support a sample (e.g., a biological substrate) on its surface. In some examples, the second substrate can include a plurality of barcoded probes and/or permeabilization reagents.

The devices for holding or supporting substrates described further include an alignment mechanism that is connected to at least one of the members and aligns the first and second members. Thus, the devices of the disclosure can advantageously align the first substrate and the second substrate and any samples, barcoded probes, or permeabilization reagents that may be on the surface of the first and second substrates. That is, the devices of the disclosure can facilitate analysis of a sample (e.g., a biological sample) by bringing the first and second substrates into contact with each other in an aligned manner. Alignment of the first and second substrates is key in spatial transcriptomics applications as the sample (e.g., a biological sample) may be required to be aligned with a barcoded area of a substrate.

Current methods of aligning biological samples with barcoded areas in spatial transcriptomics assays involve a user carefully placing the biological sample onto a substrate that includes a plurality of barcoded probes. Thus, in some examples, an advantage of the devices described is providing an alignment tool for users to align a sample with a barcoded area. The devices of the disclosure can reduce user error during the assay analysis, thereby also reducing sample analysis costs. In some examples, another advantage of the devices of the disclosure is a reduction in the number of aberrations or imaging imperfections that may arise due to user error in aligning a biological sample with a barcoded area of the substrate. In some examples, the devices of the disclosure allow for pre-screening of samples for areas of interest. In some examples, the devices of the disclosure allow for archived samples to be examined.

In one aspect, the present disclosure refers to a method of preparing a ~~biological~~tissue sample for spatial analysis, the method comprising:

mounting a first substrate and a second substrate on a support device, the support device comprising:

a first member comprising a first retaining mechanism configured to retain the first substrate comprising a ~~biological~~tissue sample;

a second member comprising a second retaining mechanism configured to retain the second substrate comprising an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain; and

an alignment mechanism connected to one or both of the first and second members; and

actuating the alignment mechanism to align~~ing~~ the first and second members such that the tissue~~biological~~ sample is aligned with at least a portion of the array when the first and second members are aligned.

In one aspect, the present disclosure refers to a method of preparing a ~~biological~~tissue sample for spatial analysis, the method comprising:

providing a support device holding a first substrate, a second substrate, and a reagent medium, wherein the support device comprises a first member, a second member, and an alignment mechanism connected to one or both of the first member and the second member, wherein the first substrate comprises a tissue~~biological~~ sample mounted thereon and the second substrate comprises an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain and wherein the tissue~~biological~~ sample comprises an analyte;

~~aligning~~actuating the alignment mechanism to align the first and second members such that~~ the first and second substrates such that~~ the ~~biological~~tissue sample is aligned with at least a portion of the array when the~~a~~ first member and the~~a~~ second member are aligned, and ~~such that~~ the reagent medium contacts both the tissue~~biological~~ sample and the array; and

~~passively or actively~~ migrating the analyte from the tissue~~biological~~ sample to the array.

In one example, this disclosure is directed to a substrate including an array disposed on a surface of the substrate. The array includes a plurality of non-metallic fluorescent markers. A non-metallic fluorescent marker of the plurality of non-metallic fluorescent markers includes a fluorescent probe. The substrate includes a plurality of metallic fiducial markers arranged on the surface in a frame pattern forming a perimeter around the array.

In some examples, the metallic fiducial markers include gold. In some examples, the metallic fiducial markers include nanoparticles. In some

In another example, this disclosure is directed to a sample holder, including a first member including a first retaining mechanism configured to retain a first substrate including a sample, a second member including a second retaining mechanism configured to retain a second substrate including a reagent medium, and an alignment mechanism connected to one or both of the first and second members, and configured to align the first and second members such that the sample contacts at least a portion of the reagent medium when the first and second members are aligned.

