CLAIMS

1. A sample holder, comprising:

a first member comprising a first retaining mechanism configured to retain a first substrate comprising a sample, the 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 a second substrate comprising a reagent medium disposed in a 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,

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; and

wherein the first substrate and the second substrate are positioned with a minimum spacing between the first substrate and the second substrate.

2. The sample holder of claim 1, further comprising 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.

3. The sample holder of claim 1, wherein the spacing member is further configured to maintain an approximately parallel arrangement of the first substrate and second substrate.

4. The sample holder of claim 1, wherein the alignment mechanism comprises:

a pin extending from the second member; and

an aperture in the first member, the aperture sized and configured to mate with the pin.

~~5. The sample holder of claim 1, further comprising 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.~~

~~6. The sample holder of claim 1, wherein the first member is further configured to retain a third substrate disposed in the first plane, the third substrate comprising a second sample.~~

~~7. The sample holder of claim 1, wherein the first retaining mechanism comprises 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.~~

~~8. The sample holder of claim 1, wherein the second retaining mechanism comprises 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.~~

~~9. The sample holder of claim 1, wherein the adjustment mechanism is coupled to the second member and comprises a linear actuator configured to move the second member along the axis orthogonal to the second plane.~~

~~10. The sample holder of claim 9, wherein the linear actuator is configured to move the second member along the axis orthogonal to the second plane at a velocity of at least 0.1 mm/sec.~~

~~11. The sample holder of claim 9, wherein the linear actuator is 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.~~

~~12. The sample holder of claim 1, wherein the second member comprises a heater configured to heat the first substrate and/or the second substrate.~~

~~13. The sample holder of claim 12, wherein the second member further comprises an insulation gasket coupled to the heater.~~

~~14.~~1. A sample holder, comprising:

a first member comprising a first retaining mechanism configured to retain a first substrate comprising a sample, the first retaining mechanism configured to retain the first substrate disposed in a first plane, the sample disposed in a first region of the first substrate;

a second member comprising a second retaining mechanism configured to retain a second substrate comprising a reagent medium disposed in a second plane and disposed in a second region of the second substrate;

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;

an adjustment mechanism configured to move the second member along the axis orthogonal to the second plane; and

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.

2. The sample holder of claim 1, further comprising a computing device communicatively coupled to the image capture device and to the sample holder, the computing device comprising a display, a data processor, and a non-transitory computer readable storage medium storing computer readable and executable instructions, which when executed cause the data processor to adjust the location of the first substrate relative to the second substrate to cause all or the portion of the first region to be aligned with the second region.

3. The sample holder of any one of the preceding claims, further comprising an LED illumination source.

~~15.~~4. The sample holder of any one of the preceding claims~~claim 16~~, wherein the image capture device is positioned inferior to the second member.

~~16.~~5. The sample holder of any one of the preceding claims~~claim 19~~, wherein the image capture device is disposed on a track configured to move the image capture device along the track.

~~17. The sample holder of claim 16, wherein the first member is 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.~~

~~18. The sample holder of any one of the preceding claims, wherein the second substrate comprises the reagent medium further disposed in a fourth region of the second substrate.~~

~~19. The sample holder of any one of the preceding claims, wherein the alignment mechanism is 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.~~

~~20. The sample holder of any one of the preceding claims, wherein the alignment mechanism is 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.~~

~~21.~~6. The sample holder of any one of the preceding claims, wherein the alignment mechanism comprises 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~~.

~~22. The sample holder of any one of the preceding claims, wherein the image capture device is 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.~~

~~23.~~7. The sample holder of any one of the preceding claims, further comprising a controller configured to control the alignment mechanism, the adjustment mechanism, and/or the image capture device.

~~24.~~8. The sample holder of ~~any one of the preceding~~ claim~~s~~ 7, wherein the controller is configured to communicate with a user interface, the user interface configured to display information about the sample holder.

