

various steps and techniques discussed herein can be performed using a variety of different devices and system components, not all of which are expressly set forth.

Furthermore, various examples of the present disclosure relate to control slides and related substrates, methods of using control slides, and control slide systems preferably intended for use in assessments of image quality and/or resolution. More specifically, examples include control slides and their substrates, methods, and systems to test and assess the quality and/or resolutions of imaging systems used in spatial gene expression technologies. In some examples, a distinct advantage of the control slides provided herein is that it can be used to assess the viability of an imaging system prior to imaging and/or analyzing a sample. For example, an imaging system may or may not be adequate to detect a sample at a pre-determined or required resolution. A control slide or substrate consistent with this disclosure may be imaged prior to processing a sample in order to determine if the imaging system provides adequate resolution. As another example, a conventional imaging acquisition method (e.g., in spatial gene expression technologies) may not have a way to verify resolution other than by imaging and/or analyzing the sample, which can be rather costly and inefficient. In other words, the conventional methods to obtain spatial gene expression images, for example, do not enable a user to verify resolution and/or quality of image prior to imaging and analyzing a sample. But a control slide and/or substrate consistent with the examples of the present disclosure may be more efficient in this situation because it can allow a user to qualitatively and/or quantitatively assess the compatibility of their imaging system and optimize their image acquisition without wasting experimental resources. Such a control slide and/or substrate would be amenable to test and/or calibrate various imaging apparatuses and systems including systems not directed to spatial gene expression imaging and analysis.

In some examples, an additional advantage of the control slides and substrates provided herein is that it provides a user the imaging capability to assess the image quality of a sample having more than one histochemical stains and/or more than one fluorescent stains. For example, the user may have a sample that contains two or more fluorophores. Therefore, the user can assess the image quality of an imaging system and make any necessary adjustments to the parameters of all fluorescence channels by simultaneously imaging one substrate that includes two or

more fluorescent markers. That is, the imaging capability can be the capability of an imaging apparatus to adequately image a sample. In some examples, an additional advantage of the substrate is that it provides a user a quick reference to a substrate region via the plurality of fiducial markers and glyphs. For example, a user utilizing
5 microscopes slides used in spatial gene expression methodologies (e.g., microscope slides having spatially-barcoded arrays) may be able to efficiently align the substrate region with a region of interest in the microscope slide (e.g., the region containing a spatially-barcoded array or a sample) during image processing. Thus, the user may be able to correctly align a sample region and/or an array region with a substrate region
10 of the substrate. Furthermore, the plurality of fiducial markers allows for rapid identification of orientation and slide placement. As such, control slides and substrates within the scope of this disclosure may be able to reduce experimentation time and improve calibration of imaging systems. Additionally, with the arrangement and positioning of the fiducials matching that of the gene expression slide, users can use
15 the control slide as a reference for the automation of image acquisition.

The devices provided herein can provide consistent and even heating to a substrate surface. Even heating can be critical to ensuring that preparative reactions performed on a sample supported by the substrate occur according to established protocols and achieve desired outcomes.

20 Further, heating to temperatures above room temperature can cause condensation to form on an upper surface of an enclosed substrate well if the substrate is heated without an upper lid. Condensation can change the composition of reaction mixtures in the substrate wells, inhibiting preparative reactions and/or producing unpredictable results. The devices described in this disclosure can be used to reduce
25 or prevent condensation from forming in substrate wells.

Certain types of thermocyclers and heating devices are purpose-built for particular types of substrates such as multi-well substrates. Loading other types of substrates such as standard microscope slides into such devices can lead to uneven substrate heating. The devices described in this disclosure can be used to support
30 substrates within heating devices that are not designed for such substrates, ensuring that adequate and even heat transfer occurs to the substrates. In particular, the devices can be used to adapt thermocyclers designed to accept multi-well substrates so that

other types of substrates can be effectively heated within the thermocyclers as part of a sample preparation protocol.

