permeabilization solutions having various detergents, buffers, proteases, and/or nucleases for different periods of time and at various temperatures.

Additionally, various methods of delivering fluids (e.g., a permeabilization solution) to a biological sample are described herein including the use of a substrate holder.

In one example, a method for delivering a fluid to biological sample disposed on a first substrate and an array disposed on a second substrate is provided. The method includes delivering the fluid to the first substrate and/or the second substrate. At least one of the first substrate and the second substrate including a spacer. The method further includes assembling, subsequent to the delivering, the chamber including the first substrate, the second substrate, the biological sample, and the spacer. The spacer is disposed between the first substrate and the second substrate. The spacer is configured to maintain the fluid within the chamber and maintain a separation distance between the first substrate and the second substrate. The spacer is positioned to at least partially surround an area on the first substrate on which the biological sample is disposed and/or at least partially surround the array disposed on the second substrate. The area of the first substrate, the spacer, and the second substrate at least partially encloses a volume including the biological sample.

In some variations of the method, the chamber includes a partially or fully sealed chamber. The second substrate includes 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 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 applying, prior to delivering the fluid, a 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. The method may further include generating a 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 may include 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 may be disposed outside an enclosed area of the second substrate. The enclosed area may be 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 disposed outside an enclosed area of the second substrate. The enclosed area may be formed by the spacer. 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.

In one example, a system is provided. The system includes a first substrate including a coating for adhering a biological sample. The system further includes a second substrate including an array. The system further includes a spacer disposed between the first substrate and the 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 further configured to maintain a separation distance between the first substrate and the second substrate, the spacer positioned to at least partially surround the biological sample on the first substrate and/or at least partially surround the array disposed on the second substrate.

In one example, a kit is provided. The kit includes a second substrate including an array. The second substrate further includes a spacer surrounding the array.

In another example, a kit is provided. The kit includes a first substrate including a biological sample. The first substrate further includes a spacer surrounding the biological sample.

In one example, a kit is provided. The kit includes a spacer configured to be disposed between a first substrate and the second substrate. The kit further includes instructions for use according to a method described herein.

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, comprising: assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological

sample, and a gasket, wherein the gasket is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the area of the first substrate, the gasket, and the second substrate at least partially encloses a volume comprising the biological sample. The method further comprises delivering the fluid to the partially sealed chamber through one or more apertures of the gasket, thereby delivering the fluid to the array and the biological sample.

In some examples, the method further includes prior to assembling, providing the biological sample on the area of the first substrate and the array disposed on the second substrate.

In some examples, the first substrate, the second substrate, or both comprises a glass surface. In some examples, the glass surface is a glass slide. In some examples, the first substrate and the second substrate are axially aligned. In some examples, the first substrate and the second substrate are in a cross-configuration.

In some examples, delivering the fluid includes flowing the fluid through the one or more apertures of the gasket. Delivering the fluid may include injecting the fluid through the one or more apertures of the gasket using a syringe. In some examples, the syringe is configured to interface or lock with one of the one or more apertures of the gasket. In some examples, delivering the fluid may be at a temperature of about 5°C to about 80°C.

In some examples, the method further includes permeabilizing the biological sample. In some examples, the second substrate may include one or more dried permeabilization reagent(s) disposed thereon. In some examples, the fluid solubilizes the one or more dried permeabilization reagent(s). In some examples, the fluid comprises one or more permeabilization reagent(s). In some examples, the permeabilizing is performed for about 1 minute to about 90 minutes. In some examples, the permeabilizing may include heating the first substrate, the second substrate, or both the first substrate and the second substrate. In some examples, the permeabilizing of the biological sample may be controlled by modulating the temperature of the fluid. In some examples, modulating the temperature of the fluid may include heating the fluid to at least about 40°C.

