a substrate comprising 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 is capable of binding specifically to the analyte; contacting the biological sample with the substrate; applying an electric field to the biological sample and the substrate, thereby transferring the analyte from the biological sample to the substrate.

In another example aspect, the present disclosure refers to a method for capturing an analyte from a biological sample, the method comprising: providing a substrate, wherein the substrate comprises a plurality of capture probes and wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain; contacting the biological sample with the substrate; and providing a diffusion-resistant medium to the biological sample, wherein the diffusion-resistant medium reduces diffusion of analytes away from the plurality of capture probes and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from the biological sample.

In another example aspect, the present disclosure refers to a method for capturing an analyte from a biological sample, the method comprising: providing a substrate comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain; contacting the biological sample with the substrate; and providing a diffusion-resistant medium comprising one or more permeabilization reagent(s) to the biological sample, wherein the one or more permeabilization reagent(s) promotes migration of the analyte present in the biological sample towards the plurality of capture probes, and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from the biological sample.

In another example aspect, the present disclosure refers to a method for capturing an analyte from a biological sample, the method comprising: providing a diffusion-resistant medium, wherein the diffusion-resistant medium comprises a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain, and wherein the diffusion-resistant medium comprises one or more permeabilization reagent(s); and contacting the diffusion-resistant medium to the biological sample, wherein the one or more permeabilization reagent(s) releases the analyte from the biological sample, and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from the biological sample.

In another example aspect, the present disclosure refers to a method for capturing an analyte from a biological sample, the method comprising: providing a first substrate, comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain, providing the biological sample on a second substrate; and sandwiching a diffusion-resistant medium between the first substrate and the biological sample, wherein the diffusion-resistant medium comprises one or more permeabilization reagent(s) that releases the analyte from the biological sample, and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from 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: (a) permeabilizing the biological sample on a substrate; (b) contacting the biological sample with 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; (c) enzymatically synthesizing a molecule comprising (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 without removing the biological sample from the substrate; and (d) determining (i) all or a portion of the sequence corresponding to the spatial barcode or the complement thereof, and (ii) all or a portion of the sequence corresponding to the analyte or the 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: (a) fixing a fresh frozen biological sample with acetone; (b) contacting the biological sample with 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 (c) 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.

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.

WHAT IS ~~ITEMIZED~~CLAIMED IS:

1. A method for generating a spatially barcoded array, the method comprising:
 - (a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe of the plurality of immobilized surface probes comprises an oligonucleotide;
 - (b) exposing the array to a concentration gradient of a first deoxyribonucleotide triphosphate (dNTP), wherein the concentration gradient of the first dNTP varies along a first direction; and
 - (c) adding one or more first nucleotides derived from the first dNTP to the oligonucleotide, wherein the number of the one or more first nucleotides that are added to the oligonucleotide correlates with the concentration of the first dNTP in the concentration gradient at the surface probe's location, thereby generating a spatially barcoded array.
2. The method of ~~claim item~~ 1, wherein the plurality of immobilized surface probes have a common sequence.
3. The method of ~~claim item~~ 1 or ~~claim item~~ 2, wherein the method further comprises exposing the array to a concentration gradient of a second dNTP, wherein the concentration gradient of the second dNTP varies along a second direction.
4. The method of ~~claim item~~ 3, wherein the first direction and the second direction forms a 90° or 180° angle.
5. The method of ~~claim item~~ 3 or ~~claim item~~ 4, wherein the method further comprises exposing the array to a concentration gradient of a third dNTP, wherein the concentration gradient of the third dNTP varies along a third direction.
6. The method of ~~claim item~~ 5, wherein the first dNTP, the second dNTP, and the third dNTP, have different concentration gradients.

7. The method of [claimitem 5](#) or [claimitem 6](#), wherein the method further comprises exposing the array to a concentration gradient of a fourth dNTP, wherein the concentration gradient of the fourth dNTP varies along a fourth direction.
- 5 8. The method of [claimitem 7](#), wherein the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP, have different concentration gradients.
9. The method of [claimitem 7](#) or [claimitem 8](#), wherein the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP, are different.
- 10 10. The method of any one of [claimitems 7-9](#), wherein the fourth dNTP is dTTP.
11. The method of [claimitem 10](#), wherein one or more dTTP form a capture domain that interacts with an analyte.
- 15 12. The method of any one of [claimitems 3-11](#), wherein the one or more second nucleotides derived from the second dNTP, are added to the oligonucleotide after the one or more first nucleotides derived from the first dNTP are added to the oligonucleotide.
- 20 13. The method of any one of [claimitem 5-12](#), wherein the one or more third nucleotides derived from the third dNTP, are added to the oligonucleotide after the one or more second nucleotides derived from the second dNTP are added to the oligonucleotide.
- 25 14. The method of any one of [claimitems 7-13](#), wherein the one or more fourth nucleotides are added to the oligonucleotide after the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide.
- 30 15. The method of any one of [claimitems 1-14](#), wherein a poly(T) oligonucleotide is added to the oligonucleotide.
16. The method of [claimitem 15](#), wherein the poly(T) oligonucleotide is added by a ligase enzyme.