5 In some examples, the devices of the disclosure allow a surface of the substrate to directly contact the surface of a heating device (e.g., a thermocycler), thereby permitting uniform heating throughout the substrate. That is, in some examples, no additional substrates or housing elements are required to be positioned in between the heat source and the substrates to be heated. In addition, because the devices of the disclosure allow the surface of the substrate to directly contact the surface of a heating device (e.g., a thermocycler), the temperature of the substrate can
10 be more easily controlled by the user and can be heated to a desired temperature in less time than when using devices that do not allow surface contact between the substrate and the heating device (e.g., a thermocycler). Thus, samples (e.g., a biological samples) on the substrate can be heated uniformly and in a controlled manner.

15 In some examples, another advantage of the devices described is that their design can be a one-piece design that facilitates set-up and reduces time spent by the user in assembling the device and substrate. In some examples, the user can easily insert a substrate into the devices described without fastening of multiple pieces. For example, the user can use a single optional tool, such as a blade, to aid in the insertion
20 of the substrate into the device or to aid in removing the substrate from the device. In some examples, the user does not use any tools to aid in the insertion of the substrate into the device or to aid in removing the substrate from the device.

In some examples, the devices can be single use devices that can be disposed after use, thereby preventing any contamination or sterility issues with samples (e.g.,
25 biological samples) that are being supported by the substrates. For example, the devices can be sterilized and pre-packaged for the user in order to decrease the risk of sample contamination.

This disclosure further describes devices for holding or supporting substrates. In particular, the devices described include a first and second members that receive a
30 first and second substrate, respectively. In some examples, the devices of the disclosure can be used for sandwiching the first and second substrates together for spatial transcriptomics applications. In some examples, the first substrate can

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.

5 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
10 (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
15 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
20 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
25 examined.

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

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

30 a first member comprising a first retaining mechanism configured to retain the first substrate comprising a biological 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

5 (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

aligning the first and second members such that the biological sample is aligned with at least a portion of the array when the first and second members are aligned.

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

providing a support device holding a first substrate, a second substrate, and a reagent medium, wherein the first substrate comprises a 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 sample comprises an analyte;

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aligning the first and second substrates such that the biological sample is aligned with at least a portion of the array when a first member and a second member are aligned, and such that the reagent medium contacts both the biological sample and the array; and

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

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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.

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

examples, the frame pattern is rectangular or square shaped. In some examples, a metallic fiducial marker of the plurality of metallic fiducial markers has a dimension of at least 0.1 mm. In some examples, the non-metallic fluorescent marker has a maximum dimension of less than 0.1 mm. In some examples, the array includes a first non-metallic fluorescent marker including a first fluorescent probe having a first average concentration and a second non-metallic fluorescent marker including a second fluorescent probe having a second average concentration; wherein the first average concentration is different than the second average concentration. In some examples, the first average concentration is from about 0.01 micromolar (μM) to about 100 μM .

In some examples, the plurality of non-metallic fluorescent markers include a first subset of non-metallic fluorescent markers having a first fluorescence intensity peak at a first excitation wavelength and a second subset of non-metallic fluorescent markers having a second fluorescence intensity peak at a second excitation wavelength. In some examples, the first excitation wavelength is different from the second excitation wavelength. In some examples, the plurality of metallic fiducial markers do not fluoresce at the first excitation wavelength. In some examples, the plurality of metallic fiducial markers do not fluoresce at the second excitation wavelength. In some examples, the first excitation wavelength is about 560 nanometers (nm) to about 610 nm. In some examples, the second excitation wavelength is about 600 nanometers (nm) to about 700 nm. In some examples, the first subset of the plurality of non-metallic fluorescent markers fluoresces at the first excitation wavelength while the second subset of the plurality of non-metallic fluorescent markers does not detectably fluoresce at the first excitation wavelength.

In some examples, the fluorescent probe is conjugated to an oligonucleotide. In some examples, the fluorescent probe includes tetramethylrhodamine (TRITC), Red Fluorescent Protein (DsRed), a dye having an absorption wavelength that peaks at about 590 nm, cyanine-3 (Cy3), a dye having an absorption wavelength that peaks at about 650 nm, cyanine-5 (Cy5), or combinations thereof.