~~25.~~9. The sample holder of ~~any one of the preceding~~ claim 8~~s~~, wherein the controller is configured to receive a user input from the user interface.

~~26. The sample holder of any one of the preceding claims, wherein the controller is configured to control the alignment mechanism, the adjustment mechanism, and/or the image capture device responsive to the user input.~~

~~27. The sample holder of any one of the preceding claims, further comprising 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.~~

~~28. The sample holder of any one of the preceding claims, wherein the first retaining mechanism is configured to retain the first substrate in a fixed position with respect to the first plane.~~

~~29.~~10. The sample holder of any one of the preceding claims, wherein the second member further comprises a spring mechanism configured to couple to at least a portion of an inferior surface of the second substrate.

~~30.~~11. The sample holder of any one of the preceding claims, wherein the second member further comprises a base configured to couple to an inferior surface of the second substrate and retain the second substrate on the second member, wherein the spring mechanism extends in a superior direction from the base.

~~31.~~12. The sample holder of any one of the preceding claims, wherein the spring mechanism is configured to dispose the second substrate along the second plane at an angle different than the base.

~~32. The sample holder of any one of the preceding claims, wherein the first member is configured to dispose the first substrate along the first plane at an angle parallel with the base.~~

~~33.~~13. The sample holder of any one of the preceding claims, wherein the adjustment mechanism is configured to move the second member toward the first member such that the first substrate contacts at least a portion of the reagent medium.

~~34. The sample holder of any one of the preceding claims, wherein the reagent medium is disposed in proximity and superior to the spring mechanism.~~

~~35.~~14. The sample holder of any one of the preceding claims, wherein the first substrate contacting the at least a portion of the reagent medium urges 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.

~~36.~~15. The sample holder of any one of the preceding claims, wherein the adjustment mechanism 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.

~~37. The sample holder of any one of the preceding claims, further comprising a sensor configured to detect a bubble in the reagent medium responsive to the sample contacting at least a portion of the reagent medium.~~

~~38. A method of capturing an analyte from a biological sample disposed in a first region of a first substrate, the method comprising:~~

~~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;~~

~~mounting a second substrate on a second member of the support device, the second substrate disposed in a second plane and comprising a second region including a plurality of second capture probes, wherein a second capture probe of the plurality of second capture probes comprises a spatial barcode and a second capture domain;~~

~~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 are vertically aligned when the first substrate is positioned superior to the second substrate;~~

~~applying a reagent medium to the first substrate and/or the second substrate, the reagent medium providing a permeabilization buffer between the biological sample and the second substrate; and~~

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, thereby allowing the analyte to migrate from the biological sample to the second substrate, the analyte binding to the second capture domain.

39. The method of claim 38, further comprising 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.

40. The method of claim 38, wherein the positioning comprises 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.

41. A method for aligning a sample area with an array area, the method comprising:

receiving a first substrate within a first retaining mechanism of a sample holder, the first substrate comprising a sample and the sample area;

receiving a second substrate within a second retaining mechanism of the sample holder, the second substrate comprising an array, wherein the second substrate or the sample holder comprises an array area indicator associated with the array area of the second substrate; and

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.

42. A method for aligning a sample area with an array area, the method comprising:

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;

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; and

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.

~~43. The method the preceding claim, wherein the first substrate comprises 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.~~

~~44. The method of any one of the preceding claims, further comprising 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.~~

~~45. The method of any one of the preceding claims, wherein the first substrate includes a fiducial mark.~~

~~46. The method any one of the preceding claims, further comprising~~

~~receiving, by a data processor of a computing device communicatively coupled to the sample holder, an image of the sample;~~

~~providing, by the data processor, the image of the sample for display via a display of the computing device; and~~

~~receiving, by the data processor, an input identifying the sample area indicator based on the provided image.~~

~~47. The method of any one of the preceding claims, wherein adjusting the location of the first substrate relative to the second substrate comprises~~

~~viewing the first substrate and the second substrate within the sample holder; and~~

~~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.~~

~~48. The method of any one of the preceding claims, wherein the array area indicator is provided on the sample holder.~~

~~49. The method of any one of the preceding claims, further comprising~~

~~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 overlaid atop the first substrate within the sample holder; and~~

providing, by the data processor, the plurality of video images for display via a display of the first computing device.