~~In one aspect, the present disclosure refers to a sample holder, comprising:~~

~~a first member comprising a first retaining mechanism configured to retain a first substrate comprising a sample;~~

~~a second member comprising a second retaining mechanism configured to retain a second substrate comprising a reagent medium; and~~

~~an alignment mechanism connected to one or both of the first and second members, and configured to align the first and second members such that the sample contacts at least a portion of the reagent medium when the first and second members are aligned.~~

In some examples, the alignment mechanism includes a rotating actuator connected to the first and second members. In some examples, the alignment mechanism includes one or more connectors positioned on one or bothof the first and second members, and one or more receivers positioned on one or bothof the first and second members, wherein the one or more receivers are positioned to engage with the one or more connectors. In some examples, the rotating actuator includes a hinge. In some examples, the rotating actuator includes a folding member. In some examples, the rotating actuator includes at least one arm. In some examples, the first retaining mechanism includes a recess dimensioned to receive the first substrate. In some examples, the sample holder further includes a gasket positioned within the recess and configured to maintain an interference fit between the recess and the first substrate. In some examples, the first retaining mechanism includes one or more members configured to apply a force to the first substrate to maintain contact between the first substrate and the first member.

In some examples, the second retaining mechanism includes a recess dimensioned to receive the second substrate. In some examples, the second retaining mechanism includes one or more members configured to apply a force to the second substrate to maintain contact between the second substrate and the second member. In some examples, the reagent medium includes at least one of: a solution including a permeabilization reagent, a solid permeabilization reagent, and a hydrogel compound including a permeabilization reagent. In some examples, the solution including the permeabilization agent includes greater than about 2 w/v % sodium dodecyl sulfate (SDS). In some examples, the solution including the permeabilization agent includes about 8 w/v % to about 12 w/v % SDS. In some examples, the solution including the permeabilization agent includes proteinase K. In some examples, the solution including the permeabilization agent includes

the sample holder further includes a second adjustment mechanism configured to adjust a distance of the separation in direction orthogonal to a surface of the first substrate that supports the sample. In some examples, the second adjustment mechanism is a component of the alignment mechanism. In some examples, the second adjustment mechanism is connected to one or both of the first member and the second member.

In another example, this disclosure is directed to a support device for a substrate including a sample. The support device includes any of the sample holder described above; and a plate including a platform, a plurality of members connected to a first surface of the platform, and a support member connected to a second surface of the platform. The plate is configured to connect to the sample holder.

~~In one aspect, the present disclosure refers to a support device for a substrate comprising a sample, the support device comprising:~~

~~the sample holder as disclosed herein; and~~

~~a plate comprising a platform, a plurality of members connected to a first surface of the platform, and a support member connected to a second surface of the platform,~~

~~wherein the plate is configured to connect to the sample holder.~~

In some examples, the sample holder and the plate are configured so that when the sample holder and the plate are connected, at least 75% of a region of the first substrate that contacts the sample is overlaid by the support member. In some examples, a member of the plurality of members is dimensioned to be received by a region of a heating device. In some examples, the region of the heating device includes a heat transfer element configured to transfer heat to a well of a multi-well substrate. In some examples, the heat transfer element includes a recess in a heating member. In some examples, the support devicer further includes an attachment mechanism configured to couple the support member to the sample holder. In some examples, the attachment mechanism includes one or both of an aperture and a recess formed in the first member of the sample holder and configured to receive the support member.

In some examples, the attachment mechanism includes one or more extension members connected to the first member of the sample holder and configured to engage with corresponding recess(es) in the support member. In some examples, the attachment mechanism includes one or more extension members connected to the support member and configured to engage with corresponding recess(es) in the first member of the sample holder. In some examples, the plurality of members form a two-dimensional array on the first surface of the platform, and the plurality of members are spaced so that they align with wells on a multi-well substrate. In some examples, the region of the first substrate that contacts the sample is completely overlaid by the support member. In some

WHAT IS CLAIMED IS:

1. A method of preparing a ~~biological~~tissue sample for spatial analysis, the method comprising:

mounting a first substrate and a second substrate on a support device, the support device comprising:

a first member comprising a first retaining mechanism configured to retain the first substrate comprising a ~~biological~~tissue sample;

a second member comprising a second retaining mechanism configured to retain the second substrate comprising an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain; and

an alignment mechanism connected to one or both of the first and second members; and

actuating the alignment mechanism to align~~ing~~ the first and second members such that the ~~biological~~tissue sample is aligned with at least a portion of the array when the first and second members are aligned.

