FLOW CELL DEVICES AND USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to, and benefit of, U.S. Provisional Application No. 63/567,218, filed on March 19, 2024, the contents of which are incorporated by reference in their entirety herein.

BACKGROUND

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

[0003] Existing NGS flow cell devices are multi-layered structures fabricated from planar surface substrates and other flow cell components, which are then bonded to form fluid flow channels. Such flow cell devices may require costly, multi-step precision fabrication techniques to achieve the required design specifications. On the other hand, inexpensive and off-the-shelf, single 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 required for applications such as NGS.

SUMMARY

[0004] Described herein are flow cell devices and systems for sequencing nucleic acids. The flow cell devices, systems, and methods described herein can advantageously achieve efficient delivery and usage of reagents to significantly lower consumable costs and reduce delivery time of reagents in sequencing analysis. The devices, systems, and methods described herein can advantageously achieve more efficient and effective cleaning and alleviate contamination caused by reagent residuals, thereby increasing accuracy and reliability of sequencing analysis. The devices, systems, and methods herein can advantageously allow or cause delivery or purging of an air gap (e.g., an amount of air, a bolus of air, quantum of air, or a flow of air or otherwise gaseous flow) between administration of two liquid reagents without impairing chemical functioning of the flow cell

device (also referred to herein as "flow cells") and its sequencing coating(s), which may not be feasible with existing flow cells and their coatings. Such air gap may greatly facilitate cleaning and thus reduces contamination by left-over or residual reagents in subsequent reactions on the flow cell. The air gap may improve a homogeneity of the reagents across a channel of the flow cell device, thereby reducing concentration gradients of the reagent and improving accuracy of sequencing.

[0005] The devices, systems, and methods herein can eliminate series of tubing (e.g., a common line for different reagents) for reagent administration such that the flow cell devices can be robust against fluidics errors and adaptable to different fluidic control and administration. As such, the flow cells disclosed herein can be flexible for various sequencing applications. For example, the flow cell devices herein may not include locked-in tubing, and therefore, errors resulting from malfunction of the tubing (e.g., a clogged tube) can be easily resolved in comparison to existing flow cell systems. As another example, the flow cell devices herein can be conveniently adapted for the addition/removal of nozzles or dispensing tips for a new sequencing application. Furthermore, the flow cell devices and systems herein can include an open landing area in combination with the air gap, which advantageously achieves a more homogenous distribution of reagents on the flow cell and with less consumption of reagents in comparison to existing devices. The flow cell devices and systems herein can separate fluidic pathways of reagents or other sequencing liquids from actuation pathways thereby avoiding errors that may be caused by leakage of reagents into such actuation pathways. The flow cell devices and system herein also may allow accurate and reliable fluidic dispensing in more than 1000 dispenses while enabling dispensing of various numbers of fluids. The flow cell devices and systems described herein are suitable for rapid DNA sequencing and can help realize more efficient use of expensive reagents and reduce the amount of time for sample pre-treatment and replication compared to other DNA sequencing techniques. Therefore, flow cell devices and systems described herein can result in a faster and more cost-effective sequencing method than other systems known in the art.

INCORPORATION BY REFERENCE

[0006] 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.

DESCRIPTION OF THE DRAWINGS

[0007] The novel features of the inventive concepts are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present inventive concepts will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the inventive concepts are utilized, and the accompanying drawings of which:

[0008] **FIG. 1** illustrates a block diagram of a computer-implemented system for performing operations in DNA sequencing and sequencing analysis, according to some embodiments.

[0009] **FIG. 2** is a schematic showing of a flow cell system, according to some embodiments.

[0010] **FIG. 3** is a schematic showing of a flow cell device, according to some embodiments.

[0011] **FIG. 4** is a schematic showing of a flow cell device, according to some embodiments.

[0012] **FIGS. 5A-5C** show a flow cell device, according to some embodiments. FIG. 5A is a perspective view of the substrates, according to some embodiments. FIG. 5B is a top view of the flow cell device in FIG. 5A. FIG. 5C is a cross-sectional view of the flow cell device at D-D' in FIG. 5B.

[0013] **FIG. 5D** is a perspective view of substrates of a flow cell device, according to some embodiments.

[0014] **FIG. 5E** is a perspective view of substrates of a flow cell device, according to some embodiments.

[0015] **FIG. 5F** shows a perspective view and a top view of a flow cell device, according to some embodiments.

[0016] **FIGS. 6A-6C** show fluidic control devices of the flow cell systems for delivery of reagents to a flow cell device, according to some embodiments. FIG. 6A shows a fluidic control device including a dispenser (vertical line) and a continuous track (left most arrow). FIG. 6B shows a fluidic control device including a dispensing plate (692) with an electrowetting surface. FIG. 6C shows a fluidic control device comprising a reagent reservoir (694) and a sipper (693).

[0017] **FIG. 7A** shows a graph illustrating contamination levels achieved by flow cell systems disclosed herein in comparison to existing flow cell systems.

[0018] **FIG. 7B** shows a table illustrating reduction of reagent consumption during a same sequencing application achieved by a flow cell system disclosed herein in comparison to an existing flow cell system.

[0019] **FIG. 8** illustrates a block diagram of a computer system for fluidic control and configured to perform sequencing and sequencing analysis, according to some embodiments.

[0020] **FIG. 9** is a schematic showing a linear single stranded library molecule (900) which comprises: a surface pinning primer binding site (920); an optional left unique identification sequence (980); a left index sequence (960); a forward sequencing primer binding site (940); an insert region having a sequence-of-interest (910); a reverse sequencing primer binding site (950); a right index sequence (970); and a surface capture primer binding site (930).

[0021] **FIG. 10** is a schematic showing a linear single stranded library molecule (900) which comprises: a surface pinning primer binding site (920); a left index sequence (960); a forward sequencing primer binding site (940); an insert region having a sequence-of-interest (910); a reverse sequencing primer binding site (950); a right index sequence (970); an optional right unique identification sequence (990); and a surface capture primer binding site (930).

[0022] **FIG. 11** is a schematic of various configurations of multivalent molecules. Left (Class I): schematics of multivalent molecules having a "starburst" or "helter-skelter" configuration. Center (Class II): a schematic of a multivalent molecule having a dendrimer configuration. Right (Class III): a schematic of multiple multivalent molecules formed by reacting streptavidin with 4-arm or 8-arm PEG-NHS with biotin and dNTPs. Nucleotide units are designated 'N', biotin is designated 'B', and streptavidin is designated 'SA'.

[0023] **FIG. 12** is a schematic of a multivalent molecule comprising a generic core attached to a plurality of nucleotide-arms.

[0024] **FIG. 13** is a schematic of a multivalent molecule comprising a dendrimer core attached to a plurality of nucleotide-arms.

[0025] **FIG. 14** shows a schematic of a multivalent molecule comprising a core attached to a plurality of nucleotide-arms, where the nucleotide arms comprise biotin, spacer, linker and a nucleotide unit.

[0026] **FIG. 15** is a schematic of a nucleotide-arm comprising a core attachment moiety, spacer, linker and nucleotide unit.

[0027] **FIG. 16** shows the chemical structure of a spacer (top), and the chemical structures of various linkers, including an 11-atom Linker, a 16-atom Linker, a 23-atom Linker and an N3 Linker (bottom).

[0028] **FIG. 17** shows the chemical structures of various linkers, including Linkers 1-9.

[0029] **FIG. 18** shows the chemical structures of various linkers joined/attached to nucleotide units.

[0030] **FIG. 19** shows the chemical structures of various linkers joined/attached to nucleotide units.

[0031] **FIG. 20** shows the chemical structures of various linkers joined/attached to nucleotide units.

[0032] **FIG. 21** shows the chemical structures of various linkers joined/attached to nucleotide units.

[0033] **FIG. 22** shows the chemical structure of a biotinylated nucleotide-arm. In this example, the nucleotide unit is connected to the linker via a propargyl amine attachment at the 5 position of a pyrimidine base or the 7 position of a purine base.

[0034] **FIG. 23** shows a schematic illustration of one embodiment of the flow cell devices in which the support comprises a glass substrate and alternating layers of hydrophilic coatings which are covalently or non-covalently adhered to the glass, and which further comprises chemically-reactive functional groups that serve as attachment sites for oligonucleotide primers.

[0035] **FIGS. 24A-24E** show an embodiment of the flow cell device depicted in FIGS. 5A-5D. FIG. 24A is a perspective view of the flow cell device. FIG. 24B is a perspective view of the flow cell device showing the top, middle and bottom substrates. FIG. 24C is a top view of the top substrate of the flow cell device. FIG. 24D is a top view of the middle substrate of the flow cell device. FIG. 24E is a top view of the bottom substrate of the flow cell device.

[0036] **FIGS. 25A-25E** show an embodiment of the flow cell device disclosed herein. FIG. 25A is a perspective view of the flow cell device. FIG. 25B is a perspective view of the flow cell device showing the top, middle and bottom substrates. FIG. 25C is a top view of the top substrate of the flow cell device. FIG. 25D is a top view of the middle substrate of the flow device. FIG. 25E is a top view of the bottom substrate of the flow cell device.

[0037] **FIGS. 26A-26C** show embodiments of flow cell devices with different landing areas, according to some embodiments. FIG. 26A is a top view of an embodiment of the flow cell device. FIG. 26B is a top view of another embodiment of the flow cell device. The flow device in FIG. 26A comprises a differently sized open landing area and inlet as compared to the flow cell device in FIG. 26B or FIGS. 24A-24E. The tapered transition portion from the cleaning outlet to the open landing area of the flow cell device in FIG. 26A is also altered from embodiments in FIG. 26B or FIGS. 24A-24E. FIG. 26C is a top view of yet another

embodiment of the flow cell device. The flow device in FIG. 26C comprises a differently sized open landing area and inlet as compared to the flow cell device in FIGS. 25A-E.

[0038] **FIGS. 27A-27G** show an embodiment of the flow cell device disclosed herein. FIG. 27A is a side view of the flow cell device. FIG. 27B shows a cross-sectional view at A-A in FIG. 27A. FIG. 27C is a top view of the flow cell device. FIG. 27D is a cross-sectional view at B-B in FIG. 27B. FIG. 27E shows an expanded view of area A in FIG. 27B. FIG. 27F shows an expanded view of area C in FIG. 27C. FIG. 27G shows an expanded view of area B in FIG. 27D.

[0039] **FIGS. 28A-28C** show an embodiment of the flow cell device in FIG. 5E in a top view (FIG. 28A), a prospective view (FIG. 28B), and a prospective view of the bottom, middle, and top substrates (FIG. 28C).

[0040] **FIG. 29** shows a graph illustrating contamination levels of individual tiles and average contamination level across multiple tiles of the flow cell device achieved by flow cell systems disclosed herein.

[0041] **FIGS. 30A-30B** show schematics of two embodiments of the fluid dispensing device herein.

[0042] **FIGS. 31A-31B** show schematics of an embodiment of the fluid dispensing device herein. The fluid dispensing device is coupled to an actuator configured to actuate the pump(s) of the fluid dispensing device. The fluid dispensing device, in this particular embodiment, includes a microfluidic chip (FIG. 31B).

[0043] **FIG. 31C** shows a schematic of the fluid dispensing device in **FIGS. 31A-31B** coupled to an actuator configured to move relative to the microfluidic chip and actuate the pump(s) of the fluid dispensing device.

[0044] **FIGS. 32A-32B** shows a schematic of the cross section of the fluid dispensing device in **FIGS. 31A-31B** with the movable pin configured to deliver fluid from the microfluidic chip to the dispensing tips. The movable pin is mechanically coupled to the actuator (FIG. 32A) or the microfluidic chip (FIG. 32B).

[0045] **FIGS. 33A-33B** shows a schematic of an embodiment of the fluid dispensing device herein with barrels and plungers. Each of the barrel and plunger pairs connects a different compartments of the fluid dispensing device to a microfluidic pathway of the microfluidic chip, and are configured to deliver fluid from the cartridge to the microfluidic chip, and then to the dispensing tips.

[0046] **FIGS. 34A-34C** show schematics of an embodiment of the fluid dispensing device herein with barrels and plungers. The barrel and plunger pair connects to individual reservoirs

and then to corresponding compartments of the fluid dispensing device through individual valves. The barrel and plunger pair is configured to deliver fluid from the cartridge to the microfluidic chip, and then to the dispensing tips through the corresponding valve.

[0047] **FIGS. 35A -35C** show schematics of an embodiment of the fluid dispensing device with the reagent cartridge, the transportation valve, and the microfluidic chip.

[0048] **FIG. 35D** shows a schematic of an embodiment of the fluid dispensing device with the reagent cartridge, the transportation valve, and the microfluidic chip.

[0049] **FIG. 35E** shows a schematic of the transportation valve in FIG. 35D.

[0050] **FIGS. 36A-36B** show schematics of a cross-sectional view of the fluidic dispensing device in FIGS. 35A-35E.

[0051] **FIG. 36C** shows a schematic of a cross-sectional view of the transportation valve, rotor, biasing element, and microfluidic chip in FIGS. 35D-35E.

[0052] **FIGS. 37A-37D** show schematics of a bottom view of the fluidic dispensing device (FIG. 37A) in relation to the flow cell device, and a bottom view of the microfluidic chip in relation to the transportation valve (FIGS. 37B-37D).

[0053] **FIGS. 37E-37F** show schematics of a bottom view of an exemplary embodiment of the microfluidic chip in relation.

[0054] **FIGS. 38A-38B** show schematics of an embodiment of the fluid dispensing device herein with the transportation valve and the microfluidic chip.

[0055] **FIGS. 39A-39C** show the at least two opening positions for reagent aspiration and dispensing and the closed position of the transportation valve in relation to the microfluidic chip.

[0056] **FIG. 40** shows a schematic of an embodiment of the fluid dispensing device herein with the transportation valve and the microfluidic chip.

[0057] **FIGS. 41A -41C** show schematics of an embodiment of the microfluidic chip.

[0058] **FIGS. 42A -42C** show schematics of an embodiment of the microfluidic chip. In this case, the fluidic flow within the microfluidic chip, among the pathways, in-chip well, valves, and between the microfluidic chip and the dispensing tips or reagent compartments.

[0059] **FIG. 42D** shows a schematic of exemplary embodiment of the microfluidic chip, in this case, the in-chip well and the fluidic pathways.

[0060] **FIG. 42E** shows a schematic of exemplary embodiment of the microfluidic chip, in this case, the in-chip well and the fluidic pathways.

[0061] **FIGS. 42F -42H** show schematics of a bottom view of an exemplary embodiment of the in-chip well and its cross section in relation to the fluidic pathway(s).

[0062] **FIGS. 43A -43B** show schematics of fluidic flow issues in existing microfluidic chips and their corresponding in-chip wells.

[0063] **FIGS. 44A-44B** show schematics of exemplary embodiments of the microfluidic chips having a single piece substrate with films (FIG. 44A) or two separated substrate halves with a mid-film in between.

[0064] **FIGS. 45A -45C** show schematics of exemplary embodiments of the in-chip well of the microfluidic chips with cut-outs.

[0065] **FIGS. 46A -46B** show schematics of an exemplary embodiment of the reagent cartridge in relation to the microfluidic chip and the dispensing tips. The microfluidic chip is in sequencing position and washing position (FIG. 46B) and transition between the sequencing position and washing position (FIG. 46A) relative to the dispensing tips.

[0066] **FIG. 46C** shows a schematic of a top view of an exemplary embodiment of the reagent cartridge with two washing positions for the microfluidic chip and the dispensing tips.

[0067] **FIGS. 47A-47D** show schematics of an exemplary embodiment of the microfluidic chip with valves that controls fluidic communication between the microfluidic chip, the reagent cartridges, and the dispensing tips.

DETAILED DESCRIPTION

[0068] Described herein are systems and devices to analyze different nucleic acid sequences e.g., from amplified nucleic acid arrays in flow cells or from an array of immobilized nucleic acids. The systems and devices described herein can also be useful in, e.g., sequencing for comparative genomics, tracking gene expression, microRNA sequence analysis, epigenomics, and aptamer and phage display library characterization, and other sequencing applications. The systems and devices herein comprise various combinations of optical, mechanical, fluidic, thermal, electrical, and computing devices/aspects. The systems and devices described herein can also be useful for imaging applications that use sequencing systems to image target analytes such as proteins or nucleic acid in cells or tissues disposed within flow cell devices. See, for example, WO2024040068, the contents of which are incorporated by reference in their entireties herein.

[0069] The advantages of the disclosed flow cell devices, fluidic control devices, and systems include, but are not limited to: significantly lower consumable costs (e.g., as compared to those for currently available nucleic acid sequencing systems); efficient and effective cleaning of flow cell devices, thereby reducing contamination of sequencing

processes by residual reagent(s); reduced delivery time of reagents, reduced washing time, and increased homogeneity of reagents on the flow cells; reduced device and system manufacturing/maintenance complexity and cost; flexible system throughput and flexible adaptation of the systems to different sequencing applications.

[0070] Capillary flow cell devices, cartridges, and systems can include, but are not limited to: an open dispensing tip in the fluidic control device and an open landing area on the flow cell device to allow open delivery of reagents without the complexity and cost of existing tubing and to enable flexibility in the systems to adapt to different sequencing applications; a slippery coating that facilitates fluidic transfer and residual cleaning from the opening landing area; a cleaning outlet in fluidic connection to the open landing area to facilitate cleaning of liquid meniscus that cannot be effectively cleaned using washing reagents alone; a channel coating that allows purging of an air gap between two fluidic reagents without damaging subsequent sequencing reactions; and compatibility with a wide variety of detection methods such as fluorescence imaging.

[0071] Although the disclosed flow cell devices and systems are described primarily in the context of their use for nucleic acid sequencing applications, various aspects of the disclosed devices and systems may be applied not only to nucleic acid sequencing but also to any other type of chemical analysis, biochemical analysis, nucleic acid analysis, cell analysis, or tissue analysis application. It shall be understood that different aspects of the disclosed devices and systems can be appreciated individually, collectively, or in combination with each other.

Sequencing systems

[0072] Disclosed herein, in some embodiments, are flow cell devices and systems that can be employed for performing or facilitating DNA sequencing analysis using sequencing systems. The sequencing systems may utilize various sequencing techniques including but not limited to the sequencing techniques disclosed herein.

[0073] FIG. 1 illustrates a block diagram of a computer-implemented system 100 for performing sequencing and sequencing analysis, according to one or more embodiments disclosed herein. The system 100 has a sequencing system 110 that includes a flow cell device 112, a sequencer 114, an imager 116, a data storage device 122, and a user interface 124. The sequencing system 110 may optionally be connected to a cloud 130 (e.g., coupled to a server, compute device, database, etc.). The sequencing system 110 may include one or more of dedicated processors 118, an integrated circuit (e.g., Field-Programmable Gate Array(s) (FPGAs)) 120, and a computer system 126.

[0074] In some embodiments, the flow cell device 112 is configured to capture DNA fragments and form DNA sequences for base-calling on the flow cell device 112. The flow cell device 112 can include a support as described herein. The support can be a solid support. The support can include a surface coating thereon as disclosed herein. The surface coating can be a polymer coating as disclosed herein. The surface coating can be disposed on a surface of the one or more channels of the flow cell device. A different or identical surface can be placed on a surface of the inlet of the flow cell device.

[0075] In some embodiments, the flow cell device 112 can include a plurality of tiles (e.g., portions, locations, areas, sections, etc.) thereon configured to be imaged by the imager 116, and each tile may be separated into a plurality of subtiles. In some embodiments, the subtiles may be organized in a grid. Each subtile can include a plurality of clusters or polonies (e.g., a collection of DNA molecules such as the concatemer template molecules disclosed herein) thereon. In some embodiments, the flow cell device 112 may include a number of tiles in a range of about 1 tile to about 2000 tiles, about 100 tile to about 1500 tiles, or about 200 tile to about 500 tiles, inclusive of all ranges and subranges therebetween. In some embodiments, each tile may be divided into a number of subtiles in a range of about 2 subtiles to about 200 subtiles, about 10 subtiles to about 100 subtiles, or about 20 subtiles to about 50 subtiles, inclusive of all ranges and subranges therebetween. In some embodiments, the subtiles may be organized in a grid that may have M by N subtiles. As a nonlimiting example, a flow cell can have 424 tiles, and each tile can be divided into a 6 x 9 grid, therefore 54 subtiles. In some embodiment, the imager 116 may be configured to obtain one or more images (hereinafter, "flow cell image(s)" of the plurality of tiles, a subset of the plurality of tiles, and/or a subset of the plurality of subtiles. The flow cell image(s) as disclosed herein can include an image including signals (e.g., fluorescence levels) of the plurality of clusters or polonies. The flow cell image can include one or more tiles of signals or one or more subtiles of signals. In some embodiments, a flow cell image can be an image that includes all the tiles and approximately all signals thereon. The flow cell image can be acquired from a channel during (i) an imaging cycle or (ii) a sequencing cycle using the imager 116. In some embodiments, each tile may include millions of polonies or clusters. As a nonlimiting example, a tile can include about 1 to 10 million clusters or polonies. Each polony can be a collection of many copies of DNA molecules.