In another example, this disclosure is directed to a substrate including an array including a plurality of fluorescent markers disposed on a surface of the substrate, the

plurality of fluorescent markers including a first subset of fluorescent markers, wherein a fluorescent marker of the first subset has a first fluorescent probe, and a second subset of fluorescent markers, wherein a fluorescent marker of the second subset has a second fluorescent probe. The first fluorescent probe is different from the second fluorescent probe.

In some examples, the first fluorescent probe or the second fluorescent probe includes a fluorescent dye, a fluorescent protein, or combinations thereof. In some examples, the first fluorescent probe or the second fluorescent probe includes a fluorescent dye that is selected from the group consisting of an acridine dye, a fluorone dye, a cyanine dye, a luciferin, an oxazine dye, a phenanthridine dye, a rhodamine dye, and combinations thereof. In some examples, the first fluorescent probe or the second fluorescent probe includes a fluorescent protein that is selected from the group consisting of a green fluorescent protein (GFP), a Tag blue fluorescent protein (TagBFP), cerulean, a cyan fluorescent protein (CFP), venus, citrine, a yellow fluorescent protein (YFP), a monomeric enhanced green fluorescent protein (EGFP), mCherry, mKate2, a photoactivable green fluorescent protein (PA-GFP), a photoactivable mCherry (PA-mCherry), a fluorescent protein-fusion protein, and combinations thereof. In some examples, the plurality of fluorescent markers are non-metallic. In some examples, a fluorescent marker of the plurality of fluorescent markers has a maximum dimension that is less than 0.1 mm.

In another example, this disclosure is directed to a substrate including an array including a plurality of fluorescent markers disposed on a surface of the substrate, and a plurality of fiducial markers arranged on the surface in a frame pattern forming a perimeter around the array. A fluorescent marker of the plurality of fluorescent markers includes a fluorescent dye.

In some examples, the fluorescent dye is conjugated to an oligonucleotide. In some examples, the fluorescent dye is selected from the group consisting of an acridine dye, a fluorone dye, a cyanine dye, a luciferin, an oxazine dye, a phenanthridine dye, a rhodamine dye, and combinations thereof. In some examples, the fluorescent dye is selected from the group consisting of tetramethylrhodamine (TRITC), Red Fluorescent Protein (DsRed), a dye having an absorption wavelength that peaks at about 590 nm, cyanine-3 (Cy3), a dye having an

absorption wavelength that peaks at about 650 nm, cyanine-5 (Cy5), and combinations thereof.

In another example, this disclosure is directed to a substrate including an array including fluorescent markers disposed on a surface of the substrate, and
5 a plurality of fiducial markers arranged on the surface in a frame pattern forming a perimeter around the array. A fluorescent marker of the plurality of fluorescent markers include a fluorescent probe. A minimum spacing between the fluorescent marker and a fiducial marker of the plurality of fiducial markers is at least 50 microns.

In some examples, the minimum spacing between the fluorescent marker and
10 the fiducial marker is at least 100 microns. In some examples, the minimum spacing between the fluorescent marker and the fiducial marker is at least 500 microns. In some examples, the minimum spacing between the fluorescent marker and the fiducial marker is at least 1,000 microns. In some examples, the minimum spacing between the fluorescent marker and the fiducial marker is at least 1,500 microns. In some
15 examples, the minimum spacing between the fluorescent marker and the fiducial marker is at least 2,000 microns. In some examples, the minimum spacing between the fluorescent marker and the fiducial marker is about 50 to about 3,000 microns. In some examples, the minimum spacing between the fluorescent marker and the fiducial marker is about 500 to about 2,000 microns. In some examples, the minimum spacing
20 between the fluorescent marker and the fiducial marker is about 1,000 to about 1,500 microns.

In another example, this disclosure is directed to a substrate including a plurality of fluorescent markers disposed on a surface of a substrate, and a plurality of fiducial markers arranged on the surface. The plurality of fiducial
25 markers includes a first subset of fiducial markers arranged in a first pattern and a second subset of fiducial markers arranged in a second pattern. The second pattern is different from the first pattern.