In some examples, the one or more permeabilization reagent(s) may be selected from the group consisting of: a detergent, an enzyme, and a buffer. In some examples, the one or more dried permeabilization reagent(s) comprises one or both of a detergent and an enzyme. In some examples, the detergent comprises one or more of SDS, N-lauroylsarcosine, saponin, or any combination thereof. In some examples, the enzyme may

include one or more of proteinase K, pepsin, collagenase, trypsin, or any combination thereof. In some examples, the buffer may include TE, TAE, TBE, or PBS.

In some examples, delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer sequentially in any order. In some examples, delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer as a mixture. In some examples, delivering may include delivering the one or more permeabilization reagent(s) two or more times to the partially sealed chamber. In some examples, the one or more apertures may include a hydrophobic coating.

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 comprising: assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a gasket, wherein a gasket is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the first substrate, the gasket, and the second substrate at least partially encloses a volume comprising the biological sample. The method further comprising delivering the fluid to the partially sealed chamber through one or more via-holes in the first substrate or one or more via-holes in the second substrate, thereby delivering the fluid to the array and the biological sample.

In some examples, the method further includes prior to assembling, providing the biological sample on the area of the first substrate and the array disposed on the second substrate.

In some examples, the first substrate, the second substrate, or both comprises a glass surface. In some examples, the glass surface is a glass slide. In some examples, the first substrate and the second substrate are axially aligned. In some examples, the first substrate and the second substrate are in a cross-configuration.

In some examples, delivering the fluid includes flowing the fluid through via-holes in the first substrate. In some examples, delivering the fluid comprises flowing the fluid through one or more via-holes in the second substrate. Delivering the fluid may include injecting the fluid through the one or more via-holes using a syringe. In some examples, the syringe is configured to interface or lock with one of the one or more via-holes in the first substrate. In some examples, the syringe is configured to interface or lock with one of the one or more via-holes in the second substrate.

In some examples, delivering the fluid comprises delivering the fluid to one or more via-holes in the first substrate by capillary flow. In some examples, delivering the fluid comprises delivering the fluid to one or more via-holes in the second substrate by capillary flow.

In some examples of delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second substrate, delivering the fluid is at temperature of about 5°C to about 80°C.

In some examples, the method includes permeabilizing the biological sample. In some examples, the substrate comprises one or more dried permeabilization reagent(s) disposed thereon. In some examples, the fluid solubilizes the one or more dried permeabilization reagent(s). In some examples, the fluid comprises one or more permeabilization reagent(s). In some examples, the permeabilizing is performed for about 1 minute to about 90 minutes. In some examples, the permeabilizing comprises heating the first substrate, the second substrate, or both the first substrate and the second substrate. In some examples, the permeabilizing of the biological sample is controlled by modulating the temperature of the fluid. In some examples, modulating the temperature of the fluid comprises heating the fluid to at least about 40°C.

In some examples, the one or more permeabilization reagent(s) may be selected from the group consisting of: a detergent, an enzyme, and a buffer. In some examples, the one or more dried permeabilization reagent(s) comprises one or both of a detergent and an enzyme. In some examples, the detergent comprises one or more of SDS, N-lauroylsarcosine, saponin, or any combination thereof. In some examples, the enzyme may include one or more of proteinase K, pepsin, collagenase, trypsin, or any combination thereof. In some examples, the buffer may include TE, TAE, TBE, or PBS.

In some examples, delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer sequentially in any order. In some examples, delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer as a mixture. In some examples, delivering may include delivering the one or more permeabilization reagent(s) two or more times to the partially sealed chamber.

Also provided herein are method 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 comprising delivering a fluid to the area on the first substrate, wherein a virtual gasket surrounds the area on the first substrate and contains the fluid within the area; and assembling

the second substrate with the first substrate, thereby delivering the fluid to the array and the biological sample.

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 comprising: delivering a fluid to the area on the second substrate, wherein a virtual gasket surrounds the area on the second substrate and contains the fluid on the array; and assembling the first substrate with the second substrate, thereby delivering the fluid to the array and the biological sample.

In some examples, prior to assembling, providing the biological sample on the area of the first substrate and the array disposed on the second substrate.