[0076] More details of the flow cell device 112 and its functional and structural elements are disclosed herein in relation to figures, e.g., FIGS. 2-4, 5A-5F, 6A-6C, and 7A-7B.

[0077] The sequencer 114 may be configured to flow mixtures of reagents onto the flow cell. Such mixtures of reagents include nucleotide mixtures, polymerases, reagents to add or cleave chain terminating moieties from the nucleotides in between nucleotide addition steps, and perform other steps for the formation of the DNA molecules suitable for sequencing applications on the flow cell 112. The nucleotides may have fluorescent elements (also referred to as "labels" or "moieties") attached that emit light or energy at a wavelength that indicates the type of nucleotide. Each type of fluorescent element may correspond to a particular nucleotide base (e.g., A, G, C, T). The fluorescent elements may emit light in visible wavelengths. In some embodiments, the sequencer 114 and the flow cell device 112 may be configured to perform various sequencing methods disclosed herein or known in the art, for example, sequencing-by-avidite, sequencing by binding or sequencing by synthesis.

[0078] For example, each nucleotide base may be assigned a color. Different types of nucleotides can have different colors. Adenine (A) may be red, cytosine (C) may be blue, guanine (G) may be green, and thymine (T) may be yellow, for example. The color or wavelength of the fluorescent element for each nucleotide may be selected so that the nucleotides are distinguishable from one another based on the wavelengths of light emitted by the fluorescent elements.

[0079] The imager 116 may be configured to capture images of the flow cell device 112 after each flowing step. In an embodiment, the imager 116 may include a camera configured to capture digital images, such as a CMOS or a CCD camera. The camera may be configured to capture images at the wavelengths of the fluorescent elements bound to the nucleotides. The images can be called flow cell images.

[0080] In some embodiments, the imager 116 can include one or more optical systems disclosed herein. The optical system(s) can be configured to capture optical signals from the flow cell and generate corresponding digital images thereof. The digital images can then be used for base calling.

[0081] In an embodiment, the images of the flow cell may be captured in groups, where each image in the group is taken at a wavelength or in a spectrum that matches or includes one of the fluorescent elements. In another embodiment, the images may be captured as single images that captures all of the wavelengths of the fluorescent elements.

[0082] The resolution of the imager 116 controls the level of detail in the flow cell images, including pixel size. In existing systems, this resolution is very important, as it controls the accuracy with which a spot-finding algorithm identifies the polony centers. In some embodiments, the image resolution of flow cell images disclosed herein can be about 10

nanometers (nm) to 900 nm, inclusive of all ranges or subranges therebetween. In some embodiments, the image resolution of the flow cell images can be between about 10 nm to about 900 nm, about 10 nm to about 500 nm, about 10 nm to about 200 nm, about 20 nm to about 500 nm, about 20 nm to about 200 nm, or any range or subrange therebetween. One way to increase the accuracy of spot finding is to improve the resolution of the imager 116, or improve the processing performed on images taken by imager 116. Detecting polony centers in pixels other than those detected by a spot-finding algorithm can be performed. Suitable spot-finding algorithms will be known to persons of ordinary skill in the art. These methods can allow for improved accuracy in detection of polony centers without increasing the resolution of the imager 116. The resolution of the imager 116 may even be less than existing systems with comparable performance, which may reduce the cost of the sequencing system 110.

[0083] The image quality of the flow cell images can control the base calling accuracy. The imager 116dislosed herein can increase the accuracy of base calling Alternatively, the processing performed on images taken by imager 116 can result in a better image quality.

[0084] After base calling is performed, a processor (e.g., dedicated processors 118, FPGA(s) 120, computer system 126, or a combination thereof) may optionally perform additional processing and/or analysis of base calling results. In some embodiments, after base calling is performed, the sequencing read(s) (processed and/or raw) can be outputted from the system to an external device (e.g., the cloud 130 and/or to a computer system 126). The sequencing read(s) herein can include a forward read (R1), a reverse read(R2), or both. The sequencing reads herein can be any orderly sequence of bases of A, T, C, and G.

[0085] In some embodiments, the sequencing read(s) can be communicated (e.g., directly or indirectly) to the computer system 126 for subsequent analysis such as adaptor trimming, or phasing, for example.

[0086] These sequencing analysis methods, including primary analysis, or secondary analysis, or combinations thereof, can be advantageously performed in parallel in the computer system 126, without interference with or delay of existing sequencing workflow of the system 100. The results of sequencing analysis can be made available for generating sequencing results for users. Some or all operations of the sequencing process can be advantageously performed by the FPGA(s) and data can be communicated between the CPU(s) and FPGA(s) to reduce the total operational time from methods operating without the FPGA(s).

[0087] The operations or actions disclosed herein may be performed by the dedicated processors 118, the FPGA(s) 120, the computer system 126, or a combination thereof. One or more operations or actions in methods disclosed herein may be performed by the dedicated processors 118, the FPGA(s) 120, the computer system 126, or a combination thereof. In some embodiments, which operations or actions are to be performed by the dedicated processors 118, the FPGA(s) 120, the computer system 126, or their combinations can be determined based on one or more of: a computation time for the specific operation(s), the complexity of computation in the specific operation(s), the need for data transmission between the hardware devices, or combinations thereof.

[0088] The computer system 126 can include one or more general purpose computers that provide interfaces to run a variety of programs in an operating system, such as Windows™ or Linux™. Such an operating system may provide great flexibility to a user.

[0089] In some embodiments, the dedicated processors 118 may be custom processors with specific hardware or instructions for performing method steps (e.g., rather than general purpose computers). Dedicated processors 118 can directly run specific software without an operating system. The lack of an operating system reduces overhead, at the cost of the flexibility in what the dedicated processors 118 may perform. A dedicated processor may make use of a custom programming language, which may be designed to operate more efficiently than the software run on general-purpose computers. This may increase the speed at which the steps are performed and allow for real time processing.

[0090] In some embodiments, the FPGA(s) 120 may be configured to perform operations of the sequencing analysis methods described herein. An FPGA is programmed as hardware that may perform a specific task. A special programming language may be used to transform software steps into hardware componentry. Once an FPGA is programmed, the hardware directly processes digital data that is provided to it without running software. The FPGA instead uses logic gates and registers to process the digital data. Because there is no overhead required for an operating system, an FPGA may process data faster than a general-purpose computer. Similar to dedicated processors 118, this is at the cost of flexibility.

[0091] The lack of software overhead may also allow an FPGA to operate faster than a dedicated processor 118, although this can depend on the exact processing to be performed and the specific FPGA 120 and dedicated processor 118.

[0092] A group of FPGA(s) 120 may be configured to perform processing steps in parallel. For example, a number of FPGA(s) 120 may be configured to perform a processing step for an image, a set of images, a subtile, or a select region in one or more images. In some

embodiments, each FPGA 120 may perform a respective step or substep of the processing steps at the same time, reducing the time needed to process data. This may allow the processing steps to be completed in real time or near real-time. Further discussion of the use of FPGAs is provided below.

[0093] Performing the processing steps in real time may allow the system 100 to use less memory, as the data may be processed as it is received rather than stored for subsequent analysis. This provides advantages over conventional systems, which may store the data before the data is processed, which may require more memory and/or accessing and communication with a computer system located in the cloud 130.

[0094] In some embodiments, the data storage device 122 is used to store information used in or obtained from sequencing analysis. For example, the DNA sequences determined after adaptor trimming may be stored in the data storage device 122. Compressed, or uncompressed, or combinations thereof, sequencing data may be stored in the data storage device 122. The FASTQ file may also be stored in the data storage device 122.

[0095] The user interface 124 may be used by a user to operate the sequencing system or access data stored in the data storage device 122 or the computer system 126.

[0096] The computer system 126 may control the general operation of the sequencing system and may be coupled to the user interface 124. In some embodiments, the computer system 126 may perform one or more steps in sequencing analysis, such as base calling, adaptor trimming, demultiplexing, phasing etc. In some embodiments, the computer system 126 may be structurally and/or functionally similar to computer system 800, as described in more detail in FIG. 8. The computer system 126 may include a memory configured to store information regarding the operation of the sequencing system 110, such as, for example, configuration information, instructions for operating the sequencing system 110, or user information. The computer system 126 may be configured to pass information between the sequencing system 110 and the cloud 130. For example, the computer system 126 may be configured to receive base calling results from the dedicated processors 118 and/or FPGA(s) and send the base calling results to the cloud 130 for storage and/or further analysis.

[0097] As discussed above, the sequencing system 110 may have dedicated processors 118, FPGA(s) 120, or the computer system 126. The sequencing system 110 may use one, two, or all of these elements to accomplish the processing described above. In some embodiments, when these elements are present together, the processing tasks are split between them. For example, the FPGA(s) 120 may be used to perform some portion or all of sequencing analysis operations, while the computer system 126 may perform other processing functions for the

sequencing system 110. The distribution of processing across the dedicated processor(s) 118, the FPGA(s) 120, and/or general purpose processors (e.g., in the computer system 126) can enable parallel processing and/or increase efficiency of processing steps. For example, complex processing steps may be allocated to the dedicated processor(s) 118 and/or FPGA(s) 120 while processing for general operation of the system 110 is carried out by the computer system 126.

[0098] Various combinations of these elements may allow various system embodiments that balance efficiency and speed of processing with cost of processing elements.

[0099] The cloud 130 may be a network, server, remote storage, or some other remote computing system separate from the sequencing system 110. The connection to cloud 130 may allow access to data stored externally to the sequencing system 110 or allow for updating of software in the sequencing system 110.

Flow cell devices

[00100] Disclosed herein, in some embodiments, are flow cell devices and systems that can be employed for performing or facilitating DNA sequencing analysis. Flow cell devices herein can be used to immobilize template nucleic acid molecules derived from biological samples and introduce a repetitive flow of sequencing reagents (e.g., sequencing-by-binding, sequencing-by-synthesis, or sequencing-by-avidite, or combinations thereof) to attach labeled nucleotides or labeled multivalent molecules to specific positions in the template sequences. A series of labeled signals are detected and decoded to reveal the nucleotide sequences of the template molecules, e.g., immobilized, or amplified, or combinations thereof, nucleic acid template molecules attached to a surface of the flow cell.

[00101] In some embodiments, flow cell devices 200, 300, 400, 500, 700, 1100, 1200, 1300, 1400 disclosed herein can comprise a support having one or more substrates, one or more channels, an inlet, and an outlet. FIGS. 2-4, 5A-5F, and 24A-28C show embodiments of flow cell devices.

[00102] In some embodiments, the flow cell devices disclosed herein (e.g., flow cell devices 200, 300, 400, 500, 700, 1100, 1200, 1300, 1400) can include a support (e.g., support 210, 510). In some embodiments, the support 210, 510 can be solid or opaque. In some embodiments, at least part of the support 210, 510 can be transparent so that light transmitting from a light source of the imager (116 in FIG. 1) can travel through the transparent portion of the support 210, 510 and reach the samples located on the flow cell device.

[00103] The support (e.g., support 210, 510) can include one or more substrates (e.g., substrates 320, 322, 330, 420, 422, 430, 520, 522, 530). As shown in FIGS. 3-4, the one or more substrates can include a top substrate 320, 420 and a bottom substrate 330, 430. When the flow cell device 112 is placed in the sequencing system 110 for imaging, the top substrate (e.g., top substrate 320, 420) can be closer to the camera of the imager 116, along the z direction (axis relative to a flow device shown in FIG. 2), than the bottom substrate (e.g., bottom substrate 330, 430). The bottom substrate (e.g., bottom substrate 330, 430) can be closer to a translation stage of the sequencing system 110 for holding and supporting the flow cell device 112 during sequencing than the top substrate (e.g., top substrate 320, 420).

[00104] In some embodiments, the flow cell devices 200, 300, 400, 500, 700 can further include a middle substrate 322, 422, 522 in between the top 320, 420, 520 and the bottom substrate 330, 430, 530 as shown in FIGS. 3-4, 5A and 5C.

[00105] Each substrate 320, 322, 330, 420, 422, 430, 520, 522, 530 can have a predetermined thickness, and different substrates can have different thickness. In some embodiments, each substrate can have a uniform thickness along the z direction. In some embodiments, each substrate can have a uniform thickness along the z direction in at least a portion of the substrate. For example, the portion with uniform thickness can encompass the channel(s) (e.g., channel(s) 250, 350, 450, 550) or the imaging areas of the flow cell device 112.

[00106] In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of about 0.2 millimeters (mm) to about 5 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top or bottom substrate can have a thickness of about 0.6 mm to about 3 mm. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of about 0.8 mm to about 2 mm, inclusive of all ranges and subrange therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of about 0.8 mm to about 1.5 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of about 0.8 mm to about 1.2 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of about 0.9 mm to about 1.1 mm, inclusive of all ranges and subranges therebetween.

[0107] In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of 0.2 mm to 5 mm, inclusive of all ranges and subranges

therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of 0.6 mm to 3 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of 0.8 mm to 2 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of 0.8 mm to 1.5 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of 0.8 mm to about 1.2 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of 0.9 mm to 1.1 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the top substrate 320, 420, 520 or bottom substrate 330, 430, 530 can have a thickness of 0.95 mm to 1.05 mm, inclusive of all ranges and subranges therebetween.

[0108] In some embodiments, the middle substrate 322, 422, 522 can have a thickness of about 40 micrometers (μm) to 200 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of about 40 μm to 150 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of about 40 μm to 70 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of about 80 μm to 120 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of about 60 μm to 90 μm, inclusive of all ranges and subranges therebetween.

[0109] In some embodiments, the middle substrate 322, 422, 522 can have a thickness of 40 μm to 200 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of 40 μm to 150 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of 40 μm to 70 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of 80 μm to 120 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the middle substrate 322, 422, 522 can have a thickness of 60 μm to 90 μm, inclusive of all ranges and subranges therebetween.

[0110] In some embodiments, the substrate(s) 320, 322, 330, 420, 422, 430, 520, 522, 530 can have an elongate shape extending along the y axis. In some embodiments, the substrate(s) can have various shapes such as rectangular, square, etc.

[0111] In some embodiments, the one or more substrates 320, 322, 330, 420, 422, 430, 520, 522, 530 can have one or more surfaces that are substantially planar. In some embodiments, the one or more substrates may contain no curvature perceivable to naked eyes (e.g., without magnification), e.g., as shown in FIGS. 2-4. In some embodiments, the flatness of the surface(s) of the substrates 320, 322, 330, 420, 422, 430, 520, 522, 530 can be measured as the height from a peak to a valley in a direction orthogonal to the surface(s). In some embodiments, he height can be less than about 0.01 mm, 0.02 mm, 0.03 mm, 0.04 mm, 0.05 mm, 0.06 mm, 0.07 mm, 0.08 mm, or 0.09 mm, e.g., along a direction orthogonal to the surface. In other words, the flat surface(s) of the substrates 320, 322, 330, 420, 422, 430, 520, 522, 530 may fit between two parallel planar 2D planes that are less than 0.01 mm, 0.02 mm, 0.03 mm, 0.04 mm, 0.05 mm, 0.06 mm, 0.07 mm, 0.08 mm, or 0.09 mm apart from each other. In some embodiments, the flatness of the surface(s) of the substrates can include a height from its peak to valley that is less than 0.01 mm, 0.02 mm, 0.03 mm, 0.04 mm, 0.05 mm, 0.06 mm, 0.07 mm, 0.08 mm, or 0.09 mm. In some embodiments, the substrates are not planar. In some embodiments, a portion or the entirety of the one or more substrates 320, 322, 330, 420, 422, 430, 520, 522, 530 can be curved.

[0112] In some embodiments, the support (e.g., support 210, 510) or the one or more substrates (e.g., substrate 320, 322, 330, 420, 422, 430, 520, 522, 530) can include any suitable material such as, for example, glass or plastic. In some embodiments, the support or one or more substrates are all-glass or all-plastic. In some embodiments, the support or the one or more substrates can include a tape such as a pressure sensitive adhesive (PSA) tape. For example, the middle substrate 322, as shown in FIG. 3, can formed from or include PSA tape. Therefore, the top substrate (e.g., top substrate 320, 420, 520) and/or the bottom substrate (e.g., bottom substrate 330, 430, 530) can be conveniently affixed to the middle substrate (e.g., middle substrate 322) to via the tape.

[0113] The substrate(s) 320, 322, 330, 420, 422, 430, 520, 522, 530 can define one or more channels 250, 350, 450, 550 of the flow cell devices 200, 300, 400, 500. The channels 250, 350, 450, 550 can allow fluid, e.g., liquid or gas, to flow therethrough.

[0114] In some embodiments, the gas can include one type of gas. In some embodiments, the gas can include a combination of different types of gases. In some embodiments, the gas includes air. In some embodiments, the gas can include dry air (e.g., air with low humidity). In some embodiments, the gas includes one or more inert gases. In some embodiments, the gas includes one or more active gases.

[0115] In some embodiments, the channels 250, 350, 450, 550 can be configured to receive reagents that are liquid. In some embodiments, the reagents can be devoid or deprived of air bubbles that are greater than a predetermined size. In some embodiments, a first reagent is configured to wet a first coating of a surface of the one or more channels (e.g., channels 250, 350, 450, 550). In some embodiments, a second reagent can be configured to re-wet the surface of the one or more channel(s) (e.g., channels 250, 350, 450, 550) after the surface of the channel(s) (e.g., channels 250, 350, 450, 550) have at least partly dried as a result of the gas gap (or gas flow) through the channel(s).

[0116] In some embodiments, the channel(s) (e.g., channels 250, 350, 450, 550) can be defined by a top interior surface 521 and a bottom interior surface 521 of the substrates 520, 530, as shown in FIG 5C. In some embodiments, the channel(s) 550 (e.g., or any of the channels 250, 350, 450) can each include a lumen 551 defined by a top interior surface 521 and a bottom interior surface 521 of the substrates 520, 530 surrounding the lumen 551, and a groove in either the top, bottom, or both surfaces, without a middle substrate.

[0117] In some embodiments, the channels 350, 450, 550 can be defined by the top and bottom substrates with an addition of a middle substrate 322, 422, 522. As shown in FIGS. 3, 4, and 5A, the middle substrate 322, 422, 522 can define a void, e.g., an elongated void, extending along a longitudinal axis, or y axis, of the middle substrate 322, 422, 522. A width of the void can define the width of the channel 350, 452, 550, along the x axis. A length of the void, along the y direction, can define the length of the channel 350, 450, 550). FIGS. 3-4 and 5A show flow cell devices with channels 350, 450, 550 defined by the top substrates 320, 420, 520, middle substrate 322, 422, 522 (e.g., the void of the middle substrate), and bottom substrate 330, 430, 530.

[0118] In some embodiments, the channels 350, 450, 550 can include microfluidic channels. In some embodiments, a gap or height (along the z direction, axis shown in FIG. 2) between the top interior surface and the bottom interior surface of the substrates that defines the channel(s) (e.g., channel 350, 450, 550) is about 150 μm, 130 μm, 120 μm, 110 μm, 100 μm, 90 μm, 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm, inclusive of all ranges and subranges therebetween. In some embodiments, the gap or height of the channel is no more than about 100 μm. In some embodiments, the gap or height of the channel is no more than about 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm, inclusive of all ranges and subranges therebetween.

[0119] In some embodiments, a gap or height between the top interior surface and the bottom interior surface of the substrates that defines the channels, along the z direction, is 150 μm, 130 μm, 120 μm, 110 μm, 100 μm, 90 μm, 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm.

In some embodiments, the gap or height of the channel is no more than 100 μm. In some embodiments, the gap or height of the channel is no more than 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm.

[0120] In some embodiments, a length of the channel, along the y direction, can be about 120 mm, 100 mm, 90 mm, 80 mm, 70 mm, 60 mm, 50 mm, 40 mm, or 30 mm, inclusive of all range and subranges therebetween. In some embodiments, the length of the channel is no more than about 100 mm. In some embodiments, the length of the channel is no more than about 80 mm, 75 mm, 70 mm, 65 mm, 60 mm, 55 mm, 50 mm, 45mm, or 40 mm, inclusive of all range and subranges therebetween.

[0121] In some embodiments, a length of the channel, along the y direction, is 120 mm, 100 mm, 90 mm, 80 mm, 70 mm, 60 mm, 50 mm, 40 mm, or 30 mm. In some embodiments, the length of the channel is no more than 100 mm. In some embodiments, the length of the channel is no more than 80 mm, 75 mm, 70 mm, 65 mm, 60 mm, 55 mm, 50 mm, 45mm, or 40 mm.

