

examples, the plurality of immobilized surface probes have a common sequence. In some examples, 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. 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 concentration gradient of a third dNTP, wherein the concentration gradient of the third dNTP varies along a third direction. In some examples, the first dNTP, the second dNTP, and the third dNTP have different concentration gradients. In some examples, 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. In some examples, the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP have different concentration gradients. In some examples, the first dNTP, the second dNTP, the third dNTP, and the fourth dNTP are different. In some examples, the fourth dNTP is dTTP. In some examples, one or more dTTP form a capture domain that interacts with an analyte. 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 more second nucleotides derived from the second dNTP are added to the oligonucleotide. In some examples, 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. In some examples, a poly(T) oligonucleotide is added to the oligonucleotide. In some examples, the poly(T) oligonucleotide is added by a ligase enzyme. In some examples, the nucleotides are added by a polymerase. In some examples, the polymerase is a terminal transferase. In some examples, the terminal transferase is 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 oligonucleotide

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
10 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,
15 thereby generating a spatially barcoded array.

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 of the plurality of immobilized surface probes comprises an oligonucleotide; (b) exposing the array to a first dNTP
20 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 oligonucleotide correlates with the concentration of the polymerase in the first concentration gradient at the surface probe's location, thereby generating
25 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
30 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
35 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
40 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

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 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 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 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 first
25 oligonucleotide and the second oligonucleotide, thereby generating a capture probe comprising the first spatial barcode and the second spatial barcode.

In another 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 spatial
30 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 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 substrate,
10 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 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
15 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
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

of first oligonucleotides are attached to one or more distinct spatial positions. In some examples, members of the plurality of first oligonucleotides comprise a first spatial barcode. In some examples, members of the plurality of second oligonucleotides are delivered to one or more distinct spatial positions. In some examples, members of the plurality of second oligonucleotides
5 comprise a second spatial barcode. In some examples, 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. In some examples, the first spatial barcode indicates the position of the first oligonucleotide along a first direction. In some examples, the second spatial barcode indicates the position of the second oligonucleotide
10 along a second direction. In some examples, the first direction intersects with the second direction at an angle greater than 0° . In some examples, the angle is about 90° . In some examples, the second oligonucleotide comprises a poly-d(T) sequence. In some examples, the second oligonucleotide comprises a capture domain, wherein the capture domain is configured to hybridize to the poly(A) tail of an mRNA. In some examples, the first oligonucleotide and/or the
15 second oligonucleotide further comprise a unique molecular identifier (UMI). In some examples, the first oligonucleotide and/or the second oligonucleotide further comprise a functional domain. In some examples, the first oligonucleotide and/or the second oligonucleotide further comprise a cleavage domain. In some examples, the method further comprises delivering a plurality of third oligonucleotides comprising a third spatial barcode to a distinct spatial position on the substrate.
20 In some examples, a third oligonucleotide of the plurality further comprises a surface linker. In some examples, the surface linker of the third oligonucleotide attaches to the substrate at a distinct spatial position. In some examples, the method further comprises delivering a plurality of fourth oligonucleotides comprising a fourth spatial barcode to a distinct spatial position on the substrate. In some examples, a fourth oligonucleotide of the plurality further comprises a surface
25 linker. In some examples, the surface linker of the fourth oligonucleotide attaches to the substrate at a distinct spatial position. In some examples, the third oligonucleotide and the second oligonucleotide are ligated. In some examples, the fourth oligonucleotide and the third oligonucleotide are ligated.

In some examples are methods of associating presence or abundance of an analyte with a
30 location in a biological sample, comprising: (a) contacting the biological sample with a

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 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 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 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 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 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 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 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 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.

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

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

DESCRIPTION OF DRAWINGS

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

FIG. 1 shows an exemplary spatial analysis workflow.

FIG. 2 shows an exemplary spatial analysis workflow.