In some examples, the plurality of fiducial markers further includes a third subset of fiducial markers arranged in a third pattern adjacent to first and second
30 patterns on the surface. In some examples, the third subset of fiducial markers is a glyph. In some examples, the third pattern is a geometric shape. In some examples, the third pattern is a circle, an hourglass, a hexagon, a square, a rectangle, a triangle, a pentagon, a heptagon, an octagon, a nonagon, a decagon, an

ellipse, or a regular polygon. In some examples, the first pattern forms a first side and the second pattern forms a second side. In some examples, the third pattern that forms a third side, wherein the third pattern is different from the first and second patterns. In some examples, the plurality of fiducial markers further includes a fourth subset of

5 fiducial markers arranged in a fourth pattern that forms a fourth side, wherein the fourth pattern is different from the first, second, and third patterns. In some examples, the first, second, third, and fourth sides are arranged to form a frame on the surface of the substrate. In some examples, the first subset of fiducial markers are spaced apart from one another at a different periodicity than the second subset of fiducial markers.

10 In some examples, the first subset of fiducial markers are spaced apart from one another on the surface of the substrate at a higher periodicity than the second subset of fiducial markers. In some examples, the first subset of fiducial markers are spaced apart from one another on the surface of the substrate at a lower periodicity than the second subset of fiducial markers. In some examples, the first subset of fiducial

15 markers are arranged on the surface of the substrate in three staggered rows that form a two-dimensional lattice structure.

In another example, this disclosure is directed to a slide including any one of the substrates described above.

In another example, this disclosure is directed to a method of assessing an

20 imaging capability of an imaging system. The method includes identifying a frame includes a plurality of metallic fiducial markers arranged on a surface of a substrate, illuminating the substrate using radiation at a first wavelength, measuring a light emitted by an array disposed on the surface within a perimeter defined by the frame, wherein the array includes a plurality of non-metallic fluorescent markers, wherein a

25 non-metallic fluorescent marker of the plurality of the non-metallic fluorescent markers includes a fluorescent probe, and determining presence or absence of the non-metallic fluorescent marker on a visual representation of an object including relative dimensional information in at least two orthogonal spatial dimensions, wherein the visual representation is generated by the imaging system based on the light emitted by

30 the array.

In another example, this disclosure is directed to a system including at least one array including a plurality of non-metallic fluorescent markers disposed on a surface of a substrate, wherein a non-metallic fluorescent marker of the plurality of non-

metallic fluorescent markers includes a fluorescent probe, a plurality of metallic fiducial markers arranged on the surface in at least one frame pattern forming a perimeter around the array, and a computing device including a processor operatively coupled to a microscope, and a non-transitory computer readable storage medium
5 with a computer program including instructions executable by the processor causing the processor to generate a visual representation of an object including relative dimensional information in at least two orthogonal spatial dimensions, wherein the object includes the at least one frame pattern and the at least one array.

In another example, this disclosure is directed to a support device for a
10 substrate including a sample region, the support device including 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, and a substrate holder including a substrate mount and an attachment mechanism to couple the substrate holder to the support member. The substrate holder is configured so that when a
15 substrate is secured by the substrate mount and the substrate holder is coupled to the support member, at least 60% of the sample region 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-
20 well substrate. In some examples, the heat transfer element includes a recess in a heating member. In some examples, the plurality of members form a two-dimensional array on the first surface, and the plurality of members are spaced so that they align with wells on a multi-well substrate. In some examples, the substrate mount includes a recess formed in the substrate holder. In some examples, the substrate mount includes
25 at least one fastener configured to secure the substrate within the recess. In some examples, the attachment mechanism includes an aperture configured to receive the support member. In some examples, the attachment mechanism includes one or more extensions configured to engage with corresponding recesses in the support member. In some examples, the sample region includes a plurality of wells on the substrate. In
30 some examples, the substrate holder includes a recess dimensioned to receive a gasket. In some examples, the substrate holder includes a gasket positioned so that when the substrate is secured by the substrate mount, a vapor-tight seal is formed between the

substrate holder and the substrate. In some examples, the sample region includes a plurality of wells on the substrate, and the gasket includes a plurality of apertures, wherein an aperture of the plurality of apertures is positioned so that when the substrate is secured by the substrate mount, the aperture is aligned with a well. In
5 some examples, the gasket is configured to prevent fluid transport between apertures when the substrate is secured by the substrate mount.