2. The method of claim 1, wherein the actuating the alignment mechanism to align~~ing~~ comprises:

(i) rotating the first member or the second member about a hinge axis; or

(ii) rotating the first and second members until the tissue~~biological~~ sample and the array are aligned; or

(iii) maintaining a separation between the first substrate and the second substrate when the first substrate and the second substrate are aligned; or

(iv) maintaining the first substrate and the second substrate in an approximately parallel relationship when the first substrate and the second substrate are aligned, wherein optionally an angle between the first substrate and the second substrate is 2 degrees or less.

3. The method of claim 1, wherein the support device further comprises one or more spacing members configured to maintain a spacing or a parallel arrangement of the first substrate and the second substrate.

4. The method of claim 1, wherein the array comprises a plurality of features, wherein:

a feature comprises a sub-plurality of capture probes, wherein each capture probe of the sub-plurality of capture probes comprises the same spatial barcode and other features of the plurality of features comprise sub-pluralities of capture probes comprising different spatial barcodes; and/or the feature comprises a printed spot, a gel bead, a gel pad, or any combination thereof.

5. The method of claim 1, further comprising acquiring an image of the tissue~~biological~~ sample aligned with at least a portion of the array.

6. The method of claim 1, further comprising capturing a first analyte, a second analyte, and/or a third analyte from the tissue~~biological~~ sample, wherein:

the capture domain of the capture probe specifically binds the first analyte;

a second capture probe of the plurality of capture probes comprises a second spatial barcode and a second capture domain, wherein the second capture domain specifically binds the second analyte; and/or

a third capture probe of the plurality of capture probes comprises a third spatial barcode and a third capture domain, wherein the third capture domain specifically binds to an analyte capture sequence of an analyte capture agent, wherein the analyte capture agent comprises an analyte binding moiety that specifically binds to the third analyte, an analyte binding moiety barcode, and the analyte capture sequence.

7. The method of claim 6, wherein:

(i) the spatial barcode, the second spatial barcode, and the third spatial barcode comprise a same sequence or different sequences; or

(ii) the first analyte and the second analyte comprise a nucleic acid, wherein optionally the nucleic acid is RNA or DNA; or

(iii) the second analyte is a ligation product resulting from ligation of a first probe and a second probe hybridized to a nucleic acid analyte from the ~~biological~~tissue sample, and wherein the method further comprises ligating the first probe and the second probe hybridized to the nucleic acid analyte; or

(iv) the analyte binding moiety comprises an antibody or an antigen-binding fragment thereof, wherein optionally the third analyte comprises a protein.

8. The method of claim 6, further comprising a step of extending a 3' end of the capture probe using the first analyte as a template, a 3' end of the second capture probe using the second analyte as a template, or a 3' end of the third capture probe using the analyte binding moiety barcode as a template.

9. The method of claim 6, wherein the capturing comprises contacting at least a portion of the ~~biological~~tissue sample and at least a portion of the array with a reagent medium.

10. The method of claim 9, wherein the reagent medium comprises:

(i) a permeabilization enzyme, a nuclease, a buffer, a detergent, or any combination thereof; or

(ii) a permeabilization reagent comprising a solid, a liquid, a gel, or a dried permeabilization reagent, wherein optionally the permeabilization reagent promotes release of analytes from the ~~biological~~tissue sample; or

(iii) one or more of sodium dodecyl sulfate (SDS), proteinase K, pepsin, N-lauroylsarcosine, RNAse, and a sodium salt thereof.