In some examples, the first substrate, the second substrate, or both comprises a glass surface. In some examples, the glass surface is a glass slide.

In some examples, the first substrate and the second substrate are axially aligned. In some examples, the first substrate and the second substrate are in a cross configuration.

In some examples, the virtual gasket comprises a hydrophobic coating. In some examples, the virtual gasket includes one or more apertures. In some examples, the hydrophobic coating is applied with a stamp. In some examples, the hydrophobic coating is a paraffin-based wax. In some examples, the hydrophobic coating covers the all, or a portion of, the first substrate outside the area on the first substrate. In some examples, the hydrophobic coating covers all, or a portion of, the second substrate outside the array on the second substrate. In some examples, the hydrophobic coating is applied in a pattern. In some examples, the hydrophobic coating comprises electrowetting.

In some examples, delivering the fluid comprises delivering the fluid to the first substrate. In some examples, delivering the fluid comprises delivering the fluid to the second substrate. In some examples, delivering the fluid comprises flowing the fluid through the one or more apertures of the virtual gasket. In some examples, delivering the fluid comprises injecting the fluid through the one or more apertures of the gasket with a syringe.

In some examples, the syringe is configured to interface or lock with one of the one or more apertures of the virtual gasket. In some examples, delivering the fluid comprises flowing the fluid by capillary flow. In some examples, delivering the fluid is at temperature of about 5°C to about 80°C.

In some examples, the method includes permeabilizing the biological sample. In some examples, the second substrate comprises one or more dried permeabilization

reagent(s) disposed thereon. In some examples, the fluid solubilizes the one or more dried permeabilization reagent(s). In some examples, the fluid comprises one or more permeabilization reagent(s). In some examples, the permeabilizing is performed for about 1 minute to about 90 minutes. In some examples, the permeabilizing comprises heating the first substrate, the second substrate, or both the first substrate and the second substrate. In some examples, the permeabilizing of the biological sample is controlled by modulating the temperature of the fluid. In some examples, modulating the temperature of the fluid comprises heating to at least about 40°C.

In some examples, the one or more permeabilization reagent(s) is/are selected from the group consisting of: a detergent, an enzyme, and a buffer. In some examples, the one or more dried permeabilization reagent(s) comprises one or both of a detergent and an enzyme. In some examples, the detergent comprises one or more of SDS, N-lauroylsarcosine, saponin, or any combination thereof. In some examples, the enzyme comprises one or more of proteinase K, pepsin, collagenase, trypsin, or any combination thereof. In some examples, the buffer comprises TE, TAE, TBE, and PBS. In some examples, delivering the one or more permeabilization reagent(s) comprises delivering the detergent, the enzyme, and the buffer sequentially in any order. In some examples, delivering the one or more permeabilization reagent(s) comprises delivering the detergent, the enzyme, and the buffer as a mixture. In some examples, delivering comprises delivering the one or more permeabilization reagent(s) two or more times to the partially sealed chamber.

In some examples, patterning the hydrophobic coating surrounds one or more regions of interest in the biological sample.

In some examples, the method includes imaging the biological sample. In some examples, the biological sample is a tissue section. In some examples, the biological sample is a fresh-frozen tissue section. In some examples, the biological sample is a fixed biological sample. In some examples, the fixed biological sample is a formalin-fixed paraffin-embedded biological sample.

In some examples, the array comprises a plurality of features. In some examples, the array comprises about 5,000 features. In some examples, a feature of the plurality of features is selected from the group consisting of a bead, a spot, an inkjet spot, a well, a hydrogel pad, and a nanoparticle. In some examples, a plurality of capture probes are attached to the bead. In some examples, a capture probe of the plurality of capture probes comprises (i) a capture domain; and (ii) a spatial barcode unique to the feature. In some examples, the capture domain of the capture probe comprises a poly(T) sequence. In some

examples, the capture probe includes one or more of: a functional domain, a cleavage domain, a unique molecular identifier, or any combination thereof.

