

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 the preceding claims, wherein the hydrophobic area comprises a hydrophobic coating.
5. The method of any one of the preceding claims, wherein the separation distance comprises a distance of at least 2 μm .
6. The method of any one of the preceding claims, wherein the separation distance comprises a distance between about 5 μm to 25 μm .

7. The method of any one of the preceding claims, wherein the second substrate comprises the spacer.
8. The method of any one of the preceding claims, wherein the first substrate comprises the spacer.
9. The method of any one of the preceding claims, 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 the preceding claims, wherein the fluid comprises a wetting agent.
11. The method of any one of the preceding claims, wherein the fluid comprises one or more permeabilization reagent(s).
12. The method of any one of the preceding claims, wherein the spacer comprises an air permeable spacer portion configured to vent a bubble from the fluid.
13. The method of any one of the preceding claims, further comprising generating vibration to the first substrate and/or the second substrate.
14. The method of any one of the preceding claims, 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 the preceding claims, 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 the preceding claims, 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 the preceding claims, 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 the preceding claims, 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 the preceding claims, 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 the preceding claims, 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 the preceding claims, wherein the spacer is formed from a uniform thickness material.
22. The method of any one of the preceding claims, wherein the spacer is formed from a material with a variable thickness.
23. The method of any one of the preceding claims, wherein the spacer is printed on the first substrate and/or the second substrate.

24. The method of any one of the preceding claims, wherein the spacer comprises a photoresist pattern.
25. The method of any one of the preceding claims, wherein the spacer comprises a beveled edge on one or more sides of the spacer.
26. A 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 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.
27. A kit comprising a second substrate comprising an array, wherein the second substrate further comprises a spacer surrounding the array.
28. A kit comprising a first substrate comprising a biological sample, wherein the first substrate further comprises a spacer surrounding the biological sample.
29. A kit comprising 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.
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:
- 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.

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 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.

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 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.

33. 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.

34. A kit comprising:

a first substrate comprising a coating for adhering a biological sample;

a second substrate comprising an array; and

a gasket.

35. 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.

36. 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.

37. A 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 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.

38. The system of claim 37, wherein the first substrate comprises the biological sample mounted thereon.

39. The system of any one of the preceding claims, further comprising a sample holder comprising:

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 a second plane,

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, and

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.

40. The system of any one of claims 26, 37, and 38, further comprising a sample holder comprising:

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.