

CLAIMS

WHAT IS CLAIMED IS:

1. A device for holding a sample comprising:
a support;
a coating coupled to the support comprising:
a matrix binding agent for attaching a synthetic three-dimensional (3D) matrix to the support; and
a sample binding agent for attaching the sample to the support.
2. The device of claim 1, wherein the matrix binding agent forms a covalent bond to the 3D matrix.
3. The device of claim 1 or claim 2, wherein the matrix binding agent binds to the 3D matrix via an interpenetrating network.
4. The device of any one of claims 1-3, wherein the matrix binding agent and the sample binding agent are different.
5. The device of any one of claims 1-3, wherein the matrix binding agent and the sample binding agent are the same.
6. The device of any one of claims 1-3, wherein the matrix binding agent comprises an acrylate.
7. The device of claim 6, wherein the acrylate is a methacrylate.
8. The device of any one of claims 1-7, wherein the matrix binding agent comprises a silane.
9. The device of claim 8, wherein the silane of the matrix binding agent comprises a methyl silane, dimethyl silane, or trimethyl silane.
10. The device of any one of claims 6-9, wherein the acrylate is bound to the silane by a C₁-C₁₅ alkyl, alkenyl, or alkynyl.
11. The device of any one of claims 1-9, wherein the matrix binding agent comprises 3-(trimethoxysilyl)propyl acrylate or 3-(trimethoxysilyl)propyl methacrylate.
12. The device of any one of claims 1-11, wherein the matrix binding agent comprises polymeric 3-(trimethoxysilyl)propyl acrylate or 3-(trimethoxysilyl)propyl methacrylate.
13. The device of any one of claims 1-12, wherein the matrix binding agent comprises methacryloxymethyltrimethoxysilane, 3-acrylamidopropyltrimethoxysilane, acryloxymethyltrimethoxysilane, (3-acryloxypropyl)trimethoxysilane, (3-methacrylamidopropyl)triethoxysilane, methacryloxymethyltriethoxysilane,

methacryloxymethyltrimethoxysilane, methacryloxypropyltriethoxysilane, or any combinations thereof.

14. The device of any one of claims 1-13, wherein the matrix binding agent comprises a hydrogel.

15. The device of claim 14, wherein a thickness of the hydrogel is at most about 300 micrometer (μm).

16. The device of claim 14 or 15, wherein the hydrogel comprises acrylamide.

17. The device of claim 16, wherein the acrylamide is a polyacrylamide.

18. The device of any one of claims 1-17, wherein the sample binding agent attaches to the sample via an electrostatic interaction.

19. The device of any one of claims 1-17, wherein the sample binding agent comprises a negative charge.

20. The device of any one of claims 1-17, wherein the sample binding agent comprises a positive charge.

21. The device of any one of claims 1-20, wherein the sample binding agent comprises a silane.

22. The device of claim 21, wherein the silane of the sample binding agent comprises a methyl silane, dimethyl silane, or trimethyl silane.

23. The device of any one of claims 1-22, wherein the sample binding agent comprises 3-aminopropyltriethoxysilane (APES).

24. The device of any one of claims 1-23, wherein the sample binding agent comprises a hydrolytic stability enhancing agent.

25. The device of any one of claims 1-24, wherein the sample binding agent comprises bis(triethoxysilyl)ethane (BTESE).

26. The device of any one of claims 1-25, wherein the sample binding agent comprises a heteropolymer comprising APES and BTESE.

27. The device of any one of claims 1-26, wherein the sample binding agent comprises poly-L-lysine.

28. The device of any one of claims 1-27, wherein the sample binding agent attaches to the sample via at least one of a hydrogen bond and a Van der Waals force.

