

Application No. 62/819,458, filed March 15, 2019, U.S. Provisional Patent Application No. 62/839,223, filed April 26, 2019, U.S. Provisional Patent Application No. 62/839,320, filed April 26, 2019, U.S. Provisional Patent Application No. 62/839,346, filed April 26, 2019, U.S. Provisional Patent Application No. 62/842,463, filed May 2, 2019, U.S. Provisional Patent Application No. 62/860,993, filed June 13, 2019, U.S. Provisional Patent Application No. 62/839,526, filed April 26, 2019 and U.S. Provisional Patent Application No. 62/858,331, filed on June 7, 2019. The contents of each of these applications are incorporated herein by reference in their entireties.

BACKGROUND

Cells within a tissue of a subject have differences in cell morphology and/or function due to varied analyte levels (e.g., gene and/or protein expression) within the different cells. The specific position of a cell within a tissue (e.g., the cell's position relative to neighboring cells or the cell's position relative to the tissue microenvironment) can affect, e.g., the cell's morphology, differentiation, fate, viability, proliferation, behavior, and signaling and cross-talk with other cells in the tissue.

Spatial heterogeneity has been previously studied using techniques that only provide data for a small handful of analytes in the contact of an intact tissue or a portion of a tissue, or provide a lot of analyte data for single cells, but fail to provide information regarding the position of the single cell in a parent biological sample (e.g., tissue sample).

SUMMARY

In one example, provided herein are methods for generating an array, the methods include: providing a first array that includes a plurality of probes on a first substrate, where a probe of the plurality includes a spatial barcode; providing a second array that includes a plurality of primers on a second substrate; generating an oligonucleotide complementary to a probe on the first array; allowing the oligonucleotide to hybridize with a primer on the second array; and extending the primer using the oligonucleotide as a template, thereby generating a probe on the second array. In one aspect, the present disclosure refers to a method for generating an array, the method comprising: providing a first array comprising a plurality of probes immobilized on a first substrate, wherein a probe of the plurality comprises a spatial barcode; providing a second array comprising a plurality of primers immobilized on a second substrate; generating an intermediate oligonucleotide complementary to a probe on the first array; releasing the intermediate oligonucleotide from the probe on the first array; allowing the intermediate oligonucleotide to hybridize with a primer on the second array; and extending the primer using the intermediate oligonucleotide as a template, thereby generating a probe on the second array.

In some examples of any of the methods for generating an array provided herein, the primer on the second array includes a sequence that is identical to a portion of the sequence of the probe on the first array. In some examples, the portion of the sequence is in a functional domain of

methods for generating an array provided herein, the second substrate includes a plurality of second features, and the primer on the second array is attached to a feature of the plurality of second features. In some examples, the plurality of first features and/or the plurality of second features are beads, wells, spots, or hydrogel pads.

5 In another example~~aspect~~, the present disclosure refers to a method of generating an array, the method comprising: providing a first array comprising a plurality of probes on a first substrate, wherein a probe of the plurality comprises a spatial barcode; providing a primer comprising a sequence complementary to a portion of a probe on the first array; allowing the primer to hybridize with the probe; extending the primer using the probe as a template to
10 generate an oligonucleotide; aligning the first substrate and a second substrate; and attaching the oligonucleotide to the second substrate.

In another example, provided herein are methods of generating an array, the methods include: providing a first array that includes a plurality of probes on a first substrate, where a probe of the plurality includes a spatial barcode; providing a primer that includes a sequence complementary to a portion of a
15 probe on the first array; allowing the primer to hybridize with the probe; extending the primer using the probe as a template to generate an oligonucleotide; aligning the first substrate and a second substrate; and attaching the oligonucleotide to the second substrate. In some examples, the primer includes a linker, and attaching the oligonucleotide to the second substrate includes attaching through the linker. In some
20 examples, the second substrate includes a plurality of features, and attaching the oligonucleotide to the second substrate includes attaching to a feature of the plurality. the plurality of features are beads, wells, spots, or hydrogel pads. In some examples, the oligonucleotide is attached directly to the second substrate. In some examples of any of the methods for generating an array provided herein, the methods further include de-hybridizing the probe on the first array from the oligonucleotide after attaching the
25 oligonucleotide to the second substrate. In some examples of any of the methods for generating an array provided herein, a probe of the plurality on the first array further includes a sequence selected from the group consisting of: a capture domain, a cleavage domain, and a UMI.

