SAMPLE HANDLING APPARATUS AND FLUID DELIVERY METHODS

BACKGROUND

[0001] Cells within a tissue of a subject have differences in cell morphology and/or function due to varied analyte levels (e.g., gene and/or protein expression) within the different cells. The specific position of a cell within a tissue (e.g., the cell's position relative to neighboring cells or the cell's position relative to the tissue microenvironment) can affect, e.g., the cell's morphology, differentiation, fate, viability, proliferation, behavior, and signaling and cross-talk with other cells in the tissue.

[0002] Spatial heterogeneity has been previously studied using techniques that only provide data for a small handful of analytes in the context of an intact tissue or a portion of a tissue, or provide a lot of analyte data for single cells, but fail to provide information regarding the position of the single cell in a parent biological sample (e.g., tissue sample).

[0003] Analytes within the biological sample are generally released through disruption (e.g., permeabilization) of the biological sample. Various methods of delivering permeabilization reagents to the biological sample are described herein.

SUMMARY

[0004] 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.

[0005] Analytes within a biological sample are generally released through disruption (e.g., permeabilization) of the biological sample or other releasing means. Various methods of disrupting a biological sample are known, including permeabilization of the cell membrane of the biological sample. Described herein are methods of delivering a fluid to the biological sample, systems for sample analysis, and sample alignment methods. Also described herein are methods of delivering a fluid, including for example, a buffer or a permeabilization solutions having various detergents, buffers, proteases, and/or nucleases for different periods of time and at various temperatures.

[0006] ~~In an aspect,~~Disclosed herein is a sample holder~~ is provided~~. In one embodiment, the sample holder can include a first member including a first retaining mechanism configured to retain a first substrate comprising a sample. The first retaining mechanism can be configured to retain the first substrate disposed in a first plane. The sample holder can further include a

second member including a second retaining mechanism configured to retain a second substrate including a reagent medium disposed in a second plane. The sample holder can further includes an alignment mechanism connected to one or both of the first member and the second member. The alignment mechanism can be configured to align the first and second members along the first plane and/or the second plane such that the sample contacts at least a portion of the reagent medium when the first and second members are aligned and within a threshold distance along an axis orthogonal to the second plane. The adjustment mechanism may be configured to move the second member along the axis orthogonal to the second plane and/or move the first member along an axis orthogonal to the first plane. The first substrate and the second substrate may be positioned with a minimum spacing between the first substrate and the second substrate.

[0006] In some variations, one or more features disclosed herein including the following features may optionally be included in any feasible combination. For example, the sample holder may further include a spacing member positioned between the first member and the second member and configured to maintain the minimum spacing between the first member and the second member. The spacing member may be further configured to maintain an approximately parallel arrangement of the first substrate and second substrate. The alignment mechanism may include a pin extending from the second member. The alignment mechanism may include an aperture in the first member, the aperture sized and configured to mate with the pin. The sample holder may further include a coupling member coupled to the first substrate and the second substrate and configured to inhibit movement between the first substrate and the second substrate. The coupling member may include a magnet. The first member may be further configured to retain a third substrate disposed in the first plane. The third substrate may include a second sample.

[0007] The first retaining mechanism may include one or more spring members configured to apply a force to the first substrate to maintain contact between the first substrate and the first member. The second retaining mechanism may include one or more spring members configured to apply a force to the second substrate to maintain contact between the second substrate and the second member. The adjustment mechanism may be coupled to the second member and may include a linear actuator configured to move the second member along the axis orthogonal to the second plane. The linear actuator may be configured to move the second member along the axis orthogonal to the second plane at a velocity of at least 0.1 mm/sec. The linear actuator may be configured to move the second member along the axis orthogonal to the second plane with an amount of force of at least 0.1 lbs. The second member may include a heater configured to heat the first substrate and/or the second substrate. The second member

may further include an insulation gasket coupled to the heater. The first retaining mechanism may be configured to retain the first substrate in a fixed position with respect to the first plane.