17. The methods of any one of [claim items](#) 1-15, wherein the nucleotides are added by a polymerase.
- 5 18. The method of [claim item](#) 17, wherein the polymerase is a terminal transferase.
19. The method of [claim item](#) 18, wherein the terminal transferase is a terminal deoxynucleotidyl transferase.
- 10 20. The method of any one of [claim items](#) 1-18, wherein the spatial barcode is formed by the one or more first nucleotides.
21. The method of any one of [claim items](#) 3-18, wherein the spatial barcode is formed by the one or more first nucleotides and the one or more second nucleotides.
- 15 22. The method of any one of [claim items](#) 5-18, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides.
- 20 23. The method of any one of [claim items](#) 7-18, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides.
- 25 24. The method of any one of [claim items](#) 1-22, wherein the oligonucleotide further comprises a cleavage domain, a functional domain, and a unique molecular identifier, or combinations thereof.
- 30 25. The method of any one of [claim items](#) 1-23, wherein the array has one or more microfluidic channels.
26. The method of any one of [claim items](#) 1-25, wherein exposing the array to a concentration gradient comprises an optically controlled reaction.

27. The method of any one of [claimitem](#) 1-26, wherein the array comprises a plurality of features, wherein the features comprise one or more pluralities of the immobilized surface probes.

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28. A method for generating a spatially barcoded array, the method comprising:

(a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe of the plurality of immobilized surface probes comprises an oligonucleotide;

10 (b) exposing the array to a first dNTP and a first concentration gradient of a polymerase, wherein the first concentration gradient of the polymerase varies along a first direction; and

(c) adding one or more first nucleotides derived from the first dNTP to the oligonucleotide, wherein the number of the one or more first nucleotides that are added to the
15 oligonucleotide correlates with the concentration of the polymerase in the first concentration gradient at the surface probe's location, thereby generating a spatially barcoded array.

20 29. The method of [claimitem](#) 28, wherein the oligonucleotide comprises a functional domain, a cleavage domain, a unique molecular identifier, or combinations thereof.

30. The method of [claimitem](#) 28 or [claimitem](#) 29, wherein the method further comprises exposing the array to a second dNTP and a second concentration gradient of the polymerase, wherein the second concentration gradient of the polymerase varies along a second
25 direction.

31. The method of [claimitem](#) 30, wherein the first direction and the second direction forms a 90° or 180° angle.

30 32. The method of [claimitem](#) 30 or [claimitem](#) 31, wherein the method further comprises exposing the array to a third dNTP and a third concentration gradient of the polymerase, wherein the third concentration gradient of the polymerase varies along a third direction.

33. The method of [claimitem](#) 32, wherein the first direction, the second direction, and the third direction of concentration gradients are different.

34. The method of [claimitem](#) 32 or [claimitem](#) 33, wherein the method further comprises exposing the array to a fourth dNTP and a fourth concentration gradient of the polymerase, wherein the fourth concentration gradient of the polymerase varies along a fourth direction.

35. The method of [claimitem](#) 34, wherein the first direction, the second direction, the third direction, and the fourth direction of concentration gradients are different.

36. The method of any one of [claimitems](#) 30-35, wherein the one or more second nucleotides derived from the second dNTP, are added to the oligonucleotide after the one or more first nucleotides derived from the first dNTP are added to the oligonucleotide.

37. The method of any one of [claimitems](#) 32-36, wherein the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide after the one or more second nucleotides derived from the second dNTP are added to the oligonucleotide.

38. The method of any one of [claimitems](#) 34-37, wherein the one or more fourth nucleotides derived from the fourth dNTP are added to the oligonucleotide after the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide.

39. The method of any one of [claimitem](#) 34-38, wherein the fourth dNTP is deoxythymidine triphosphate (dTTP).

40. The method of any one of [claimitem](#) 28-39, wherein a poly(T) oligonucleotide is added to the oligonucleotide.

41. The method of any one of [claimitems](#) 28-40, wherein the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides are added by a polymerase.

42. The method of ~~claim~~item 41, wherein the polymerase is a terminal deoxynucleotidyl transferase.

5 43. The method of any one of ~~claim~~items 28-42, wherein the spatial barcode is formed by the one or more first nucleotides.

44. The method of any one of ~~claim~~items 30-42, wherein the spatial barcode is formed by the one or more first nucleotides, and the one or more second nucleotides.

10 45. The method of any one of ~~claim~~items 32-42, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides.

15 46. The method of any one of ~~claim~~items 34-42, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides.

20 47. The method of any one of ~~claim~~items 28-46, wherein the array comprises a plurality of features, wherein the features comprise one or more pluralities of immobilized surface probes.

25 48. The method of any one of ~~claim~~items 28-47, wherein exposing the array to a concentration gradient comprises an optically controlled reaction.

49. A method for generating a spatially barcoded array, the method comprising:

- a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe comprises an oligonucleotide;
- b) exposing the array to a concentration gradient of a first deoxyribonucleotide triphosphate (dNTP), wherein the concentration gradient of the first dNTP varies along a first direction;
- 30 c) exposing the array to a concentration gradient of a second dNTP, wherein the concentration gradient of the second dNTP varies along a second direction, wherein the first direction and the second direction are different; and

d) adding one or more first nucleotides derived from the first dNTP and one or more second nucleotides derived from the second dNTP to the oligonucleotide, wherein the percentage of the one or more first nucleotides and the percentage of the one or more second nucleotides correlate with concentration of the first dNTP and the second dNTP at the surface probe's location, thereby generating the spatially barcoded array.