30 In another example~~aspect~~, the present disclosure refers to a method of generating an array, the method comprising: providing a first array comprising a plurality of probes on a first substrate, wherein a probe of the plurality comprises a spatial barcode; providing a second array comprising a plurality of primers on a second substrate, wherein a primer of the plurality comprises a sequence complementary to a portion of a probe on the first array; aligning the first array and the second array, thereby allowing the primer to hybridize with the probe; extending the primer using the probe as a template to generate an oligonucleotide; and de-hybridizing the oligonucleotide from the probe, thereby generating an array.

35 In another example, provided herein are methods of generating an array, the methods include: providing a first array that includes a plurality of probes on a first substrate, where a probe of the plurality includes a spatial barcode; providing a second array that includes a plurality of primers on a second substrate, where a primer of the plurality includes a sequence complementary to a portion of a probe on the first array; aligning the first array and the second
40 array, thereby allowing the primer to hybridize with the probe; extending the primer using the probe as a template to generate an oligonucleotide; and de-hybridizing the oligonucleotide from the probe, thereby generating an array. In some examples, the primer includes a linker, and the primer is attached to the second substrate through the linker. In some examples, the primer is attached directly to the second substrate. In some examples of any of the methods
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WHAT IS CLAIMED IS:

1. A method for generating an array, the method comprising:
 - providing a first array comprising a plurality of probes immobilized on a first substrate, wherein a probe of the plurality comprises a spatial barcode;
 - providing a second array comprising a plurality of primers immobilized on a second substrate;
 - generating an intermediate oligonucleotide complementary to a probe on the first array;
 - releasing the intermediate oligonucleotide from the probe on the first array;
 - allowing the intermediate oligonucleotide to hybridize with a primer on the second array; and
 - extending the primer using the intermediate oligonucleotide as a template, thereby generating a probe on the second array.
2. The method of claim 1, wherein the primer on the second array comprises a sequence that is identical to a portion of the sequence of the probe on the first array.
3. The method of claim 2, wherein the portion of the sequence is in a functional domain of the probe.
4. The method of claim 2, wherein the probe has a free 5' end and the portion of the sequence comprises a sequence at the ~~free~~-5' end of the probe.
5. The method of any one of the preceding claims, wherein the plurality of primers on the second array comprise a common sequence.
6. The method of any one of the preceding claims, wherein the probe on the first array is attached to the first substrate via its 3' end.

7. The method of any one of the preceding claims, wherein the spatial barcode comprises 6 to 25 nucleotides.
8. The method of any one of the preceding claims, wherein the spatial barcode is unique to a particular region on the first array.
9. The method of claim 8, wherein probes associated with a particular region on the first array comprise a common spatial barcode.
10. The method of any one of the preceding claims, wherein the plurality of probes on the first array comprise a common functional domain.
- ~~11. The method of any one of the preceding claims, further comprising, following generating the oligonucleotide, releasing the oligonucleotide from the probe on the first array.~~
- ~~12.~~11. The method of ~~any one of the preceding claims~~claim 11, wherein releasing the intermediate oligonucleotide comprises de-hybridizing the intermediate oligonucleotide from the probe using heat.
- ~~13.~~12. The method of ~~any one of the preceding claims~~claim 11 or 12, further comprising, following releasing the intermediate oligonucleotide from the probe, facilitating migration of the released intermediate oligonucleotide to the second array.
- ~~14.~~13. The method of claim ~~12~~13, wherein facilitating migration of the released intermediate oligonucleotide comprises applying an electric field to the first and second arrays, wherein the electric field increases diffusion of the released intermediate oligonucleotide.
- ~~15.~~14. The method of any one of the preceding claims, wherein the density of the plurality of probes on the first array is about equal to or higher than the density of the plurality of primers on the second array.

