

CLAIMS

WHAT IS CLAIMED IS:

1. A flow cell device, comprising:
 - (a) a first reservoir housing a first solution and having an inlet end and an outlet end, wherein the first agent flows from the inlet end to the outlet end in the first reservoir;
 - (b) a second reservoir housing a second solution and having an inlet end and an outlet end, wherein the second agent flows from the inlet end to the outlet end in the second reservoir;
 - (c) a central region having an inlet end fluidically coupled to the outlet end of the first reservoir and the outlet end of the second reservoir through at least one valve;
wherein the volume of the first solution flowing from the outlet of the first reservoir to the inlet of the central region is less than the volume of the second solution flowing from the outlet of the second reservoir to the inlet of the central region.
2. The device of claim 1, wherein the first solution is different from the second solution.
3. The device of claim 1, wherein the second solution comprises at least one reagent common to a plurality of reactions occurring in the central region.
4. The device of claim 1, wherein the second solution comprises at least one reagent selected from the list consisting of a solvent, a polymerase, and a dNTP.
5. The device of claim 1, wherein the second solution comprise low cost reagents.
6. The device of claim 1 wherein the first reservoir is fluidically coupled to the central region through a first valve and the second reservoir is fluidically coupled to the central region through a second valve.
7. The device of claim 1, wherein the valve is a diaphragm valve.
8. The device of claim 1, wherein the first solution comprises a reagent and the second solution comprises a reagent and the reagent in the first solution is more expensive than the reagent in the second solution.
9. The device of claim 1, wherein the first solution comprises a reaction-specific reagent and the second solution comprises nonspecific reagent common to all reaction occurring in the central region, and wherein the reaction specific reagent is more expensive than the nonspecific reagent.
10. The device of claim 1, wherein the first reservoir is positioned in close proximity to the inlet of the central region to reduce dead volume for delivery of the first solutions.

11. The device of claim 1, wherein the first reservoir is placed closer to the inlet of the central region than the second reservoir.
12. The device of claim 1, wherein the reaction-specific reagent is configured in close proximity to the second diaphragm valve so as to reduce dead volume relative to delivery of the plurality of nonspecific reagents from the plurality of reservoirs to the first diaphragm valve.
13. The device of claim 1, wherein the central region comprises a capillary tube.
14. The device of claim 13, wherein the capillary tube is an off-shelf product.
15. The device of claim 13, wherein the capillary tube is removable from the device.
16. The device of claim 13, wherein the capillary tube comprises an oligonucleotide population directed to sequence a eukaryotic genome.
17. The device of claim 1, wherein the central region comprises a microfluidic chip.
18. The device of claim 17, wherein the microfluidic chip comprises a single etched layer.
19. The device of claim 17, wherein the microfluidic chip comprises at least one chip channel.
20. The device of claim 19, wherein the channel has an average depth in the range of 50 to 300 μm .
21. The device of claim 19, wherein the channel has an average length in the range of 1 to 200 mm.
22. The device of claim 19, wherein the channel has an average width in the range of 0.1 to 30 mm.
23. The device of claim 19, wherein the channel is formed by laser irradiation.
24. The device of claim 17, wherein the microfluidic chip comprises one etched layer.
25. The device of claim 17, wherein the microfluidic chip comprises one non-etched layer, and wherein the etched layer is bonded with the non-etched layer.
26. The device of claim 17, wherein the microfluidic chip comprises two non-etched layers, and wherein the etched layer is positioned between the two non-etched layers.
27. The device of claim 17, wherein the microfluidic chip comprises at least two bonded layers.
28. The device of claim 17, wherein the microfluidic chip comprises quartz.