[0008] In ~~another~~one aspect, a sample holder is provided. In an embodiment, the sample holder may include a first member including a first retaining mechanism configured to retain a first substrate comprising a sample. The first retaining mechanism may be configured to retain the first substrate disposed in a first plane. The sample may be disposed in a first region of the first substrate. The sample holder may also include a second member including a second retaining mechanism configured to retain a second substrate including a reagent medium disposed in a second plane and disposed in a second region of the second substrate. The sample holder may also include an alignment mechanism connected to one or both of the first member and the second member, and configured to optically align the first region with the second region along the first plane and/or the second plane such that the sample contacts at least a portion of the reagent medium when the first region and the second region are aligned and when the first substrate and the second substrate are separated by a threshold distance. The sample holder may further include an adjustment mechanism configured to move the second member along the axis orthogonal to the second plane. The sample holder may also include an image capture device configured to capture, responsive to the alignment and during a time when the sample is in contact with at least a portion of the reagent medium, an image of the first region and the second region.

[0009] In some variations, one or more features disclosed herein including the following features may optionally be included in any feasible combination. For example, the sample holder may include a spacing member positioned between the first member and the second member and configured to provide a minimum spacing between the first substrate and the second substrate. The spacing member may be further configured to maintain an approximately parallel arrangement of the first substrate and the second substrate. The image capture device may be positioned inferior to the second member. The image capture device may be disposed on a track configured to move the image capture device along the track. The first member may be further configured to retain a third substrate disposed in the first plane, the third substrate comprising a second sample disposed in a third region of the third substrate. The second substrate may include the reagent medium further disposed in a fourth region of the second substrate. The alignment mechanism may be further configured to optically align the third region with the fourth region along the first plane and/or the second plane such that the second sample contacts at least a portion of the reagent medium when the third region and the fourth region are aligned and when the third substrate and the second substrate are separated by the threshold distance. The alignment mechanism may be further configured to optically

align, responsive to optically aligning the first region with the second region, the third region with the fourth region along the first plane and/or the second plane. The alignment mechanism may include a sensor configure to determine a threshold alignment of the first region with the second region and a threshold alignment of the third region with the fourth region. The image capture device may be further configured to capture, responsive to the alignment of the third region with the fourth region and during a time when the second sample is in contact with at least a portion of the reagent medium, an image of the third region and the fourth region. The sample holder may further include a controller configured to control the alignment mechanism, the adjustment mechanism, and/or the image capture device. The controller may be configured to communicate with a user interface, the user interface configured to display information about the sample holder. The controller may be configured to receive a user input from the user interface. The controller may be configured to control the alignment mechanism, the adjustment mechanism, and/or the image capture device responsive to the user input. The sample holder may further include a sensor, wherein the controller is further configured to control, based on the sensor, the alignment mechanism, the adjustment mechanism, and/or the image capture device. The first retaining mechanism may be configured to retain the first substrate in a fixed position with respect to the first plane. The second member may further include a spring mechanism configured to couple to at least a portion of an inferior surface of the second substrate. The second member may further include a base configured to couple to an inferior surface of the second substrate and retain the second substrate on the second member. The spring mechanism may extend in a superior direction from the base. The spring mechanism may be configured to dispose the second substrate along the second plane at an angle different than the base. The first member may be configured to dispose the first substrate along the first plane at an angle parallel with the base. The adjustment mechanism may be configured to move the second member toward the first member such that the first substrate contacts at least a portion of the reagent medium. The reagent medium may be disposed in proximity and superior to the spring mechanism. The first substrate contacting the at least a portion of the reagent medium may urge the reagent medium in an opposite direction such that the reagent medium is displaced over the second substrate and/or the first substrate as the second member moves toward the first member and the second substrate and the first substrate become substantially parallel. The adjustment mechanism may be configured to move the second member toward the first member such that the spring mechanism compresses so that the first substrate, the second substrate, and the base are substantially parallel. The sample holder may further include a sensor configured to detect a bubble in the reagent medium responsive to the sample contacting at least a portion of the reagent medium.