[0122] In some embodiments, a width of the channel, along the x direction, can be about 50 mm, 40 mm, 30 mm, 25 mm, 20 mm, 15 mm, 10 mm, 8 mm, or 5 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the width of the channel is no more than about 10 mm or about 7 mm, inclusive of all range and subranges therebetween. In some embodiments, the width of the channel is no more than about 30 mm, 25 mm, 20 mm, 15 mm, 10 mm, or 5 mm, inclusive of all range and subranges therebetween.

[0123] In some embodiments, a width of the channel, along the x direction, is 50 mm, 40 mm, 30 mm, 25 mm, 20 mm, 15 mm, 10 mm, 8 mm, or 5 mm. In some embodiments, the width of the channel is no more than 10 mm or 7 mm. In some embodiments, the width of the channel is no more than 30 mm, 25 mm, 20 mm, 15 mm, 10 mm, or 5 mm.

[0124] In some embodiments, the distance between two adjacent channels or the distance from an edge of the channel to the edge of the flow cell device, along the x axis, is about 0.5 mm to about 15 mm, inclusive of all range and subranges therebetween. In some embodiments, the distance between two adjacent channels or the distance from an edge of the channel to the edge of the flow cell device, along the x axis, is about 1 mm to about 8 mm, inclusive of all range and subranges therebetween. In some embodiments, the distance between two adjacent channels or the distance from an edge of the channel to the edge of the flow cell device, along the x axis, is about 2 mm to 6 mm, inclusive of all range and subranges therebetween.

[0125] In some embodiments, the distance between two adjacent channels or the distance from an edge of the channel to the edge of the flow cell device, along the x axis, is 0.5 mm to 15 mm, inclusive of all range and subranges therebetween. In some embodiments, the distance between two adjacent channels or the distance from an edge of the channel to the edge of the flow cell device, along the x axis, is 1 mm to 8 mm, inclusive of all range and subranges therebetween. In some embodiments, the distance between two adjacent channels or the distance from an edge of the channel to the edge of the flow cell device, along the x axis, is 2 mm to 6 mm, inclusive of all range and subranges therebetween.

[0126] In some embodiments, the flow cell devices can have more than one channel. In some embodiments, the flow cell devices can have more than one channel and some or all of the channels can have a unform size and shape. FIGS. 5A, 5E, and 5F show embodiments of flow cell devices 500, 1300, 1400 with two channels of identical size and shape. In some embodiments, the flow cell devices can have channels of different sizes, or shapes, or combinations thereof. FIGS. 3-4 and 5D show embodiments of flow cell devices 300, 400 with similar channel length but different channel widths.

[0127] In some embodiments, the channels may include a tapered portion that connects the open landing area to the body of the channel (e.g., FIGS. 2-4, 5A-5B, 5D-5F). The tapered area and its taper angle can be determined by the size of the open landing area to which the tapered area connects and/or the width of the channel body (e.g., along the x-axis). The size (e.g., an area) of the tapered area and a degree of the taper angle can be adjusted to facilitate efficient fluid transfer from the open landing area to the body of the channel. FIG. 4 shows an embodiment of the flow cell device 400 with a tapered transition portion 451 connecting the open landing area 441 to the body of the channel 452. A second tapered area 453 can be used to connect the body of the channel 452 to the outlet 460. Whiles described with respect to FIG. 4, any of the flow cell devices 100, 200, 300, 500, 700 described herein can have a first tapered transition portion and/or a second tapered area.

[0128] The size and shape of the tapered transition portion 451 may be varied depending on the applications of the flow cell device.

[0129] FIGS. 27A-27G show an embodiment of a flow cell device 700 with the size and dimensions of the tapered transition portion connecting the body of the channel to the outlet.

[0130] In some embodiments, the flow cell device 700 can have a first end at which the inlet is near and a second end opposite the first end at which the outlet is near. In some embodiments, the tapered transition portion may be disposed at a predetermined distance from the first end of the flow cell device. In some embodiments, the tapered transition portion

from the outlet to the body of the channel can be about 3 mm to about 15 mm along the y axis (e.g., from the first end of the flow cell device 700), inclusive of all ranges and subranges therebetween. In some embodiments, the tapered transition portion from the outlet to the body of the channel can be about 5 mm to about 12 mm along the y axis, inclusive of all range and subranges therebetween. In some embodiments, the tapered transition portion from the outlet to the body of the channel can be about 6 mm to about 9 mm along the y axis, inclusive of all range and subranges therebetween. In some embodiments, the tapered transition portion from the outlet to the body of the channel can be 3 mm to 15 mm along the y axis, inclusive of all range and subranges therebetween. In some embodiments, the tapered transition portion from the outlet to the body of the channel can be 5 mm to 12 mm along the y axis, inclusive of all range and subranges therebetween. In some embodiments, the tapered transition portion from the outlet to the body of the channel can be 6 mm to 9 mm along the y axis, inclusive of all range and subranges therebetween.

[0131] In some embodiments, the tapered angle, e.g., the acute angle between an edge of the flow cell device and an edge of the tapered area is about 25.1 degrees, as shown in FIG. 27B. In some embodiments, the tapered angle can be in the range of about 15 degrees to about 40 degrees, inclusive of all range and subranges therebetween. In some embodiments, the tapered angle can be in the range of about 20 degrees to about 30 degrees, inclusive of all range and subranges therebetween. In some embodiments, the tapered angle can be in the range of 15 degrees to about 40 degrees, inclusive of all range and subranges therebetween. In some embodiments, the tapered angle can be in the range of 20 degrees to about 30 degrees, inclusive of all range and subranges therebetween.

[0132] In some embodiments, each channel has its own corresponding open landing area, or inlet, or combinations thereof, e.g., as shown in FIGS. 4, 5A, 5F and 25A-25F. In some embodiments, two or more channels share a single open landing area 1341, or inlet, or combinations thereof, e.g., as shown in FIGS. 5E and 28A-28C.

[0133] In some embodiments, the open landing area is directly connected to the body of the channel. In some embodiments, the open landing area is connected to the body of the channel without a tapered transition portion in between. FIGS. 25A-25E show an embodiment of the flow cell device 700. The flow cell device 700 can be structurally and/or functionally similar to the flow cell devices 200, 300, 400, 500, and therefore, certain details of the flow device 700 are not described again herein. The flow cell device 700 includes a circular open landing area 741 that is directly connected to the body portion of the channel 752 without a tapered transition portion. As shown, the channel 750 can begin where the open landing area ends

741. In some embodiments, the channel width can be substantially equivalent or exactly equivalent to the diameter of the opening landing area 741. As shown in FIG. 26C, the size of the open landing area can be different from the body of the channel (in contrast to the embodiment in FIGS. 25A-25E) either in one channel or in both channels. For example, the diameter of the open landing area can be smaller than the width of the channel along the x axis. When the diameter of the open landing area is smaller than the width of the channel, the flow device 1200 can be a tapered transition region 1251 between the open landing area and the body of the channel.

[0134] The flow cell devices 200, 300, 400, 500, 700 can include one or more inlets and one or more outlets. For example, as shown in FIG. 5C, the flow cell device can have inlet 540 and outlet 560. A channel 550 can run from its corresponding inlet 540 to its corresponding outlet 560, thereby allowing fluid communication from the inlet 540 to the outlet 560. Sequencing reagents can be introduced to the flow cell device 200, 300, 400, 500 via the inlet 540, flow through the channels 550 and interact with samples located therein, and exit from the outlet 560.

[0135] The size and shape of the inlet and outlet can be customized to suit various sequencing applications. For example, the size and shape can be determined based on the specific sequencing application(s), such as, a minimal flush volume, a contamination threshold, the parameters of the flow cell (e.g., the size of the flow cell channels), or the parameters of the dispenser (e.g., the size of the dispensing tip). As a nonlimiting example, the inlet can be cylindrical as shown in FIG. 5C with walls defining the hole or void extending along the z direction and orthogonal to the substrates. At the bottom of the cylindrical void/ hole, the inlet 440 can be connected to a cleaning outlet (e.g., cleaning outlet 470, 570). The inlet can be shaped differently. For example, the inlet can have an inverted cone shape with a wider opening at the top and can narrow down toward the channel to reduce residual of reagents from remaining in the inlet. FIG. 5D shows an embodiment with an inlet as a cylindrical shape. Without the cleaning outlet, the inlet has no connection to the cleaning outlet. In another embodiment, as in FIG. 5E, an inlet 1340 can be part or all of the open landing pad. In some embodiments, the inlet 1340 can comprise the open landing pad or a portion thereof but no other structural elements in the flow cell device. In another embodiment of the flow cell device, as in FIG. 5F, an inlet 1440 may be a groove of various sizes or shapes defined in the middle substrate, or defined in the middle and the bottom substrates, which is in fluidic connection to the channels. FIGS. 28A-28C shows the embodiment in FIG. 5E from different views. FIG. 28A is a top view of the flow cell device

1300. FIG. 28B shows three different substrates in a prospective view, and FIG. 28C shows the bottom 1330, middle 1322, and top substrates 1320.

[0136] In some embodiments, the diameter of the inlet (e.g., inlet 340, 440, 540) (e.g., the widest dimension in the x-y plane, can be in the range of about 3 mm to about 11 mm, inclusive of all range sand subranges therebetween. The height of the inlet, along the z direction, can be defined as the total height of the top substrate and the middle substrate. In some embodiments, the height of the inlet can be in the range of about 1 mm to 12 mm, inclusive of all range sand subranges therebetween.

[0137] The diameter of the outlet (e.g., outlet 360, 460, 560) or the cleaning outlet (e.g., cleaning outlet 470, 570, 770), in the x-y plane, can be in the range of about 0.3 mm to about 4 mm, inclusive of all range sand subranges therebetween. In some embodiments, the diameter of the outlet can be in the range of about 0.4 mm to about 2 mm, inclusive of all range sand subranges therebetween, and the outlet can be a cylindrical shape.

[0138] The diameter of the inlet, e.g., the widest dimension in the x-y plane, can be in the range of 3 mm to 11 mm. As another example, the height of the inlet, along the z direction, can be the total height of the top substrate and the middle substrate, and it can be in the range of 1 mm to 12 mm.

[0139] The diameter of the outlet or the cleaning outlet, in the x-y plane, can be in the range of 0.3 mm to 4 mm. In some embodiments, the diameter of the outlet can be in the range of 0.4 mm to 2 mm, and the outlet can be a cylindrical shape.

[0140] The size and shape of the inlet and outlet can be customized to suite various sequencing applications. For example, the size and shape can be determined based on the specific sequencing application(s), such as, a minimal flush volume, a contamination threshold, the parameters of the flow cell, e.g., the size of the flow cell channels, or the parameters of the dispenser, e.g., the size of the dispensing tip. As a nonlimiting example, the inlet can be cylindrical as shown in FIG. 5C with walls extending along the z direction and orthogonal to the substrates. At the bottom of the cylindrical void/ hole, the inlet 440, 540 can be connected to a cleaning outlet 470, 570. The inlet can be shaped differently. For example, the inlet can have an inverted cone shape with wider openings at the top and narrows down toward the channel to reduce the residuals of reagents that can remain in the inlet. FIG. 5D shows an embodiment with an inlet as a cylindrical shape. Without the cleaning outlet, the inlet has no connection to the cleaning outlet. In another embodiment, as in FIG. 5E, the inlet 1340 can comprise the open landing pad or a portion thereof but no other structural elements in the flow cell device. FIGS. 28A-28C shows the embodiment in FIG. 5E from different

views. FIG. 28A is a top view of the flow cell device 1300. FIG. 28B shows three different substrates in a prospective view, and FIG. 28C show the bottom 1330, middle 1322, and top substrates 1320. In yet another embodiment of the flow cell device, as in FIG. 5F, the inlet 1440 may be a grove of various sizes or shape defined in the middle substrate, or in the middle and the bottom substrates that are in fluidic connection to the channels.

[0141] FIGS. 2- 4, and 5A-5F show flow cell devices 200, 300, 400, 500 with two to three substrates forming one or two channels, and each channel having a corresponding inlet and outlet. However, the number of substrates, channels, inlets, and outlets can vary in different embodiments. In some embodiments, the number of substrates, channels, inlets and outlets can be any integer number that is greater than 0. In some embodiments, flow cell devices can include 2, 4, 6, 8, 10, inclusive of all ranges and subranges therein, or even more channels.

[0142] FIGS. 24A-24E show different views of the flow cell device 500 as described herein with respect to FIGS. 5A-5C. FIGS. 25A-25E show different views of a flow cell device 700, according to an embodiment. Structural elements of the flow cell devices disclosed herein can have varying sizes. Such structural elements can include, but are not limited to, the inlet, the open landing area, the outlet, the tapered transition portion from the cleaning outlet to the open landing area or the inlet, the tapered transition portion from the inlet to the corresponding channel, and the tapered transition portion from the channel to the corresponding outlet.

[0143] FIGS. 26A-26C show embodiments of flow cell devices 1100, 500, 1200 with different sizes or dimensions of the open landing area, the tapered transition portion from the cleaning outlet to the inlet or the open landing area (e.g., tapered transition portion 1154, 554, 1251), the tapered transition portion from the inlet or the opening landing area to the channel, or their combinations. The flow cell devices 1100, 1200 may have features that are structurally and/or functionally similar to any of the flow cell devices 200, 300, 400, 500, 700, and therefore, certain features of the flow cell devices 1100, 1200 are not described in further detail herein.

[0144] FIGS. 27A-27G show embodiments of the flow cell device 700. In these particular embodiments, as shown in FIG. 27A, the total thickness of the flow cell device is 2.07 mm. The top and the bottom substrates have thicknesses of 1 mm.

[0145] The flatness of surface A in FIG.27A from its peak to valley, e.g., the difference between the highest and lowest points on the surface, is less than 0.02 mm. The flatness of surface B in FIG. 27B from its peak to valley, e.g., the difference between the highest and lowest points on the surface, is less than 0.02 mm. FIG. 27B shows that each channel edge to

the edge of the flow cell device 700 along x axis can be about 2.24 mm. The channels can have a width of 8.5 mm. The gap between the two channels along the x axis can be 3.5 mm. Each lane starts at 11 mm away from the closest edge of the flow cell device along the y axis. FIG. 27C shows that the cleaning outlet is 6.5 mm away from the edge of the flow cell device along the x axis, and the two cleaning outlets are 12 mm apart from each other along x axis. The cleaning outlets are 3 mm away from the closest edge of the flow cell device along the y axis. The diameter of the cleaning outlet is 0.81 mm. Alignment element "1" in FIG. 27C is configured to align the flow cell device 700 to the moving stage that can hold the flow cell device 700 and move it relative to the optical system, which is positioned 11.25 mm from one edge of the flow cell device 700 and 13.75 mm from the other edge of the flow cell device 700 along the x axis, and positioned between the opening landing areas. As shown in FIG. 27C, the outlets are of the same dimension as the cleaning outlets. The total width of the flow cell device 700 is 25 mm. The total length of the flow cell device 700 is 75 mm. The cleaning outlets are 3 mm away from one edge of the flow cell device 700 along the y axis. The outlets are 3 mm away from the opposite edge of the flow cell device along the y axis. FIG. 27G shows the middle substrate of 0.07 mm. FIGS. 27E and 27F show that the open landing area has a circular shape with a diameter of 8.52 mm. The tapered transition from the outlet to the body of the channel includes a curved portion that is a portion of a circular shape with a radius of 0.5 mm, and the angle defined between the tapered transition portion is 50.2 degrees.

[0146] In some embodiments, the curved portion of the tapered transition portion, as shown in FIG. 27E can be a portion of a circular shape with a radius in the range of about 0.2 mm to about 1.5 mm. In some embodiments, the curved portion can be a portion of a circular shape with a radius in the range of about 0.3 mm to about 0.9 mm. In some embodiments, the curved portion can be a portion of a circular shape with a radius in the range of about 0.4 mm to about 0.7 mm.

[0147] In some embodiments, the curved portion can be a portion of a circular shape with a radius in the range of 0.2 mm to 1.5 mm. In some embodiments, the curved portion can be a portion of a circular shape with a radius in the range of 0.3 mm to 0.9 mm. In some embodiments, the curved portion can be a portion of a circular shape with a radius in the range of 0.4 mm to 0.7 mm.

Channel coatings and air gaps

[0148] As shown in FIG. 5C, one or more of the interior surfaces 521 can be coated with a first coating 522.

[0149] In some embodiments, the channel(s) 550 are configured to allow fluids (e.g., liquid reagents) and an air gap (e.g., a flow or bolus of air between the flow of fluids) to flow therethrough. In some embodiments, the air gap can comprise a bolus of gas. The air gap can be introduced similarly as the liquid reagents, e.g., via the inlet 540, through the channel(s) 550, and out of the outlet 560. Alternatively, the air gap can be introduced from other openings such as the outlet 560 or the cleaning outlet 570 of the flow cell device 500. The air gap can be driven mechanically by one or more structural elements of the fluidic control device herein. As an example, the air gap can be sucked into the channel(s) 550 via the inlet 540 by a mechanical force applied at the outlet 560, e.g., by a pump or a vacuum. As another example, the air gap may be purged or caused to flow into the channel(s) 550 by a pump or the like via the inlet 540. While described with reference to flow cell device 500, it should be appreciated that any of the flow devices 100, 200, 300, 400, 700 described herein can be configured to cause an air gap to flow therethrough as described herein with reference to FIG. 5C.

[0150] The volume of the air gap can vary depending on the geometry, size, or combinations thereof, of the flow cells and channels. For example, the volume of air gap can be selected to fill up about 30%, 40%, 50%, 60%, or 70% of a total volume of each channel. As another example, the volume of the air gap can be adjusted based on the subsequent reagent to be administered. For example, the air gap can be increased if higher cleaning or reduction in contamination is desired.

[0151] The air gap that flows through the one or more channels 550 can be configured to push existing reagents in the channel(s) 550 toward the outlet 560 and exit from outlet 560. Subsequent delivery of reagent can achieve high homogeneity in the flow cells. In other words, the air gap can effectively eliminate a first reagent from the channel 550 to prepare for a delivery a second reagent. Known flow cells devices rely solely on washing buffer(s) between delivery of sequencing reagents, which results in mixing of the sequencing reagents with washing liquid(s) can cause a concentration gradient of the sequencing reagent with higher concentration of the sequencing reagent at an end of the channel 550 closer to the landing area 541, and lower concentration at an opposite end closer to the outlet 560. Such gradient or inhomogeneity can be gradually reduced by repeated washing but remains difficult to completely eliminate. The gradient of concentration or inhomogeneity in concentration of reagents may cause sequencing analysis of tiles toward the opposite end of the flow cell 500 (e.g., the end near the outlet 560) to be less accurate and unreliable at least partly due to inhomogeneous reaction, or attachment, or combinations thereof, of compounds

in the reagent to the polonies. In addition, known flow cell devices cand methods can cause an introduction of air bubbles into the channels between reagents, which can damage the channel coating, the polonies tethered thereon and being imaged, or combinations thereof, thereby impairing the sequencing process. The flow cell devices herein can utilize the air gap to minimize or eliminate concentration gradient or inhomogeneity in the flow cells (e.g., the channels of the flow cells), along the y axis (axis shown in FIG. 2).

[0152] In some embodiments, the air gap and washing liquid(s) can be combined to achieve optimal cleaning of the channel(s). In some embodiments, the air gap can be used alone to achieve optimal cleaning of the channels. In some embodiments, a washing scheme, using air gap, washing liquid(s), or both, can be determined based on the contamination level of the reagent to be delivered. In some embodiments, the washing scheme, using air gap, washing liquid(s), or both, can be determined based on the cost of the reagent, alone or in combination with other factors such as contamination levels. In some embodiments, the order of using air gap and washing liquid(s) can vary when the two are combined in the washing scheme. The air gap can be applied after or before any number of flushing with washing liquid(s). In some embodiments, the air gap can be purged in between any selected flushing of washing liquids.

[0153] The air gap that flows through the one or more channels may dry (e.g., at least partially dry) the coating of the one or more channels, but the functionality of the coating can remain unaltered after one or more air gaps flow therethrough. In some embodiments, the air gap that flows through the one or more channels may dry the polonies tethered on the channel coating. However, the air gap (e.g., one or more parameters of the airgap) can be configured to prevent damage to the polonies and ensures proper sequencing reaction of the polonies when a subsequent liquid reagent is flushed through the channel(s). As such, the flow cell devices with such channels can be cleaned by delivering (e.g., purging, flowing, expelling, moving) air gaps into the channels, alone or in combination with washing with reagents. Usage of the air gap for cleaning can increase the efficiency and effectiveness of cleaning the channels while simultaneously reducing the costs by reducing a number of reagents for washing and performing sequencing analysis, while satisfying a predetermined contamination requirement. The predetermined contamination required may be customized to be at various levels. For example, the predetermined contamination level may be based on the sequencing application and the reagent(s) being applied. As a nonlimiting example, the contamination level may be below 0.1%, 0.01%, 0.005%, or 0.001%.