29. The device of claim 17, wherein the microfluidic chip comprises borosilicate glass.
30. The device of claim 19, wherein the chip channel comprises an oligonucleotide population directed to sequence a prokaryotic genome.
31. The device of claim 19, wherein the chip channel comprises an oligonucleotide population directed to sequence a transcriptome.
32. The device of claim 19, wherein the chip channel is formed by laser irradiation.
33. The device of claim 19, wherein the chip channel has an open top.
34. The device of claim 19, wherein the chip channel is positioned between a top layer and a bottom layer.
35. The device of claim 19, wherein the chip channel is positioned adjacent to a top layer.
36. The device of claim 1, wherein the central region comprises a window that allows at least a part of the central region to be illuminated and imaged.
37. The device of claim 13, wherein the capillary tube comprises a window that allows at least a part of the capillary tube to be illuminated and imaged.
38. The device of claim 19, wherein the etched channel comprises a window that allows at least a part of the chip channel to be illuminated and imaged.
39. The device of claim 1, wherein the central region comprises a surface having at least one oligonucleotide tethered thereto.
40. The device of claim 39, wherein the surface is an interior surface of channel or capillary tube.
41. The device of claim 39 or 40, wherein the surface is a locally planar surface.
42. The device of claim 39, wherein the oligonucleotide is directly tethered to the surface.
43. The device of claim 39, wherein the oligonucleotide is tethered to the surface through an intermediate molecule.
44. The device of claim 39, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a eukaryotic genomic nucleic acid segment.
45. The device of claim 39, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a prokaryotic genomic nucleic acid segment.
46. The device of claim 39, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a viral nucleic acid segment.
47. The device of claim 39, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a transcriptome nucleic acid segment.

48. The device of claim 1, wherein the central region comprises an interior volume suitable for sequencing a eukaryotic genome.
49. The device of claim 1, wherein the central region comprises an interior volume suitable for sequencing a prokaryotic genome.
50. The device of claim 1, wherein the central region comprises an interior volume suitable for sequencing a transcriptome.
51. The device of claim 1, comprises a temperature modulator thermally coupled to the central region.
52. The device of claim 1, wherein the temperature modulator comprises a heat block.
53. The device of claim 1, wherein the temperature modulator comprises a vent.
54. The device of claim 1, wherein the temperature modulator comprises a course for air flow.
55. The device of claim 1, wherein the temperature modulator comprises a fan.
56. A flow cell device comprising:
- (d) a framework;
 - (e) a plurality of reservoirs harboring reagents common to a plurality of reactions compatible with the flow cell;
 - (f) a single reservoir harboring a reaction-specific reagent;
 - (g) a removable capillary having 1) a first diaphragm valve gating intake of a plurality of nonspecific reagents from the plurality of reservoirs, and 2) a second diaphragm valve gating intake of a single reagent from a source reservoir in close proximity to the second diaphragm valve.
57. The flow cell device of claim 56, wherein the framework comprises a thermal modulator.
58. The flow cell device of claim 57, wherein the thermal modulator comprises a heat block.
59. The flow cell device of claim 57, wherein the thermal modulator comprises a vent.
60. The flow cell device of claim 57, wherein the thermal modulator comprises a course for air flow.
61. The flow cell device of claim 57, wherein the thermal modulator comprises a fan.
62. The capillary flow cell device of claim 56, wherein the framework comprises a light detection access region.

63. The flow cell device of claim 62, wherein the light detection access region allows exposure of the removable capillary to an excitation spectrum.

64. The flow cell device of claim 62, wherein the light detection access region allows detection of an emission spectrum arising from the removable capillary.

65. The flow cell device of claim 56, wherein the reagents common to a plurality of reactions comprise at least one reagent selected from the list consisting of a solvent, a polymerase, and a dNTP.

66. The flow cell device of claim 56, wherein the reagents common to a plurality of reactions comprise low cost reagents.

67. The flow cell device of claim 56, wherein the reagents common to a plurality of reactions are directed to the first diaphragm valve through a first channel that is longer than a second channel connecting the second diaphragm valve to the single reservoir.

68. The flow cell device of claim 56, wherein the reaction-specific reagent is more expensive than any one nonspecific reagent.

69. The flow cell device of claim 56, wherein the reaction-specific reagent is more expensive than all nonspecific reagents.

70. The flow cell device of claim 56, wherein the reaction-specific reagent is configured in close proximity to the second diaphragm valve so as to reduce dead volume relative to delivery of the plurality of nonspecific reagents from the plurality of reservoirs to the first diaphragm valve.