[0010] ~~In another aspect,~~Disclosed herein is a method of capturing an analyte from a biological sample disposed in a first region of a first substrate ~~is provided~~. In an embodiment, the method may include mounting the first substrate on a first member of a support device, the first substrate disposed in a first plane, the first member configured to retain the first substrate in a fixed position with respect to the first plane. The method can further include mounting a second substrate on a second member of the support device. The second substrate can be disposed in a second plane and comprising a second region including a plurality of second capture probes. A second capture probe of the plurality of second capture probes can comprises a spatial barcode and a second capture domain. The method can further include aligning, along the first plane and/or the second plane, the first region with the second region such that the first region and the second region can be vertically aligned when the first substrate is positioned superior to the second substrate. The method can further include applying a reagent medium to the first substrate and/or the second substrate. The reagent medium can provide a permeabilization buffer between the biological sample and the second substrate. The method can further include positioning, responsive to the aligning and the applying, the second substrate such that the biological sample contacts at least a portion of the reagent medium when the first and second members are aligned and within a threshold distance along an axis orthogonal to the second plane allowing the analyte to migrate from the biological sample to the second substrate. The analyte can bind to the second capture domain.

[0011] In some variations, one or more features disclosed herein including the following features may optionally be included in any feasible combination. For example, the method may further include capturing, responsive to the positioning and during a time when the biological sample is in contact with at least a portion of the reagent medium, an image of the first region and the second region. The positioning may include compressing the reagent medium between the first substrate and the second substrate by moving the second member toward the first member to a minimum separation distance maintained by a spacing member positioned between the first substrate and the second substrate.

[0012] ~~In another aspect,~~Disclosed herein is a method for aligning a sample area with an array area ~~is provided~~. In an embodiment, the method may include receiving a first substrate within a first retaining mechanism of a sample holder. The first substrate may include a sample and the sample area. The method may also include receiving a second substrate within a second retaining mechanism of the sample holder. The second substrate may include an array, wherein the second substrate or the sample holder comprises an array area indicator associated with the array area of the second substrate. The method may also include adjusting a location of the

first substrate relative to the second substrate to cause all or a portion of the sample area to be aligned with the array area.

[0013] ~~In another aspect,~~Disclosed herein is a method for aligning a sample area with an array area~~ is provided~~. In an embodiment, the method may include receiving a first substrate within a first retaining mechanism of a sample holder. The first retaining mechanism comprises an array area indicator associated with the array area. The method may also include receiving a second substrate within a second retaining mechanism of the sample holder. The second substrate comprising an array, wherein the second substrate comprising a sample and the sample area. The method may further include adjusting a location of the first substrate relative to the second substrate to cause all or a portion of the sample area to be aligned with the array area.

[0014] In some variations, one or more features disclosed herein including the following features may optionally be included in any feasible combination. For example, the first substrate may include a sample area indicator associated with the sample area of the first substrate and the adjusting further causes the sample area indicator to be aligned with respect to the array area indicator. The method may also include applying the sample area indicator to a first side of the first substrate prior to applying the sample to the first side of the first substrate. The sample area indicator may include a drawing applied to the first substrate by a user. The method may also include applying the sample area indicator to a second side of the first substrate after applying the sample to the first side of the first substrate. The sample area indicator may include a stamp or a sticker. Applying the sample area indicator to the second side of the first substrate may include drawing the sample area indicator with a marker by a user. The first substrate includes a fiducial mark.

[0015] The method can further include receiving, by a data processor of a computing device communicatively coupled to the sample holder, an image of the sample. The method can also include providing, by the data processor, the image of the sample for display via a display of the computing device. The method can further include receiving, by the data processor, an input identifying the sample area indicator based on the provided image. The received input may be provided to the computing device by a user, or by a remote computing device communicatively coupled to the computing device. The method may also include automatically determining, by the data processor, the sample area indicator, based on the image.