11. The method of claim 9, further comprising adding the reagent medium to the second substrate, wherein optionally the addition of the reagent medium occurs before the aligning of the first and second members.

12. The method of claim 1, wherein the aligning further comprises viewing or imaging through one or more apertures defined by the first and/or second members.

13. The method of claim 1, wherein the first and/or second substrates comprise one or more fiducial markings and wherein:

(i) the aligning comprises adjusting a position of the first and/or second substrates so that the one or more fiducial markings are aligned with one another; or

(ii) the aligning comprises adjusting a position of the first and/or second members so that the ~~biological~~tissue sample is aligned with the one or more fiducial markings of the first and/or second members.

14. A method of preparing a ~~biological~~tissue sample for spatial analysis, the method comprising:

providing a support device holding a first substrate, a second substrate, and a reagent medium, wherein the support device comprises a first member, a second member, and an alignment mechanism connected to one or both of the first member and the second member, wherein the first substrate comprises a tissue~~biological~~ sample mounted thereon and the second substrate comprises an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain and wherein the ~~biological~~tissue sample comprises an analyte;

~~aligning~~actuating the alignment mechanism to align the first and second members such that ~~the first and second substrates such that~~ the ~~biological~~tissue sample is aligned with at least a portion of the array when ~~a~~the first member and ~~a~~the second member are aligned, and ~~such that~~ the reagent medium contacts both the ~~biological~~tissue sample and the array; and

~~passively or actively~~ migrating the analyte from the ~~biological~~tissue sample to the array.

~~15. A sample holder, comprising:~~

~~a first member comprising a first retaining mechanism configured to retain a first substrate comprising a sample;~~

~~a second member comprising a second retaining mechanism configured to retain a second substrate comprising a reagent medium; and~~

~~an alignment mechanism connected to one or both of the first and second members, and configured to align the first and second members such that the sample contacts at least a portion of the reagent medium when the first and second members are aligned.~~

~~16. The sample holder of claim 15, wherein the alignment mechanism comprises:~~

(a) a rotating actuator connected to the first and second members; and/or

(b) one or more connectors positioned on one or both of the first and second members; and one or more receivers positioned on one or both of the first and second members, wherein the one or more receivers are positioned to engage with the one or more connectors.

17. The sample holder of claim 16, wherein the rotating actuator comprises a hinge or a folding member or at least one arm.

18. The sample holder of any one of claims 15-17, wherein:

(a) the first retaining mechanism comprises:

(i) a recess dimensioned to receive the first substrate, wherein optionally the sample holder further comprises a gasket positioned within the recess and configured to maintain an interference fit between the recess and the first substrate; or

(ii) one or more members configured to apply a force to the first substrate to maintain contact between the first substrate and the first member;

and/or

(b) the second retaining mechanism comprises:

(i) a recess dimensioned to receive the second substrate; or

(ii) one or more members configured to apply a force to the second substrate to maintain contact between the second substrate and the second member;

and/or

(c) the reagent medium comprises at least one of: a solution comprising a permeabilization reagent, a solid permeabilization reagent, and a hydrogel compound comprising a permeabilization reagent, wherein optionally the solution comprising the permeabilization agent comprises:

(i) greater than about 2 w/v % sodium dodecyl sulfate (SDS), wherein optionally the permeabilization agent comprises about 8 w/v % to about 12 w/v % SDS; or

(ii) proteinase K; or

(iii) greater than 2 w/v % N-lauroylsarcosine or a sodium salt thereof;

and/or

(d) the first member comprises an aperture positioned so that when the first substrate is retained, the aperture is aligned with a sample region of the first substrate;

and/or

(e) the second member comprises at least one aperture positioned so that when the first substrate is retained and the first and second members are aligned by the alignment mechanism, aperture of the at least one aperture is aligned with at least a portion of a sample region of the first substrate, wherein optionally the sample holder further comprises a reagent well formed by one or more bounding surfaces of the at least one aperture and by a back surface of the second substrate, wherein a reagent solution added to the reagent well is contained by the bounding surfaces and permeates through the back surface of the second substrate, and wherein optionally the back surface of the second substrate is opposite to a front surface of the second substrate that faces the sample on the first substrate.