50. The method of ~~claimitem~~ 49, wherein the method further comprises exposing the array to a concentration gradient of a third dNTP, wherein the concentration gradient of the third dNTP varies along a third direction, and wherein the third direction is different than the first direction and the second direction.

51. The method of ~~claimitem~~ 50, wherein the method further comprises exposing the array to a concentration gradient of a fourth dNTP, wherein the concentration gradient of the fourth dNTP, varies along a fourth direction, and wherein the fourth direction is different than the first direction, the second direction, and the third direction.

52. The method of ~~claimitem~~ 50 or ~~claimitem~~ 51, wherein one or more third nucleotides derived from the third dNTP, are added to the oligonucleotide in about the same time when one or more first nucleotides and the one or more second nucleotides are added to the oligonucleotides.

53. The method of any one of ~~claimitems~~ 50-52, wherein one or more fourth nucleotides derived from the fourth dNTP are added to the oligonucleotide in about the same time when the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides are added to the oligonucleotide.

54. The method of any one of ~~claimitems~~ 51-53, wherein the percentages of the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides correlate with the concentration gradient of the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP, thereby generating a spatially barcoded array.

55. The method of any one of ~~claim~~items 49-54, wherein a poly(T) oligonucleotide is added to the oligonucleotide after the one or more first nucleotides, the one or more second
5 nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides are added to the oligonucleotide.

56. The method of any one of ~~claim~~items 51-55, where the fourth dNTP is dTTP.

10 57. A method of generating an array, the method comprising:

- (a) providing a plurality of first oligonucleotides on a substrate, wherein a first oligonucleotide of the plurality comprises a first spatial barcode and is attached to the substrate;
- (b) delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode; and
- 15 (c) ligating the first oligonucleotide and the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.

58. A method of generating an array, the method comprising:

- 20 (a) providing a plurality of first oligonucleotides on a substrate, wherein a first oligonucleotide of the plurality is attached to the substrate and comprises a first spatial barcode;
- (b) delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode, a surface linker that binds to the substrate, and a cleavage domain;
- (c) attaching the second oligonucleotide to the substrate;
- 25 (d) ligating the first oligonucleotide and the second oligonucleotide; and
- (e) cleaving the cleavage domain in the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.

59. A method of generating an array, the method comprising:

- 30 (a) delivering a plurality of first oligonucleotides to a substrate, wherein a first oligonucleotide of the plurality comprises a first spatial barcode and a surface linker that binds to the substrate;

- (b) delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode; and
- (c) ligating the first oligonucleotide and the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.

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60. A method of generating an array, the method comprising:

- (a) delivering a plurality of first oligonucleotides to a substrate, wherein a first oligonucleotide comprises a spatial barcode and a surface linker that binds to the substrate;
- (b) delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide comprises a spatial barcode, a surface linker that binds to the substrate, and a cleavage domain;
- (c) ligating the first oligonucleotide and the second oligonucleotide; and
- (d) cleaving the cleavage domain in the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.

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61. The method of any one of [claim items](#) 57-60, wherein the surface linker of the first oligonucleotide is attached to the substrate prior to the delivery of the plurality of second oligonucleotide.

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62. The method of any one of [claim items](#) 57-61, wherein the first oligonucleotide is attached at a first distinct spatial position on the substrate.

63. The method of any one of [claim items](#) 57-62, wherein the second oligonucleotide is delivered to a distinct spatial position on the substrate.

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64. The method of any one of [claim items](#) 57-63, wherein the second oligonucleotide attaches to the substrate prior to ligating the first oligonucleotide and the second oligonucleotide.

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65. The method of any one of [claim items](#) 57-64, wherein the first oligonucleotide comprises an overhanging sequence.

66. The method of any one of [claimitem](#)s 57-65, wherein the first oligonucleotide comprises an overhanging sequence and the second oligonucleotide comprises an overhanging sequence, wherein the overhanging sequence of the first oligonucleotide and the overhanging sequence of the second oligonucleotide hybridize to each other.

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67. The method of any one of [claimitem](#)s 57-66, wherein the substrate is washed prior to ligating the first oligonucleotide and the second oligonucleotide.

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68. The method of any one of [claimitem](#)s 57-67, wherein members of the plurality of first oligonucleotides are attached to one or more distinct spatial positions.

69. The method of any one of [claimitem](#)s 57-68, wherein members of the plurality of first oligonucleotides comprise a first spatial barcode.

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70. The method of any one of [claimitem](#)s 57-69, wherein members of the plurality of second oligonucleotides are delivered to one or more distinct spatial positions.

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71. The method of any one of [claimitem](#)s 57-70, wherein members of the plurality of second oligonucleotides comprise a second spatial barcode.

72. The method of any one of [claimitem](#)s 57-71, wherein a capture probe at a distinct spatial position comprises a different first spatial barcode and a different second spatial barcode compared to another capture probe at a different distinct spatial position.

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73. The method of any one of [claimitem](#)s 69-72, wherein the first spatial barcode indicates the position of the first oligonucleotide along a first direction.

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74. The method of any one of [claimitem](#)s 71-73, wherein the second spatial barcode indicates the position of the second oligonucleotide along a second direction.

75. The method of [claimitem](#) 73 or 74, wherein the first direction intersects with the second direction at an angle greater than 0°.