29. The device of any one of claims 1-27, wherein the sample binding agent comprises a hydrogel.

30. The device of claim 29, wherein the hydrogel comprises acrylamide.

31. The device of claim 30, wherein the acrylamide is a polyacrylamide.

32. The device of any one of claims 29-31, wherein a thickness of the hydrogel is at most about 300 μm .
33. The device of claim 32, wherein the sample binding agent is the same as the matrix binding agent.
34. The device of any one of claims 1-33, wherein a surface of the support is hydrophilic.
35. The device of any one of claims 1-34, wherein the support is a solid support or a semi-solid support.
36. The device of any one of claims 1-35, wherein the support comprises a plate, a slide, a coverslip, a flow cell, a microchip, a microcentrifuge tube, a test tube, or a well.
37. The device of any one of claims 1-35, wherein the support comprises glass, microspheres, inert particles, magnetic particles, plastic, polysaccharide, nylon, nitrocellulose, ceramic, resin, silica, silicon, modified silicon, polytetrafluoroethylene, or metal.
38. The device of claim 37, wherein the glass comprises modified glass, functionalized glass, or inorganic glass.
39. The device of claim 37, wherein the plastic comprises acrylic, polystyrene, polypropylene, polyethylene, polybutylene, or polyurethane.
40. The device of any one of claims 1-39, wherein at least a portion of the support is covered by a removable mask.
41. The device of claim 40, wherein the removable mask is or is substantially impermeable to formaldehydes, waxes, polyolefins, alcohols or glycols.
42. The device of claim 40 or 41, wherein the removable mask is attached to the support using an adhesive.
43. The device of any one of claims 1-39, wherein a removable boundary is attached to a surface of the support, wherein the removable boundary comprises a side wall and wherein the surface of the support and the removable boundary form a well.
44. The device of claim 43, wherein the removable boundary is attached to the surface of the support with an adhesive, wherein the adhesive forms a seal between the removable boundary and a surface of the support.
45. The device of claim 43 or 44, wherein the removable boundary is a sample cassette.
46. The device of claim 45, wherein the support is sandwiched in between a top piece and a bottom piece of the sample cassette.
47. The device of any one of claims 1-46, wherein the support comprises a sample binding area.

48. The device of claim 47, wherein the sample binding area is identified with a visible marking.
49. The device of claim 47 or claim 48, wherein the sample binding area is transparent.
50. The device of any one of claims 1-49, wherein the support or the removable boundary comprises a machine-readable identification tag.
51. The device of claim 50, wherein the machine-readable identification tag provides the device with a unique identifier.
52. The device of claim 50 or 51, wherein the machine-readable identification tag identifies a sample attached to the device.
53. The device of any one of claims 50-52, wherein the machine-readable identification tag provides a system preparing or analyzing a sample attached to the device with instructions for preparing or analyzing the sample.
54. The device of any one of claims 50-53, wherein the machine-readable identification tag comprises a quick response (QR) code, a data matrix, a radio-frequency identification (RFID) tag, or a near-field communication (NFC) chip.
55. The device of any one of claims 1-54, wherein the support comprises at least one fiducial marker.
56. The device of any one of claims 1-55, wherein the support comprises the sample attached thereto.
57. The device of any one of claims 1-56, wherein the support comprises a positive charge.
58. The device of claim 57, wherein the positive charge of the support is reduced or neutralized.
59. The device of claim 57 or 58, wherein the positive charge of the support comprises an amine group.
60. The device of claim 59, wherein the amine group is neutralized by an n-hydroxysuccinimide (NHS) ester.
61. The device of any one of claims 1-60, wherein the support further comprises the synthetic 3D matrix attached thereto.
62. The device of claim 61, wherein a thickness of the synthetic 3D matrix is at least about 100 μm .
63. The device of any one of claims 1-62, wherein the sample is a biological sample.
64. The device of claim 63, wherein the biological sample is a cell or a tissue.