In some examples, the substrate holder includes a plurality of apertures, wherein an aperture of the plurality of apertures is aligned with an aperture of the gasket. In some examples, the sample region is completely overlaid by the support
10 member. In some examples, the substrate holder is configured so that when a substrate is secured by the substrate mount and the substrate holder is coupled to the support member, at least a portion of the substrate contacts the support member. In some examples, the portion of the substrate that contacts the support member includes at least a part of the sample region or a part of the substrate that is on an opposite side of
15 the substrate from the sample region. In some examples, the support member contacts all of the substrate. In some examples, the attachment mechanism is configured so that the substrate holder couples to the support member in a single orientation.

In some examples, the substrate holder includes a first member, a second member, and an engagement mechanism configured to secure the first member to the
20 second member. The substrate mount is positioned in the first member or the second member. In some examples, the engagement mechanism is adjustable. In some examples, the engagement mechanism includes one or more thumbscrews. In some examples, the first member includes the substrate mount, and wherein the substrate mount includes a recess formed in the first member. In some examples, the first
25 member includes at least one fastener configured to secure the substrate within the recess. In some examples, the first member includes the attachment mechanism, and wherein the attachment mechanism includes an aperture configured to receive the support member. In some examples, the second member includes a recess dimensioned to receive a gasket. In some examples, the second member includes a
30 gasket positioned so that when the substrate is secured by the substrate mount and the first member is secured to the second member, a vapor-tight seal is formed between the substrate holder and the substrate.

In some examples, the sample region includes a plurality of wells on the substrate, the gasket includes a plurality of apertures, wherein an aperture of the plurality of apertures is positioned so that when the substrate is secured by the substrate mount, the aperture is aligned with a well. In some examples, the gasket is configured to prevent fluid transport between gasket apertures when the substrate is secured by the substrate mount and the first member is secured to the second member. In some examples, the second member includes a plurality of apertures, wherein an aperture of the plurality of apertures is aligned with an aperture of the plurality of apertures of the gasket.

In another example, this disclosure is directed to a method of incubating a sample disposed on a sample region of a substrate. The method includes mounting the substrate on a support device, positioning the substrate and the support device in a heating apparatus, and activating the heating apparatus to transfer heat to the sample. The support device includes 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, and a substrate holder including a substrate mount and an attachment mechanism to couple the substrate holder to the support member. The substrate holder is configured so that when the substrate is secured by the substrate mount and the substrate holder is coupled to the support member, at least 60% of the sample region is overlaid by the support member.

In another example, this disclosure is directed to a support device for a substrate including a sample region. The support device includes 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, a substrate mount including a first surface and a second surface, the first surface being coupled to the support member, and a substrate holder including an attachment mechanism to couple the substrate mount to the substrate holder. The second surface of the substrate mount is a substrate for receiving a sample.

In some examples, the substrate mount is a glass slide. In some examples, when the substrate holder is coupled to the support member, at least 60% of the sample region is overlaid by the support member. In some examples, when the substrate holder is coupled to the support member, at least 75% of the sample region is overlaid by the support member. In some examples, when the