76. The method of [claimitem](#) 75, wherein the angle is about 90°.

77. The method of any one of [claimitems](#) 57-76, wherein the second oligonucleotide comprises a poly-d(T) sequence.

78. The method of any one of [claimitems](#) 57-77, wherein the second oligonucleotide comprises a capture domain, wherein the capture domain is configured to hybridize to the poly(A) tail of an mRNA.

79. The method of any one of [claimitems](#) 57-78, wherein the first oligonucleotide and/or the second oligonucleotide further comprise a unique molecular identifier (UMI).

80. The method of any one of [claimitems](#) 57-79, wherein the first oligonucleotide and/or the second oligonucleotide further comprise a functional domain.

81. The method of any one of [claimitems](#) 57-80, wherein the first oligonucleotide and/or the second oligonucleotide further comprise a cleavage domain.

82. The method of any one of [claimitems](#) 57-81, wherein the method further comprises delivering a plurality of third oligonucleotides comprising a third spatial barcode to a distinct spatial position on the substrate.

83. The method of [claimitem](#) 82, wherein a third oligonucleotide of the plurality further comprises a surface linker.

84. The method of [claimitem](#) 83, wherein the surface linker of the third oligonucleotide attaches to the substrate at a distinct spatial position.

85. The method of any one of [claimitem](#)s 82-84, wherein the method further comprises delivering a plurality of fourth oligonucleotides comprising a fourth spatial barcode to a distinct spatial position on the substrate.

5 86. The method of [claimitem](#) 85, wherein a fourth oligonucleotide of the plurality further comprises a surface linker.

87. The method of [claimitem](#) 86, wherein the surface linker of the fourth oligonucleotide attaches to the substrate at a distinct spatial position.

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88. The method of any one of [claimitem](#)s 82-87, wherein the third oligonucleotide and the second oligonucleotide are ligated.

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89. The method of any one of [claimitem](#)s 85-88, wherein the fourth oligonucleotide and the third oligonucleotide are ligated.

90. A method of associating presence or abundance of an analyte with a location in a biological sample, comprising:

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(a) contacting the biological sample with an array produced by any one of [claimitem](#)s 1-89;

(b) releasing an analyte from the biological sample, wherein the analyte is bound by a capture probe at a distinct spatial position of the substrate;

(c) detecting the bound analyte; and

(d) correlating the analyte with a barcode of the capture probe at the distinct spatial position of the substrate,

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thus associating presence or abundance of an analyte with a location in the biological sample.

What is claimed is:

1. A method for generating a spatially barcoded array, the method comprising:
 - (a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe of the plurality of immobilized surface probes comprises an oligonucleotide;
 - (b) exposing the array to a concentration gradient of a first deoxyribonucleotide triphosphate (dNTP), wherein the concentration gradient of the first dNTP varies along a first direction; and
 - (c) adding one or more first nucleotides derived from the first dNTP to ~~at the~~ the 3' terminus of the oligonucleotide using a terminal transferase, wherein the number of the one or more first nucleotides that are added to the oligonucleotide correlates with the concentration of the first dNTP in the concentration gradient at the surface probe's location, thereby generating a spatially barcoded array.
2. The method of claim 1, wherein the plurality of immobilized surface probes have a common sequence.
3. The method of claim 1 or claim 2, wherein the method further comprises exposing the array to a concentration gradient of a second dNTP, wherein the concentration gradient of the second dNTP varies along a second direction.
4. The method of claim 3, wherein the first direction and the second direction forms a 90° or 180° angle.
5. The method of claim 3 or claim 4, wherein the method further comprises exposing the array to a concentration gradient of a third dNTP, wherein the concentration gradient of the third dNTP varies along a third direction.
6. The method of claim 5, wherein the first dNTP, the second dNTP, and the third dNTP, have different concentration gradients.

7. The method of claim 5 or claim 6, wherein the method further comprises exposing the array to a concentration gradient of a fourth dNTP, wherein the concentration gradient of the fourth dNTP varies along a fourth direction.
8. The method of claim 7, wherein the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP, have different concentration gradients.
9. The method of claim 7 or claim 8, wherein the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP, are different.
10. The method of any one of claims 7-9, wherein the fourth dNTP is dTTP.
11. The method of claim 10, wherein one or more dTTP form a capture domain that interacts with an analyte.
12. The method of any one of claims 3-11, wherein the one or more second nucleotides derived from the second dNTP, are added to the oligonucleotide after the one or more first nucleotides derived from the first dNTP are added to the oligonucleotide.
13. The method of any one of claim 5-12, wherein the one or more third nucleotides derived from the third dNTP, are added to the oligonucleotide after the one or more second nucleotides derived from the second dNTP are added to the oligonucleotide.
14. The method of any one of claims 7-13, wherein the one or more fourth nucleotides are added to the oligonucleotide after the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide.
15. The method of any one of claims 1-14, wherein a poly(T) oligonucleotide is added to the oligonucleotide after the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, or the one or more fourth nucleotides are added to the oligonucleotide.

16. The method of claim 15, wherein the poly(T) oligonucleotide is added by a ligase enzyme.

~~17. The methods of any one of claims 1-15, wherein the nucleotides are added by a polymerase.~~

~~18. The method of claim 17, wherein the polymerase is a terminal transferase.~~

~~19~~17. The method of any one of claims 1-16~~18~~, wherein the terminal transferase is a terminal deoxynucleotidyl transferase.

~~20~~18. The method of any one of claims 1-~~18~~16, wherein the spatial barcode is formed by the one or more first nucleotides.