65. The device of any one of claims 1-64, wherein the sample comprises one or more nucleic acid molecules.
66. A system for analyzing a sample comprising a stage for holding a device of any one of claims 1-65.
67. A system for analyzing a sample comprising:
a first module comprising a first housing comprising:
a stage configured to retain a sample comprising a plurality of nucleic acid molecules in a three dimensional (3D) matrix, which plurality of nucleic acid molecules have a relative 3D spatial relationship; and
a detector configured to detect one or more signals from the sample;
and
a second module comprising a second housing comprising:
a computer operatively coupled to the detector, wherein the computer is configured to:
(a) bring said plurality of nucleic acid molecules or derivatives thereof in contact with detectable moieties, and (ii) use said detector to obtain signals corresponding to said detectable moieties from a plurality of planes of said 3D matrix, and
(b) use said signals obtained by said detector to generate a 3D volumetric representation of said plurality of nucleic acid molecules, which 3D volumetric representation identifies said relative 3D spatial relationship of said plurality of nucleic acid molecules,
a stage;
wherein the first housing is physically distinct from the second housing.
68. The system of claim 67, wherein the first module further comprises a fluidic waste extraction tube positioned above the stage.
69. The system of claim 67 or 68, wherein the second module further comprises a reagent reservoir interface.
70. The system of any one of claims 67-69, wherein the stage comprises at least one recess for holding a device for retaining the sample, wherein the device comprises a support.
71. The system of claim 70, wherein the stage comprises a lid for securing the device.
72. The system of claim 71, wherein the lid comprises a hinge.
73. The system of any one of claims 70-72, wherein the device for retaining the sample is a device of any one of claims 1-65.

74. The system of any one of claims 70-72, further comprising the device of any one of claims 1-65.
75. The system of any one of claims 70-74, wherein the at least one recess comprises a sample position controller that positions the sample within the at least one recess.
76. The system of claim 75, wherein the sample position controller comprises at least one mechanical linkage that positions the sample within the at least one recess.
77. The system of claim 75 or 76, wherein the at least one mechanical linkage comprises a cam.
78. The system of any one of claims 75-77, wherein sample position controller comprises at least one pin that positions the sample within the at least one recess.
79. The system of any one of claims 75-78, wherein the sample position controller comprises at least one X pin, at least one Y pin, and at least one Z pin.
80. The system of any one of claims 67-79, wherein the stage further comprises a temperature controller.
81. The system of claim 80, wherein the temperature controller comprises a Peltier element.
82. The system of any one of claims 67-81, wherein the stage is a motorized stage that moves in an x, y, and z direction relative to the detector.
83. The system of any one of claims 67-82, wherein the first module comprises a machine-readable identification tag reader.
84. The system of claim 83, wherein the machine-readable identification tag reader comprises at least one of a quick response (QR) code reader, a data matrix reader, a radio-frequency identification (RFID) tag reader, and a near-field communication (NFC) chip reader.
85. The system of claim 83 or 84, wherein the stage comprises the machine-readable identification tag reader.
86. The system of any one of claims 83-85, wherein the machine-readable identification tag reader reads a machine-readable identification tag on a device for retaining a sample.
87. The system of any one of claims 67-85, wherein the first module comprises an optical assembly that comprises the detector.
88. The system of claim 87, wherein the detector is a camera.
89. The system of claim 88, wherein the camera comprises a CMOS or sCMOS sensor.
90. The system of any one of claims 87-89, wherein the optical assembly comprises an objective lens.

