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, wherein the spacer comprises an aperture positioned within a wall of the spacer surrounding the chamber, wherein the fluid is delivered into the aperture, and wherein the spacer is 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 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.

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,

wherein the spacer comprises an aperture positioned within a wall of the spacer surrounding the chamber, wherein the fluid is delivered into the aperture, and

wherein the spacer is 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 any one of claims 1 to 2, wherein the separation distance comprises a distance of at least 2 μm.

4. The method of any one of claims 1 to 3, wherein the separation distance comprises a distance between 5 μm to 25 μm.

5. The method of any one of claims 1 to 4, wherein the second substrate comprises the spacer.

6. The method of any one of claims 1 to 5, wherein the first substrate comprises the spacer.

7. The method of any one of claims 1 to 6, further comprising, prior to delivering the fluid:

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

8. The method of any one of claims 1 to 7, wherein the fluid comprises a wetting agent.

9. The method of any one of claims 1 to 8, wherein the fluid comprises one or more permeabilization reagent(s).

10. The method of any one of claims 1 to 9, 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.

11. The method of any one of claims 1 to 10, 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.

12. The method of any one of claims 1 to 11, 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.

13. The method of any one of claims 1 to 12, wherein the spacer is formed from a uniform thickness material.

14. The method of any one of claims 1 to 13, wherein the spacer is formed from a material with a variable thickness.

15. The method of any one of claims 1 to 14, wherein the spacer is printed on the first substrate and/or the second substrate.

16. The method of any one of claims 1 to 15, wherein the spacer comprises a photoresist pattern.

17. The method of any one of claims 1 to 16, wherein the spacer comprises a beveled edge on one or more sides of the spacer.

18. 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 17.

19. 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 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.

20. The method of claim 1, wherein the array is a spatial array comprising a plurality of discrete features each comprising a plurality of capture probes attached to the second substrate, wherein a capture probe of the plurality of capture probes comprises (i) a capture domain and (ii) a spatial barcode unique to the feature.

21. The method of claim 20, further comprising:

migrating one or more molecules from the biological sample toward the plurality of capture probes via the fluid; and

binding the one or more molecules from the biological sample to one or more capture probes of the plurality of capture probes.

22. The method of claim 21, wherein the migrating comprises active migration via electrophoresis.

23. The method of claim 21, wherein the migrating comprises passive migration via diffusion.

24. The method of any one of claims 21 to 23, wherien the binding occurs via hybridization.

25. The method of any one of claims 21 to 24, wherein the one or more molecules comprise nucleic acids.

26. The method of any one of claims 20 to 25, wherien a feature of the plurality of discrete features is a spot.

27. The method of any one of claims 20 to 26, wherein the capture domain of the capture probe comprises a poly(T) sequence.

28. The method of claim 1, wherein the spacer comprises a material selected from graphite, polyester, Polytetrafluoroethylene (PTFE), polyamide, polychlorotrifluoroethylene (PCTFE), poly(ethene-co-tetrafluoroethene) (ETFE), and PEEK (Polyether ether ketone).