[0154] Referring back to FIGS. 5A-5F, in some embodiments, the surface if the channel(s) 550 can be passivated for the first coating 522. In some embodiments, the surface is

passivated with the first coating 522 that immobilizes surface capture primers, nucleic acid template molecules, or both for capturing polynucleotides thereon. In some embodiments, during sequencing, the surface of the channel(s) 550 can include polynucleotides captured thereon. In some embodiments, the polynucleotides captured thereon are configured to be imaged in a sequencing cycle.

[0155] In some embodiments, the first coating 522 of the surface can include one or more layers. In some embodiments, the first coating 522 can include one or more hydrophilic polymer coating layers. The first coating 522 can comprise a plurality of oligonucleotide molecules attached to at least one hydrophilic polymer coating layer. The hydrophilic polymer coating layer(s) can comprise polyethylene glycol (PEG). The hydrophilic polymer layer(s) can comprise a branched hydrophilic polymer. In some embodiments, the branched hydrophilic polymer can include at least 8 branches. In some embodiments, the hydrophilic polymer coating layer(s) can include a water contact angle of no more than about 50 degrees.

[0156] In some embodiments, the surface of the channel 550 can include at least one discrete region that comprises a plurality of clonally-amplified sample nucleic acid molecules that have been annealed to the plurality of attached oligonucleotide molecules (e.g., attached surface capture primers). In some embodiments, at least one of the plurality of the clonally-amplified sample nucleic acid molecules comprises a concatemer molecule annealed to at least one of the plurality of attached oligonucleotides.

[0157] In some embodiments, the at least one of the plurality of sample nucleic acid molecules comprises a single-stranded multimeric nucleic acid molecule comprising repeats of a regularly occurring monomer unit. The single-stranded multimeric nucleic acid molecules can be at least 10 kilobases in length. In some embodiments, the at least one of the plurality of sample nucleic acid molecules further comprises a double-stranded monomeric copy of the regularly occurring monomer unit. The plurality of oligonucleotide molecules can be present at a substantially uniform surface density across the surface of the channel 550. The plurality of oligonucleotide molecules can be present at a local surface density of at least about 100,000 molecules/μm^2 at a first position on the surface, and at a second local surface density at a second position on the surface. In some embodiments, the plurality of oligonucleotide molecules is present at a surface density of at least about 1,000 molecules/m^2.

[0158] In some embodiments, the samples disclosed herein on the flow cell device may be two dimensional (2D) or three dimensional (3D) samples. The sample(s) may include in situ samples such as cells or tissue.

[0159] In some embodiments, the sample(s) may be immobilized on the support of the flow cell device. In some embodiments, the sequencing system including an optical system can advantageously enable sequencing and imaging of target analyte(s) (proteins, nucleic acids, lipids, polysaccharides and the like) or features while they remain inside the intact cell or tissue. In some embodiments, the cell or tissue and the targets (e.g., target analytes, structure elements, organelles, etc.) therewithin remain intact during sequencing and/or imaging. In some embodiments, the one or more samples being imaged using the optical systems herein can be 2D or 3D samples. The 2D sample(s) may include traditional nucleotide acid molecules extracted from various sources. The 3D samples can include samples in which polonies within the sample do not lie in a single z plane while keeping the polonies in focus. The 3D samples may include in situ samples such as cells and/or tissues. In some embodiments, the cells or tissue samples can be immobilized on the flow cell device or substrate for sequencing and/or imaging without modifying the spatial locations of targets within the cells or tissue. In some embodiments, the cells or tissue samples are immobilized on the flow cell device or otherwise substrate for sequencing or imaging without modifying the spatial relationship of targets or target analytes within the cells or tissue. In some embodiments, the cells and/or tissue are immobilized with the morphological features, RNA, mRNA, and protein targets of the samples intact inside the cell(s) or tissue during sequencing and/or imaging. In some embodiments, the spatial locations or relationships of the target analytes or targets remain intact during sequencing and/or imaging. In some embodiments, the spatial locations or relationships of the target analytes or targets during sequencing and/or imaging are not manually reconstructed using artificially added structure or features in the sample. For example, the nucleus, cell membrane, mitochondria, cytoskeleton and extracellular matrix can retain their relative spatial relationship to each other in the sample(s) during imaging and/or sequencing.

[0160] In some embodiments, the one or more samples herein may include a cell or cells cultured on a support, e.g., a flow cell, or on a surface that is transferred to a support, e.g., the flow cell. In some embodiments, a cell may be an adherent cell. In some embodiments, a cell may be a confluent cell. In some embodiments, a cell may be a suspended cell. In some embodiments, a suspended cell may be adhered to the surface by a specific capture mechanism such as an antigen-antibody interaction, or a receptor-ligand interaction, including without limitation an interaction of a known surface receptor with a known ligand; an unknown surface receptor with a known ligand, an known ligand with an unknown ligand, or an unknown receptor with an unknown ligand. In some embodiments, a suspended cell

may be adhered to a surface by interaction with a specific carbohydrate binding interaction, a specific protein or peptide binding interaction, or a specific lipid-lipid interaction, lipid-peptide interaction, or lipid-carbohydrate interaction. In some embodiments, a suspended cell may be adhered to a surface by a nonspecific interaction with said surface, such as by use of a charged surface (e.g., a polylysine, poly argininine, polyglutamic acid, polyaspartic acid surface or the like, or a charged polymer surface, such as a polyethylenimine surface; or a plasma-treated or ion-treated glass or polystyrene surface, or the like). It will be understood by one of skill in the art that in addition to surfaces disclosed herein, any surface useful for, or conventionally used for, cell culture, will be useful for capture of adherent cells. In particular embodiments, a surface useful for capture of adherent cells will comprise at least one of polyethylene oxide, streptavidin, protein A, or any combination thereof. In some embodiments, a suspended cell may be introduced to a flow cell by flow of a liquid suspension comprising the cell through the flow cell, by direct pipetting or liquid transfer onto a surface of the flow cell, by gravitational precipitation, by centrifugation, or by any method known in the art for bringing cells into contact with a surface.

[0161] In some embodiments, the one or more samples include biological analytes (e.g., target analyte(s) as described herein) that are located inside the sample(s) or on the membrane of the sample(s). In some embodiments, the one or more samples include target analyte(s) that are on the exterior or interior surface of the cell. In some embodiments, the one or more samples include target analyte(s) that are on the exterior or interior surface of the cell membrane. In some embodiments, In some embodiments, the one or more samples include target analyte(s) that are part of the extracellular matrix. In some embodiments, the one or more samples include target analyte(s) that are part of and/or located on one or more organelles within the cell or tissue. In some embodiments, the one or more samples include target analytes that are on or in the glycocalyx or belong to part of the glycocalyx.

[0162] In some embodiments, the biological analyte(s) or target analyte(s) comprise at least one polypeptide, lipid, nucleic acid or polysaccharide. In some embodiments, the target analyte(s) comprise at least one polypeptide, enzyme or lipid located anywhere in the sample(s) including the cytoplasm and nucleus. In some embodiments, the target analyte(s) comprise at least one polypeptide, enzyme or lipid located in or on a cellular structure including without limits any cellular membrane, nucleus, nucleolus, mitochondria, chloroplast, Golgi apparatus, ribosome, endoplasmic reticulum, microtubules, actin filaments, peroxisomes and lysosomes.

[0163] In some embodiments, the one or more samples herein include analytes (e.g., nucleic acids, DNA, RNA, mRNA, and/or proteins) obtained from cell or tissue with preserved spatial information to undergo sequencing and/or imaging outside the cell or tissue. In some embodiments, the one or more samples herein include analytes (e.g., nucleic acids, DNA, RNA, and/or proteins) removed from cell or tissue so that the analytes are not inside the cell or tissue anymore to undergo sequencing and/or imaging outside the cell or tissue, while keeping the rest of the cell or tissue, e.g., the structure of the cell or tissue, intact while the analytes are outside. In some embodiments, the one or more samples include analytes (e.g., nucleic acids, DNA, RNA, and/or proteins) transferred to the outside of the cell or tissue with artificially reconstructed spatial information to undergo sequencing and/or imaging outside the cell or tissue.

[0164] In some embodiments, the one or more sample(s) comprises a cell, a plurality of cells, a section of a cell, an intact tissue, an organ, a tissue section, an intact tumor, or a tumor section. In some embodiments, the sample(s) comprises a fresh cellular sample, a freshly-frozen cellular sample, a sectioned cellular sample, or an FFPE cellular sample. In some embodiments, the sample(s) comprises one or more living cells or non-living cells. In some embodiments, the sample(s) can be obtained from a virus, fungus, prokaryote or eukaryote. In some embodiments, the sample(s) can be obtained from an animal, fungus, plant, yeast, or bacterium. In some embodiments, the animal is a mammal or an insect. In some embodiments, the sample(s) comprises one or more virally-infected cells. In some embodiments, the sample(s) comprises transfected cells or displaced cells. In some embodiments, the sample(s) comprises mammalian transfected or displaced cells. In some embodiments, the sample(s) comprises a biofilm, i.e. a consortium of microorganisms that adhere together. In some embodiments, the sample(s) can be obtained from any organism including human, simian, ape, canine, feline, bovine, equine, murine, porcine, caprine, lupine, ranine, piscine, plant, insect, or bacterium. In some embodiments, the sample(s) can be obtained from any organ including head, neck, brain, breast, ovary, cervix, colon, rectum, endometrium, gallbladder, intestines, bladder, prostate, testicles, liver, lung, kidney, esophagus, pancreas, thyroid, pituitary, thymus, skin, heart, larynx, or other organs.

[0165] The methods, devices, and systems disclosed herein may allow sequencing and analysis of various samples and sources. The samples may include nucleic acids extracted from any of a variety of biological samples, e.g., blood samples, saliva samples, urine samples, cell samples, tissue samples, and the like. In some embodiments, the samples here may include a variety of different cell, tissue, or sample types known to those of skill in the

art. For example, the sample(s) may be from eukaryotes (such as animals, plants, fungi, protista), archaebacteria, or eubacteria. In some embodiments, the sample(s) may include prokaryotic or eukaryotic cells, such as adherent or non-adherent eukaryotic cells. In some embodiments, the sample(s) may be from, for example, primary or immortalized rodent, porcine, feline, canine, bovine, equine, primate, or human cell lines. In some embodiments, the sample(s) may include a variety of different cell, organ, or tissue types (e.g., white blood cells, red blood cells, platelets, epithelial cells, endothelial cells, neurons, glial cells, astrocytes, fibroblasts, skeletal muscle cells, smooth muscle cells, gametes, or cells from the heart, lungs, brain, liver, kidney, spleen, pancreas, thymus, bladder, stomach, colon, or small intestine). In some embodiments, the sample(s) may include normal or healthy cells. Alternately or in combination, the sample(s) may include diseased cells, such as cancerous cells, or from pathogenic cells that are infecting a host. In some embodiments, the sample(s) may include a distinct subset of cell types, e.g., immune cells (such as T cells, cytotoxic (killer) T cells, helper T cells, alpha beta T cells, gamma delta T cells, T cell progenitors, B cells, B-cell progenitors, lymphoid stem cells, myeloid progenitor cells, lymphocytes, granulocytes, Natural Killer cells, plasma cells, memory cells, neutrophils, eosinophils, basophils, mast cells, monocytes, dendritic cells, and/or macrophages, or any combination thereof), undifferentiated human stem cells, human stem cells that have been induced to differentiate, rare cells (e.g., circulating tumor cells (CTCs), circulating epithelial cells, circulating endothelial cells, circulating endometrial cells, bone marrow cells, progenitor cells, foam cells, mesenchymal cells, or trophoblasts). Other cells are contemplated and consistent with the disclosure herein.

[0166] In some embodiments, the sample(s) harbors a plurality of biological analytes or target analytes including polypeptides, lipids, nucleic acids and polysaccharides, or a mixture thereof. In some embodiments, the sample(s) harbors 2-10,000 different biological or target analytes. In some embodiments, the target analytes comprise a plurality of target polypeptides. In some embodiments, the plurality of target polypeptides have different sequences. In some embodiments, the sample(s) harbors 1-25 different target polypeptide, or harbors 25-50 different target polypeptides, or harbors 50-75 different target polypeptides, or harbors 75-100 different target polypeptides, or harbors any range therebetween of different target polypeptides. In some embodiments, the sample(s) harbors more than 100 different target polypeptides, or more than 250 different target polypeptides, or more than 500 different target polypeptides, or more than 1000 different target polypeptides. In some embodiments, the sample(s) harbors more than 10,000 different target polypeptides.

[0167] In some embodiments, the sample(s) can be deposited (e.g., seeded) onto a support which is passivated with a coating that promotes cell adhesion. In some embodiments, the sample(s) can be deposited on a support that lacks immobilized capture primers which can bind target polynucleotide analytes from the sample(s). In some embodiments, the support can be coated with one or more compounds that generate a charged coated surface. In some embodiments, the support is coated with a lysine compound, poly-lysine compound, arginine compound, poly-arginine compound, or an amino-terminated compound (e.g., including amino-terminated PEG). The support can be coated with an unbranched compound, a branched compound, or a mixture of unbranched and branched compounds. In some embodiments, the support can be coated with modified peptides, including, for example and without limitation, cationic anti-microbial peptides or dual surface anti-microbial peptides. In some embodiments, the support can be coated with polycyclic peptide antibiotics comprising thioether amino acids lanthionine or methyllanthionine and/or unsaturated amino acids dehydroalaine and 2-aminoisobutryic acid. In some embodiments, the support can be coated with at least one small peptide such as melittin. In some embodiments, the support can be coated with a compound that promotes integrin-mediated cell adhesion. For example, and without limitation, the support can be coated with tripeptide arginyl-glycyl-aspartic acid (Arg-Gly-Asp; also known as RGD). In some embodiments, the support can be coated with amines or polymers having -NH2 groups which promote cell adhesion, including for example polyethyleneimine (PEI) or polydopamine (PDA).

[0168] Depending on the sample(s) immobilized on the support (e.g., a flow cell), the flow cell images may include single or multiple z locations along an z axis orthogonal to the image plane of the flow cell images. In particular, for three dimensional samples, e.g., cells, tissues, or other in situ samples, the flow cell images herein can include multiple z-levels (i.e., axial locations) in order to cover the whole sample(s) in 3D. The z axis can extend from the objective lens of the optical system disclosed herein to the support, e.g., flow cell. The z axis can be orthogonal to the image plane of the flow cell images. Each z location of flow cell images may be separated from the adjacent z location(s) for a predetermined distance, for example, for about 0.1 um to about 15 ums. Each z location of flow cell images may be separated from the adjacent level(s) for 0.5 um to 10 ums. Each z location of flow cell images may be separated from the adjacent level(s) for 0.2 um to 2 ums. At each z location, flow cell images can be acquired from one or more sequencing cycles and/or one or more channels. Each flow cell image may include in its field of view at least part of one or more tiles or subtiles of the flow cell. The image plane is defined by the x and y axis. And the axial axis

(i.e., z axis) is orthogonal to the x-y plane. Preparation of the 3D sample(s) and immobilization on flow cells for sequencing reactions and imaging using the optical systems herein are disclosed in U.S. Patent Publication US20230326065A1 and spatially resolved capture of nucleic acids are disclosed in WO2024040068, the contents of which are herein incorporated by reference in their entirety.

[0169] In some embodiments, the first coating 522 can comprise multiple hydrophilic polymer coating layers. The first coating can include a first layer comprising a monolayer of polymer molecules tethered to the surface of the substrate 520, 522, 530. The first coating can further include a second layer comprising a second monolayer of polymer molecules tethered to the polymer molecules of the first layer; and a third layer comprising a third monolayer of polymer molecules tethered to the polymer molecules of the second layer, wherein at least one of the first layer, the second layer, or the third layer comprises branched polymer molecules.

[0170] In some embodiments, the third layer can comprise oligonucleotides (e.g., surface capture primers) tethered to the polymer molecules of the third layer. The oligonucleotides tethered to the polymer molecules of the third layer can be distributed at a plurality of depths (e.g., along the z-axis) throughout the third layer.

[0171] In some embodiments, the first coating can comprise a fourth layer comprising branched polymer molecules tethered to the polymer molecules of the third layer, and a fifth layer comprising polymer molecules tethered to the branched polymer molecules of the fourth layer. In some embodiments, the polymer molecules of the fifth layer further comprise oligonucleotides (e.g., surface capture primers) tethered to the polymer molecules of the fifth layer. The oligonucleotides tethered to the polymer molecules of the fifth layer can be distributed at a plurality of depths (e.g., along the z-axis) throughout the fifth layer.

[0172] In some embodiments, the hydrophilic polymer coating layer of the first coating can comprise a molecule selected from the group consisting of: polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), poly(vinyl pyridine), poly(vinyl pyrrolidone) (PVP), poly(acrylic acid) (PAA), polyacrylamide, poly(N-isopropylacrylamide) (PNIPAM), poly(methyl methacrylate) (PMA), poly(2-hydroxylethyl methacrylate) (PHEMA), poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA), polyglutamic acid (PGA), poly-lysine, poly-glucoside, streptavidin, and dextran.

[0173] In some embodiments, when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, an image of the surface exhibits a ratio of fluorescence intensities for the clonally-amplified, Cyanine dye-3-labeled

sample nucleic acid molecules, or complementary sequences thereof, and nonspecific Cyanine dye-3 dye adsorption background (B_{inter}) of at least 3:1.

[0174] In some embodiments when the image of the surface exhibits a ratio of fluorescence intensities for clonally amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and a combination of nonspecific Cyanine dye-3 dye adsorption background and nonspecific amplification background ($B_{inter}+B_{intra}$) of at least 3:1.

[0175] In some embodiments, when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, the image of the surface exhibits a ratio of fluorescence intensities for clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and nonspecific dye adsorption background (B_{inter}) of at least 5:1.

[0176] In some embodiments, when the image of the surface exhibits a ratio of fluorescence intensities for clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and a combination of nonspecific Cyanine dye-3 dye adsorption background and nonspecific amplification background ($B_{inter}+B_{intra}$) of at least 5:1.

[0177] In some embodiments, when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, the fluorescence image of the surface exhibits a contrast-to-noise ratio (CNR) of at least 20 when the fluorescence image is acquired using an inverted microscope equipped with a 20× objective, NA=0.75, dichroic mirror optimized for 532 nm light, a bandpass filter optimized for Cyanine dye-3 emission, and a camera under non-signal saturating conditions, while the surface is immersed in a buffer.

[0178] In some embodiments, one or more of the interior surfaces 521 (hereinafter, "surface") can be coated, in combination with the first coating 522, a second coating, and a third coating of fluorescent beads (not shown).

[0179] The fluorescent beads can be chemically immobilized to the surface of the channel 550. In some embodiments, the fluorescent beads can be covalently immobilized to the surface. The fluorescent beads can be immobilized or fixedly attached to the surface by forming a coating thereon, e.g., a third coating, so that the fluorescent beads remain fixed or immobilized relative to the surface 521. The third coating can be applied directly to and in contact with the surface 521. Alternatively, the third coating can be applied indirectly to or not in direct contact with the surface 521. In some embodiments, the third coating can be applied in between the surface 521 and the first coating 522.

[0180] In some embodiments, the fluorescent beads can be chemically immobilized to the surface. In some embodiments, the fluorescent beads can be covalently immobilized to the surface. In some embodiments, the fluorescent beads can be pre-activated to enable chemical attachment to the surface. In some embodiments, the fluorescent beads can be pre-activated to enable covalent attachment to the surface. In some embodiments, the clusters or polonies of polynucleotides captured thereon and the fluorescent beads can be imaged simultaneously in one or more sequencing cycles using the sequencing system 110.

Open landing areas

[0181] The flow cell devices, fluidic control devices, and systems can include an open landing area. FIGS. 2-4, and 5A-5F shows flow cell devices with an open landing area 341, 441, 541 the for one or more channels 350, 450, 550.

[0182] The open landing area 341, 441, 541 can be part of the inlet 340, 440, 540. The open landing area 341, 441, 541 can be on a bottom substrate 330, 430, 530. The open landing area 341, 441, 541 can be in fluidic connection with its corresponding channel(s) 350, 450, 550 to allow flow of fluid (e.g., reagents or air gap) from the open landing area 341, 441, 541 through the channel(s) 350, 450, 550.