91. The system of claim 90, wherein the objective lens is a water immersion lens, oil immersion lens, water dipping lens, air lens, or lens with an adjustable refractive index.
92. The system of claim 90 or 91, wherein the objective lens is an autofocus objective lens.
93. The system of any one of claims 90-92, further comprising an autofocus controller.
94. The system of claim 93, wherein the autofocus controller comprises an integrated circuit, a computer, or a field-programmable gate array (FPGA).
95. The system of claim 93 or 94, wherein the autofocus controller is a reflection-based autofocus controller.
96. The system of any one of claims 67-95, wherein the first module comprises a light source.
97. The system of claim 96, wherein the light source comprises a laser, light-emitting diode, or incandescent lamp.
98. The system of claim 96 or 97, wherein the light source comprises a spectral filter.
99. The system of any one of claims 67-98, wherein the fluidic waste extraction tube is a sipper tube.
100. The system of any one of claims 68-99, wherein the first module further comprises a sensor that detects a position of the fluidic waste extraction tube.
101. The system of claim 100, wherein the sensor is among a plurality of sensors.
102. The system of claim 101, wherein the plurality of sensors comprises a plurality of photointerruptors.
103. The system of any one of claims 100-102, wherein the position of the fluidic waste extraction tube comprises a position of a tip of the fluidic waste extraction tube.
104. The system of any one of claims 100-103, wherein the position of the fluidic waste extraction tube comprises a position of the fluidic waste extraction tube relative to a device for retaining the sample or a position of the fluidic waste extraction tube relative to the sample.
105. The system of any one of claims 67-104, wherein the second module comprises a user interface.
106. The system of claim 105, wherein the user interface comprises a touchscreen.
107. The system of any one of claims 69-106, wherein the second module further comprises a reagent reservoir loaded onto the reagent reservoir interface.
108. The system of claim 107, wherein the reagent reservoir comprises a plurality of reagent reservoirs.

109. The system of any one of claims 69-108, wherein the reagent reservoir interface is in fluidic communication with the first module.
110. The system of claim 109, wherein the reagent reservoir interface is in fluidic communication with sample in the first module.
111. The system of any one of claims 107-110, wherein the reagent reservoir comprises a machine-readable identification tag.
112. The system of any one of claims 69-111, wherein the reagent reservoir interface comprises a machine-readable identification tag reader.
113. The system of claim 112, wherein the machine-readable identification tag reader comprises at least one of a quick response (QR) code reader, a data matrix reader radio-frequency identification (RFID) tag reader, and near-field communication (NFC) chip reader.
114. The system of any one of claims 111-113, wherein the machine-readable identification tag reader reads the machine-readable identification tag on the reagent reservoir.
115. The system of any one of claims 67-114, wherein the system further comprises a reagent cartridge interface for fluidically connecting the system to a reagent cartridge comprising a plurality of chambers for holding reagents.
116. The system of claim 115, wherein the reagent cartridge interface comprises a plurality of first tubular bodies for introducing gas into the chambers for holding reagents.
117. The system of claim 115 or 116, wherein the plurality of first tubular bodies comprise piercing elements for piercing a plurality of upper seals on the top of the reagent cartridge.
118. The system of claim 117, wherein the piercing elements are needles.
119. The system of any one of claims 116-118, further comprising a tank of pressurized argon gas fluidically connected to the plurality of first tubular bodies.
120. The system of any one of claims 88-119, wherein the reagent cartridge interface comprises a lid.
121. The system of claim 120, wherein the lid comprises the plurality of first tubular bodies.
122. The system of any one of claims 115-121, wherein the reagent cartridge interface comprises a plurality of second tubular bodies on the bottom for removing reagents from the chambers for holding reagents.
123. The system of claim 122, wherein the plurality of second tubular bodies comprise piercing elements for piercing a lower seal on the bottom of the chambers for holding reagents.