Also provided herein are kits including a first substrate comprising a coating for adhering a biological sample; a second substrate comprising an array; and a gasket. In some kits, the gasket comprises no apertures. In some examples, the gasket comprises one aperture for fluid delivery. In some kits, the gasket comprises two apertures for fluid delivery. In some kits, the first substrate comprises one or more via-holes for fluid delivery. In some kits, the second substrate comprises one or more via-holes for fluid delivery. In some kits, the kit includes a paraffin-based crayon. In some kits, the kit includes a hydrophobic coating stamp. In some kits, the kit includes a reverse transcriptase and a nuclease. In some kits, the kit includes one or more permeabilization agent(s). In some kits, the first substrate, the second substrate, and the gasket are assembled into a partially sealed chamber. In some kits, the kit includes instructions for assembling a partially sealed chamber.

Also provided herein is a method for delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second area of a second substrate. The method includes assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a spacer, wherein the spacer is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the area of the first substrate, the spacer, and the second substrate at least partially encloses a volume comprising the biological sample. The method further includes delivering the fluid to the partially sealed chamber, thereby delivering the fluid to the array and the biological sample.

In some variations of the method, the method includes prior to assembling, providing the biological sample on the area of the first substrate and providing the array disposed on the second substrate. The first substrate, the second substrate, or both may include a glass surface. The glass surface may include a glass slide. The first substrate and the second substrate may be axially aligned. The first substrate and the second substrate may be in a cross configuration. Delivering the fluid comprises flowing the fluid through the one or more apertures of the spacer. In some examples, delivering the fluid comprises injecting the fluid through the one or more apertures of the spacer using a syringe. The syringe may be configured to interface or lock with one of the one or more apertures of the spacer. Delivering the fluid may be at a temperature of about 5°C to about 80°C. The method may include permeabilizing the biological sample. The second substrate may include one or more dried permeabilization reagent(s) disposed thereon. The fluid may solubilize the one or more dried permeabilization reagents(s).

The fluid may include one or more permeabilization reagent(s). The permeabilizing may be performed for about 1 minute to about 90 minutes. The permeabilizing may include heating the first substrate, the second substrate, or both the first substrate and the second substrate. The permeabilizing of the biological sample may be controlled by modulating the temperature of the fluid. Modulating the temperature of the fluid may include heating to at least about 40°C. The one or more permeabilization reagent(s) may be selected from the group consisting of: a detergent, an enzyme, and a buffer. The one or more dried permeabilization reagent(s) may include one or both of a detergent and an enzyme. The detergent may include one or more of SDS, N-lauroylsarcosine, saponin, or any combination thereof. The enzyme may include one or more of proteinase K, pepsin, collagenase, trypsin, or any combination thereof. The buffer may include TE, TAE, TBE, and PBS. Delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer sequentially in any order. Delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer as a mixture. Delivering may include delivering the one or more permeabilization reagent(s) two or more times to the partially sealed chamber. The one or more apertures may include a hydrophobic coating.

Also provided herein is a method for delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second area of a second substrate. The method includes delivering a fluid to the second area on the second substrate. A spacer surrounds the second area on the second substrate and contains the fluid on the array. The method further includes assembling the first substrate with the second substrate, thereby delivering the fluid to the array and the biological sample.

In some variations of the method, the method includes, prior to assembling, providing the biological sample on the area of the first substrate and providing the array disposed on the second substrate. The first substrate, the second substrate, or both may include a glass surface. The glass surface may include a glass slide. The first substrate and the second substrate may be axially aligned. The first substrate and the second substrate may be in a cross configuration. Delivering the fluid may include flowing the fluid through one or more via-holes in the first substrate. Delivering the fluid may include flowing the fluid through one or more via-holes in the second substrate. In some examples, delivering the fluid comprises injecting the fluid through the one or more via-holes using a syringe. The syringe may be configured to interface or lock with one of the one or more via-holes in the first substrate. The syringe may be configured to interface or lock with one of the one or more via-holes in the second substrate. Delivering the fluid may include delivering the fluid to one or more via-holes in the first