[0016] Adjusting the location of the first substrate relative to the second substrate can include viewing the first substrate and the second substrate within the sample holder. Adjusting the location of the first substrate relative to the second substrate can also include adjusting the first

retaining mechanism and/or the second retaining mechanism to cause all or the portion of the sample area to be aligned with the array area. The array area indicator may be provided on the sample holder. The second substrate may be fixed in place within the sample holder and the first retaining mechanism is adjusted to cause all or the portion of the sample area to be aligned with the array area. The array area location indicator may be provided on a transparent surface of the second retaining mechanism. The array area location indicator may be provided on a first surface of the second retaining mechanism. The first surface may be opposite a second surface of the second retaining mechanism at which the second substrate is received.

[0017] The method may further include receiving, by a first data processor of a first computing device communicatively coupled to the sample holder, a plurality of video images acquired via a microscope coupled to the first computing device. The plurality of video images displaying the second substrate may be overlaid atop the first substrate within the sample holder. The method may also include providing, by the data processor, the plurality of video images for display via a display of the first computing device. The method may further include adjusting the first retaining mechanism to cause the sample area to be aligned with the array area. The method may also include providing the plurality of video images to a second data processor of a second computing device remote from and communicatively coupled to the first computing device. The second computing device configured to provide the plurality of video images for display. The second computing device may be further configured to receive an input from a user identifying the sample area indicator. The data processor of the second computing device may be further configured to control adjusting the location of the first substrate and/or the second substrate to cause the sample area to be aligned with the array area via a controller coupled to the sample holder and to the first computing device.

[0018] The method may also include receiving, by a first data processor of a first computing device communicatively coupled to the sample holder, a plurality of video images acquired via a microscope coupled to the computing device. The plurality of video images displaying the second substrate overlaid atop the first substrate within the sample holder. The method may further include automatically determining, by the first data processor, a sample area indicator on the first substrate based on the plurality of video images. The method may also include performing the adjusting automatically, by the first data processor, based on the automatically determined sample area indicator. Performing the aligning automatically may further include determining, by the first data processor, an area of the sample relative to an area of the array. Performing the aligning automatically may also include automatically determining a sample area indicator on the first substrate responsive to determining the area of the sample is less than the area of the array. Performing the aligning automatically may also include providing, by

the first data processor, the sample area indicator as an outline of the sample. Performing the aligning automatically may further include performing the adjusting automatically, by the first data processor, based on the outline of the sample.

[0019] Performing the adjusting automatically may further include determining, by the first data processor, a fiducial mark located on the first substrate. Performing the adjusting automatically may also include performing the adjusting automatically, by the first data processor, based on the determined fiducial mark.

[0020] The method may also include receiving, by the data processor of the first computing device, an image of a sample and a sample area indicator from a second computing device communicatively coupled to the first computing device. The second computing device configured to acquire and provide the image of the sample and the sample area indicator to the data processor of the first computing device. The method may further include registering, by the data processor of the first computing device, the received image of the sample and the sample area indicator with at least one video image of the plurality of video images. The method may also include providing, by the data processor of the first computing device and based on the registering, a registered sample image via a display of the first computing device. The method may further include receiving an input to the first computing device identifying the sample area indicator based on the registered sample image. The method may also include performing the adjusting automatically based on the received input identifying the sample area indicator.

[0021] The array may be configured to capture analytes from the sample. The array area indicator may be located on the second substrate. The second substrate may include a reagent medium, and all or the portion of the sample area are aligned with the array area without the reagent medium contacting the sample.

