FLOW CELL DEVICE AND USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/776,827, filed December 7, 2018 and U.S. Provisional Application No. 62/892,419, filed August 27, 2019, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Flow-cell devices are widely used in chemistry and biotechnology applications. Particularly in next-generation sequencing (NGS) systems, such devices are used to immobilize template nucleic acid molecules derived from biological samples and then introduce a repetitive flow of sequencing-by-synthesis reagents to attach labeled nucleotides to specific positions in the template sequences. A series of label signals are detected and decoded to reveal the nucleotide sequences of the template molecules, e.g., immobilized and/or amplified nucleic acid template molecules attached to an internal surface of the flow cell.

[0003] Typical NGS flow cells are multi-layer structures fabricated from planar surface substrates and other flow cell components (see, for example, U.S. Patent Application Publication No. 2018/0178215 A1), which are then bonded through mechanical, chemical, or laser bonding techniques to form fluid flow channels. Such flow cells typically require costly multi-step, precision fabrication techniques to achieve the required design specifications. On the other hand, inexpensive and off-the-shelf, single lumen (flow channel) capillaries are available in a variety of sizes and shapes but are generally not suited for ease of handling and compatibility with the repetitive switching between reagents that are required for application such as NGS.

SUMMARY

[0003a] In one aspect, the present disclosure refers to 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, wherein a capillary lumen of the one or more capillaries comprises a low nonspecific binding coating, and wherein the low nonspecific binding coating comprises covalently-tethered oligonucleotide primers.

[0004] Described herein are novel flow cell devices and systems for sequencing nucleic acids. The devices and systems described herein can achieve a more efficient use of the reagents help reduce the cost and time of the DNA sequencing process. The devices and systems can utilize a commercially-available, off-the-shelf capillaries or a micro or nano scale fluidic chip with a selected pattern of channels. The flow cell devices and systems described herein are suitable for rapid DNA sequencing and can help achieve more efficient use of expensive reagents and reduce the amount of time required for sample pre-treatment and replication compared to other DNA sequencing techniques. The result is a much faster and cost-effective sequencing method.

[0005] Some examples relate to a flow cell device, comprising: 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; 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; 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

[0006] Some examples relate to a flow cell device comprising: a framework; a plurality of reservoirs harboring reagents common to a plurality of reactions compatible with the flow cell; a single reservoir harboring a reaction-specific reagent; 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.

[0007] Some examples relate to a flow cell device comprising: a flame work; a plurality of reservoirs harboring reagents common to a plurality of reactions compatible with the flow cell; a single reservoir harboring a reaction-specific reagent; a removable or non-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. 3) optionally, a mounting example whereby capillaries are affixed / mounted to glass substrate via an index mounting media.

[0008] Some examples relate to 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, optionally, a mounting example whereby capillaries are affixed / mounted to glass substrate via an index mounting media

[0009] Some examples relate to a method of sequencing a nucleic acid sample and a second nucleic acid sample, comprising: delivering a plurality of oligonucleotides to an interior surface of an at least partially transparent chamber; delivering a first nucleic acid sample to the interior surface; delivering a plurality of nonspecific reagents through a first channel to the interior surface; delivering a specific reagent through a second channel to the interior surface, wherein the second channel has a lower volume than the first channel; visualizing a sequencing reaction on the interior surface of the

at least partially transparent chamber; and replacing the at least partially transparent chamber prior to a second sequencing reaction.

[0010] Some examples relate to a method of reducing a reagent used in a sequencing reaction, comprising: providing a first reagent in a first reservoir; providing a second reagent in a first second reservoir, wherein each of the first reservoir and the second reservoir are fluidicly coupled to a central region, and wherein the central region comprises a surface for the sequencing reaction; and 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.

[0011] Some examples relate to a method of increasing the efficient use of a regent in a sequencing reaction, comprising: providing a first reagent in a first reservoir; providing a second reagent in a first second reservoir, wherein each of the first reservoir and the second reservoir are fluidicaly coupled to a central region, and wherein the central region comprises a surface for the sequencing reaction; and 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.

INCORPORATION BY REFERENCE

[0012] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0014] Some novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0015] FIG. 1 illustrates one embodiment of a single capillary flow cell having 2 fluidic adaptors.

CLAIMS

WHAT IS CLAIMED IS:

1. 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.

wherein a capillary lumen of the one or more capillaries comprises a low nonspecific binding coating, and wherein the low nonspecific binding coating comprises covalently-tethered oligonucleotide primers.

2. The flow cell device of claim 1, wherein at least a portion of at least one of the one or more capillaries is optically transparent.