substrate by capillary flow. Delivering the fluid may include delivering the fluid to one or more via-holes in the second substrate by capillary flow. Delivering the fluid may be at a temperature of about 5°C to about 80°C. The method may include permeabilizing the biological sample. The second substrate may include one or more dried permeabilization reagent(s) disposed thereon. The fluid may solubilize the one or more dried permeabilization reagent(s). The fluid may include one or more permeabilization reagent(s). The permeabilizing may be performed for about 1 minute to about 90 minutes. The permeabilizing may include heating the first substrate, the second substrate, or both the first substrate and the second substrate. The permeabilizing of the biological sample may be controlled by modulating the temperature of the fluid. Modulating the temperature of the fluid may include heating to at least about 40°C. The one or more permeabilization reagent(s) may be selected from the group consisting of: a detergent, an enzyme, and a buffer. The one or more dried permeabilization reagent(s) may include one or both of a detergent and an enzyme. The detergent may include one or more of SDS, N-lauroylsarcosine, saponin, or any combination thereof. The enzyme may include one or more of proteinase K, pepsin, collagenase, trypsin, or any combination thereof. The buffer may include TE, TAE, TBE, and PBS. Delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer sequentially in any order. Delivering the one or more permeabilization reagent(s) may include delivering the detergent, the enzyme, and the buffer as a mixture. Delivering may include delivering the one or more permeabilization reagent(s) two or more times to the partially sealed chamber.

Also provided herein is a system. The system comprising a first substrate for adhering a biological sample. The system further comprising a second substrate comprising an array. The system further comprising a spacer disposed between the first substrate and the 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 further configured to maintain a separation distance between the first substrate and the second substrate, the spacer positioned to at least partially surround the biological sample on the first substrate and/or the array disposed on the second substrate.

In some variations of the system, the first substrate comprises the biological sample mounted thereon. The system of any one of the preceding examples may further include a sample holder. In some examples, the sample holder comprises a first member comprising a first retaining mechanism configured to retain the first substrate disposed in a first plane, a second member comprising a second retaining mechanism configured to retain the second substrate disposed in the second plane, and an alignment mechanism connected to one or both of the first

member and the second member, and 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 sample holder further comprises an adjustment mechanism 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 to assemble the chamber comprising the first substrate, the second substrate, the biological sample, and the spacer when the first and second members are aligned and within the threshold distance along an axis orthogonal to the second plane.

In some variations, the systems provided herein further comprise a sample holder. For example, the system comprising: a first substrate comprising a coating for adhering a biological sample; a second substrate comprising an array; and a spacer disposed between the first substrate and the second substrate may further comprise the sample holder. Further, the system comprising: a first substrate for adhering a biological sample; a second substrate comprising an array; and a spacer disposed between the first substrate and the second substrate may further comprise the sample holder. The sample holder comprises a first member comprising a retaining mechanism that retains the first substrate, a second member comprising a second retaining mechanism that retains the second substrate, and an alignment mechanism that is connected to at least one of first and second members or to both first and second members and configured to align the first and second members such that the biological sample (or a portion thereof) on the first substrate is aligned with and contacts the array of the second substrate.

In one aspect, the present disclosure relates to a method for delivering a fluid to a biological sample disposed on a first substrate and an array disposed on a second substrate, the method comprising: 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; and assembling, subsequent to the delivering, a chamber comprising the first substrate, the second substrate, the biological sample, and the spacer, wherein the spacer is disposed between the first substrate and second substrate and is configured to maintain the fluid within the chamber and maintain a separation distance between the first substrate and the second substrate, the spacer positioned to at least partially surround an area on the first substrate on which the biological sample is disposed and/or the array disposed on the second substrate, wherein the area of the first substrate, the spacer, and the second substrate at least partially encloses a volume comprising the biological sample.

In another aspect, the present disclosure relates to a kit comprising a spacer configured to be disposed between a first substrate and second substrate, and instructions for use according to the method as disclosed herein.