~~21~~19. The method of any one of claims 3-~~18~~16, wherein the spatial barcode is formed by the one or more first nucleotides and the one or more second nucleotides.

~~22~~20. The method of any one of claims 5-~~18~~16, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides.

~~23~~21. The method of any one of claims 7-~~18~~16, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides.

~~24~~22. The method of any one of claims 1-~~22~~20, wherein the oligonucleotide further comprises a cleavage domain, a functional domain, and a unique molecular identifier, or combinations thereof.

~~25~~23. The method of any one of claims 1-~~23~~21, wherein the array has one or more microfluidic channels.

~~26~~24. The method of any one of claims 1-~~25~~23, wherein exposing the array to a concentration gradient comprises an optically controlled reaction.

~~27~~25. The method of any one of claims 1-~~26~~24, wherein the array comprises a plurality of features, wherein the features comprise one or more pluralities of the immobilized surface probes.

~~28~~26. A method for generating a spatially barcoded array, the method comprising:

- (a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe of the plurality of immobilized surface probes comprises an oligonucleotide;
- (b) exposing the array to a first dNTP and a first concentration gradient of a polymerase, wherein the first concentration gradient of the polymerase varies along a first direction, and wherein the polymerase is a terminal transferase; and
- (c) adding one or more first nucleotides derived from the first dNTP to the 3' terminus of the oligonucleotide, wherein the number of the one or more first nucleotides that are added to the oligonucleotide correlates with the concentration of the polymerase in the first concentration gradient at the surface probe's location, thereby generating a spatially barcoded array.

~~29~~27. The method of claim ~~28~~26, wherein the oligonucleotide comprises a functional domain, a cleavage domain, a unique molecular identifier, or combinations thereof.

~~30~~28. The method of claim ~~28~~26 or claim ~~29~~27, wherein the method further comprises exposing the array to a second dNTP and a second concentration gradient of the polymerase, wherein the second concentration gradient of the polymerase varies along a second direction.

~~31~~29. The method of claim ~~30~~28, wherein the first direction and the second direction forms a 90° or 180° angle.

3230. The method of claim 3028 or claim 3129, wherein the method further comprises exposing the array to a third dNTP and a third concentration gradient of the polymerase, wherein the third concentration gradient of the polymerase varies along a third direction.

3331. The method of claim 3230, wherein the first direction, the second direction, and the third direction of concentration gradients are different.

3432. The method of claim 3230 or claim 3331, wherein the method further comprises exposing the array to a fourth dNTP and a fourth concentration gradient of the polymerase, wherein the fourth concentration gradient of the polymerase varies along a fourth direction.

3533. The method of claim 3432, wherein the first direction, the second direction, the third direction, and the fourth direction of concentration gradients are different.

3634. The method of any one of claims 2830-3533, wherein the one or more second nucleotides derived from the second dNTP, are added to the oligonucleotide after the one or more first nucleotides derived from the first dNTP are added to the oligonucleotide.

3735. The method of any one of claims 3032-3634, wherein the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide after the one or more second nucleotides derived from the second dNTP are added to the oligonucleotide.

3836. The method of any one of claims 3234-3735, wherein the one or more fourth nucleotides derived from the fourth dNTP are added to the oligonucleotide after the one or more third nucleotides derived from the third dNTP are added to the oligonucleotide.

3937. The method of any one of claim 3234-3836, wherein the fourth dNTP is deoxythymidine triphosphate (dTTP).

4038. The method of any one of claim 2628-3937, wherein a poly(T) oligonucleotide is added to the oligonucleotide after the one or more first nucleotides, the one or more second

nucleotides, the one or more third nucleotides, or the one or more fourth nucleotides are added to the oligonucleotide.

~~41. The method of any one of claims 28-40, wherein the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides are added by a polymerase.~~

~~4239.~~ The method of any one of claims 26-3841, wherein the terminal transferasepolymerase is a terminal deoxynucleotidyl transferase.

~~4340.~~ The method of any one of claims 2628-3942, wherein the spatial barcode is formed by the one or more first nucleotides.

~~4441.~~ The method of any one of claims 2830-3942, wherein the spatial barcode is formed by the one or more first nucleotides, and the one or more second nucleotides.

~~4542.~~ The method of any one of claims 3032-3942, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides.

~~4643.~~ The method of any one of claims 3234-3942, wherein the spatial barcode is formed by the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides.

~~4744.~~ The method of any one of claims 2628-4346, wherein the array comprises a plurality of features, wherein the features comprise one or more pluralities of immobilized surface probes.

~~4845.~~ The method of any one of claims 2628-4744, wherein exposing the array to a concentration gradient comprises an optically controlled reaction.

4946. A method for generating a spatially barcoded array, the method comprising:

- a) providing an array comprising a plurality of immobilized surface probes, wherein an immobilized surface probe comprises an oligonucleotide;
- b) exposing the array to a concentration gradient of a first deoxyribonucleotide triphosphate (dNTP), wherein the concentration gradient of the first dNTP varies along a first direction;
- c) exposing the array to a concentration gradient of a second dNTP, wherein the concentration gradient of the second dNTP varies along a second direction, wherein the first direction and the second direction are different; and
- d) adding one or more first nucleotides derived from the first dNTP and one or more second nucleotides derived from the second dNTP to the 3' terminus of the oligonucleotide using a terminal transferase, wherein the percentage of the one or more first nucleotides and the percentage of the one or more second nucleotides correlate with concentration of the first dNTP and the second dNTP at the surface probe's location, thereby generating the spatially barcoded array.