71. The flow cell device of claim 56, wherein the capillary comprises a locally planar surface.

72. The flow cell device of claim 71, wherein the locally planar surface is at least partially transparent to an excitation wavelength.

73. The flow cell device of claim 71, wherein the locally planar surface is at least partially transparent to an emission wavelength.

74. The flow cell device of claim 71, wherein the locally planar surface comprises an oligonucleotide tethered thereto.

75. The flow cell device of claim 74, wherein the oligonucleotide is directly tethered to the surface.

76. The flow cell device of claim 74, wherein the oligonucleotide is tethered to the surface through an intermediate molecule.

77. The flow cell device of claim 74, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a eukaryotic genomic nucleic acid segment.

78. The flow cell device of claim 74, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a prokaryotic genomic nucleic acid segment.

79. The flow cell device of claim 74, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a viral nucleic acid segment.

80. The flow cell device of claim 74, wherein the oligonucleotide exhibits a segment that specifically hybridizes to a transcriptome nucleic acid segment

81. The flow cell device of claim 56, wherein the capillary comprises an interior volume suitable for sequencing a eukaryotic genome.

82. The flow cell device of claim 56, wherein the capillary comprises an interior volume suitable for sequencing a prokaryotic genome.

83. The flow cell device of claim 56, wherein the capillary comprises an interior volume suitable for sequencing a transcriptome.

84. The flow cell device of claim 56, wherein the capillary comprises a tube.

85. The flow cell device of claim 84, wherein the tube is an off-shelf product.

86. The capillary flow cell device of claim 85, wherein the tube is manufactured to match specifications of the framework.

87. The flow cell device of claim 85, wherein the tube comprises an oligonucleotide population directed to sequence a eukaryotic genome.

88. The flow cell device of claim 56, wherein the device comprises a microfluidic chip.

89. The flow cell device of claim 88, wherein the microfluidic chip comprises a single etched layer.

90. The flow cell device of claim 88, wherein the microfluidic chip comprises at least one chip channel.

91. The flow cell device of claim 88, wherein the microfluidic chip comprises one etched layer.

92. The flow cell device of claim 91, wherein the microfluidic chip comprises one non-etched layer

93. The flow cell device of claim 91, wherein the microfluidic chip comprises two non-etched layers.

94. The flow cell device of claim 91, wherein the microfluidic chip comprises at least two bonded layers.

95. The flow cell device of claim 88, wherein the microfluidic chip comprises quartz.

96. The flow cell device of claim 88, wherein the microfluidic chip comprises borosilicate glass.

97. The flow cell device of claim 90, wherein the chip channel comprises an oligonucleotide population directed to sequence a prokaryotic genome.
98. The flow cell device of claim 90, wherein the chip channel comprises an oligonucleotide population directed to sequence a transcriptome.
99. A flow cell device comprising:
- a) one or more capillaries, wherein the one or more capillaries are replaceable;
 - b) two or more fluidic adaptors attached to the one or more capillaries and configured to mate with tubing that provides fluid communication between each of the one or more capillaries and a fluid control system that is external to the flow cell device; and
 - c) optionally, a cartridge configured to mate with the one or more capillaries such that the one or more capillaries are held in a fixed orientation relative to the cartridge, and wherein the two or more fluidic adaptors are integrated with the cartridge.
100. The flow cell device of claim 99, wherein at least a portion of the one or more capillaries are optically transparent.
101. The flow cell device of claim 99 or claim 100, wherein the one or more capillaries are fabricated from glass, fused-silica, acrylic, polycarbonate, cyclic olefin copolymer (COC), cyclic olefin polymer (COP), or any combination thereof.
102. The flow cell device of any one of claims 99 to 101, wherein the one or more capillaries have a circular, square, or rectangular cross-section.
103. The flow cell device of any one of claims 99 to 102, wherein the largest internal cross-sectional dimension of a capillary lumen is between about 10 μm to about 1 mm.
104. The flow cell device of any one of claims 99 to 103, wherein the largest internal cross-sectional dimension of a capillary lumen is less than about 500 μm .
105. The flow cell device of any one of claims 99 to 104, wherein the two or more fluidic adaptors are fabricated from polydimethylsiloxane (PDMS; elastomer), polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), polyethyleneimine (PEI), polyimide, cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyethylene terephthalate (PET), epoxy resin, or any combination thereof.
106. The flow cell device of any one of claims 99 to 105, wherein the cartridge is fabricated from polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), polyethyleneimine (PEI), polyimide, cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyethylene terephthalate (PET), epoxy resin, or any combination thereof.