substrate holder is coupled to the support member, at least 90% of the sample region is overlaid by the support member. In some examples, when the substrate holder is coupled to the support member, the sample region is fully overlaid by the support member. In some examples, a member of the plurality of members is dimensioned to
5 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 elements includes recesses in a heating member. In some examples, the plurality of members form a two-dimensional array on the first surface, and the plurality of members are spaced so that
10 they align with wells on a multi-well substrate. In some examples, the attachment mechanism includes a fastener configured to engage the substrate mount. In some examples, the attachment mechanism includes an aperture configured to receive the support member. In some examples, the attachment mechanism includes one or more tabs configured to engage with the substrate mount. In some examples, the sample
15 region includes a plurality of wells on the substrate. In some examples, the substrate holder includes a gasket positioned so that when the substrate is secured by the substrate mount, a vapor-tight seal is formed between the substrate holder and the substrate. In some examples, the sample region includes a plurality of wells on the substrate, and wherein the gasket includes a plurality of apertures, wherein an aperture
20 of the plurality of apertures is positioned so that when a substrate is secured by the substrate mount, the aperture of the gasket is aligned with a well. In some examples, the gasket is configured to prevent fluid transport between apertures of the gasket when the substrate is secured by the substrate mount. In some examples, the substrate holder includes a plurality of apertures, wherein an aperture of the plurality of
25 apertures of the substrate holder is aligned with an aperture of the gasket. In some examples, the sample region is completely overlaid by the support member. In some examples, the substrate holder is configured so that when the substrate is held by the substrate mount and the substrate holder is coupled to the support member, at least a portion of the substrate contacts the support member. In some examples, the portion of
30 the substrate that contacts the support member includes at least a part of the sample region or a part of the substrate that is on an opposite side of the substrate from the sample region. In some examples, the support member contacts the entire substrate. In some

examples, the attachment mechanism is configured so that the substrate holder couples to the support member in a single orientation.

In another example, this disclosure is directed to support device for a substrate including a sample region. The support device includes a substrate mount including a first surface and a second surface, the first surface coupled to the support member, a substrate holder including an attachment mechanism to couple the substrate mount to the substrate holder, a gasket, a plurality of ribs extending perpendicular from a bottom surface of the substrate holder, and an engagement mechanism configured to secure the substrate mount to the gasket. The second surface of the substrate mount is a substrate for receiving a sample, and the gasket is positioned between the substrate mount and the bottom surface of the substrate holder.

In some examples, the engagement mechanism is adjustable. In some examples, the engagement mechanism includes one or more tabs. In some examples, the engagement mechanism includes one or more press latches. In some examples, the substrate holder includes at least one fastener configured to secure the substrate mount within the recess. In some examples, the substrate holder includes a recess dimensioned to receive the gasket. In some examples, the sgasket is positioned so that when the substrate mount is secured by the substrate holder, a vapor-tight seal is formed between the substrate holder and the substrate mount. In some examples, the sample region includes a plurality of wells on the substrate mount, and the gasket includes a first plurality of apertures, wherein an aperture of the first plurality of apertures is positioned so that when the substrate mount is secured by the substrate holder, the aperture is aligned with a well. In some examples, the gasket is configured to prevent fluid transport between the first plurality of apertures when the substrate mount is secured by the substrate holder. In some examples, the substrate holder includes a second plurality of apertures, wherein an aperture of the second plurality of apertures is aligned with an aperture of the first plurality of apertures.

In another example, this disclosure is directed to a method of incubating a sample disposed on a sample region of a substrate. The method includes mounting the substrate on a support device, positioning the substrate and support device in a heating apparatus, and activating the heating apparatus to transfer heat to the sample. The support device includes 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, a substrate mount including a first surface and a second surface, the first surface being coupled to the support member, and a substrate holder including an attachment mechanism to couple the substrate mount to the substrate holder. The second surface of the substrate mount includes a substrate for receiving a sample.

In some examples, the substrate mount is a glass slide. In some examples, when the substrate holder is coupled to the support member, at least 60% of the sample region is overlaid by the support member.

In another example, this disclosure is directed to a support device for a substrate comprising a sample region. The support device including a substrate mount comprising a first surface and a second surface, the second surface of the substrate mount is a substrate configured for receiving a sample, a substrate holder including a first portion configured to receive a gasket, the first portion comprising a plurality of ribs extending from a surface of the substrate holder; and a second portion configured to receive a substrate mount. The first and second portions are coupled together by a hinge such that when the substrate holder is in a closed state, the first portion is configured to fold over the second portion to secure the substrate mount between the first and second portions.