50. The method of any one of the preceding claims, further comprising adjusting the first retaining mechanism to cause the sample area to be aligned with the array area.

51. The method of any one of the preceding claims, further comprising

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;

automatically determining, by the first data processor, a sample area indicator on the first substrate based on the plurality of video images; and

performing the adjusting automatically, by the first data processor, based on the automatically determined sample area indicator.

52. The method of any one of the preceding claims, wherein performing the aligning automatically further comprises

determining, by the first data processor, an area of the sample relative to an area of the array;

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;

providing, by the first data processor, the sample area indicator as an outline of the sample; and

performing the adjusting automatically, by the first data processor, based on the outline of the sample.

53. The method of any one of the preceding claims, wherein performing the adjusting automatically further comprises

determining, by the first data processor, a fiducial mark located on the first substrate; and

performing the adjusting automatically, by the first data processor, based on the determined fiducial mark.

54. The method of any one of the preceding claims, further comprising

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;

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;

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;

receiving an input to the first computing device identifying the sample area indicator based on the registered sample image; and

performing the adjusting automatically based on the received input identifying the sample area indicator.

55. A system for aligning a sample area with an array area, the system comprising:

a sample holder comprising

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;

a second retaining mechanism configured to retain a second substrate received within the second retaining mechanism, the second substrate comprising 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 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;

a microscope operatively coupled to the sample holder and configured to view the first substrate and the second substrate within the sample holder; and

a first computing device communicatively coupled to the microscope and to the sample holder, the computing device comprising 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.~~

~~56. 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.~~

~~57. 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.~~

~~58. The method of any one of claims 56-57, wherein the first substrate, the second substrate, or both comprises a glass surface.~~

~~59. The method of any one of claims 56-58, wherein the first substrate and the second substrate are axially aligned.~~

~~60. The method of any one of claims 56-58, wherein the first substrate and the second substrate are in a cross configuration.~~

~~61. The method of any one of claims 56-60, wherein the virtual gasket comprises a hydrophobic coating.~~

~~62. 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.

63. The method of claim 62, wherein the chamber comprises a partially or fully sealed chamber.

64. The method of claim 62, 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.

65. The method of any one of the preceding claims, wherein the hydrophobic area comprises a hydrophobic coating.

66. The method of any one of the preceding claims, wherein the separation distance comprises a distance of at least 2 μm.

67. The method of any one of the preceding claims, wherein the separation distance comprises a distance between about 5 μm to 25 μm.

68. The method of any one of the preceding claims, wherein the second substrate comprises the spacer.

69. 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.

~~70. The method of claim 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.~~

~~71. The method of claim 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.~~

~~72. The method of claim 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.~~

~~73. 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.~~

~~74. 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.~~

~~75. 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.~~

~~76. A kit comprising a second substrate comprising an array, wherein the second substrate further comprises a spacer surrounding the array.~~

~~77. A kit comprising a first substrate comprising a biological sample, wherein the first substrate further comprises a spacer surrounding the biological sample.~~

~~78. 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.~~

~~79. A system for aligning a sample area with an array area, the system comprising:~~

~~a sample holder comprising~~

~~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;~~

~~a second retaining mechanism configured to retain a second substrate received within the second retaining mechanism, the second substrate comprising 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 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;~~

~~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; and~~

~~a first computing device communicatively coupled to the at least one image capture device and to the sample holder, the computing device comprising 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.~~

~~80. The system or sample holder of any one of the preceding claims, wherein the first retaining mechanism comprises a first set of alignment marks associated with a first portion of the sample area.~~