107. The flow cell device of any one of claims 99 to 106, wherein the cartridge further comprises one or more miniature valves, miniature pumps, temperature control components, or any combination thereof.

108. The flow cell device of any one of claims 99 to 107, wherein a capillary lumen of the one or more capillaries comprises a low nonspecific binding coating.

109. The flow cell device of claim 108, wherein the low nonspecific binding coating further comprises covalently-tethered oligonucleotide primers.

110. The flow cell device of claim 109, wherein the covalently-tethered oligonucleotides are tethered at a surface density of about 1000 per μm^2 .

111. The flow cell device of any one of claims 108 to 110, wherein a surface property of low nonspecific binding coating is adjusted to provide optimal performance of a solid-phase nucleic acid amplification method performed within the one or more capillaries.

112. The flow cell device of any one of claims 108 to 110, wherein the flow cell device comprises two or more capillaries, and wherein the low nonspecific binding coating of the two or more capillaries is the same.

113. The flow cell device of any one of claims 108 to 112, wherein the flow cell device comprises two or more capillaries, and wherein the low nonspecific binding coating of one or more capillaries is different from that of the other capillaries.

114. The flow cell device of any one of claims 1 to 113, wherein the flow cell device comprises an interior surface that is passivated.

115. The flow cell device of claim 114, wherein the interior surface comprises:

- a) a substrate;
- b) at least one hydrophilic polymer coating layer;
- c) a plurality of oligonucleotide molecules attached to at least one hydrophilic polymer coating layer; and
- d) at least one discrete region of the surface that comprises a plurality of clonally-amplified, sample nucleic acid molecules that have been annealed to the plurality of attached oligonucleotide molecules,

wherein a fluorescence image of the surface exhibits a contrast-to-noise ratio (CNR) of at least 20.

116. The flow cell device of claim 115, wherein the hydrophilic polymer coating layer has a water contact angle of less than 50 degrees.

117. The flow cell device of claim 114-116, wherein the substrate is glass or plastic.

118. A system comprising:
- a) one or more of the flow cell devices of any one of claims 99 – 113;
 - b) a fluid flow controller; and
 - c) optionally, a temperature controller or an imaging apparatus.
119. The system of claim 118, wherein the fluid flow controller comprises one or more pumps, valves, mixing manifolds, reagent reservoirs, waste reservoirs, or any combination thereof.
120. The system of claim 118 or claim 119, wherein the fluid flow controller is configured to provide programmable control of fluid flow velocity, volumetric fluid flow rate, the timing of reagent or buffer introduction, or any combination thereof.
121. The system of any one of claims 118 to 120, wherein the temperature controller comprises a metal plate positioned so that it makes contact with the one or more capillaries, and a peltier or resistive heater.
122. The system of claim 121, wherein the metal plate is integrated into the cartridge.
123. The system of any one of claims 118 to 122, wherein the temperature controller comprises one or more air delivery devices configured to direct a stream of heated or cooled air such that it makes contact with the one or more capillaries.
124. The system of any one of claims 121 to 123, wherein the temperature controller further comprises one or more temperature sensors.
125. The system of claim 124, wherein the one or more temperature sensors are integrated into the cartridge.
126. The system of any one of claims 118 to 125, wherein the temperature controller allows the temperature of the one or more capillaries to be held at a fixed temperature.
127. The system of any one of claims 118 to 126, wherein the temperature controller allows the temperature of the one or more capillaries to be cycled between at least two set temperatures in a programmable manner.
128. The system of any one of claims 118 to 127, wherein the imaging apparatus comprises a microscope equipped with a CCD or CMOS camera.
129. The system of any one of claims 118 to 128, wherein the imaging apparatus comprises one or more light sources, one or more lenses, one or more mirrors, one or more prisms, one or more bandpass filters, one or more long-pass filters, one or more short-pass filters, one or more dichroic reflectors, one or more apertures, and one or more image sensors, or any combination thereof.