In some examples, the substrate mount is a glass slide. In some examples, the substrate holder comprises a gasket disposed between the first portion and the second portion of the substrate holder. In some examples, the first portion of the substrate holder comprises a releasable engagement mechanism configured to secure the first portion to the second portion when the substrate holder is in the closed state. In some examples, the first surface of the substrate mount engages with at least one of the plurality of ribs extending from a surface of the substrate holder. In some examples, the second portion defines a recessed cavity formed in the substrate holder configured to receive the substrate mount. In some examples, the second portion defines a cavity configured to receive the substrate mount. In some examples, the second portion defines an opening within the recessed cavity, and wherein the opening exposes at least a portion of one side of the substrate mount when the substrate holder is in the closed state.

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 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. 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

greater than 2 w/v %N-lauroylsarcosine or a sodium salt thereof. In some examples, the first member includes an aperture positioned so that when the first substrate is retained, the aperture is aligned with a sample region of the first substrate. In some examples, the second member includes 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, an aperture of the at least one aperture is aligned with at least a portion of a sample region of the first substrate.

In some examples, the sample holder further includes 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. In some examples, 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. In some examples, the sample holder further includes 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. In some examples, the alignment mechanism is configured to maintain a separation between the first and second substrates when the first and second substrates are aligned. In some examples, 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. In some examples, 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.

In some examples, the separation between the first and second substrates is between 50 microns and 500 microns. In some examples, 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. In some examples, the angle is 0.5 degrees or less. In some examples, the sample holder further includes 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. In some examples,

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 device 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

examples, 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. In some examples, the portion of the first substrate that contacts the support member includes 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. In some examples, the portion of the first substrate that contacts the support member includes at least 90% of a surface of the substrate that is opposite to a surface of the first substrate that contacts the sample. In some examples, the attachment mechanism is configured so that the sample holder couples to the support member in a single orientation.

In another example, this disclosure is directed to a method for providing a visual guide for printed array location including using an informational label with printed guides to place a biological sample on a solid support, and analyzing the sample, wherein the printed guides provide a visual guide for the printed array location.

In another example, this disclosure is directed to a method for placing a biological sample on an array, including using an informational label with printed guides to place the biological sample on a solid support, analyzing the biological sample, wherein the printed guides provide a visual guide for the printed array location; and removing the informational label from the solid support.

In some examples, the informational label is transparent. In some examples, the step of removing the informational label occurs before the step of analyzing the biological sample. In some examples, the solid support is a slide. In some examples, the biological sample is a tissue section. In some examples, the informational label is removable. In some examples, the informational label is mechanically adhered. In some examples, the informational label is printed with ink. In some examples, the ink is white ink, black ink, colored ink, fluorescent ink, or a combination thereof. In some examples, the informational label is matte. In some examples, the informational label is glossy. In some examples, the informational label includes holes or cutout in the interior of the informational label. In some examples, the informational label is capable of thermal and electrical conductivity. In some examples, the printed guides are fiducial markers. In some examples, the fiducial markers include a box surrounding the array and a dot identifying the center of the array. In some examples, the informational label

contains metadata. In some examples, the informational label occupies part of the solid support. In some examples, the informational label occupies all of the solid support.

In one example, a method for generating a spatial RNA integrity number for a location on an array includes: (a) contacting a tissue sample stained with a histology stain with an array, wherein the array includes a capture probe attached to a location on the array, and the capture probe comprises a capture domain that specifically binds to a biological analyte from the tissue sample; (b) generating a cDNA molecule from the biological analyte specifically bound to the capture domain; (c) labeling the cDNA by hybridizing a labeled oligonucleotide probe to the cDNA; (e) generating an image of the labeled cDNA and an image of the histology stain, and using the image of the labeled cDNA and the image of the histology stain to generate a spatial RNA integrity number for the location on the array.