3. The flow cell device of claim 1 or claim 2, 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.

4. The flow cell device of any one of claims 1 to 3, wherein the cartridge further comprises one or more miniature valves, miniature pumps, temperature control components, or any combination thereof.

5. The flow cell device of claim 1, wherein the covalently-tethered oligonucleotide primers are tethered at a surface density of at least 1000 molecules per μm^2.

6. The flow cell device of any one of claims 1 to 5, wherein a surface property of the 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.

7. The flow cell device of any one of claims 1 to 6, 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.

8. The flow cell device of any one of claims 1 to 6, 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.

9. The flow cell device of any one of claims 1 to 8, wherein the coating comprises one or more layers of a hydrophilic polymer and has a water contact angle of less than 50 degrees.

10. The flow cell device of any one of claims 1 to 9, wherein the capillary lumen of the one or more capillaries further comprises at least one discrete region on a surface of the capillary lumen that comprises a plurality of clonally-amplified, sample nucleic acid molecules that have been annealed to the covalently-tethered oligonucleotide primers, and wherein a fluorescence image of the surface exhibits a contrast-to-noise ratio (CNR) of at least 20 when the clonally-amplified, sample nucleic acid molecules have been directly or indirectly labeled with a fluorophore.

11. The flow cell device of claim 10, wherein the at least one discrete region comprises a concatemer sequence that has been annealed to the covalently-tethered oligonucleotide primers.

12. The flow cell device of claim 10, wherein the at least one discrete region comprises a population of oligonucleotide molecules directed to sequence a eukaryotic genome, a prokaryotic genome, or a transcriptome.

13. The flow cell device of claim 10, wherein the plurality of clonally-amplified, sample nucleic acid molecules, or complementary sequences thereof, are labeled with a fluorophore such that a fluorescence image of the surface exhibits a contrast-to-noise ratio (CNR) of at least 20 when the fluorophore is Cyanine dye-3 and the fluorescence image is acquired using an Olympus IX83 inverted fluorescence microscope equipped with a 20X objective, a 532 nm light source, a bandpass and dichroic mirror filter set optimized for 532 nm long-pass excitation light, an emission bandpass filter optimized for Cyanine dye-3 emission, and an Andor sCMOS, Zyla 4.2 camera, under non-signal saturating conditions while the surface is immersed in 25 mM ACES, pH 7.4 buffer.

14. The flow cell device of any one of claims 1 to 13, further comprising:

a) a first reservoir configured to house a first solution; and

b) a second reservoir configured to house a second solution;

wherein an outlet of the first reservoir and an outlet of the second reservoir are fluidically-coupled to an inlet of the one or more capillaries through at least one valve such that the volume

of the first solution flowing from the outlet of the first reservoir to the inlet of the one or more capillaries is less than the volume of a second solution flowing from the outlet of the second reservoir to the inlet of the one or more capillaries.

15. The flow cell device of claim 14, wherein the at least one valve is a diaphragm valve.

16. The flow cell device of claim 14 or claim 15, wherein the outlet of the first reservoir is fluidically-coupled to the inlet of the one or more capillaries through a first valve, and the outlet of the second reservoir is fluidically-coupled to the inlet of the one or more capillaries through a second valve.

17. The flow cell device of any one of claims 14 to 16, wherein the outlet of the first reservoir is positioned close to the inlet of the one or more capillaries to reduce a dead volume for delivery of the first solution from the first reservoir to the one or more capillaries.

18. The flow cell device of any one of claims 14 to 17, wherein the outlet of the first reservoir is positioned closer to the inlet of the one or more capillaries region than is the outlet of the second reservoir.

19. The flow cell device of claim 14, wherein the outlet of the first reservoir is positioned close to the second valve to reduce a dead volume for delivery of the first solution to the one or more capillaries relative to a dead volume for delivery of the second solution to the one or more capillaries.

20. The flow cell device of any one of claims 16 to 19, wherein the first solution comprises a reaction-specific reagent, and wherein the second solution comprises at least one reagent common to a plurality of reactions occurring in the one or more capillaries.

21. The flow cell device of any one of claims 16 to 20, wherein the second solution comprises at least one reagent selected from the list consisting of a solvent, a polymerase, and a dNTP.

22. The flow cell device of any one of claims 1 to 21, wherein the one or more capillaries are off-the-shelf products.

23. The flow cell device of any one of claims 1 to 22, wherein the cartridge comprises a temperature control component that is thermally coupled to the one or more capillaries.

24. The flow cell device of claim 23, wherein the temperature control component comprises a heat block, a vent, a course for air flow, or a fan.