19. The sample holder of any one of claims 16-18, further comprising a first adjustment mechanism connected to the first member and configured to translate the first substrate in at least one direction parallel to a surface of the first substrate that supports the sample.

20. The sample holder of any one of claims 16-19, wherein the alignment mechanism is configured to maintain a separation between the first and second substrates when the first and second substrates are aligned, wherein optionally:

(i) the alignment mechanism is configured to maintain the separation such that at least a portion of the sample on the first substrate contacts at least a portion of the reagent medium on the second substrate; or

(ii) the separation between the first and second substrates is between 50 microns and 1 mm, measured in a direction orthogonal to a surface of the first substrate that supports the sample; or

(iii) the separation between the first and second substrates is between 50 microns and 500 microns; or

(iv) the alignment mechanism is configured to maintain the first and second substrates approximately parallel when the first and second substrates are aligned so that an angle between the first and second substrates is two degrees or less, wherein optionally the angle is 0.5 degrees or less and wherein optionally the sample holder further comprises one or more spacing members connected to one or both of the first and second members positioned so that when the first and second substrates are aligned, the one or more spacing members are between the first and second members.

21. The sample holder of claim 20, further comprising a second adjustment mechanism configured to adjust a distance of the separation in direction orthogonal to a surface of the first substrate that supports the sample; wherein optionally the second adjustment mechanism is:

(i) a component of the alignment mechanism; or

(ii) connected to one or both of the first member and the second member.

22. A support device for a substrate comprising a sample, the support device comprising:

the sample holder of any one of claims 16-21; and

a plate comprising a platform, a plurality of members connected to a first surface of the platform, and a support member connected to a second surface of the platform, wherein the plate is configured to connect to the sample holder.

23. The support device of claim 22, wherein:

(a) the sample holder and the plate are configured so that when the sample holder and the plate are connected, at least 75% of a region of the first substrate that contacts the sample is overlaid by the support member;

and/or

(b) a member of the plurality of members is dimensioned to be received by a region of a heating device, wherein optionally the region of the heating device comprises a heat transfer element configured to transfer heat to a well of a multi-well substrate, and wherein optionally the heat transfer element comprises a recess in a heating member;

and/or

(c) the support device further comprises an attachment mechanism configured to couple the support member to the sample holder, wherein optionally:

(i) the attachment mechanism comprises one or both of an aperture and a recess formed in the first member of the sample holder and configured to receive the support member; or

(ii) the attachment mechanism comprises one or more extension members connected to the first member of the sample holder and configured to engage with corresponding recess(es) in the support member; or

(iii) the attachment mechanism comprises one or more extension members connected to the support member and configured to engage with corresponding recess(es) in the first member of the sample holder;

and/or

(d) the plurality of members form a two-dimensional array on the first surface of the platform, and the plurality of members are spaced so that they align with wells on a multi-well substrate;

and/or

(e) the region of the first substrate that contacts the sample is completely overlaid by the support member, wherein optionally the sample holder and the plate are configured so that when the sample holder and the plate are connected, at least a portion of the first substrate contacts the support member, wherein optionally the portion of the first substrate that contacts the support member comprises a part of the substrate that is on an opposite side of the first substrate from a region of the first substrate that contacts the sample, and wherein optionally the portion of the first substrate that contacts the support member comprises at least 90% of a surface of the substrate that is opposite to a surface of the first substrate that contacts the sample;

~~and/or~~

~~(f) the attachment mechanism is configured so that the sample holder couples to the support member in a single orientation.~~