[0022] ~~In another aspect,~~Disclosed herein is a system for aligning a sample area with an array area~~ is provided~~. In an embodiment, the system may include a sample holder. The sample holder may include a first retaining mechanism configured to retain a first substrate received within the first retaining mechanism. The first substrate comprising a sample and the sample area. The sample holder may also include a second retaining mechanism configured to retain a second substrate received within the second retaining mechanism. The second substrate may include an array, wherein the second substrate or the sample holder comprises an array area indicator associated with the array area of the second substrate or the sample holder. The sample holder may be configured to adjust a location of the first substrate relative to the second substrate to cause all or a portion of the sample area to be aligned with the array area. The sample holder may also include an image capture device (e.g., microscope) operatively coupled

to the sample holder and configured to view the first substrate and the second substrate within the sample holder. The sample holder may also include a first computing device communicatively coupled to the image capture device (e.g., microscope) and to the sample holder. The computing device may include a display, a first data processor, and a non-transitory computer readable storage medium storing computer readable and executable instructions, which when executed cause the first data processor to adjust the location of the first substrate relative to the second substrate to cause all or the portion of the sample area to be aligned with the array area.

[0023] In some variations, one or more features disclosed herein including the following features may optionally be included in any feasible combination. For example, the first substrate may include a sample area indicator associated with the sample area of the first substrate and the adjusting further causes the sample area indicator to be aligned with respect to the array area indicator. The sample area indicator may be applied to a first side of the first substrate prior to applying the sample to the first side of the first substrate. The sample area indicator may include a drawing applied to the first substrate by a user. The sample area indicator may be applied to a second side of the first substrate after applying the sample to the first side of the first substrate. The sample area indicator may include a stamp or a sticker. Applying the sample area indicator to the second side of the first substrate may include drawing the sample area indicator with a marker by a user. The first substrate may include a fiducial mark. The instructions may further cause the first data processor to perform operations including receiving an image of the sample acquired via the microscope. The instructions may further cause the first data processor to perform operations including providing the image of the sample for display via the display of the first computing device. The instructions may further cause the first data processor to perform operations including receiving an input identifying the sample area indicator based on the provided image.

[0024] The received input may be provided to the first computing device by a user, or by a remote computing device communicatively coupled to the first computing device. The instructions may further cause the first data processor to perform operations including automatically determining the sample area indicator, based on the image. Adjusting the location of the first substrate relative to the second substrate may include viewing the first substrate and the second substrate within the sample holder via the microscope. Adjusting the location of the first substrate relative to the second substrate may also include adjusting the first retaining mechanism and/or the second retaining mechanism to cause all or the portion of the sample area to be aligned with the array area. The array area indicator may be provided on the sample holder. The second substrate may be fixed in place within the sample holder and

the first retaining mechanism is adjusted to cause all or the portion of the sample area to be aligned with the array area. The array area location indicator may be provided on a transparent surface of the second retaining mechanism. The array area location indicator may be provided on a first surface of the second retaining mechanism, the first surface opposite a second surface of the second retaining mechanism at which the second substrate is received.

[0025] The instructions may further cause the first data processor to perform operations including receiving a plurality of video images acquired via the microscope, the plurality of video images displaying the second substrate overlaid atop the first substrate within the sample holder. The instructions may further cause the first data processor to perform operations including providing the plurality of video images for display via the display of the first computing device. The instructions may further cause the first data processor to adjust the location of the first substrate relative to the second substrate by adjusting the first retaining mechanism to cause the sample area to be aligned with the array area location. The instructions may further cause the first data processor to provide the plurality of video images to a second data processor of a second computing device remote from and communicatively coupled to the first computing device. The second computing device may be configured to provide the plurality of video images for display.

[0026] The second computing device may be further configured to receive an input from a user identifying the sample area indicator. The second data processor may be further configured to control adjusting the location of the first substrate and/or the second substrate to cause the sample area to be aligned with the array area via a controller coupled to the sample holder and to the first computing device. The instructions may further cause the first data processor to perform operations including receiving a plurality of video images acquired via the image capture device (e.g., microscope), the plurality of video images displaying the second substrate overlaid atop the first substrate within the sample holder. The instructions may further cause the first data processor to perform operations including automatically determining a sample area indicator on the first substrate based on the plurality of video images. The instructions may further cause the first data processor to perform operations including automatically adjusting the location of the first substrate relative to the second substrate to cause all or the portion of the sample area to be aligned with the array area based on the automatically determined sample area indicator.