~~16.15.~~ The method of any one of the preceding claims, wherein the number of the plurality of probes on the first array is about equal to or higher than the number of the plurality of primers on the second array.

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~~17.16.~~ The method of any one of the preceding claims, further comprising releasing the intermediate oligonucleotide from the probe generated on the second array.

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~~18.17.~~ The method of claim ~~16~~~~17~~, wherein releasing the intermediate oligonucleotide comprises de-hybridizing the intermediate oligonucleotide from the probe using heat.

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~~19.18.~~ The method of any one of the preceding claims, wherein the first substrate comprises a plurality of first features, and wherein the probe of the plurality is attached to a feature of the plurality of the first features.

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~~20.19.~~ The method of claim ~~18~~~~19~~, wherein the spatial barcode is unique to the feature.

~~21.20.~~ The method of any one of the preceding claims, wherein the second substrate comprises a plurality of second features, and wherein the primer on the second array is attached to a feature of the plurality of second features.

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~~22.21.~~ The method of any of claims ~~18~~~~19~~~~21~~~~20~~, wherein the plurality of first features and/or the plurality of second features are beads, wells, spots, or hydrogel pads.

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~~23. A method of generating an array, the method comprising:~~
~~providing a first array comprising a plurality of probes on a first substrate,~~
~~wherein a probe of the plurality comprises a spatial barcode;~~
~~providing a primer comprising a sequence complementary to a portion of a probe~~
~~on the first array;~~
~~allowing the primer to hybridize with the probe;~~
~~extending the primer using the probe as a template to generate an oligonucleotide;~~

aligning the first substrate and a second substrate; and
attaching the oligonucleotide to the second substrate.

24. The method of claim 23, wherein the primer comprises a linker, and wherein attaching
the oligonucleotide to the second substrate comprises attaching through the linker.

25. The method of claim 23 or 24, wherein the second substrate comprises a plurality of
features, and wherein attaching the oligonucleotide to the second substrate comprises
attaching to a feature of the plurality.

26. The method of claim 25, where the plurality of features are beads, wells, spots, or
hydrogel pads.

27. The method of claim 23, wherein the oligonucleotide is attached directly to the second
substrate.

28. The method of any one of the preceding claims, further comprising de-hybridizing the
probe on the first array from the oligonucleotide after attaching the oligonucleotide to the
second substrate.

29. The method of any one of the preceding claims, wherein a probe of the plurality on the
first array further comprises a sequence selected from the group consisting of: a capture
domain, a cleavage domain, and a UMI.

30. A method of generating an array, the method comprising:
providing a first array comprising a plurality of probes on a first substrate,
wherein a probe of the plurality comprises a spatial barcode;
providing a second array comprising a plurality of primers on a second substrate,
wherein a primer of the plurality comprises a sequence complementary to a portion of a
probe on the first array;

aligning the first array and the second array, thereby allowing the primer to
hybridize with the probe;
extending the primer using the probe as a template to generate an oligonucleotide;
and
de-hybridizing the oligonucleotide from the probe, thereby generating an array.

31. The method of claim 30, wherein the primer comprises a linker, and wherein the primer
is attached to the second substrate through the linker.

32. The method of claim 30, wherein the primer is attached directly to the second substrate.

33. The method of any one of claims 30-32, wherein the first substrate comprises a plurality
of first features, and wherein the probe of the plurality is attached to a feature of the
plurality of the first features.

34. The method of any one of claims 30-33, wherein the second substrate comprises a
plurality of second features, and wherein the primer of the plurality is attached to a
feature of the plurality of the second features.

35. The method of any one of claims 30-34, wherein de-hybridizing the oligonucleotide from
the probe comprises applying heat.