In some examples, the tissue sample comprises a tissue section, a region within a tissue, or a single cell within a tissue. In some examples, the histology stain is hematoxylin and eosin. In some examples, the capture domain comprises a poly(T) sequence. In some examples, the biological analyte is 18S rRNA. In some examples, the labeled oligonucleotide probe is fluorescently labeled. In some examples, the step (c) comprises hybridizing at least four different labeled oligonucleotide probes to the cDNA. In some examples, the at least four labeled oligonucleotide probes are sequentially hybridized at different sites within the cDNA. In some examples, the labeled oligonucleotide probes are fluorescently labeled. In some examples, the step of generating an image of the labeled cDNA comprises measuring a fluorescent signal for each of the at least four labeled oligonucleotide probes. In some examples, the fluorescent signal from each of the at least four labeled oligonucleotide probes is used to generate a spatial RNA integrity number for the location on the array.

In another example, a method of determining process bias in a spatial analysis workflow includes: (a) providing a substrate comprising one or more test analytes, wherein the one or more test analytes are disposed on the substrate in a known amount at a known location; (b) contacting the substrate with an array comprising one or more capture probes under conditions that allow the one or more capture probes to interact with the one or more test analytes, wherein a capture probe

comprises a spatial barcode and a capture domain; (c) detecting the one or more test analytes that interact with the one or more capture probes; and (d)

determining whether the spatial analysis workflow accurately detects the presence, amount, location, or combinations thereof, of the one or more test analytes based on the detecting in step (c), thereby determining process bias in the spatial analysis workflow.

In some examples, the substrate comprises a semi-porous material. In some examples the semi-porous material comprises at least one of a nitrocellulose membrane, a hydrogel, a nylon filter, or combinations thereof. In some examples, the one or more test analytes comprises at least one of nucleic acid, a protein, a lipid, or combinations thereof. In some examples, the one or more test analytes is RNA. In some examples, the one or more test analytes are disposed on the substrate in a defined pattern. In some examples, the defined pattern comprises one or more spots.

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.

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

5 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.

10 **FIG. 2** shows an exemplary spatial analysis workflow.

FIG. 3 shows an exemplary spatial analysis workflow.

FIG. 4 shows an exemplary spatial analysis workflow.

FIG. 5 shows an exemplary spatial analysis workflow.

15 **FIG. 6** is a schematic diagram showing an example of a barcoded capture probe, as described herein.

FIG. 7 is a schematic illustrating a cleavable capture probe, wherein the cleaved capture probe can enter into a non-permeabilized cell and bind to target analytes within the sample.

20 **FIG. 8** is a schematic diagram of an exemplary multiplexed spatially-barcoded feature.

FIG. 9 is a schematic diagram of an exemplary analyte capture agent.

FIG. 10 is a schematic diagram depicting an exemplary interaction between a feature-immobilized capture probe **1024** and an analyte capture agent **1026**.

25 **FIG. 11A, 11B, and 11C** are schematics illustrating how streptavidin cell tags can be utilized in an array-based system to produce a spatially-barcoded cells or cellular contents.

FIG. 12 is a schematic showing the arrangement of barcoded features within an array.

30 **FIG. 13** is a schematic illustrating a side view of a diffusion-resistant medium, e.g., a lid.

WHAT IS CLAIMED IS:

1. A method of preparing a biological 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 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
 - aligning the first and second members such that the biological 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 aligning comprises:
 - (i) rotating the first member or the second member about a hinge axis; or
 - (ii) rotating the first and second members until the 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 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 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 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 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 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 sample is aligned with the one or more fiducial markings of the first and/or second members.

14. A method of preparing a biological sample for spatial analysis, the method comprising:
- providing a support device holding a first substrate, a second substrate, and a reagent medium, wherein the first substrate comprises a 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 sample comprises an analyte;
 - aligning the first and second substrates such that the biological sample is aligned with at least a portion of the array when a first member and a second member are aligned, and such that the reagent medium contacts both the biological sample and the array; and
 - passively or actively migrating the analyte from the biological 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.