[0183] The inlet 340, 440, 540 can comprise a void or hole in the top substrate 320, 420, 520 that is located above at least part of the open landing area 341,441, 541. The air gap, or liquid reagents, or combinations thereof, can be introduced via the void or hole of the inlet 340, 440, 540 to reach the open landing area 341, 441, 541, and then transfer from the open landing area 341, 441, 541 into the corresponding channel(s) 350, 450, 550. In some embodiments, the void or hole can have a cross-sectional area in the x-y plane that is equivalent or substantially equivalent to a cross-sectional area of the open landing area 341, 441, 541, e.g., as in FIGS. 3-4, 5A and 5F. In some embodiments, the void or hole can have a cross-sectional area in the x-y plane that is greater than the area of the open landing area, e.g., as shown in FIG. 5E. The void or hole in FIG. 5E can form a rectangular cross-section in the x-y plane. In some embodiments, the cross-sectional area of the rectangular void or hole can be as wide as the flow cell device along the x-axis (axis shown in FIG. 2).

[0184] The inlet 340, 440, 540 and the open landing area 341, 441, 541 can advantageously enable open administration of liquids or gas to the flow cell devices 300, 400, 500. The open administration enabled by the flow cell devices herein can remove series of closed tubing or locked-in tubing, thereby greatly reducing system complexity and cost and allowing more flexible adaptation of the systems and devices for various sequencing applications.

[0185] The size and shape of the hole or void, and the size and shape of the open landing area can vary in different embodiments. The sizes and shapes may be determined based on parameters in the specific sequencing application(s), such as, a flush volume, a contamination threshold, the dimensions of the flow cell (e.g., the width of the flow cell channels), or the parameters of the dispenser (e.g., the size of the dispensing tip). As a nonlimiting example, the hole or void can be cylindrical as shown in FIG. 5C with walls defining the hole or void extending along the z direction and orthogonal to the substrates. However, the hole or void can be shaped, or sized, or combinations thereof, differently. For example, the hole or void can have an inverted cone shape with wider openings at the top and narrows down toward the channel to reduce the residual reagent from remaining in the inlet. In some embodiments, a larger open landing area may better facilitate reagent transfer into the channels. In some embodiments, a ratio of the area of the open landing area to the width of the channels can be kept in a predetermined range to facilitate reagent transfer into the channels. As a nonlimiting example, the diameter or width of the opening area, (e.g., a maximum dimension in the x-y plane) can be in the range of about 3 mm to about 40 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the diameter of the opening area is substantially equivalent or equivalent to the width of the corresponding channel. In some embodiments, the diameter or width of the opening area is about 10%, 20%, 30%, 40% or 50% less than the width of the corresponding channel. As a nonlimiting example, the diameter or width of the hole or void(e.g., the maximum dimension in the x-y plane) can be in the range of about 3 mm to about 40 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the diameter of the hole or void is substantially equivalent or equivalent to the width of the corresponding channel. In some embodiments, the diameter or width of the hole or void is about 10%, 20%, 30%, 40% or 50% less than the width of the corresponding channel.

[0186] In some embodiments, the flow cell system may include a fluidic operation device which may comprise a dispenser 280, 580 (shown in FIG. 2 and FIG. 5C) that is configured to openly dispense one or more reagents to the inlet 540 and onto the open landing area 541. The dispenser can openly dispense from a tip, via the void or hole of the inlet 540, to the open landing area 541. In some embodiments, the flow cell device may not include tubing connecting the dispenser (e.g., dispenser 580) and the inlet (e.g., inlet 540). In some embodiments, the dispenser 580 can directly contact a portion of the inlet 540 (e.g., the landing area 541, or a wall of the void) to openly dispense the reagents (e.g., without tubing). In some embodiments, the dispenser 580 does not directly contact any physical part of the

inlet 540, but a tip of the dispenser 580 may at least partially extend into the void or hole of the inlet 540. In some embodiments, at least part of the tip of the dispenser 580 is in contact with the open landing area 541. In some embodiments, the tip of the dispenser 580 is not in direct physical contact with the open landing area 541.

[0187] In some embodiments, the dispenser may include more than one dispensing tips, e.g., pipette tips, so that each reagent can be dispensed from a respective dispensing tip without mixing of reagents occurring in the dispenser or the dispensing tips. The dispensers disclosed herein may use separate dispensing tips for dispensing different reagents to eliminate the problem of residual contamination in a common line (e.g., a single line shared for dispensing all reagents) that occurs in existing dispensing devices and to reduce or remove dead volume in the common line (e.g., the volume that stays in the common line and needs washing if a different reagent is going to be dispensed). Using different dispensing tips for different reagents can reduce dead volume and/or contamination in the common line because when dispensing, the volumes left in the dispensing tips or the separate line leading to the tip may be used in contrast to the dead volume. Therefore, dispensers disclosed herein can reduce consumption of reagents for identical sequencing processes when compared to existing systems. Further, removal of the common line and usage of separate dispensing tips reduces mixing and contamination of reagents dispensed into the flow cell devices.

[0188] In some embodiments, the dispenser and dispenser tip(s) may be manually operated to cause movement and/or dispensing. In some embodiments, the dispenser and dispenser tip(s) may be automatically operated to cause movement and/or dispensing. For example, the dispenser may include an array of dispensing tips, each in fluidic communication with a reagent reservoir in a cartridge, and a robotic arm can move (e.g., automatically without manual control) the array to position a corresponding dispenser tip above the open landing area and controls the dispensing (e.g., automatically without manual control). When a subsequent reagent is to be delivered, the robotic arm can withdraw the previous dispensing tip and position the subsequent dispensing tip in the array (e.g., holding the subsequent reagent) above the open landing area for dispensing.

[0189] In some embodiments, the flow cell devices 400, 500, 700 further comprise a cleaning outlet 470, 570, 770. The cleaning outlet 470, 570, 770 can be defined in the one or more substrates, for example, in the bottom substrate 430, 530. In some embodiments, the cleaning outlet 470, 570, 770 may be located on a top substrate or in a middle substrate as a side port (not shown). The cleaning outlet 470, 570, 770 can be in fluidic connection with the inlet 440, 540. In some embodiments, the cleaning outlet 470, 570, 770 is configured to be

coupled with a fluid driving device, e.g., a pump or vacuum 471 of the fluidic control device. The pump 471 may be in addition to or instead of the pump 472 coupled to the outlet 460. In some embodiments, a same fluid driving device, e.g., pump, can be coupled to the outlet 460, 560 and the cleaning outlet 470.

[0190] A distance (e.g., within the x-y plane) from the cleaning outlet 470, 570, 770 to the inlet 440, 540 can be shorter than a distance from the cleaning outlet 470, 570, 770 to the outlet 460, 560. The shorter distance from the cleaning outlet 470, 570, 770 to the inlet 440, 540 can facilitate transfer of liquid or gas from the open landing area 441, 541 to the cleaning outlet efficiently.

[0191] In some embodiments, a position of the cleaning outlet 470, 570, 770 relative to the inlet or open landing area 441, 541, 741can be different. In some embodiments, the cleaning outlet 770 can be directly underneath the open landing area 741, e.g., in FIGS. 25A-25E and 26C. In such embodiments, the cleaning outlet 770 is directly connected to the open landing area.

[0192] In some embodiments, the cleaning outlet 470, 570 may not be directly beneath the open landing area 441, 541 but at a distance from the open landing area, e.g., in FIGS. 4, 5A, 24A-E, and 26A-B. In such embodiments, the cleaning outlet 470, 570 cannot be directly connected to the corresponding open landing area 441, 541, but instead connected via a tapered transition portion 454, 554 therebetween.

[0193] The distance from the cleaning outlet (e.g., cleaning outlet 470, 570) to the closest edge or the center of the open landing area (e.g., open landing area 441, 541) can be 0 mm or about 3 mm, inclusive of all ranges and subranges therebetween. The distance from the cleaning outlet to the closest edge or the center of the open landing area, when the cleaning outlet is not directly underneath the open landing area, can be from about 0 mm to about 20 mm, inclusive of all ranges and subranges therebetween. The distance from the cleaning outlet to the closest edge or the center of the open landing area, when the cleaning outlet is not directly underneath the open landing area, can be from about 0 mm to about 15 mm, inclusive of all ranges and subranges therebetween. The distance from the cleaning outlet to the closest edge or the center of the open landing area, when the cleaning outlet is not directly underneath the open landing area, can be from about 0 mm to about 10 mm, inclusive of all ranges and subranges therebetween. The distance from the cleaning outlet to the closest edge or the center of the open landing area, when the cleaning outlet is not directly underneath the open landing area, can be from about 3 mm to about 10 mm, inclusive of all ranges and subranges therebetween.

[0194] The distance from the cleaning outlet to the closest edge or the center of the open landing area, when the cleaning outlet is not directly underneath the open landing area, can be from 0 mm to 15 mm, inclusive of all ranges and subranges therebetween. The distance from the cleaning outlet to the closest edge or the center of the open landing area, when the cleaning outlet is not directly underneath the open landing area, can be from 0 mm to 10 mm, inclusive of all ranges and subranges therebetween. The distance from the cleaning outlet to the closest edge or the center of the open landing area, when the cleaning outlet is not directly underneath the open landing area, can be from 3 mm to 10 mm, inclusive of all ranges and subranges therebetween.

[0195] In some embodiments, there can be residuals of reagents, such as meniscus, as shown in the bottom panel of FIG. 4, that remains on the landing pad, or on the wall(s) of the hole of the inlet 440, or combinations thereof. Such residuals, if not removed, may cause unintended mixing when a subsequent reagent is delivered to the open landing area and consequently contaminate sequencing reactions in the channels. Washing with liquid(s) alone may not be effective in removing such residual reagents as meniscus, and therefore, known flow cell devices may require multiple flushes of washing liquids to completely remove the residual reagents, thereby increasing washing time and washing costs. The cleaning outlet 470, 570 in fluidic connection with the inlet 440, 540 can advantageously facilitate time- and cost- effective removal of such residuals. In some embodiments, a mechanical driving force can be applied, e.g., by a pump or an inlet vacuum, via the cleaning outlet 470, 570, to completely remove such residual of reagents on the landing pad. As such, the required time and washing volume to remove the residuals to achieve a predetermined contamination level can be effectively improved from existing flow cell devices.

[0196] The size and shape of the cleaning outlet (e.g., cleaning outlet 470, 570, 770) may be customized to suit different sequencing applications. Although the cleaning outlet is shown as a cylinder in FIG. 5C, it can be made in different shapes, such as a cone, an inverted cone, etc. In some embodiments, the size and shape of the cleaning outlet can be identical to that of the outlet. In some embodiments, the size of the cleaning outlet can be no more than about 10%, 20%, or 30% different from that of the outlet. In some embodiments, the diameter of the cleaning outlet in the x-y plane is about 0.3 mm to about 10 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the height of the cleaning outlet in the z direction is the same as the height of the bottom substrate. In some embodiments, the height of the cleaning outlet is about 0.3 mm to about 3 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the height of the cleaning

outlet is about 0.5 mm to about 1 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the diameter of the cleaning outlet in the x-y plane is 0.3 mm to 10 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the height of the cleaning outlet is 0.3 mm to 3 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the height of the cleaning outlet is 0.5 mm to 1 mm, inclusive of all ranges and subranges therebetween.

Slippery coatings

[0197] In some embodiments, part of the substrate (e.g., substrates 320, 322, 330, 420, 422, 430, 520, 522, 530) can be covered with a second coating, e.g., a slippery coating, to facilitate transfer of fluids on the coating, either alone or in combination with the first coating disclosed herein. In some embodiments, the second coating may be disposed on part of the substrate other than an interior surface of the channels of the substrate. In some embodiments, second coating can be different from the first coating of the channels. In some embodiments, the second coating can be applied directly to the substrate(s) without application of the first coating. In some embodiments, the second coating can be applied to the substrate(s) on top of the application of the first coating.

[0198] The thickness of the second coating along the z axis may be configured such that the second coating does not interfere or reduce fluidic communication speed, or other fluidic parameter(s), or combinations thereof, to the channels in comparison to flow cell devices without the second coating. The thickness of the second coating along the z axis may be configured to increase or facilitate a speed of fluid flow, or other fluidic parameter(s), or combinations thereof, to the channels in comparison to flow cell devices without the second coating.

[0199] In some embodiments, the open landing area 341 can be covered with the second coating 342. FIG. 3 shows an embodiment of the second coating 342 on the open landing area 341, the rest of the landing pad 343, and the part of the top substrate that is above the landing pad 343. The right panel of FIG. 3 shows a schematic drawing of the second coating 342 with a liquid droplet of a reagent thereon. In some embodiments, the second coating 342 can be applied to at least part of the open landing area 341. In some embodiments, the second coating 342 can be applied to any combination of surfaces of the substrates except the interior surfaces defining the lumen of the channels. The second coating 342 can effectively facilitate liquid transfer from the open landing area 341 to the channels 350or to a cleaning outlet (not shown) to exit the flow cell device 300. For example, the second coating 342 may help reduce the volume of residual reagents on the landing pad 341 when the reagent(s) is

transferred into the channels 350. In some embodiments, the second coating 342 may facilitate complete removal of the residual reagents on the landing pad 341, when an inlet vacuuming force is applied via the cleaning outlet.

[0200] In some embodiments, the second coating 342 can include any liquid-repelling coating. In some embodiments, the second coating 342 can include an omniphobic coating. In some embodiments, the second coating 342 comprises a slippery omniphobic covalently attached liquid (SOCAL) coating. In some embodiments, the second coating 342 comprises a liquid-like polymer brush surface that is covalently attached to the one or more substrates. In some embodiments, the second coating 342 is formed by acid-catalyzed graft polycondensation of one or more saline monomers. The one or more saline monomers can comprise dimethyldimethoxysilane (PDMS). In some embodiments, the one or more saline monomers can have a low surface energy that is below about 10, 15, 20, 25, or 20 mJ/m^2.

[0201] The second coating 342 can be formed using various methods. For example, the second coating 342 can be formed by impregnating lubricants in one or more porous surfaces. In some embodiments, the coating comprises a slippery liquid-infused porous surface (SLIPS). In some embodiments, the lubricants comprise a liquid with a low surface energy, where the low surface energy is below a predetermined threshold. The predetermined threshold can be about 20 milliJoule per square meter (mJ/m^2). In some embodiments, the predetermined threshold can be about 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30 mJ/m^2, inclusive of all ranges and subranges therebetween. In some embodiments, the lubricants comprise a silicone oil. In some embodiments, the coating comprises low surface energy that is below about 10, 15, 20, 25, or 20 mJ/m^2, inclusive of all ranges and subranges therebetween.

Contamination levels and cost of goods sold (COGS) savings

[0202] In some embodiments, one or more of the open landing area with open dispensing (e.g., dispensing without tubing through open air), the channel coating (e.g., the first and/or third coating), the slippery coating (e.g., the second coating) of the landing pad, and the cleaning outlet and vacuuming can be used alone or in different combinations to achieve cleaning of the flow cell devices. FIGS. 2-4, 5A-5F show nonlimiting embodiments of the combination of one or more of these features in the flow cell devices 200, 300, 400, 500.

[0203] FIG. 7A shows the contamination level of flow cell devices disclosed herein, in comparison to existing flow cell devices. Images of the flow cell channels are acquired per flush volume. The flushing in this embodiment is about 60 microliter (μL), determined based on at least the channel size and geometry. The contamination of flow cell channels, on

average, is about 1% for all three flow cell devices and two existing flow cells. The contamination level starts to decrease as the number of flush volume increases. When the flush factor reaches 5, the total volume of flushed reagents reaches about 300 μL. At this flushing factor, the contamination level of three flow cell devices disclosed herein, with SLIPS coating on the open landing read, with inlet vacuum from the cleaning outlet, or their combinations, exhibit a contamination level of lower than 0.01%, while the contamination level of existing flow cells are significantly higher, at above 0.1%. It takes more than 10 flush factors or over 600 μL of washing reagents for the existing flow cell devices to achieve similar contamination level as in the three flow cell devices disclosed herein. In some embodiments, flushing with more than 300 μL does not further decrease the contamination level to a significant level, e.g., 5x or more. The contamination level of about 0.001% can be satisfactory for all of the reagents used in the NGS sequencing application. The three flow cell devices (e.g., labeled as "SLIPS" 1002, "Inlet Vacuum" 1004, and "SLIPS + Inlet Vacuum" 1006) advantageously achieved contamination levels for accurate and reliable sequencing process with a significant reduction in Cost of Goods Sold (COGS) than existing methods. The three flow cell devices corresponds to flow cell device 300 in FIG. 3 and its variations, with SLIP coating only, with an inlet vacuum only, and with both the SLIP coating and inlet vacuum.

[0204] FIG. 29 shows residual level or contamination level that is averaged among different tiles of a flow cell device with an open landing area and inlet vacuum as shown in flow cell device 500 disclosed herein and with and a hydrophobic coating over the open landing area. Tile contamination variation across the flow cell device can be caused by the spatial location of the tile on the flow cell and its relative position to the inlet, or the outlet, or combinations thereof. Average contamination levels of different tiles of the flow cell devices disclosed herein are effectively reduced to be less than 1% by the first cycle of flush volume. By the third flush volume or cycle, the residual or contamination level of different tiles are all reduced to below 0.001% (e.g., the target threshold as shown by the dotted line). The average tile contamination (shown by the solid square) is below the level of 0.001% by the third flush volume. The individual tile contaminations across the flow cell are below the level of 0.001% by the third or fourth flush volume. The flush volume is about 60 μL so that the contamination level for individual tiles regardless of the spatial location on the flow cell is reduced to be below 0.001% with a total flush volume of reagents or washing buffer of 240 μL (e.g., almost two times lower than that needed in existing flow cell systems). Each individual flush volume can be optimized based on the channel size and volume of the flow

cell device. In some embodiments, each individual flush volume can be in a range from 0.5x to 2x of the volume of the channel to be washed.

[0205] In some embodiments such as flow cell device 500), the volume of sequencing-by-avidite reagents for stepping, cleaving, and imaging are all significantly reduced by using the flow cell devices disclosed herein. In existing systems, the stepping reagent requires a volume of about 430 μL, and in flow devices described herein the volume was reduced to about 90 μL with active volume reduction (AVR) to recycle a certain portion of the reagents. The AVR can be used in both existing flow cells systems and the flow cell systems disclosed herein. In some embodiments, the AVR can be about 40%, 50%, 60%, 70%, 80%, or 90% of the total volume that is required with respect to a sequencing application (e.g., only using 40%, 50%, 60%, 70%, 80%, or 90% of the total flow volume needed in identical sequencing runs but without AVR). In some embodiments, the total volume can be a volume without AVR. In some embodiments, the reagents saving (e.g., a reduction in the total volume of reagents) with AVR can be about 5x in comparison to existing flow cell devices. Without AVR, the reduction can still be about 2.5x in comparison to existing flow cell devices. In some embodiments, the cleaving, trapping, and imaging reagents are reduced from about 300 μL to about 60 μL, with AVR. Table 1. in FIG. 7B shows the volume of sequencing reagents required in using an existing flow cell system and COGS saving or reduced volume of reagents required using a flow cell device disclosed herein. Additionally, a total volume of reagents used in the flow cell devices disclosed herein to achieve a target contamination level (e.g., 0.001% or lower) is lower than a total volume of reagents needed in existing flow cell devices to achieve the target contamination level.

Fluidic control devices

[0206] Disclosed herein are fluidic control devices that can be coupled to the flow cell devices and actively apply mechanical forces for dispensing or collecting liquids, or gas, or combinations thereof, from the flow cell devices.

[0207] In some embodiments, the fluidic control devices can comprise a pump, a vacuum, or any other device that can actively apply a mechanical force to the lumen of the channels, or the open landing area, or combinations thereof. In some embodiments, the fluidic control devices apply a mechanical force to the channels via the outlet or cleaning outlet. FIG. 4 shows a fluidic control device with a vacuum 472 that is coupled to all outlets 460 (e.g., each outlet coupled to each channel) of the flow cell device 400. FIG. 4 shows another vacuum 471 that is coupled to the cleaning outlet 470 of the flow cell device 400. In some embodiments, the vacuums 471 and 472 can be the same vacuum or pump.

[0208] In some embodiments, the fluidic control devices can comprise a dispenser (e.g., dispensers 280, 580 shown in FIG. 2 and FIG. 5C) with one or more dispensing tips. The dispenser can be configured to dispense preset amounts of reagents within a certain time window to the inlet.

[0209] In some embodiments, the fluidic control devices can comprise a robotic arm that controls movement of the dispenser. In some embodiments, the robotic arm can move the dispenser in 3D space so that the dispensing tip can reach a specific location (e.g., a specific position relative to the open landing area or channel) before the dispensing tip starts dispensing. In some embodiments, the robotic arm can retrieve a dispensing tip after the dispensing tip has dispenses fluid and move a second dispending tip to a location (e.g., a position relative to the open landing area or channel) to subsequently dispense fluid.