5047. The method of claim **4649**, wherein the method further comprises exposing the array to a concentration gradient of a third dNTP, wherein the concentration gradient of the third dNTP varies along a third direction, and wherein the third direction is different than the first direction and the second direction.

5148. The method of claim **5047**, wherein the method further comprises exposing the array to a concentration gradient of a fourth dNTP, wherein the concentration gradient of the fourth dNTP, varies along a fourth direction, and wherein the fourth direction is different than the first direction, the second direction, and the third direction.

5249. The method of claim **5047** or claim **5148**, wherein one or more third nucleotides derived from the third dNTP, are added to the oligonucleotide in about the same time when one or more first nucleotides and the one or more second nucleotides are added to the oligonucleotides.

~~5350~~. The method of any one of claims ~~4750-4952~~, wherein one or more fourth nucleotides derived from the fourth dNTP are added to the oligonucleotide in about the same time when the one or more first nucleotides, the one or more second nucleotides, and the one or more third nucleotides are added to the oligonucleotide.

~~5451~~. The method of any one of claims ~~4851-5053~~, wherein the percentages of the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, and the one or more fourth nucleotides correlate with the concentration gradient of the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP, thereby generating a spatially barcoded array.

~~5552~~. The method of any one of claims ~~4649-5154~~, wherein a poly(T) oligonucleotide is added to the oligonucleotide after the one or more first nucleotides, the one or more second nucleotides, the one or more third nucleotides, ~~or~~ and the one or more fourth nucleotides are added to the oligonucleotide.

~~5653~~. The method of any one of claims ~~4851-5255~~, where the fourth dNTP is dTTP.

~~57. A method of generating an array, the method comprising:~~

- ~~(a) providing a plurality of first oligonucleotides on a substrate, wherein a first oligonucleotide of the plurality comprises a first spatial barcode and is attached to the substrate;~~
- ~~(b) delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode; and~~
- ~~(c) ligating the first oligonucleotide and the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.~~

~~58. A method of generating an array, the method comprising:~~

- ~~(a) providing a plurality of first oligonucleotides on a substrate, wherein a first oligonucleotide of the plurality is attached to the substrate and comprises a first spatial barcode;~~

- (b) ~~delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode, a surface linker that binds to the substrate, and a cleavage domain;~~
- (c) ~~attaching the second oligonucleotide to the substrate;~~
- (d) ~~ligating the first oligonucleotide and the second oligonucleotide; and~~
- (e) ~~cleaving the cleavage domain in the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.~~

59. A method of generating an array, the method comprising:

- (a) ~~delivering a plurality of first oligonucleotides to a substrate, wherein a first oligonucleotide of the plurality comprises a first spatial barcode and a surface linker that binds to the substrate;~~
- (b) ~~delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide of the plurality comprises a second spatial barcode; and~~
- (c) ~~ligating the first oligonucleotide and the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.~~

60. A method of generating an array, the method comprising:

- (a) ~~delivering a plurality of first oligonucleotides to a substrate, wherein a first oligonucleotide comprises a spatial barcode and a surface linker that binds to the substrate;~~
- (b) ~~delivering a plurality of second oligonucleotides to the substrate, wherein a second oligonucleotide comprises a spatial barcode, a surface linker that binds to the substrate, and a cleavage domain;~~
- (c) ~~ligating the first oligonucleotide and the second oligonucleotide; and~~
- (d) ~~cleaving the cleavage domain in the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.~~

61. The method of any one of claims 57-60, wherein the surface linker of the first oligonucleotide is attached to the substrate prior to the delivery of the plurality of second oligonucleotide.

- ~~62. The method of any one of claims 57-61, wherein the first oligonucleotide is attached at a first distinct spatial position on the substrate.~~
- ~~63. The method of any one of claims 57-62, wherein the second oligonucleotide is delivered to a distinct spatial position on the substrate.~~
- ~~64. The method of any one of claims 57-63, wherein the second oligonucleotide attaches to the substrate prior to ligating the first oligonucleotide and the second oligonucleotide.~~
- ~~65. The method of any one of claims 57-64, wherein the first oligonucleotide comprises an overhanging sequence.~~
- ~~66. The method of any one of claims 57-65, wherein the first oligonucleotide comprises an overhanging sequence and the second oligonucleotide comprises an overhanging sequence, wherein the overhanging sequence of the first oligonucleotide and the overhanging sequence of the second oligonucleotide hybridize to each other.~~
- ~~67. The method of any one of claims 57-66, wherein the substrate is washed prior to ligating the first oligonucleotide and the second oligonucleotide.~~
- ~~68. The method of any one of claims 57-67, wherein members of the plurality of first oligonucleotides are attached to one or more distinct spatial positions.~~
- ~~69. The method of any one of claims 57-68, wherein members of the plurality of first oligonucleotides comprise a first spatial barcode.~~
- ~~70. The method of any one of claims 57-69, wherein members of the plurality of second oligonucleotides are delivered to one or more distinct spatial positions.~~
- ~~71. The method of any one of claims 57-70, wherein members of the plurality of second oligonucleotides comprise a second spatial barcode.~~