[0027] Automatically adjusting the location of the first substrate relative to the second substrate may also include automatically determining a sample area on the first substrate indicator responsive to determining the sample area of the first substrate is smaller than the array area of the second substrate. Automatically adjusting the location of the first substrate

relative to the second substrate may also include providing the sample area indicator as an outline of the sample via the display. Automatically adjusting the location of the first substrate relative to the second substrate may also include performing the adjusting automatically based on the outline of the sample. Automatically adjusting the location of the first substrate relative to the second substrate may also include determining a fiducial mark located on the first substrate. Automatically adjusting the location of the first substrate relative to the second substrate may also include performing the adjusting automatically based on the determined fiducial mark.

[0028] The instructions may further cause the first data processor to perform operations including receiving an image of the sample and the sample area indicator from a second computing device communicatively coupled to the first computing device. The second computing device configured to acquire and provide the image of the sample and the sample area indicator to the first data processor of the first computing device. The instructions may further cause the first data processor to perform operations including registering the received image of the sample and the sample area indicator with at least one video image of the plurality of video images. The instructions may further cause the first data processor to perform operations including providing a registered sample image based on the registering, the registered sample image provided via the display of the first computing device. The instructions may further cause the first data processor to perform operations including receiving an input to the first computing device identifying the sample area indicator within the registered sample image. The instructions may further cause the first data processor to perform operations including performing the adjusting automatically based on the identified sample area indicator.

[0029] The array may be configured to capture analytes from the sample. The array area indicator may be located on the second substrate. The second substrate may include a reagent medium, and all or the portion of the sample area are aligned with the array area without the reagent medium contacting the sample.

[0030] Also provided herein are methods for delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second substrate. In an embodiment, the method can include delivering a fluid to the area on the first substrate. A virtual gasket can surround the area on the first substrate and can contain the fluid within the area. The method can also include assembling the second substrate with the first substrate, thereby delivering the fluid to the array and the biological sample.

[0031] Also provided herein are methods for delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second substrate. The method can include delivering a fluid to the area on the second substrate. A virtual gasket can surround

the area on the second substrate and contains the fluid on the array. The method can also include assembling the first substrate with the second substrate, thereby delivering the fluid to the array and the biological sample.

[0032] In some embodiments, the first substrate, the second substrate, or both can include a glass surface. In some embodiments delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second substrate, the glass surface can be a glass slide.

[0033] In some embodiments, the first substrate and the second substrate can be axially aligned. In some embodiments, the first substrate and the second substrate can be in a cross configuration. In some embodiments, the virtual gasket may include a hydrophobic coating.

[0034] ~~In another aspect,~~Disclosed herein are a method for delivering fluid to a biological sample disposed on a first substrate and an array disposed on a second substrate ~~are provided~~. In embodiment, the method may include delivering the fluid to the first substrate and/or the second substrate, wherein at least one of the first substrate and the second substrate comprising a spacer. The method may also include assembling, subsequent to the delivering, a chamber including the first substrate, the second substrate, the biological sample, and the spacer. The spacer may be disposed between the first substrate and second substrate and may be configured to maintain the fluid within the chamber and maintain a separation distance between the first substrate and the second substrate. The spacer may be positioned to at least partially surround an area on the first substrate on which the biological sample may be disposed and/or the array disposed on the second substrate. The area of the first substrate, the spacer, and the second substrate may at least partially encloses a volume comprising the biological sample.

[0035] In some variations, one or more features disclosed herein including the following features may optionally be included in any feasible combination. For example, the chamber may include a partially or fully sealed chamber. The second substrate may include a hydrophobic area positioned away from a region of interest. The hydrophobic area may be configured to remove bubbles in the fluid from the chamber. The region of interest may include an area where the biological sample and the array overlap. The hydrophobic area may include a hydrophobic coating. The separation distance may include a distance of at least 2 μm. The separation distance may include a distance between about 5 μm to 25 μm. The second substrate may include the spacer. The first substrate may include the spacer. The method may further include, prior to delivering the fluid, applying the hydrophilic coating to the first substrate and/or the second substrate. The fluid may include a wetting agent. The fluid may include one or more permeabilization reagent(s). The spacer may include an air permeable spacer portion configured to vent a bubble from the fluid.

