

WHAT IS CLAIMED IS:

1. A sample holder, comprising:
 - a first member comprising a first retaining mechanism configured to retain a
5 first substrate comprising a sample;
 - a second member comprising a second retaining mechanism configured to retain a second substrate comprising a reagent medium; and
 - an alignment mechanism connected to one or both of the first and second
10 members, and configured to align the first and second members such that the sample contacts at least a portion of the reagent medium when the first and second members are aligned.
2. The sample holder of claim 1, wherein the alignment mechanism comprises:
 - (a) a rotating actuator connected to the first and second members; and/or
15 (b) one or more connectors positioned on one or both of the first and second members; and one or more receivers positioned on one or both of the first and second members, wherein the one or more receivers are positioned to engage with the one or more connectors.
- 20 3. The sample holder of claim 2, wherein the rotating actuator comprises a hinge or a folding member or at least one arm.
4. The sample holder of any one of claims 1-3, wherein:
 - 25 (a) the first retaining mechanism comprises:
 - (i) a recess dimensioned to receive the first substrate, wherein optionally the sample holder further comprises a gasket positioned within the recess and configured to maintain an interference fit between the recess and the first substrate; or
 - 30 (ii) one or more members configured to apply a force to the first substrate to maintain contact between the first substrate and the first member;and/or
 - (b) the second retaining mechanism comprises:

- (i) a recess dimensioned to receive the second substrate; or
- (ii) one or more members configured to apply a force to the second substrate to maintain contact between the second substrate and the second member;

5 and/or

(c) the reagent medium comprises at least one of: a solution comprising a permeabilization reagent, a solid permeabilization reagent, and a hydrogel compound comprising a permeabilization reagent, wherein optionally the solution comprising the permeabilization agent comprises:

- 10 (i) greater than about 2 w/v % sodium dodecyl sulfate (SDS), wherein optionally the permeabilization agent comprises about 8 w/v % to about 12 w/v % SDS; or
- (ii) proteinase K; or
- (iii) greater than 2 w/v % N-lauroylsarcosine or a sodium salt thereof;

15 and/or

(d) the first member comprises an aperture positioned so that when the first substrate is retained, the aperture is aligned with a sample region of the first substrate;

and/or

20 (e) the second member comprises at least one aperture positioned so that when the first substrate is retained and the first and second members are aligned by the alignment mechanism, aperture of the at least one aperture is aligned with at least a portion of a sample region of the first substrate, wherein optionally the sample holder further comprises a reagent well formed by one or more

25 bounding surfaces of the at least one aperture and by a back surface of the second substrate, wherein a reagent solution added to the reagent well is contained by the bounding surfaces and permeates through the back surface of the second substrate, and wherein optionally the back surface of the second substrate is opposite to a front surface of the second substrate that faces the

30 sample on the first substrate.

5. The sample holder of any one of claims 2-4, further comprising a first adjustment mechanism connected to the first member and configured to translate the first substrate in at least one direction parallel to a surface of the first substrate that supports the sample.

5

6. The sample holder of any one of claims 2-5, wherein the alignment mechanism is configured to maintain a separation between the first and second substrates when the first and second substrates are aligned, wherein optionally:

- 10 (i) the alignment mechanism is configured to maintain the separation such that at least a portion of the sample on the first substrate contacts at least a portion of the reagent medium on the second substrate; or
- (ii) the separation between the first and second substrates is between 50 microns and 1 mm, measured in a direction orthogonal to a surface of the first substrate that supports the sample; or
- 15 (iii) the separation between the first and second substrates is between 50 microns and 500 microns; or
- (iv) the alignment mechanism is configured to maintain the first and second substrates approximately parallel when the first and second substrates are aligned so that an angle between the first and second substrates is two
- 20 degrees or less, wherein optionally the angle is 0.5 degrees or less and wherein optionally the sample holder further comprises one or more spacing members connected to one or both of the first and second members positioned so that when the first and second substrates are aligned, the one or more spacing members are between the first and second
- 25 members.

7. The sample holder of claim 6, further comprising a second adjustment mechanism configured to adjust a distance of the separation in direction orthogonal to a surface of the first substrate that supports the sample; wherein optionally the second adjustment mechanism is:

30

- (i) a component of the alignment mechanism; or
- (ii) connected to one or both of the first member and the second member.

8. A support device for a substrate comprising a sample, the support device comprising:

the sample holder of any one of claims 2-7; and

5 a plate comprising a platform, a plurality of members connected to a first surface of the platform, and a support member connected to a second surface of the platform, wherein the plate is configured to connect to the sample holder.

9. The support device of claim 8, wherein:

10 (a) the sample holder and the plate are configured so that when the sample holder and the plate are connected, at least 75% of a region of the first substrate that contacts the sample is overlaid by the support member;

and/or

15 (b) a member of the plurality of members is dimensioned to be received by a region of a heating device, wherein optionally the region of the heating device comprises a heat transfer element configured to transfer heat to a well of a multi-well substrate, and wherein optionally the heat transfer element comprises a recess in a heating member;

and/or

20 (c) the support device further comprises an attachment mechanism configured to couple the support member to the sample holder, wherein optionally:

(i) the attachment mechanism comprises one or both of an aperture and a recess formed in the first member of the sample holder and configured to receive the support member; or

25 (ii) the attachment mechanism comprises one or more extension members connected to the first member of the sample holder and configured to engage with corresponding recess(es) in the support member; or

30 (iii) the attachment mechanism comprises one or more extension members connected to the support member and configured to engage with corresponding recess(es) in the first member of the sample holder;

and/or

(d) the plurality of members form a two-dimensional array on the first surface of the platform, and the plurality of members are spaced so that they align with wells on a multi-well substrate;

and/or

5 (e) the region of the first substrate that contacts the sample is completely overlaid by the support member, wherein optionally the sample holder and the plate are configured so that when the sample holder and the plate are connected, at least a portion of the first substrate contacts the support member, wherein optionally the portion of the first substrate that contacts
10 the support member comprises a part of the substrate that is on an opposite side of the first substrate from a region of the first substrate that contacts the sample, and wherein optionally the portion of the first substrate that contacts the support member comprises at least 90% of a surface of the substrate that is opposite to a surface of the first substrate that contacts the
15 sample;

and/or

(f) the attachment mechanism is configured so that the sample holder couples to the support member in a single orientation.