- ~~72. The method of any one of claims 57-71, wherein a capture probe at a distinct spatial position comprises a different first spatial barcode and a different second spatial barcode compared to another capture probe at a different distinct spatial position.~~
- ~~73. The method of any one of claims 69-72, wherein the first spatial barcode indicates the position of the first oligonucleotide along a first direction.~~
- ~~74. The method of any one of claims 71-73, wherein the second spatial barcode indicates the position of the second oligonucleotide along a second direction.~~
- ~~75. The method of claim 73 or 74, wherein the first direction intersects with the second direction at an angle greater than 0°.~~
- ~~76. The method of claim 75, wherein the angle is about 90°.~~
- ~~77. The method of any one of claims 57-76, wherein the second oligonucleotide comprises a poly-d(T) sequence.~~
- ~~78. The method of any one of claims 57-77, wherein the second oligonucleotide comprises a capture domain, wherein the capture domain is configured to hybridize to the poly(A) tail of an mRNA.~~
- ~~79. The method of any one of claims 57-78, wherein the first oligonucleotide and/or the second oligonucleotide further comprise a unique molecular identifier (UMI).~~
- ~~80. The method of any one of claims 57-79, wherein the first oligonucleotide and/or the second oligonucleotide further comprise a functional domain.~~
- ~~81. The method of any one of claims 57-80, wherein the first oligonucleotide and/or the second oligonucleotide further comprise a cleavage domain.~~

- ~~82. The method of any one of claims 57-81, wherein the method further comprises delivering a plurality of third oligonucleotides comprising a third spatial barcode to a distinct spatial position on the substrate.~~
- ~~83. The method of claim 82, wherein a third oligonucleotide of the plurality further comprises a surface linker.~~
- ~~84. The method of claim 83, wherein the surface linker of the third oligonucleotide attaches to the substrate at a distinct spatial position.~~
- ~~85. The method of any one of claims 82-84, wherein the method further comprises delivering a plurality of fourth oligonucleotides comprising a fourth spatial barcode to a distinct spatial position on the substrate.~~
- ~~86. The method of claim 85, wherein a fourth oligonucleotide of the plurality further comprises a surface linker.~~
- ~~87. The method of claim 86, wherein the surface linker of the fourth oligonucleotide attaches to the substrate at a distinct spatial position.~~
- ~~88. The method of any one of claims 82-87, wherein the third oligonucleotide and the second oligonucleotide are ligated.~~
- ~~89. The method of any one of claims 85-88, wherein the fourth oligonucleotide and the third oligonucleotide are ligated.~~
- ~~90. A method of associating presence or abundance of an analyte with a location in a biological sample, comprising:~~
~~(a) contacting the biological sample with an array produced by any one of claims 1-89;~~

- (b) releasing an analyte from the biological sample, wherein the analyte is bound by a capture probe at a distinct spatial position of the substrate;
 - (c) detecting the bound analyte; and
 - (d) correlating the analyte with a barcode of the capture probe at the distinct spatial position of the substrate,
- thus associating presence or abundance of an analyte with a location in the biological sample.

91. A method for transferring an analyte from a biological sample to a substrate, the method comprising:

- providing a substrate comprising 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 is capable of binding specifically to the analyte;
- contacting the biological sample with the substrate;
- applying an electric field to the biological sample and the substrate, thereby transferring the analyte from the biological sample to the substrate.

92. The method of claim 91, wherein the analyte is negatively charged.

93. The method of claim 91 or 92, wherein the analyte comprises DNA or RNA.

94. The method of claim 91 or 92, wherein the analyte comprises a protein.

95. The method of any one of claims 91-94, wherein the electric field is applied by an anode and a cathode.

96. The method of any one of claims 91-95, wherein the electric field is applied using a spatially addressable microelectrode array.

97. A method for capturing an analyte from a biological sample, the method comprising:

providing a substrate, wherein the substrate comprises a plurality of capture probes and wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;

contacting the biological sample with the substrate; and

providing a diffusion-resistant medium to the biological sample, wherein the diffusion-resistant medium reduces diffusion of analytes away from the plurality of capture probes and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from the biological sample.

98. The method of claim 97, wherein the diffusion-resistant medium is a hydrogel.

99. The method of claim 98, wherein the hydrogel comprises one or more permeabilization reagent(s).

100. The method of claim 99, wherein the contacting step results in permeabilization of the biological sample.

101. The method of claim 99 or 100, wherein the substrate comprises a well that is filled with the hydrogel.

102. The method of claim 97, wherein the diffusion-resistant medium is a glass slide.

103. The method of claim 97, wherein the diffusion-resistant medium is a porous membrane.

104. The method of claim 98 or claim 103, wherein a permeabilization reagent is flowed through a microfluidic chamber or channel over the diffusion-resistant medium.

105. The method of any one of claims 97-104, wherein a solution separates the biological sample and the diffusion-resistant medium.