130. The system of any one of claims 118 to 129, wherein the imaging apparatus is configured to acquire bright-field images, dark-field images, fluorescence images, two-photon fluorescence images, or any combination thereof.

131. The system of any one of claims 118 to 130, wherein the imaging apparatus is configured to acquire video images.

132. A flow cell device comprising a one-piece or unitary flow cell construction.

133. The flow cell device of claim 132, wherein the one-piece or unitary flow cell construction comprises a glass or polymer capillary.

134. The flow cell device of claim 132 or claim 133, wherein in a surface of a fluid channel within the device comprises a low nonspecific binding coating.

135. A method of sequencing a nucleic acid sample and a second nucleic acid sample, comprising:

- a) delivering a plurality of oligonucleotides to an interior surface of an at least partially transparent chamber;
- b) delivering a first nucleic acid sample to the interior surface;
- c) delivering a plurality of nonspecific reagents through a first channel to the interior surface;
- d) delivering a specific reagent through a second channel to the interior surface, wherein the second channel has a lower volume than the first channel;
- e) visualizing a sequencing reaction on the interior surface of the at least partially transparent chamber; and
- f) replacing the at least partially transparent chamber prior to a second sequencing reaction.

136. The method of claim 135, comprising flowing an air current past an exterior surface of the at least partially transparent surface.

137. The method of claim 135, comprising selecting the plurality of oligonucleotides to sequence a eukaryotic genome.

138. The method of claim 137, comprising selecting a prefabricated tube as the at least partially transparent chamber.

139. The method of claim 135, comprising selecting the plurality of oligonucleotides to sequence a prokaryotic genome.

140. The method of claim 135, comprising selecting the plurality of oligonucleotides to sequence a transcriptome.

141. The method of claim 139, comprising selecting a capillary tube as the at least partially transparent chamber.
142. The method of claim 140, comprising selecting a microfluidic chip as the at least partially transparent chamber.
143. A method of making a microfluidic chip in a flow cell device of claim 1, comprising:
- providing a surface; and
 - etching the surface to form at least one channel.
144. The method of claim 143, wherein the etching is performed using laser radiation.
145. The method of claim 143, wherein the channel has an average depth of 50 to 300 μm .
146. The method of claim 143, wherein the channel has an average width of 0.1 to 30 mm.
147. The method of claim 143, wherein the channel has an average length in the range of 1 to 200 mm.
148. The method of claim 143, further comprising bonding a first layer to the etched surface.
149. The method of claim 143, further comprising bonding a second layer to the etched surface, wherein the etched surface is positioned between the first layer and the second layer.
150. A method of reducing a reagent used in a sequencing reaction, comprising:
- (a) providing a first reagent in a first reservoir;
 - (b) providing a second reagent in a first second reservoir, wherein each of the first reservoir and the second reservoir are fluidically coupled to a central region, and wherein the central region comprises a surface for the sequencing reaction; and
 - (c) sequentially introducing the first reagent and the second reagent into a central region of the flow cell device, wherein the volume of the first reagent flowing from the first reservoir to the inlet of the central region is less than the volume of the second reagent flowing from the second reservoir to the central region.
151. A method of increasing the efficient use of a reagent in a sequencing reaction, comprising:
- (a) providing a first reagent in a first reservoir;
 - (b) providing a second reagent in a first second reservoir, wherein each of the first reservoir and the second reservoir are fluidically coupled to a central region, and wherein the central region comprises a surface for the sequencing reaction; and

(c) maintaining the volume of the first reagent flowing from the first reservoir to the inlet of the central region to be less than the volume of the second reagent flowing from the second reservoir to the central region.

152. The method of claim 150 or 151, wherein the first reagent is more expensive than the second agent.

153. The method of claim 150 or 151, wherein the first reagent is selected from the group consisting of a polymerase, a nucleotide, and a nucleotide analog.