[0210] In some embodiments, the fluidic control devices can comprise a dispensing roller (e.g., conveyer, track, etc.) configured to dispense the reagents as shown in FIG. 6A. The reagents can be dispensed by the dispenser 680 onto a continuous track 691 rolled on one or more wheels. As the wheels rotate, the track 691 can move the reagents to an open landing area of the flow cell. In some embodiments, the inlet can be a side-port at an edge of the substrates. In some embodiments, an active force can be applied at the outlet (e.g., via a vacuum or suction force) to facilitate delivery of the reagents from the track to the inlet and into the channels.

[0211] In some embodiments, the fluidic control devices can comprise a dispensing plate 692 with an electrowetting surface as shown in FIG. 6B. As shown, the dispensing plate 692 can be translated, thereby translating the reagents dispensed thereon to the inlet, which in this embodiment, is a side-port at an edge of the substrates.

[0212] In some embodiments, the fluidic control devices can comprise a reagent reservoir and a sipper as shown in FIG. 6C. In some embodiments, one end of the sipper 693 can be inserted in a reagent reservoir 694, and the other end of the sipper can point to or be in contact with the inlet. The reagent can be removed (e.g., sucked out) in a controlled fashion to the open landing area of the flow cell. For example, the sipper may include a lumen configured to apply a suction force (e.g., via a vacuum at the outlet) to draw the fluid out of the reagent reservoir. The open landing area can face downward (e.g., toward the reservoir). For example the hole or void of the inlet can be defined in the bottom substrate. Various mechanisms can be used to control the sipping action. For example, an active mechanical force can be applied from the outlet to sip a predetermined amount of reagent from the

reservoir. A different sipper can be used for a different reagent to avoid unintended mixing of reagents in the sipper 693.

Fluid dispensing devices

[0213] In some embodiments, the sequencing system herein may work together with a fluid dispensing device disclosed herein for various NGS sequencing applications. FIGS. 30A-47D show embodiments of fluid dispensing devices, according to embodiments. In some embodiments, the fluidic control devices herein may include the fluid dispensing device. In some embodiments, the fluidic dispensing device can be utilized with various flow cell devices to allow fluidic flow of reagents and otherwise fluids or gas to be introduced to the flow cell device and from the flow cell device during various applications including NGS sequencing. In some embodiments, the fluid dispensing device can be utilized with any of the flow cell devices(e.g., 200, 300, 400, 500, 700) disclosed herein. In some embodiments, the fluidic dispensing device can be utilized with other flow cell devices with more than two surfaces that are displaced from each other along the z-direction that is orthogonal to the x-y plane. Details of such flow cell devices are disclosed in PCT application No. PCT/US2023/081406, and are incorporated herein by references in its entirety.

[0214] In some embodiments, the dispensing module may be disposable. In some embodiments, the dispensing module may be removable from the sequencing system or flow cell device. In some embodiments, the dispensing module may be physically separated and moved relative to the sequencing system or flow cell device.

[0215] In some embodiments, the dispensing module 3100, 4100 is reversibly coupled to the one or more actuators 3500, 4500, as shown in FIGS. 30A-30B. In some embodiments, the user may manually couple the dispensing module 3100, 4100 to the actuator 3500, 4500 or physically remove the dispensing module 3100, 4100 from the actuator(s) 3500, 4500 when needed without damaging the functionality of either one of them.

[0216] In some embodiments, the dispensing module 3100, 4100 may be removably coupled to a flow cell device for sequencing analysis of a sample immobilized on the flow cell device. In some embodiments, the dispensing module 3100, 4100 may be removably coupled to a sequencing system (e.g., NGS system) for sequencing analysis of the sample immobilized on the flow cell device. For example, the dispensing module 3100, 4100 may be removed and disposed of by a user after a first sequencing application of a first number of samples is completed, and a new dispensing module may be coupled to the sequencing system for a next sequencing application. The sample(s) may be nucleotide acid template molecules immobilized on the flow cell device and can be sequenced after contacting them

with predetermined sequencing reagents and/or buffers by introducing the fluid(s) via the dispensing tips to the flow cell device, and allowing the fluid(s) to travel to and contact the sample(s), as described herein.

[0217] FIGS. 30A and 30B show two exemplary embodiments of fluid dispensing devices 3000, 4000. The fluid dispensing devices 3000, 4000 may comprise a dispensing module 3100, 4100. The dispensing module 3100, 4100 can include a reagent cartridge 3200, 4200 with one or more compartments 3210, 4210; a microfluidic chip 3300, 4300 in fluidic communication with each of the one or more compartments 3210, 4210; one or more dispensing tips 3400, 4400; or their combinations. The one or more compartments 3210, 4210 may hold fluids therein, e.g., reagents.

[0218] In some embodiments, the cartridge 3200, 4200 may include a single housing for containing the compartment(s) 3210, 4210 as a single unit. In some embodiments, the one or more compartments 3210, 4210 can be of various 3D geometrical shapes and sizes. In some embodiments, the one or more compartment 3210, 4210 may include various sizes to include predetermined volumes of different reagent. In some embodiments, at least two compartments are of different sizes. In some embodiments, all the compartments may include a dimension (e.g., height), which is identical, as shown in FIGS. 30A-30B. As a non-limiting example, the single housing in FIG. 30A may be 8 inches x 4 inches x 3.5 inches. In some embodiments, the compartments 3210, 4210 are not in fluidic communication with each other within the cartridge 3200, 4200. In some embodiments, the compartments 3210, 4210 can be fluidically isolated from one another within the cartridge 3200, 4200. In some embodiments, one or more compartments 3210, 4210 can be used to hold a same type of liquid or reagent, e.g., with identical concentration and/or mixture of biological or chemical compounds therewithin. In some embodiments, two or more compartments 3210, 4210 can be used to hold different type of liquids or reagent, e.g., a washer buffer and a sequencing reaction reagent, or a clean washing buffer and a contaminated washing buffer, etc.

[0219] In some embodiments, at least one compartment 3210, 4210 may be in fluidic communication with only one dispensing tip to avoid cross-contamination of different fluids at the dispensing tip, thereby avoiding consequent cross-contamination at the flow cell device (e.g., flow cell devices 300, 400, 500). For example, a first compartment or set of compartments 3210, 4210 holding a first type of reagent may be in fluid communication with a first dispensing tip, and second compartment or set of compartments 3210, 4210 holding a second type of reagent may be in fluid communication with a second dispensing tip (e.g., isolated from the first dispensing tip). In some embodiments, each different fluidic reagent or

each mixture of reagents is configured to travel from its corresponding compartment to its corresponding dispensing tip via its corresponding pathway to avoid cross contamination with other fluidic reagent or mixture in other compartments.

[0220] In some embodiments, at least one compartment 3210, 4210 may be in fluidic communication with two or more dispensing tips to allow simultaneously dispensing of different or identical reagent to different locations at the flow cell device 300, 400, 500. For example, two compartments 3210, 4210 may be holding same washing buffer therewithin for dispensing washing buffer simultaneously to two inlets which lead to different microfluidic channels of the flow cell device 300, 400, 500.

[0221] In some embodiment, each compartment 3210, 4210 may include various sizes that can fit into the single housing. In some embodiments, each dimension (i.e., length, width, or height) may be within 0.1 inches to 8 inches, inclusive of all ranges and subranges therebetween.

[0222] FIGS. 32A-32B show schematics of fluid dispensing devices 5000, 5000' according to an embodiment. In some embodiments, each of the one or more compartments may comprise a corresponding compartment outlet 5220 that allows the fluid to travel from a corresponding compartment to the microfluidic chip 5300, and then to the dispensing tip(s) 5400, as shown in FIG. 32A. The corresponding compartment outlet 5220 may be fluidically connected with a corresponding microfluidic pathway in a fluidically sealed fashion to prevent leakage from the connection, e.g., 5230' in FIG. 32B. In some embodiments, the compartment outlet 5220 and the corresponding inlet 5321 of the microfluidic pathway can be permanently and non-reversibly connected in a fluidically sealed fashion to avoid leakage from the connection. In embodiments with the dispensing module being disposable, the need for a user to disconnect the cartridge and the microfluidic chip and reconnect a new cartridge to the microfluidic chip is eliminated. Eliminating disconnection and reconnection of cartridges can advantageously eliminate possible leakage that can occur at the connection 5230 between the cartridge 5200 and the other part of the dispensing system, e.g., the microfluidic pathways, after a manual connection by the user.

[0223] The fluidic dispensing device 5000 may include the microfluidic chip 5300. In some embodiments, the microfluidic chip 5300 may be fixedly or permanently coupled to the reagent cartridge 5200. In some embodiments, the microfluidic chip 5300 may not be fixedly or permanently coupled to the reagent cartridge 5200. In such embodiments, spatial displacement of the microfluidic chip 5300 relative to the reagent cartridge 5200 may occur. Certain details of the fluid dispensing devices 5000, 5000' are structurally and/or functionally

similar to the fluid dispensing devices 3000, 4000, and therefore, certain details of the fluid dispensing devices 5000, 5000' are not described herein with respect to FIGS. 32A-32B.

[0224] FIGS. 46A-46B shows an exemplary embodiment in which the fluidic pathway between the microfluidic chip 9300 and the reagent cartridge 9200 may include one or more sippers 9280. Each sipper 9280 may be inserted into a corresponding compartment of the reagent cartridge 9210. Each sipper 9280 may be configured to allow fluidic communication between the reagent cartridge 9200 and the microfluidic chip 9300. Certain details of the fluid dispensing device 9000 including reagent cartridge 9200 and microfluidic chip 9300 may be structurally and/or functionally similar to the fluid dispensing devices 3000, 4000, 5000 including reagent cartridges 3200, 4200, 5200 and microfluidic chips 3300, 4300, 5300, and therefore, certain details of the fluid dispensing device 9000, reagent cartridge 9200, and microfluidic chip 9300 are described herein again.

[0225] As shown, the fluidic dispensing device 9000 can include one or more pumps 9340 that are configured to actuate fluidic communication from the reagent cartridge 9200 to the microfluidic chip 9300 via the sippers 9280. The same or different pump(s) may be used to enable dispensing of fluids via the dispensing tips 9400 to the flow cell device. In some embodiments, positive pressure (e.g., via pushing) and/or negative pressure (e.g., via vacuuming) may be used to enable dispensing of fluids via the dispensing tips and flow into the channels of the flow cell device. In some embodiments, positive pressure may be applied in order to push the reagent or otherwise fluids or gas out from the dispensing tip. Gravity may be relied on at least partly for causing the reagent or otherwise fluids or gas from the dispensing tip to flow to the flow cell device. In some embodiments, positive pressure may be applied to the reagent or otherwise fluids or gas to cause them to flow from the dispensing tip to the flow cell device (e.g., flow cell device 200, 300, 400, 500, e.g., the open landing area 341, 441, 541). In some embodiments, such positive pressure may be applied in an open (e.g., not enclosed) fluidic connection between the dispensing tip and the flow cell device, e.g., FIGS. 2 and 5C. In some embodiments, such positive pressure may be applied in a closed or guided fluidic connection between the dispensing tip and the flow cell device, e.g., via a fitted gasket positioned above the open landing area of flow cell device. The dispensing tip can go through a guiding gasket (e.g., touching the landing area) and arrive (e.g., contact, touch, align with, be aligned relative to) at the open landing area. The closed or guided fluidic connection may be fluidically sealed. The closed or guided fluidic connection may not be fluidically sealed but may allow some leakage to occur at the interface of the tip and the flow cell device. The leakage may be cleaned via various mechanisms, e.g., vacuuming. The

dispensing tip may be aligned to such that the dispensing tip can be inserted at least partly through the gasket before dispensing to the flow cell device. In some embodiments, negative pressure, alone or in combination with positive pressure, may be used to facilitate flow to the flow cell device. In some embodiments, the negative pressure may be a pulling or sucking force at the outlet of the channels of the flow cell device. In some embodiments, the negative pressure may be a vacuum force at the cleaning outlet of the flow cell device. Various actuators may be used for generating the positive and/or negative pressure disclosed herein, e.g., a syringe pump, a peristaltic pump, or a pneumatic pump.

[0226] In some embodiments, the reagent cartridge 9200 may include one or more compartments for containing washing buffers therewithin. In some embodiments, the fluidic dispensing device herein may advantageously enable automatic washing of one or more of: the dispensing tips 9400, the microfluidic chip 9300 and fluidic pathways therewithin, and the sippers 9280 without the need for any manual loading of washing buffers and manual disposal of washing waste. In some embodiments, the fluid dispensing device can be washed without manual operation from the user. No manual operation from the user is needed for the washing to be completed in the fluidic dispensing device.

[0227] In some embodiments, the washing may occur as needed (e.g., rather than continuously). For example, washing may occur between two different sequencing runs. As another example, washing may be performed while a sequencing run is still in progress for some or all of the dispensing tips, e.g., for trouble shooting or cleaning of possible contamination. In some embodiments, the automatic washing may be achieved by shifting the nozzle position by a short distance to reach the washing reservoirs. As shown in FIG. 46B, the washing waste may be dispensed onto an absorbent material or waste containers 9290 so that no manual waste collection is required.

[0228] In some embodiments, the user may only load the cartridge to start the first sequencing run, and after the sequencing run is completed, the washing may automatically occur (e.g., without further user input). After the sipper, the microfluidic chip, and/or the dispensing tips are washed, the user may dispose the disposable cartridge and load a second cartridge for a new sequence run. The top panel of FIG. 46B shows the reagent cartridge in the sequencing position relative to the dispensing tips. For washing, the sippers, microfluidic chip, and/or the pump(s) are lifted up and away from the reagent cartridge 9200 as shown in FIG. 46A. The connecting tubing (6 straight tubing connecting to the dispensing tips) to the dispensing tips are shown in FIG. 46B but not in FIG. 46A. The connection tubing may be rigid as shown in FIG. 46B. In some embodiments, the connection tubing may be flexible and

longer to allow easy movement of the sippers (on the left side of the tubing in FIG. 46B) and the tubing between the sequencing and washing positions. Such motion may be actuated by an actuator such as, for example, A motor. The sippers, microfluidic chip, and/or the pump(s) that are lifted up above the reagent cartridge may move within the x-y plane a washing distance (e.g., a distance between the sequencing position and the washing position of the microfluid chip) so that the sippers are positioned above the corresponding compartments for containing washing buffers. The sippers may then be lowered into fluidic communication with the washing buffers, as shown at the bottom of FIG. 46B. The one or more pumps may be configured to pump washing buffer from the compartments to the waste container. In some embodiments, the washing distance may be about 50mm. In some embodiments, the washing distance may be customized based on the size of the reagent cartridge, and the number of dispensing tips, and other features of the fluidic dispensing device to be within various ranges. In the embodiment as shown in FIGS. 46A-46B, the washing distance is greater than the distance between two adjacent dispensing tips along the x axis.

[0229] In some embodiments, the washing distance may be in the range from about 1 mm to about 20 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the washing distance may be in the range from about 10 mm to about 100 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the washing distance may be along the x axis, y axis, or any direction within the x-y plane. After washing, the sippers may be lifted up again and move the washing distance backwards (e.g., towards the resting position of the reagent cartridge) to be connected to the dispensing tips as shown in the top panel of FIG. 46B.

[0230] In some embodiments, the fluidic dispensing device may include more than one washing position. Each different washing position may be spatially displaced from an adjacent washing position or sequencing position by a washing distance within the x-y plane. FIG. 46C shows an exemplary embodiment with two washing positions, and the two washing positions are spatially displaced from each other along the x axis. In the embodiment as shown in FIG. 46C, the washing distance is smaller than the distance between two adjacent dispensing tips, e.g., dd, along the x axis. In other words, one or more washing positions are positioned between the distance between two adjacent dispensing tips, e.g., dd.

[0231] In some embodiments, the microfluidic chip 9300 may be positioned on top of the reagent cartridge 9200, as shown in FIG. 46A. In some embodiments, the microfluidic chip 3300, 4300, 5200 may be positioned underneath the reagent cartridge 3200, 4200, 5200 as shown in FIGS. 30A and 33A.

[0232] The one or more pumps (e.g., pumps 6340) may be positioned in various positions relative to the reagent cartridge, as shown in FIGS. 33A-33B. In some embodiments, the pump(s) 6340 may be disposed underneath the reagent cartridge 6200 as shown in FIGS. 33A and 33B. In some embodiments, the one or more pumps 9340 may be above the reagent cartridge as shown in FIG. 46A. In some embodiments, the one or more pumps 9340' may be positioned on the side of the reagent cartridge 9200', as shown in FIG. 46C.

[0233] The microfluidic chip may include one or more microfluidic pathways (5320 in FIG. 31B) therewithin. The microfluidic chip may have various sizes and/or geometrical shapes. For example, as shown in FIG. 31B, the microfluidic chip 5300 has a rectangular shaped cross-section, e.g., in the x-y plane, and a height that is orthogonal to the cross section, which makes the microfluidic chip substantially a cuboid in 3D. At least a portion of the pathways 5320 can include hollow regions contained between a top surface 5311 and a bottom surface 5312 (shown in FIG. 31A) of the microfluidic chip. In some embodiments, at least some portion of the top and/or bottom surface 5311, 5312 may be flat. In some embodiments, the top surface 5311 may be flat except for the curved portion(s) that define the pathways 5320.

[0234] In some embodiments, the top and/or bottom surface 5311, 5312 may be planar surfaces, and the flatness of the surface(s) measured from a peak to a valley of the surface (in a direction orthogonal to the planar surface) can be less than about 0.05 mm, 0.01 mm, 0.02 mm, 0.03 mm, 0.04 mm, 0.05 mm, 0.06 mm, 0.07 mm, 0.08 mm, or 0.09 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the flatness of the surface(s) of the substrates from the peak to valley can be less than 0.01 mm, 0.02 mm, 0.03 mm, 0.04 mm, 0.05 mm, 0.06 mm, 0.07 mm, 0.08 mm, or 0.09 mm, inclusive of all ranges and subranges therebetween. Each of the microfluidic pathway 5320 may fluidically connect a corresponding pathway inlet 3321 and a corresponding pathway outlet 5322 in the microfluidic chip 5300, shown in FIG. 31B.

[0235] At least one of the microfluidic pathway 5320 may include an elongated portion 5323 between the corresponding pathway inlet 5321 and outlet 5322. At least part of the elongated portion 5323 may be straight. The elongate portion 5323 extends within the x-y plane, and may include a region that can bulge out from the top surface, the bottom surface, or both. In some embodiments, the bulging out portion may extend outward from the x-y plane at least in a direction that is orthogonal to the x-y plane of the microfluidic chip 5300.

[0236] At least one of the microfluidic pathway 5320 may include a curved portion 5324 between the corresponding pathway inlet 5321 and outlet 5322. Some part of the elongated

portion 5323 and/or the curved portion 5324 may extend higher than the height of the microfluidic chip 5300 in the direction orthogonal to the x-y plane. In other words, some part of the elongated portion 5323 and/or the curved portion 5324 may bulge out from the top, bottom surface 5311, 5312, or both.

[0237] In some embodiments, the curved portion 5324 is curved within the x-y plane of the microfluidic chip, e.g., as shown in FIG. 31B. In some embodiments, the curved portion may extends out from the x-y plane at least in a direction that is orthogonal to the x-y plane of the microfluidic chip. For example, the curved portion may be of a 3D shape like a half or 3/4 donut, e.g., in FIG. 31B.

[0238] In some embodiments, at least one pathway lacks any curved portion between its corresponding inlet and outlet and within the x-y plane. In some embodiments, at least one pathway comprises a substantially circular shape between the corresponding inlet and outlet.

[0239] In some embodiments, some part of the elongated portion 5323, the circular portion, and/or the curved portion 5324 may be made of flexible or collapsible materials so that under pressure or force, the flexible material can collapse or otherwise deform to transfer such pressure or force to the fluid(s) within the microfluidic pathways, thereby moving the fluid(s) in one or both directions in the pathways. At least a part of the microfluidic chip may be made of various materials that are flexible or collapsible under pressure. As a non-limiting example, the surface of the chip that is configured to contact the push pin which exert pressure on the chip may be made of flexible plastic and the other surface may be made of non-flexible material such as glass.

[0240] FIGS. 43A-43B show exemplary fluidic issues that may occur with microfluidic chips that include traditional in-chip chambers or in-chip wells. Such fluidic issues may occur with various reagents and/or washing buffers. In some embodiments, the reagent(s) with higher viscosity may further exaggerate the fluidic issues with traditional microfluidic chips and in-chip wells. For example, as shown in FIG. 43A, air bubbles may be generated when the off-chip pump pushes the fluid in the in-well chamber toward the dispensing tips. The air bubbles may cause potential inaccuracy or errors in sequencing reactions. FIG. 43B shows that liquid residual or liquid splitting may be generated at the air-liquid interface potentially causing inaccuracy or errors in sequencing reactions. Disclosed herein are microfluidic chips with in-chip wells that reduce or eliminate the fluidic issues in traditional microfluidic chips.