In another aspect, the present disclosure relates to a method 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 comprising:assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a gasket, wherein the gasket is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the area of the first substrate, the gasket, and the second substrate at least partially encloses a volume comprising the biological sample; and delivering the fluid to the partially sealed chamber through one or more apertures of the gasket, thereby delivering the fluid to the array and the biological sample.

In another aspect, the present disclosure relates to a method 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 comprising:assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a gasket, wherein a gasket is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the first substrate, the gasket, and the second substrate at least partially encloses a volume comprising the biological sample; and, delivering the fluid to the partially sealed chamber through one or more via-holes in the first substrate or one or more via-holes in the second substrate, thereby delivering the fluid to the array and the biological sample.

In another aspect, the present disclosure relates to a method 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 comprising: delivering a fluid to the area on the first substrate, wherein a virtual gasket surrounds the area on the first substrate and contains the fluid within the area; and assembling the second substrate with the first substrate, thereby delivering the fluid to the array and the biological sample.

In another aspect, the present disclosure relates to a method 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 comprising: delivering a fluid to the area on the second substrate, wherein a virtual gasket surrounds the area on the second substrate and contains the fluid on

the array; and assembling the first substrate with the second substrate, thereby delivering the fluid to the array and the biological sample.

In yet another aspect, the present disclosure relates to a method 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 comprising: assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a spacer, wherein the spacer is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the area of the first substrate, the spacer, and the second substrate at least partially encloses a volume comprising the biological sample; and delivering the fluid to the partially sealed chamber, thereby delivering the fluid to the array and the biological sample.

In yet another aspect, the present disclosure relates to method for delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second area of a second substrate, the method comprising: delivering a fluid to the second area on the second substrate, wherein a spacer surrounds the second area on the second substrate and contains the fluid on the array; and assembling the first substrate with the second substrate, thereby delivering the fluid to the array and the biological sample.

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.

Use of ordinal terms such as "first", "second", "third", etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements. Similarly, the use of these terms in the specification does not by itself connote any required priority, precedence, or order.

In the descriptions above and in the claims, phrases such as "at least one of" or "one or more of" may occur followed by a conjunctive list of elements or features. The term "and/or"

CLAIMS

1. A method for delivering a fluid to a biological sample disposed on a first substrate and an array disposed on a second substrate, the method comprising:

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; and

assembling, subsequent to the delivering, a chamber comprising the first substrate, the second substrate, the biological sample, and the spacer, wherein the spacer is disposed between the first substrate and second substrate and is configured to maintain the fluid within the chamber and maintain a separation distance between the first substrate and the second substrate, the spacer positioned to at least partially surround an area on the first substrate on which the biological sample is disposed and/or the array disposed on the second substrate, wherein the area of the first substrate, the spacer, and the second substrate at least partially encloses a volume comprising the biological sample.

2. The method of claim 1, wherein the chamber comprises a partially or fully sealed chamber.

3. The method of claim 1 or 2, wherein the second substrate comprises a hydrophobic area positioned away from a region of interest, the hydrophobic area configured to remove bubbles in the fluid from the chamber, the region of interest comprising an area where the biological sample and the array overlap.

4. The method of any one of claims 1 to 3, wherein the hydrophobic area comprises a hydrophobic coating.

5. The method of any one of claims 1 to 4, wherein the separation distance comprises a distance of at least 2 μm.

6. The method of any one of claims 1 to 5, wherein the separation distance comprises a distance between 5 μm to 25 μm.

7. The method of any one of claims 1 to 6, wherein the second substrate comprises the spacer.

8. The method of any one of claims 1 to 7, wherein the first substrate comprises the spacer.

9. The method of any one of claims 1 to 8, further comprising, prior to delivering the fluid:

    applying the hydrophilic coating to the first substrate and/or the second substrate.