124. The system of claim 122 or 123, wherein the plurality of second tubular bodies are in fluidic communication with the device for retaining the sample.
125. The system of any one of claims 122-124, wherein the plurality of second tubular bodies are in fluidic communication with a sample.
126. The system of any one of claims 122-125, wherein the plurality of second tubular bodies comprise a plurality of sprung needle shrouds or a sprung needle shroud plate.
127. The system of claim 126, wherein the plurality of second tubular bodies become exposed when the lid of the reagent cartridge interface secures a reagent cartridge.
128. The system of claim 127, wherein the plurality of second tubular bodies are configured to pierce a seal on the reagent cartridge.
129. The system of any one of claims 116-128, wherein the reagent cartridge interface comprises a machine-readable identification tag reader.
130. The system of claim 129, wherein the machine-readable identification tag reader comprises at least one of a quick response (QR) code reader, a data matrix reader, radio-frequency identification (RFID) tag reader, and near-field communication (NFC) chip reader.
131. The system of any one of claims 129-130, wherein the machine-readable identification tag reader reads a machine-readable identification tags on a reagent cartridge connected to the reagent cartridge interface.
132. The system of any one of claims 115-128, wherein the first module comprises the reagent cartridge interface.
133. The system of any one of claims 115-128, wherein the second module comprises the reagent cartridge interface.
134. The system of any one of claims 115-133, further comprising a reagent cartridge comprising a main reservoir body comprising a plurality of chambers for holding reagents comprising upper seals and lower seals.
135. The system of claim 134, wherein the upper seals comprise a foil or a septum.
136. The system of claim 134 or 135, wherein the lower seals comprise a foil or a septum.
137. The system of any one of claims 134-136, wherein the chambers further comprise a filter between a cavity of the chamber and the septum.
138. The system of claim 137, wherein the filter is at most a 60 μm filter.
139. The system of claim 137 or claim 138, wherein the filter is separated from the septum by a void for a second tubular body of the plurality of second tubular bodies to withdraw reagent from the chamber.

140. The system of any one of claims 134-139, wherein the plurality of chambers hold a plurality of reagents, wherein at least one of the plurality of reagents comprises an enzyme, a buffer, a detection probe, and a nucleic acid.
141. The system of any one of claims 134-140, wherein the reagent cartridge further comprises a machine-readable identification tag.
142. The system of claim 141, wherein the machine-readable identification tag comprises at least one of a quick response (QR) code, a data matrix, radio-frequency identification (RFID) tag, and near-field communication (NFC) chip.
143. The system of claim 141 or 142, wherein the machine-readable identification tag is informative of the contents of the reagent cartridge.
144. The system of any one of claims 67-143, wherein the second module further comprises a waste reservoir in fluidic communication with the fluidic waste extraction tube.
145. The system of claim 144, wherein the second module further comprises a sensor to detect a presence of the waste reservoir.
146. The system of claim 144 or 145, wherein the second module further comprises a sensor to detect a level of fluid in the waste reservoir.
147. The system of any one of claims 67-146, wherein the second module comprises a digital processing device comprising: at least one processor, an operating system configured to perform executable instructions, a memory, and a computer program including instructions executable by the digital processing device to provide an application comprising:
- a first software module programmed to (i) repeatedly scan a three-dimensional sub-volume of the sample, the repeated scans including temporal data, and (ii) process data from the repeated scans including the temporal data to generate a three-dimensional map of the sub-volume of the sample.
148. The system of claim 147, wherein the three-dimensional map comprises a coordinate system.
149. The system of claim 147 or 148, wherein the digital processing device comprises a second software module programmed to detect a position of a fiducial marker associated with a scan of the repeated scans and adjust the three-dimensional map of the sub-volume of the sample to compensate for the position of the fiducial marker.
150. The system of any one of claims 147-149, wherein the digital processing device comprises a third software module programmed to control the timing of fluidic, optical, and motion-related events occurring in the first module.