[0241] FIGS. 41A-41C and 42A-42C shows an embodiment of the microfluidic chip 8300. FIGS. 41A-41B are perspective views of the microfluidic chip 8300, and FIG. 41C is a side view of the exemplary embodiment of the microfluidic chip 8300. In this particular

embodiment, the microfluidic chip 8300 may include some structural elements of the microfluidic chip in other embodiments disclosed herein (e.g., microfluidic chip 3300, 4300, 5300, 6300, 7300). As shown in FIG. 42A, the microfluidic chip 8300 may include a vertical in-chip well 8360. The vertical well 8360 can be a well that has an height, e.g., extending between the top surface and the bottom surface of the microfluidic chip 8300. The height of the vertical well 8360 may be larger compared to the in-chip chamber of traditional microfluidic chips. The vertical well 8360 can advantageously facilitate fluidic communication from the well to the dispensing tips, thereby providing convenience and efficiency in delivering reagents using the microfluidic chip 8300 in sequencing reactions without fluidic issues in traditional microfluidic chips. In some embodiments, the vertical well 8360 can advantageously stabilize fluidic communication from the well to the dispensing tips by reducing or eliminating issues associated with traditional in-chip wells. In some embodiments, the vertical in-chip well 8360 can remove flow issues related to interfacial tension and contact angle of the fluid with the chamber without having a wide (e.g., greater than 1.5 mm in x-y plane) in-chip chamber. In some embodiments, the vertical in-chip well 8360 can advantageously reduce or minimize corner flow by including rounded edges (e.g., a cylindrical shape). In some embodiments, the vertical in-chip well 8360 can advantageously reduce or minimize air bubbles in fluid transferring into fluidic pathways from the in-chip well 8360. In some embodiments, the vertical in-chip well 8360 can advantageously reduce or minimize residuals at the air-liquid interface by improving the interfacial tension and contact angle from traditional in-chip wells 8360.

[0242] As shown in FIGS. 42A-42C, a fluidic pathway (8320_2) between the input port connecting to the reagent cartridge and the well, a fluidic pathway (8320_3) between the well and the dispensing tips can share a first portion (8320_a) and a second portion (8320_b). The first portion (8320_a) and the second portion (8320_b) of the fluidic pathways (8320_2), (8320_3) may be displaced from each other at least along a direction orthogonal to a chip substrate of the microfluidic chip, as shown in FIG. 42C. The first portion (8320_a) and the second portion (8320_b) may be connected to each other with a third portion (8320_c) that extends at least along a direction orthogonal to the microfluidic chip substrate.

[0243] In some embodiments, the fluidic pathway between the first in-chip valve 8351 and the in-chip well 8360 may enable bi-directional fluidic communication therein. The fluid may go from the first valve 8351 (valve in the open position) to the well 8360 to store the reagents therein while a second valve 8352 is closed. During dispensing, the first valve 8451 may be closed. The fluid may go from the well 8360 toward the first valve 8351, and due to the

closure of the first valve 8351, the fluid may travel through the second valve 8352 (opened) to the dispensing tips.

[0244] In some embodiments, actuation pathways (e.g., air-only pathways) for actuating the flow within the microfluidic chip may cause problems when leakage occurs from the fluid containing portion of the fluidic pathway into such actuation pathways, thereby causing dysfunction of the actuation and/or inaccurate flow into the in-chip well and/or from the dispensing tips.

[0245] In some embodiments, the microfluidic chip comprises a chip substrate and one or more films sealed to the chip substrate. The chip substrate and the one or more films in combination form the one or more fluidic pathways and the in-chip wells of the microfluidic chip. The actuation pathway(s) may be external to the microfluidic chip such that the actuators and corresponding actuation force/pressure do not directly come in contact with the reagent flows within the microfluidic chip, rather the actuators and corresponding actuation force indirectly actuate reagent via a deformable membrane (e.g., a film sealed to the chip substrate).

[0246] FIGS. 47A-47D show an embodiment of the microfluidic chip 2300 disclosed herein in which the actuation pathway(s) are not included in the microfluidic chip. FIG. 47A is a top view of a chip substrate 2301 of the microfluidic chip 2300, and FIG. 47B is a cross-sectional view of the microfluidic chip 2300 at plane CC' with amembrane or top film 2302 that is sealed to the chip substrate. The cross-sectional view also shows a portion of the fluidic pathway 2319 from the cartridge to an opening of the fluidic pathway, which is not at plane CC', but rather a curved cross section of the fluidic pathway that extends from the gear shape representing the port 2309 for connecting to the reagent cartridge (shown at the bottom of FIG. 47A) and the dotted fluidic pathway to the opening 2305b surrounded by the seal marked as 2391 in FIG. 47A. FIG. 47C is a cross-sectional view of the microfluidic chip 2000 with respect to first, second, and third valves that are configured to generate the first, second, and third seals respectively, and the one or more dispensing tips.

[0247] Referring to FIG. 47A, the microfluidic chip 2300 may include a microfluidic chip substrate 2301. The chip substrate 2301 may have customized shapes and size. For example, the thickness of the chip substrate may be customized in order to form the in-chip wells 2360 of a customized depth e.g., in a range from 0.5mm to 5mm, inclusive of all ranges and subranges therebetween. The chip substrate may include one or more alignment features like the groves on the side and/or holes 2394 to align the microfluidic chip relative to other

structural elements of the fluidic dispensing device, such as the dispensing tips, the press plate, etc.

[0248] The microfluidic chip 2300 may include one or more fluidic pathways 2320 that allow fluidic communication between (i) the outlet of the reagent compartments 2210 to the corresponding in-chip well 2360, and (ii) the corresponding in-chip well 2360 to the one or more dispensing tips 2340. The fluidic pathways 2320 may be formed in the chip substrate 2301, such as hollow cavities with elongated shapes within the chip substrate, as shown in FIGS. 47A-47B. The fluidic pathways 2320 may be formed by indentation(s) or grove(s) in the chip substrate and corresponding covering of the top film 2302 and/or bottom film 2303 to the chip substrate. As an example, FIG. 47B shows the fluidic pathway 2320 that is formed by the chip substate 2301 and a bottom film 2303. The films herein may be deformable via pressure or force.

[0249] The membrane or top film 2302 may be sealed to the chip substrate along a sealed contour 2304 (e.g., a first sealed contour). The bottom film 2303 may be sealed to the chip substrate along a second sealed contour (not shown). In some embodiments, the films may be permanently sealed at the sealed contour 2304 and the second sealed contour. The sealed contour 2304 or the second sealed contour may be a closed contour enclosing an area within the x-y plane. In some embodiments, the size of the enclosed area may be smaller than the area of the chip substrate in the x-y plane. Reducing the size of the enclosed area can enable a shorter sealed contour, thereby reducing the possibility of leakage at the sealed contour. When viewed in an x-y plane, e.g., as in FIG. 47A, the in-chip wells, and various openings of the fluidic pathways 2320 may be included within or encompassed in the contour 2304. Such openings, e.g., opening 2305a, 2305b, and/or 2306, may include openings in the fluidic pathway 2320_2 and the fluidic pathway 2320_3 to allow fluidic flow along the fluidic pathways. In some embodiments, one or more of the openings 2305a, 2305b, 2306 may lead to (i) a portion of the fluidic pathway 2320_2 that flows fluid toward the bottom of the chip substrate (FIG. 47B) and (ii) other portions of the fluidic pathway formed by indentation on the top surface of the chip substrate (FIG. 47A, between openings 2305a, 2305b, and 2306 corresponding to a same fluidic pathway).

[0250] In some embodiments, some portion of the fluidic pathway 2320, e.g., light grey dotted line in FIG. 47A, may be outside of the sealed contour 2304, when viewed in a x-y plane, to reduce the size of the area within the sealed contour, and enable a shorter sealed contour in comparison to having a sealed contour enclosing all fluidic pathways, in which the sealed contour 2304 is longer. In some embodiments, the sealed contour 2304 may prevent

fluid leakage from between the film and the microfluidic chip. In some embodiments, fluidic flow within the sealed contour 2304, e.g., between openings enclosed in the sealed contour 2304, is not blocked by the sealed contour 2304. For example, the sealed contour 2304 is disposed at or near the perimeter of the film such that fluid flow through pathways within a sealed area defined by the sealed contour is not blocked.

[0251] In some embodiments, the sealed contour 2304 alone does not block fluidic communication among the reagent cartridge (e.g., holding reagent tanks 2210), the microfluidic chip 2300, and the one or more dispensing tips 2400.

[0252] In some embodiments, one or more openings 2305a, 2305b, 2306 and the in-chip well 2360 may each be sealed independently to control fluidic communication within the microfluidic chip and to control fluidic communication between the reagent cartridge, the chip 2300, and the dispensing tips 2400. In some embodiments, the first, second, and/or third seals 2391-2393 may be generated independently on the microfluidic chip, e.g., FIG. 47B, to control fluidic communication within the microfluidic chip and between the reagent cartridge, the chip 2300, and the dispensing tips 2400. The first seal 2391 may be configured to be formed around an opening 2305b (e.g., when a press plate described below presses on the microfluidic chip). In some embodiments, fluid may be allowed to flow to the in-chip well 2360 from the reagent cartridge, when the first seal 2391 is not formed. The second seal 2392 may be configured to be formed around a second opening 2305a, and the third seal 2393 may be configured to be formed around, the in-chip well 2360. Fluid may be allowed to flow to exit the in-chip well 2360 and toward the dispensing tips 2400, when at least one of the second seal 2392 or the third seal 2393 are not sealed.

[0253] In some embodiments, a seal (e.g., the contour seal or sealed contour) formed between the membrane or film and the chip substrate at the sealed edge 2304 may be permanent. In contrast, the first, second, and/or third seals 2391-2393 may not be permanent and can be formed and removed to form corresponding fluid pathways in the microfluidic chip. For example, the first, second, and/or third seals may be reversible. In some embodiments, forming and/or removing the first, second, and/or third seal may be controlled by one or more actuators. Various types of actuators may be used to generate the seals disclosed herein. For example, force or pressure may be applied to generate the seals 2391-2393such as, for example, pneumatic, peristaltic force or pressure.

[0254] In some embodiments, the fluidic dispensing system 2000 may include a press plate 2390 that is configured to press on the top film or membrane 2302 of the microfluidic chip 2300. The perspective view of the press plate 2390 is shown in FIG. 47D. The press plate

2390 may include geometric shapes that, when exposed to force or pressure, e.g., pneumatic pressure, may press on the top film or membrane and make contact with the chip substrate to form a seal between the top film or membrane and the microfluidic chip. The geometric shapes may protrude from a surface of the press plate toward the chip substrate to facilitate sealing at or near the edges of such geometrical shapes. In some embodiments, the press plate (e.g., the geometric shape) may be configured such that sealing occurs at or near the edges of the geometrical shapes and not outside the geometrical shapes. A perspective view of an embodiment of the press plate and the geometrical shapes are shown in FIG. 47D.

[0255] When the first, second, or third seal is formed, fluids (e.g., liquids reagents) within the seal may not be allowed to flow through the seal (e.g., may be blocked from flowing through or past the first, second, or third seal). Similarly, fluids outside the edge of the seal may not flow through or past the seal disposed around the edge of the microfluidic chip (e.g., the contour seal 2304). In some embodiments, the first, second, or third seal is formed at the edge of the geometrical shape (e.g., not within the edge of the shape) and fluid flow within the geometrical shape may still occur. The seal only at the edge of the geometrical shape is efficient and easy to achieve using the press plate compared with sealing a whole area within the geometrical shape. . In some embodiments, the seal is formed at the edge of the geometrical shape and is formed within at least part of the area included in the geometrical shape. In the particular embodiment in FIGS. 47A-47D, pneumatic pressure may be applied to the press plate to cause pressing that forms the first, second, and/or third seals. In this particular embodiment as shown in FIG. 47A, the first seal 2391 can be formed around a first opening 2305a, the second seal 2392 can be formed around the second opening 2305a different from the first opening, and the third seal 2393 can be formed around the in chip well. The exact shape or size of the seals may be customized as long as the shape and size enable formation of a complete seal with no leakage in the fluidic pathways. In some other embodiments, each of first and second seals may include just one opening 2305a (as shown by the dotted line around the first opening 2305a in the middle fluidic pathway in FIG. 47A). In some embodiments, the seal may include the openings 2305b and 2306, as shown in FIG. 47A (left most pathway). For example, the shape of the first seal around the opening may be circular or oval or other suitable shapes. As another example, the second seal of the in-chip well may have a size configured to ensures complete seal at the edge(s) of the well. In some embodiments, the first, second, and third seals are enclosed within the sealed contour 2304 that is permanent between the top film or membrane and the microfluidic chip.

[0256] In some embodiments, the force or pressure to form the seals (e.g., the first seal, the second seal, and/or the third seal) may be generated via one or more valves, e.g., 2351', 2352',2353' in FIG. 47C. The one or more valves may comprise one or more actuation pathways (e.g., 3320' in FIG. 47C). The actuation pathways may be pathways that deliver actuation force or pressure to the press plate when the corresponding valve(s) is in its open position(s). The one or more valves may be at positions that are fixed and not movable in the x-y plane relative to the top film or membrane and the microfluidic chip. In some embodiments, the one or more valves may be fixed in the x-y plane via one or more fastening elements 2354-2356. In some embodiments, the one or more fastening element may include a valve retaining plate 2355 and a valve manifold 2354. The valve manifold may support and allow permanent or removable attachment of the press plate 2390. The valve(s) may include pathways that extend through the manifold to the press plate for application of force or pressure, e.g., pneumatic pressure, on the press plate via the pathways. The one or more fastening element may include an interface that allows connection of the valve(s) to one or more power sources and/or one or more actuators, e.g., for generating pneumatic pressure to be delivered via the valves. In embodiments where the positions of the one or more valves are fixed and not movable in the x-y plane, each valve may be used to form a seal relative to a respective structure of the microfluidic chip. For example, a first opening 2305a may correspond to (e.g., align with or interact with) a first fluidic pathway that communicates with one or more first dispensing tips. The first opening may not align with or interact with any other structures of the microfluidic chip, e.g., a second opening 2305b of a second fluidic pathway that has an offset from the first opening 2305a. In other words, the first, second, and third valves may only facilitate formation of seals along a first fluidic pathway. In some embodiments, a fourth, a fifth, and a sixth valve may be used to generate seals for a second fluidic pathway. In some embodiments, the fluid dispensing device includes 6 fluidic pathways that are independent, and therefore the fluid dispensing device includes 6x 3 (e.g., 18) valves for generating the corresponding 3 seals for each fluidic pathway.

[0257] In some embodiments, the pneumatic pressure for pressing the press plate and generating the first, second, or third seal using corresponding pump 2352'-2353' may be in a range from 1 kPa to 200 kPa, inclusive of all ranges and subranges therebetween. In some embodiments, the pneumatic pressure for pressing the press plate and generating the first, second, or third seal using corresponding pump 2352'-2353' may be in a range from 10 kPa to 150 kPa, inclusive of all ranges and subranges therebetween. In some embodiments, the pneumatic pressure for pressing the press plate and generating the first, second, or third seal

using corresponding pump 2352'-2353' may be in a range from 20 kPa to 100 kPa, inclusive of all ranges and subranges therebetween. In some embodiments, the pneumatic pressure for pressing the press plate and generating the first, second, or third seal using corresponding pump 2352'-2353' may be in a range from 30 kPa to 80 kPa, inclusive of all ranges and subranges therebetween.

[0258] In some embodiments, the one or more valves may be movable in the x-y plane relative to the top film or membrane and the microfluidic chip via one or more fastening elements 2354-2356. The movement may be spatial displacement or translation of the valves relative to the microfluidic chip. Such movement may be enabled through actuating the one or more fastening elements, such as the valve retaining plate 2355 and the valve manifold 2354. In some embodiments, the microfluidic chip shown in FIG. 47A may include fastening elements configured to fix the valve relative to the microfluidic chip. In some embodiments, the microfluidic chip shown in FIG. 47A may include fastening elements configured to allow displacement of the one or more valves relative to the microfluidic chip (e.g., such that the one or more valves can cause an actuation force on different portions of the microfluidic chip). For example, , in embodiments where the three valves are movable along the x-y plane, the three valves may be at a first position generating the first, second, and/or third seals 2391-2393 (e.g., on afar right side of the chip) to control flow of a first reagent in the first fluidic pathway (e.g., located on the far right side of the chip). The three valves may then be moved laterally in a direction orthogonal to the CC' line (e.g., toward a left side of the chip). The three valves may be moved a distance to the second fluidic pathway. When the three valves are in a predetermined position relative to the second fluidic pathway, the valves can generate three seals to control a second reagent in the second fluidic pathway. In some embodiments, the three valves may move at the largest distance to the left most fluidic pathway, and control flow of a sixth reagent or otherwise liquids in the sixth fluidic pathway. Although shown with six fluidic pathways, it should be appreciated that the microfluidic chip can include any suitable number of fluidic pathways (e.g., 1 pathway to 20 pathways, inclusive of all ranges and subranges therebetween), and the fluid dispensing device may include a corresponding number of valves and/or valve positions such that fluid can be flowed through each fluidic pathway using the valves.

[0259] Referring to FIG. 47B, for refilling of reagents from the reagent cartridge (bottom row in FIG. 47B), the first and third seals 2391, 2393 are removed temporarily while the second seal 2392 is applied to the opening 2305a leading to the one or more dispensing tips 2400, thus sealing fluidic flow from exiting or entering from the opening 2305a. Therefore,

the second seal 2392 may block fluid from flowing from the sealed opening 2305a to the dispensing tips 2400. The first seal 2391, when not temporarily removed, may seal fluid from exiting or entering the second opening 2305b, thereby blocking fluid flow from the sealed second opening 2305b to the corresponding opening 2306 that connects the second opening 2305b to the corresponding in-chip well 2360. The third seal 2393 may seal around the in-chip well 2360 thereby blocking fluid flow from exiting or entering the in-chip well. The first and third seals 2391, 2393 are removed temporarily to allow reagent to flow from the corresponding compartment 2210 through a second opening 2305b and the opening 2306 to the in-chip well 2360.

[0260] Although the first seal of the middle pathway as shown in FIG. 47A, are shown to surround only corresponding openings 2305b, it should be appreciated that the first or the second seal, e.g., 2391 or 2392, may be surrounding not only the opening 2305a, 2305b also its corresponding opening 2306 in fluidic communication therewith, e.g., the far left fluidic pathway as shown in FIG. 47A.

[0261] It is worth noting that openings 2305a, 2305b, or openings 2306 may share same shape, size, and functions, but may be placed at different locations along the fluidic pathways in the microfluidic chips. For example, as shown in FIG. 47A, each fluidic pathway 2320 may include two different sets of opening: 2305a and opening 2306, or 2305b and opening 2306.

[0262] For dispensing of the reagents, (middle row in FIG. 47B), the first and third seals 2391, 2393 are temporarily sealed while the second seal 2392 is removed. When sealed, the first seal 2391 completely blocks reagent flow from the corresponding compartment 2210 through an opening 2305b and opening 2306 to the in-chip well 2360. When sealed, the third seal 2393 squeezes the in-chip well until a complete seal of the well is formed, resulting in the reagent within the well to flow toward the second seal, which is removed, and exit towards the dispensing tips. In other words, when the first seal and the third seal form seals with the microfluidic chip, fluid flows from the in-chip well 2360 past the second open seal and to the dispensing tips (e.g., “dispense”). When the first seal and the third seal are open and the second seal forms a seal with the microfluidic chip, fluid flows from the reagent tanks 2210 to the in chip well (e.g., “refill”)

[0263] The embodiment in FIGS. 47A-47D advantageously separates the actuation pathways from the fluidic pathways and remove the need to include fluidic valves or air-only pathways in the microfluidic chip. The actuation pathways can be external to the microfluidic chip. For example, the air pathways for generating the first, second, or third seals are part of

the valves within the valve manifold 2354. As a result, such embodiment of actuating the fluidic flow eliminates the problems that may occur when reagents may overflow into the actuation pathways, e.g., air-only pathways that are at least partly within the microfluidic chip, and cause errors in reagent refill or dispensing.