10. The method of any one of claims 1 to 9, wherein the fluid comprises a wetting agent.

11. The method of any claims 1 to 10, wherein the fluid comprises one or more permeabilization reagent(s).

12. The method of any one of claims 1 to 11, wherein the spacer comprises an air permeable spacer portion configured to vent a bubble from the fluid.

13. The method of any one of claims 1 to 12, further comprising generating vibration to the first substrate and/or the second substrate.

14. The method of any one of claims 1 to 13, wherein the delivering the fluid to the first substrate and/or the second substrate comprises adjusting a humidity of the chamber.

15. The method of any one of claims 1 to 14, wherein the delivering the fluid to the first substrate and/or the second substrate comprises generating a vacuum to a region proximate to the first substrate and/or the second substrate.

16. The method of any one of claims 1 to 15, wherein the delivering the fluid to the first substrate and/or the second substrate comprises delivering the fluid to a region of the second substrate, the spacer comprising three sides partially surrounding the fluid.

17. The method of any one of claims 1 to 16, wherein the delivering the fluid to the first substrate and/or the second substrate comprises 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.

18. The method of any one of claims 1 to 17, wherein the delivering the fluid to the first substrate and/or the second substrate comprises 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.

19. The method of any one of claims 1 to 18, wherein assembling the chamber comprises 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 urging the fluid toward the three sides partially surrounding the fluid.

20. The method of any one of claims 1 to 19, wherein the chamber comprises 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.

21. The method of any one of claims 1 to 20, wherein the spacer is formed from a uniform thickness material.

22. The method of any one of claims 1 to 21, wherein the spacer is formed from a material with a variable thickness.

23. The method of any one of claims 1 to 22, wherein the spacer is printed on the first substrate and/or the second substrate.

24. The method of any one of claims 1 to 23, wherein the spacer comprises a photoresist pattern.

25. The method of any one of claims 1 to 24, wherein the spacer comprises a beveled edge on one or more sides of the spacer.

26. A kit comprising a spacer configured to be disposed between a first substrate and second substrate, and instructions for use according to the method of any one of claims 1 to 25.

27. A method 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 comprising:

assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a gasket, wherein the gasket is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the area of the first substrate, the gasket, and the second substrate at least partially encloses a volume comprising the biological sample; and

delivering the fluid to the partially sealed chamber through one or more apertures of the gasket, thereby delivering the fluid to the array and the biological sample.

28. A method 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 comprising:

assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a gasket, wherein a gasket is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the first substrate, the gasket, and the second substrate at least partially encloses a volume comprising the biological sample; and,

delivering the fluid to the partially sealed chamber through one or more via-holes in the first substrate or one or more via-holes in the second substrate, thereby delivering the fluid to the array and the biological sample.

29. A method 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 comprising:

delivering a fluid to the area on the first substrate, wherein a virtual gasket surrounds the area on the first substrate and contains the fluid within the area; and

assembling the second substrate with the first substrate, thereby delivering the fluid to the array and the biological sample.

30. A method 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 comprising:

delivering a fluid to the area on the second substrate, wherein a virtual gasket surrounds the area on the second substrate and contains the fluid on the array; and

assembling the first substrate with the second substrate, thereby delivering the fluid to the array and the biological sample.

31. A method 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 comprising:

assembling a partially sealed chamber comprising the first substrate, the second substrate, the biological sample, and a spacer, wherein the spacer is disposed between the first substrate and second substrate, and surrounds the area on the first substrate and/or the array disposed on the second substrate, wherein the area of the first substrate, the spacer, and the second substrate at least partially encloses a volume comprising the biological sample; and

delivering the fluid to the partially sealed chamber, thereby delivering the fluid to the array and the biological sample.

32. A method for delivering a fluid to a biological sample disposed on an area of a first substrate and an array disposed on a second area of a second substrate, the method comprising:

delivering a fluid to the second area on the second substrate, wherein a spacer surrounds the second area on the second substrate and contains the fluid on the array; and

assembling the first substrate with the second substrate, thereby delivering the fluid to the array and the biological sample.