151. The system of claim 150, wherein the third software module programmed to control the timing of fluidic, optical, and motion-related events occurring in the first module is programmed to control motors, cameras, optical tuning systems, optical gating systems, and sensors.
152. The system of any one of claims 147-151, wherein the digital processing device comprises a fourth software module programmed to select or suggest a protocol for processing or analyzing a sample based on detection by the system of a machine-readable identification tag present on at least one of a sample, a reagent reservoir, and a reagent cartridge.
153. The system of any one of claims 147-152, wherein the digital processing device comprises a user interface.
154. The system of claim 153, wherein the user interface comprises a touchscreen.
155. The system of any one of claims 67-154, wherein the first module comprises a fluidic cooling system.
156. The system of any one of claims 67-155, wherein the first module does not comprise a fan for cooling.
157. The system of any one of claims 67-156, wherein the second module comprises a fan for cooling.
158. A method of analyzing a sample comprising attaching the sample to the sample binding agent of a device of any one of claims 1-65.
159. The method of claim 158, further comprising contacting the sample attached to the sample binding agent with a matrix-forming material.
160. The method of claim 159, wherein the matrix-forming material comprises acrylamide.
161. The method of claim 160, wherein the acrylamide is propargyl acrylamide.
162. The method of any one of claims 159-161, wherein the matrix-forming material further comprises a crosslinker.
163. The method of claim 162, wherein the crosslinker is N,N'-methylenebisacrylamide (BIS), piperazine diacrylate (PDA), N,N'-bisacrylylcystamine (BAC), or N,N'-diallyltartardiamide (DATD).
164. The method of any one of claims 159-163, wherein the matrix-forming material further comprises an activator or an inhibitor, which activator or inhibitor controls a rate of polymerization of the matrix-forming material.
165. The method of any one of claims 159-164, further comprising generating the synthetic 3D matrix from the matrix-forming material.

166. The method of claim 165, wherein the generating comprises polymerizing or crosslinking the matrix-forming material.
167. The method of claim 165 or 166, wherein generating the synthetic 3D matrix from the matrix-forming material is performed in an oxygen-free environment.
168. The method of any one of claims 158-167, further comprising attaching the synthetic 3D matrix to the matrix binding agent of the device.
169. The method of claim 159, wherein attaching the synthetic 3D matrix to the matrix binding agent comprises crosslinking the synthetic 3D matrix to the matrix binding agent.
170. The method of claim 169, wherein the crosslinking comprises physical crosslinking or chemical crosslinking.
171. The method of claim 169 or claim 170, wherein the crosslinking comprises free-radical polymerization, chemical conjugation, or bioconjugation reactions.
172. The method of any one of claims 158-171, wherein the crosslinking comprises photopolymerization.
173. The method of claim 172, wherein the photopolymerization is initiated by single-photon or multiphoton excitation systems.
174. The method of claim 173, wherein the photopolymerization is initiated by manipulation of light to form specific two-dimensional (2D) or 3D patterns.
175. The method of claim 173, wherein the photopolymerization is initiated by a spatial light modulator.
176. The method of claim 175, wherein the spatial light modulator is a digital spatial light modulator.
177. The method of claim 176, wherein the spatial light modulator employs a transmissive liquid crystal, reflective liquid crystal on silicon (LCOS), digital light processing, or a digital micromirror device (DMD).
178. The method of any one of claims 158-177, wherein the 3D matrix comprises a polymeric material.
179. The method of claim 178, wherein the synthetic 3D matrix comprises an additional polymeric material cross-linked to said polymeric material.
180. The method of claim 179, wherein said additional polymeric material comprises polyacrylamide, poly-ethylene glycol (PEG), poly(acrylate-co-acrylic acid) (PAA) or poly(N-isopropylacrylamide) (NIPAM).
181. The method of any one of claims 158-180, wherein said synthetic 3D matrix is configured to expand.

182. The method of any one of claims 158-181, further comprising obtaining a three-dimensional map of the sample.
183. The method of claim 182, wherein the three-dimensional map comprises a three-dimensional map of a plurality of nucleic acid sequences present in the sample.
184. The method of any one of claims 158-183, wherein the method comprises performing a FISSEQ protocol on the biological sample.
185. The method of any one of claims 158-184, wherein at least a portion of the method is performed by a system of any one of claims 67-157.
186. A kit comprising a device of any one of claims 1-65.
187. A kit comprising a device of any one of claims 1-42.
188. The kit of claim 187, further comprising a sample cassette.
189. The kit of any one of claims 186-188, further comprising an informational material that directs a user to perform a method for attaching a sample to the device.