~~81. The system or sample holder of the preceding claim, wherein the first retaining mechanism comprises a second set of alignment marks associated with a second portion of the sample area, wherein the second portion of the sample area is smaller than the first portion of the sample area.~~

~~82. The system or sample holder of any one of claims 81-82, wherein the first retaining mechanism comprises a third set of alignment marks indicating an exclusion zone on the first substrate.~~

~~83. The system or sample holder of any one of the preceding claims, wherein the sample holder comprises a first member and a second member, the first member or the second member being independently articulable.~~

~~84. The system or sample holder of the preceding claim, wherein the first member and the second member articulate to provide a uniform separation distance between the first substrate and the second substrate.~~

~~85. The system or sample holder of any one of the preceding claims, wherein the at least one image capture device is positioned inferior to the second member.~~

~~86. The system or sample holder of any one of the preceding claims, wherein the sample holder comprises a plurality of adjustable feet on a base of the sample holder, the plurality of adjustable feet configured to level the sample handling apparatus relative to a surface on which the sample holder is located, optionally comprising a leveling mode user interface for providing feedback to a user when leveling the sample holder.~~

~~87. The system or sample holder of any one of the preceding claims, wherein the sample holder comprises a clip constraining the second substrate within the second member.~~

~~88. The system or sample holder of any one of the preceding claims, wherein the sample holder comprises a plurality of image capture devices.~~

~~89. The system or sample holder of any one of the preceding claims, wherein the sample holder comprises an LED illumination source.~~

~~90. The system or sample holder of any one of the preceding claims, wherein the sample holder comprises a spring between the first member and the second member.~~

~~91. The system or sample holder of any one of the preceding claims, wherein the sample holder comprises a sensor configured to determine a home position for a top portion of the sample holder relative to a bottom portion of the sample holder as the top portion is closed upon the bottom portion.~~

~~92. The system or sample holder of the preceding claim, wherein the sensor comprises an optical beam-break sensor or an impedance sensor.~~

~~93. The system or sample holder of any one of the preceding claims, wherein the at least one image capture device comprises a focus motor configured to actuate the at least one image capture device.~~

~~94. The system or sample holder of the preceding claim, wherein the focus motor comprises a cam configured to actuate the at least one image capture device in a horizontal manner.~~

~~95. The system or sample holder of any one of the preceding claims, wherein (i) the first retaining mechanism further comprises a trough, optionally wherein the trough extends around an area of the retaining mechanism onto which the first substrate is received, or (ii) the second retaining mechanism further comprises a trough, optionally wherein the trough extends around an area of the retaining mechanism onto which the second substrate is received, or (iii) the first retaining mechanism further comprises a first trough and the second retaining mechanism further comprises a second trough, optionally wherein the first trough extends around an area of the retaining mechanism onto which the first substrate is received, optionally wherein the second trough extends around an area of the retaining mechanism onto which the second substrate is received.~~

~~96. The system or sample holder of any one of the preceding claims, further comprising a hinge resistance mechanism that provides resistance upon closure of the first member and the second member of the sample holder.~~

~~97. The system or sample holder of any one of the preceding claims, further comprising a closure feedback mechanism configured in a top portion of the sample holder, the closure feedback mechanism received within a receiving portion of a bottom portion of the sample holder when the top portion is brought into contact with the bottom portion.~~

~~98. The system or sample holder of the preceding claim, wherein the receiving portion includes a slot to receive the closure feedback mechanism.~~

~~99. The system or sample holder of any one of the preceding claims, wherein closure feedback mechanism includes a hole that can be received within and engage the receiving portion.~~

~~100. The system or sample holder of any one of the preceding claims, wherein the closure feedback mechanism is secured to the top portion of the sample holder via a pin.~~

~~101. The system or sample holder of any one of the preceding claims, further comprising a gasket configured on the second retaining mechanism of the sample holder.~~

508834099v.1