106. — A method for capturing an analyte from a biological sample, the method comprising:

~~providing a substrate comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;~~
~~contacting the biological sample with the substrate; and~~
~~providing a diffusion-resistant medium comprising one or more permeabilization reagent(s) to the biological sample, wherein the one or more permeabilization reagent(s) promotes migration of the analyte present in the biological sample towards the plurality of capture probes, and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from the biological sample.~~

~~107. A method for capturing an analyte from a biological sample, the method comprising:~~

~~providing a diffusion-resistant medium, wherein the diffusion-resistant medium comprises a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain, and wherein the diffusion-resistant medium comprises one or more permeabilization reagent(s); and~~

~~contacting the diffusion-resistant medium to the biological sample, wherein the one or more permeabilization reagent(s) releases the analyte from the biological sample, and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from the biological sample.~~

~~108. The method of claim 107, wherein the diffusion-resistant medium is a hydrogel.~~

~~109. The method of claim 108, wherein the hydrogel is attached to a substrate.~~

~~110. The method of claim 109, wherein the substrate is a slide.~~

~~111. The method of claim 110, wherein the slide is a glass slide.~~

~~112. The method of claim 111, wherein the glass slide is an acrylated glass slide.~~

~~113. The method of claim 107 or 108, wherein the biological sample is provided on a substrate.~~

114. A method for capturing an analyte from a biological sample, the method comprising:
providing a first substrate, comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;
providing the biological sample on a second substrate; and
sandwiching a diffusion-resistant medium between the first substrate and the biological sample, wherein the diffusion-resistant medium comprises one or more permeabilization reagent(s) that releases the analyte from the biological sample, and allowing the capture domain to specifically bind to the analyte from the biological sample, thereby capturing the analyte from the biological sample.

115. The method of claim 114, wherein the diffusion-resistant medium is a hydrogel comprising one or more permeabilization reagent(s).

116. The method of claim 114, wherein the diffusion-resistant medium is a porous membrane comprising one or more permeabilization reagent(s).

117. A method for determining a location of an analyte in a biological sample, the method comprising:

- (a) permeabilizing the biological sample on a substrate;
- (b) contacting the biological sample with 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;
- (c) enzymatically synthesizing a molecule comprising (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 without removing the biological sample from the substrate; and
- (d) determining (i) all or a portion of the sequence corresponding to the spatial barcode or the complement thereof, and (ii) all or a portion of the sequence corresponding to the analyte or the complement thereof, and using the determined sequences of (i) and (ii) to determine the location of the analyte in the biological sample.

118. The method of claim 117, wherein the method further comprises, before step (a), staining and/or imaging the biological sample on the substrate.

119. A method for determining a location of an analyte in a biological sample, the method comprising:

- (a) fixing a fresh frozen biological sample with acetone;
- (b) contacting the biological sample with 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
- (c) 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.

120. The method of claim 119, wherein the biological sample is not subjected to permeabilization reagents.

121. The method of claim 119 or claim 120, wherein the method further comprises, between steps (a) and (b), a step of staining and/or imaging the biological sample.

122. The method of any one of claims 117–121, further comprising selecting a region of interest in the biological sample.

123. The method of any one of claims 117–122, wherein the substrate comprises an array of features, wherein a feature comprises the plurality of capture probes.

124. The method of claim 123, wherein the array comprises at least 4,000 features.

125. The method of claim 123 or 124, wherein the array comprises approximately 5,000 features.

~~126. The method of any one of claims 123-125, wherein the features of the array are between approximately 50 micrometers and approximately 80 micrometers in diameter.~~

~~127. The method of claim 126, wherein the features of the array are between approximately 60 micrometers and 70 micrometers in diameter.~~

~~128. The method of any one of claims 123-127, wherein the features of the array are patterned.~~

~~129. The method of any one of claims 123-128, wherein the center of one feature of the array is between 80 micrometers and 120 micrometers from the center of another feature of the array.~~

~~130. The method of any one of claims 123-129, wherein the array is approximately 8 mm by 8 mm.~~

~~131. The method of any one of claims 123-130, wherein the array is smaller than 8 mm by 8 mm.~~

~~132. The method of any one of claims 123-131, wherein the array is surrounded by fiducial markers.~~

~~133. The method of claim 132, wherein the fiducial markers allow for detection of mirroring.~~

~~134. The method of any one of claims 117-133, wherein the capture probe comprises a unique molecular identifier (UMI).~~

~~135. The method of any one of claims 117-134, wherein the capture probe comprises a cleavage domain.~~

~~136. The method of claim 135, wherein the cleavage domain comprises a sequence recognized and cleaved by a uracil-DNA glycosylase, an apurinic/apyrimidinic (AP) endonuclease (APE1), a uracil-specific excision reagent (USER), and/or an endonuclease VIII.~~

~~137. The method of claim 136, wherein the cleavage domain comprises a photolabile bond, a thermosensitive bond, or a chemical sensitive bond.~~

~~138. The method of any one of claims 117-134, wherein the capture probe is not cleaved from the array.~~

~~139. The method of any one of claims 117-138, wherein the analyte comprises DNA or RNA.~~

~~140. The method of claim 139, wherein analyte comprises RNA.~~

~~141. The method of claim 140, wherein a cDNA molecule is generated from the RNA specifically bound to the capture domain, by extending the capture probe enzymatically using the RNA specifically bound to the capture domain as a template.~~

~~142. The method of any one of claims 117-141, wherein the analyte comprises a protein.~~

~~143. The method of claim 118 or 121, wherein staining the biological sample comprises hematoxylin and eosin staining.~~

~~144. The method of claim 118 or 121, wherein staining the biological sample comprises Periodic Acid Schiff staining.~~

~~145. The method of any one of claims 117-144, further comprising applying to the biological sample probes that selectively hybridize to ribosomal RNA.~~

~~146. The method of any one of claims 117-125, further comprising treating the biological sample with a duplex-specific nuclease.~~