[0036] The method may include generating vibration to the first substrate and/or the second substrate. Delivering the fluid to the first substrate and/or the second substrate may include adjusting a humidity of the chamber. Delivering the fluid to the first substrate and/or the second substrate may include generating a vacuum to a region proximate to the first substrate and/or the second substrate. Delivering the fluid to the first substrate and/or the second substrate may include delivering the fluid to a region of the second substrate, the spacer comprising three sides partially surrounding the fluid. Delivering the fluid to the first substrate and/or the second substrate may include delivering the fluid to a region of the second substrate, the region outside an enclosed area of the second substrate, the enclosed area formed by the spacer. Delivering the fluid to the first substrate and/or the second substrate may include delivering the fluid to a region of the spacer, the region outside an enclosed area of the second substrate, the enclosed area formed by the spacer.

[0037] Assembling the chamber may include positioning, responsive to the delivering, the first substrate at an angle such that a dropped side of the first substrate contacts at least a portion of the fluid when the first substrate and the second substrate are within a threshold distance along an axis orthogonal to the second substrate. The dropped side may urge the fluid toward the three sides partially surrounding the fluid. The chamber may include a hydrophobic pattern at least partially surrounding the fluid and positioned to at least partially surround the area on the first substrate and/or the array disposed on the second substrate. The spacer may be formed from a uniform thickness material. The spacer may be formed from a material with a variable thickness. The spacer may be printed on the first substrate and/or the second substrate. The spacer may include a photoresist pattern. The spacer may include a beveled edge on one or more sides of the spacer.

[0038] ~~In another aspect,~~Disclosed herein is a system ~~is provided~~. In an embodiment, the system may include a first substrate comprising a coating for adhering a biological sample. The system may also include a second substrate comprising an array. The system may further include a spacer disposed between the first substrate and second substrate and configured to maintain a fluid within a chamber comprising the first substrate, the second substrate, the biological sample, and the spacer. The spacer may be further configured to maintain a separation distance between the first substrate and the second substrate. The spacer may be positioned to at least partially surround the biological sample on the first substrate and/or the array disposed on the second substrate.

[0039] ~~In another aspect,~~Disclosed herein is a kit ~~is provided~~. In an embodiment, the kit may include a second substrate comprising an array. The second substrate may further include a spacer surrounding the array. In another embodiment, a kit is provided. In an embodiment,

the kit may include a first substrate comprising a biological sample. The first substrate may further include a spacer surrounding the biological sample. In another embodiment, a kit is provided. In an embodiment, the kit may include a spacer configured to be disposed between a first substrate and second substrate, and instructions for use according to a method of any one of the preceding claims.

[0040] ~~In another aspect,~~Disclosed herein is a system for aligning a sample area with an array area~~ is provided~~. In an embodiment, the system may include a sample holder. The sample holder may include a first retaining mechanism configured to retain a first substrate received within the first retaining mechanism, the first substrate comprising a sample and the sample area. The sample holder may also include a second retaining mechanism configured to retain a second substrate received within the second retaining mechanism. The second substrate may include an array, wherein the second substrate or the sample holder comprises an array area indicator associated with the array area of the second substrate or the sample holder. The sample holder may be configured to adjust a location of the first substrate relative to the second substrate to cause all or a portion of the sample area to be aligned with the array area. The sample holder may further include at least one image capture device operatively coupled to the sample holder and configured to view the first substrate and the second substrate within the sample holder. The sample holder may also include a first computing device communicatively coupled to the at least one image capture device and to the sample holder. The computing device may include a display, a first data processor, and a non-transitory computer readable storage medium storing computer readable and executable instructions, which when executed cause the first data processor to