[0264] In some embodiments, some or all parts of the fluidic dispensing system 2000 may be made disposable so that the user may disconnect the parts that are disposable from the sequencing system 110 after one or more cycles or after one or more sequencing runs and connect corresponding new parts replacing the disposable part to the rest of the sequencing system for performing sequencing in new cycle(s) or sequencing run(s). The disconnection of the disposable parts and connection of the new parts may be via simple connecting mechanisms that allow sealed fluidic connection. For example, the connecting mechanism may include a push and click. As another example, the connection mechanism may include a push and application of a mechanical lock. The sample(s) to be sequenced may not have to be changed with disposal of some or all of the fluidic dispensing system 2000. In some embodiments the disposable parts of the fluidic dispensing system may include one or more of: the reagent cartridge with its compartments, the microfluidic chip including the chip substrate, the top and bottom films, and the one or more dispensing tips. In some embodiments, the parts that are not disposable include one or more of: the press plate, the first, second, and third valves for generating the first, second, and third seals, respectively, the one or more fastening element of the valves including the valve manifold, and the one or more actuators. In some embodiments, the microfluidic chip may be permanently connected to the reagent cartridge to ensure physical and/or mechanical uniformity of the two so that they can be inserted or removed as a single piece.

[0265] Referring to FIG. 47A, the microfluidic chip 2300 may include one or more in-chip wells 2360 configured to hold reagents before dispensing them through one or more dispensing tips 2340.

[0266] In some embodiments, one or more in-chip wells 2360 may be in fluidic communication with more than one fluidic pathway connecting the reagent cartridge to a single in-chip well. Although only one fluidic pathway is shown in FIG. 47A connecting the reagent cartridge to a single in-chip well, one or more other fluidic pathways may be connected to the single in-chip well via different openings 2305a, 2305b, and 2306. Each of the different openings 2305a, 2305b, 2306 may be controlled by a separate seal. For example, a fourth seal may be formed around a second opening different from an existing opening to block fluidic flow from a compartment of the reagent cartridge. In order to mix two reagents

from two different compartment, the first and fourth seal may be removed to allow simultaneous flow of the two reagents via different fluidic pathways into the same in-chip well. Alternatively, the fluidic pathway going in to an in-chip well may be shared by two different reagent compartments to allow sequential flow of the two different reagents into the same in-chip well. Alternatively, the fluidic pathway going to the in-chip well may be bifurcated to allow different reagents to flow through the bifurcation and mix into a common portion of the fluidic pathway and then flow into the same in-chip well. In some embodiments, the second valve corresponding to the in-chip well may be controlled to mix reagents or otherwise fluids from different compartments. For example, the valve may generate a pneumatic pressure (e.g., less pressure than when forming the second seal) pressing on the fluids to facilitate mixing.

[0267] In some embodiments, alone or in combination with mixing within the in-chip wells, the fluids may be mixed in at least some portions of the fluidic pathways in the microfluidic chips.

[0268] In some embodiments, one or more of: the compartments, the microfluidic chip, the dispensing tips may be thermally regulated to achieve designed temperature of the reagents before the reagents exit the dispensing tip to ensure proper temperature for sequencing reactions and to reduce time needed for heating or cooling the reagents to proper temperature for sequencing reactions.

[0269] FIGS. 47A-47D only shows an embodiment where the three valves are positioned above the microfluidic chip, and the seals are generated by pressing the top film down to the chip substrate, embodiments that change the relative location of the three valves may be derived based on the embodiment in FIGS. 47A-47D, and still be functional for identical purposes and functions of the fluidic dispensing device disclosed herein. For example, in some embodiments, the microfluidic chip may be positioned above the valves (e.g., and flipped above the x-y plane) such that the press plate moves upward to push the microfluidic chip. The chip may also be flipped (e.g., about the x-y plane), so that the press plate may press on the top film (which is now at the bottom) to form seals with the chip substrate. The one or more dispensing tips may be oriented the same in such embodiments.

[0270] In some embodiments, the press plate can be attached to the valve manifold with a PSA gasket. In some embodiments, each one of the one or more geometrical shapes comprises one or more ridges, and the one or more ridges (and not the rest of the press plate) presses on the film and results in seal between the film and the chip substrate. In some embodiments, the size and shape of the seal(s) are determined by the size and shape of the

ridges. In some embodiments, the first, second, or third seal is a pneumatic seal. In some embodiments, the press plate is removably attached to the valve manifold. In some embodiments, a press plate may be removed and a different press plate may be attached to the valve manifold in order to generate different seals, e.g., for a different microfluidic chip with a distinct layout from a previously installed microfluidic chip. Such fluidic dispensing device advantageously provide flexibility and convenience for a user to select different microfluidic chips that may be more efficient for a sequencing application. For example, microfluidic chips may have different numbers of fluidic pathways depending on the number of reagents required. The microfluidic chip may also have different sized in-chip wells, thus may need different sized seal around them, for reagents needing different dispensing volumes.

[0271] In some embodiments, some structural elements of the fluidic dispensing device comprises poly carbonate, e.g., the one or more compartments of the reagent cartridge.

[0272] In some embodiments, the one or more fluidic pathways may have a cross section, e.g., cross section of the dotted pathways in FIG. 47A. in a range from 0.1 mm^2 to 5 mm^2, inclusive of all ranges and subranges therebetween. In some embodiments, the one or more fluidic pathways may have a cross section in a range from 0.2 mm^2 to 4 mm^2, inclusive of all ranges and subranges therebetween. In some embodiments, the one or more fluidic pathways may have a cross section in a range from 0.3 mm^2 to 2 mm^2, inclusive of all ranges and subranges therebetween. In some embodiments, the cross section areas disclosed herein may reduce flow resistance by 1.2x, 1.5x, 1.8x, 2x, 5x, or more than existing microfluidic chips in traditional fluidic dispensing devices. In some embodiments, the length of the one or more of the fluidic pathways may be identical to control reagent dispensing time to be similar. In some embodiments, the connection between the reagent cartridge and the microfluidic chip is at 2201. The connection may include a port (gear shapes in FIG. 47A) and the port may be configured to receive various connectors including but not limited to the bonded Luer connectors. In some embodiments, adhesive material, e.g., UV cured adhesive, may be used for secure connection without leakage at the connection between the reagent cartridge and the microfluidic chips. The connection may be identical for each of the one or more fluidic pathways. In some embodiments, structural elements of the fluidic dispensing device may comprise one or more of: polyether ether ketone (PEEK), polycarbonate, thermoplastic elastomer (TPE), polyphenylsulfone, high performance thermoplastic, resin, glass, and rubber.

[0273] In some embodiments, the fluid dispensing device is configured to dispense a volume with less than ±1%, ±2%, ±3%, ±4%, ±5%, ±6%, ±7%, ±8%, ±9%, or ±10% of

volume difference from a predetermined dispensing volume, e.g., 60 uL, in more than 100, 200, 400, 500, 800, 1000, 2000, 4000, 8000, 10000, 15000, 20000 or more dispenses.

[0274] In some embodiments, the fluid dispensing device is configured to dispense a volume with less than ±1%, ±2%, ±3%, ±4%, ±5%, ±6%, ±7%, or ±8% of volume difference from a predetermined dispensing volume, e.g., 60 uL, in all the number of dispenses for sequencing a sample in more than 20, 40, 60, 80, 100, 150, or 200 sequencing cycles.

[0275] In some embodiments, the precision of dispensing volume of the fluidic dispensing device facilitates accurate and reliable sequencing reactions at the flow cell, thereby reduce the time and cost that may incur due to dispensing errors during sequencing.

[0276] In some embodiments, time duration for dispensing from the reagent cartridge through the microfluidic chip, and then through the dispensing tips to the flow cell device may be less than 100 ms, 200 ms, 400 ms, 600 ms, 800 ms, 1 second, 1.2 seconds, 1.5 seconds, or 2 seconds.

[0277] In some embodiments, the first, second, and third valves are actuated by one or more actuators that are controlled by a computer processor disclosed herein. In some embodiments, the first, second, and third valves are actuated via automatically generated actuation force or pressure at the actuator(s).

[0278] The in-chip well (e.g., any in-chip well described herein) may have various sizes or shapes. The microfluidic chip may have multiple in-chip wells, and some of them can have identical sizes and/or shapes or different sizes and/or shapes. For example, the microfluidic chip may have a first in-chip well with about 100 µl to 300 µl in volume, inclusive of all ranges and subranges therebetween, and a cylindrical shape extending from the top surface to the bottom surface of the microfluidic chip. The microfluidic chip may have a second in-chip well with 50-150 µl in volume, inclusive of all ranges and subranges therebetween, and a spherical shape extending from the top surface to the bottom surface of the microfluidic chip. In some embodiments, the in-chip well may have a height (extending along a direction orthogonal to the chip substrate) equal to or less than the height of the chip substrate. For example, the in-chip well may have a height of about 1 mm to 15 mm, inclusive of all ranges and subranges therebetween,. In some embodiments, the in-chip well may have a height of about 4 mm to 10 mm, or 5 mm to 11 mm, 5 to 9 mm, or 5 to 8 mm, inclusive of all ranges and subranges therebetween. In some embodiments, the in-chip well may have a volume of about 20 µl to 1000 µl, inclusive of all ranges and subranges therebetween. In some embodiments, the in-chip well may have a volume of about 20 µl to 600 µl. In some embodiments, the in-chip well may have a volume of about 40 µl to 500 µl. In some

embodiments, the in-chip well may have a volume of about 100 µl to 400 µl, inclusive of all ranges and subranges therebetween. In some embodiments, the in-chip well may have a volume of about 100 µl to 300 µl, inclusive of all ranges and subranges therebetween.

[0279] In some embodiments, the volume of the in-chip well is calculated so that reagent flow from the pathway(s) to the well for a predetermined period of time with a predetermined flow rate fills less than the entire volume of the in-chip well. The predetermined period of time and the predetermined flow rate may be varied depending on various sequencing applications. For example, the maximum level of reagent in the in-chip well can be 1 mm, 1.5 mm, 2 mm, 2.5 mm, 3 mm, 3.5 mm, 4 mm, 4.5 mm, or 5 mm lower than the height of the in-chip well, inclusive of all ranges and subranges therebetween. Keeping the fluidics lower than the maximal height of the in-chip well may prevent liquid leaking into the air-only pathway 8320_1, 8320_1' (e.g., with gaseous flow but not liquid flow) connecting the in-chip well and the off-chip pump thereby avoiding contamination and/or damages by the overflow to other structural elements of the fluid dispensing device. The size, shape, and dimension of the air-only pathway may be customized to be in various ranges.

[0280] FIGS. 42D-42E shows exemplary embodiments of the in-chip well with differently shaped air-only pathways 8320_1, 8320_1' The in-chip well structure may be compatible with existing injection molding chip manufacturing process which reduces the cost and complexity of manufacturing the microfluidic chip. In some embodiments, the liquid level inside the in-chip vertical well 8360 may rise when the air pressure inside the well decreases. The liquid level may decrease when the air pressure inside the well is increased from the air flow pathway 8320_1, 8320_1'. In the embodiment as shown in FIG. 42D, the air-only pathway is on the left side of the cross section while the liquid enters and exits from the bottom of the in-chip well 8360. In the embodiment as shown in FIG. 42E, the air-only pathway is at the center while the liquid enters from a side of the well 8360. This structure can help avoid liquid entering the air-only pathway and reduce the risk of bubble formation in the fluidic pathway 8320_2 (shown in FIG. 42). In some embodiments, the pathways for actuating the fluidic flow in fluidic pathways, e.g., air-only pathways 8320_1 in FIGS. 42A-42E, are actuation pathways, that are not for fluidic communication in the fluid dispensing device, but only for actuation of the flows.

[0281] FIG. 42F shows a bottom view of an embodiment of vertical in-chip wells 8360 with various shapes and sizes and the corresponding fluidic pathways 8320. In some embodiments, the in-chip well may include rounded edges and no sharp corners. In some embodiments, the in-chip well may include a narrower top and a wider bottom. In some

embodiments, the in-chip well may have a diameter (the widest dimension in the x-y plane) from about 1mm to 20 mm, and depth (along z axis) from about 1 mm to 20 mm, inclusive of all ranges and subranges therebetween. The in-chip well may include a shape including round, oval and triangular cross-sectional shapes (as shown). In some embodiments, the in-chip well may have a diameter (the widest dimension in the x-y plane) from about 1 mm to 15 mm, and depth (along z axis) from about 3 mm to 12 mm, inclusive of all ranges and subranges therebetween. The in-chip well may include a shape including round, oval and triangular cross-sectional shapes. FIG. 42G shows an embodiment of the in-chip well, and the cross-section at AA is shown in FIG. 42H. As shown, at least part of the fluidic pathways 8320_2 and 8320_1 have a cross section in a tapered shape.

[0282] In some embodiments, the in-chip well, e.g., in FIG. 37E -37F, may have a depth along z axis that is less than 10mm, 8mm, 6 mm, 5mm, 4 mm, 3mm, 2mm, 1mm, or less, inclusive of all ranges and subranges therebetween.

[0283] In some embodiments, the in-chip well may include one or more cut-outs C that may facilitate blocking of corner flows that may go faster, as shown in FIG. 45A. For example, the cut-outs may guide corner flow toward the center to maintain a consistent flow rate of fluid across the fluidic pathways. In some embodiments, the in-chip well may include one or more cut-outs as shown in FIGS. 45B-45C. The cut-out(s) C may be located near the end of the in-chip well that is connected to the air-only pathway. The cut-out(s) may include various shapes and/or sizes to reduce corner flow of the liquid(s) that may move faster toward the air-only pathway than the liquid closer to the center of the in-chip well. FIG. 45B shows a bottom view of the in-chip well with different cut-out designs. FIG. 45C shows corresponding cross-sections of the in-chip well in FIG. 45B.

[0284] In some embodiments, during reagent aspiration, a force or pressure can be applied to the reagent in the reagent cartridge to push the reagent downward (e.g., on top of the gravity) into the first portion (8320_a) of the fluidic pathways (8320_2), then downward in the third portion (8320_c) of the fluidic pathway, and then into the second portion (8320_b) of the fluidic pathway and the in-chip well, e.g., as shown in FIGS. 42A-42C.

[0285] In some embodiments, during dispensing, the dispensing force or pressure can be applied to the in-chip well in the microfluidic chip to push the reagent in the in-chip well downward (e.g., on top of the gravity) into the second portion (8320_b) of the fluidic pathways (8320_3), then upward in the third portion (8320_c) of the fluidic pathway, and then into the first portion (8320_a) of the fluidic pathway and toward the dispensing tips, as shown in FIGS. 42A-42C.

[0286] The one or more fluidic reservoirs may be in fluidic communication with the one or more microfluidic pathways. In some embodiments, the one or more fluidic reservoirs or in-chip wells 8360 may be fluidically sealed except at the inlet and outlet that are in fluidic communication to one or more microfluidic pathways. The fluidic pathway connecting a top portion of the well to the off-chip pump may be air-only. A filter, e.g., a porous frit, may be used to prevent reagent from entering the air-only pathway and contaminating or damaging the pump port.

[0287] The microfluidic chip 8300 may comprise a top film, a chip substrate, or both bonded or otherwise attached and sealed to a substrate positioned underneath the top film, above the chip substrate, and/or between the top and chip substrate, thereby forming the microfluidic pathways and/or the one or more reservoirs within the microfluidic chip. In some embodiments, the top film, chip substrate, and/or the substrate in between the films may include polypropylene, cyclic olefin copolymer, cyclic olefin polymers, and/or various other materials. The top film and/or chip substrate may cover at least some portion of the substrate. For example, the top and/or chip substrate may cover more than 90% of the area of the substrate, e.g., FIG.41A. For example, the top and/or chip substrate may cover less than 20% of the area of the substrate, e.g., FIG. 41B. In some embodiments, one or more of the films may seal one or more portions of the microfluidic pathways (e.g., fluidically seal).

[0288] In some embodiments, the fluidic pathways (8320_2 and 8320_3) comprise the first portion (8320_a) that is between a top film and a chip substrate, and a second portion (8320_b) that is between the chip substrate and a chip substrate of the microfluidic chip.

[0289] FIGS. 44A-44B shows two different embodiments of a microfluidic chip that may achieve similar or comparable results in resolving the fluidic problems in existing microfluidic chips. Such different embodiments may be selected based on different needs of the application, for example, manufacturing capabilities, manufacturing cost, material availability, material cost, etc. FIG. 44A shows an exemplary embodiment of the substrate (molded with cavities for pathways) and the top and bottom film layers.

[0290] In some embodiments, the microfluidic chip may have a mid-film sandwiched between two substrate pieces, alone or in combination with the top and/or bottom film(s). For example, in FIG. 44B, only a mid-film is used to provide sealing to areas of both the top half of the substrate and the bottom half of the substrate. The mid-film can define a hole to enable fluidic connection between the pathway(s) defined in the top half of the chip and the pathway(s) defined in the bottom half of the chip.

[0291] The microfluidic chip may include multiple in-chip wells, each well can be positioned in a corresponding microfluidic pathway between the corresponding inlet and dispending tips of a corresponding reagent compartment (e.g., see FIGS. 34A-34C). In other words, different reagent travels through different pathways to different dispensing tips to avoid cross-contamination.

[0292] In some embodiments, the microfluidic chip may include one or more valves (e.g., valve 3350) in the one or more microfluidic pathways. The one or more valves can be two way or 3-way valves that can switch between a first configuration or position and a second configuration or position. The valve(s) can be controlled by an electrical current or voltage, a mechanical motor, a pneumatic pressure, a magnetic force, or different actuating sources. Non-limiting examples of the valves include: a solenoid valve, a pneumatic valve, a rotary valve, or a membrane valve. In some embodiments, the one or more valves may be replaced with other structures that can functionally enable: (1) fluidic communications from the reservoir via the inlets 3321 to the compartment(s) but not to the dispensing tip(s); and (2) fluidic communications from the reservoir to the dispensing tip(s) via the outlets 3322, but not to the compartment(s).

[0293] The fluid dispensing device 3000 may further comprise one or more actuators 3500 that actuate a movable pin, plunger, or syringe for pumping the fluidic reagents in one or both directions. In some embodiments, the reagents are pushed from the one or more compartments 3210 to the one or more dispensing tips 3400 through the one or more microfluidic pathways (e.g., see FIGS. 30A-30B). In some embodiments, the reagents are pushed from the one or more dispensing tips 3400 to the one or more compartments 3210 through the one or more microfluidic pathways.

[0294] In some embodiments, the fluid dispensing device 3000 further comprises one or more movable pins 3600 that are configured to move and apply pressure or force on various portion(s) of the corresponding pathway 3320 (e.g., the curved portion or bulge portion on the microfluidic chip 3300) to move reagent therein in a predetermined direction. In some embodiments, the movable pin 3600 may be of various 3D sizes and geometrical shapes. For example, it may be of a disk shape in FIG. 30A.

[0295] The movable pin may comprise various movement to actuate the reagents, such as rotation, translation, or both. The movable pin 3600 may rotate about a rotational axis that is orthogonal to the x-y plane of the microfluidic chip 3300 to move fluid in the microfluidic pathway in one or both directions. Alternatively, a movable pin 3600 herein may translate toward the microfluidic chip 3300 to move fluid in the microfluidic pathway(s) e.g., in the

curved portion, in one or both directions. In some embodiments, a movable pin 3600 herein may, without rotation, translate relative to the microfluidic chip 3300 to move fluid in the microfluidic pathway(s) e.g., in the curved portion, in one or both directions. A movable pin 3600 may, alone or in combination with other motions, translate in a direction that is parallel to the x-y plane of the microfluidic chip 3300 to move fluid in the curved portion in one or both directions.

[0296] In some embodiments, the movable pin(s) 3600 is removably coupled to the one or more actuators (e.g., actuators 3500). In some embodiments, the movable pin(s) 3600 may function to seal the fluid(s) in the microfluidic chip 3300 when the moveable pin(s) are physically coupled to the microfluidic chip 3300. In some embodiments, the movable pin(s) 3600 is coupled to the microfluidic chip 3300 but separable from the actuator(s) to seal the corresponding fluid reagent in the one or more microfluidic pathways within the microfluidic chip 3300 to prevent leakage of the dispensing module. As such, no leakage occurs even if the dispensing module is physically separated from the actuators, as shown in FIG.32B. In embodiments when the movable pin(s) 3600 is actuated and move relative to the microfluidic chip 3300, the moveable pin(s) 3600 can be removably coupled to the one or more actuators 3500. In other words, the moveable pin 3600 can be configured to form and maintain a seal with a specific microfluidic pathway to prevent leakage of fluid. The actuator 3500 can be removably coupleable to the moveable pin 3600 (e.g., the actuator can couple to the moveable pin 3600 when fluid from the specific microfluidic channel is to be actuated and decoupled from the moveable pin 3600 when fluid from the specific microfluidic channel is not to be actuated). When the dispensing module is uncoupled from the sequencing system or the flow cell device and disposed of, the movable pin may be uncoupled as a part of the dispensing module and disposed of together the dispensing module is disposed of.

[0297] In some embodiments, the movable pin(s) 5600 is fixedly coupled to the one or more actuator 5500. FIG. 32B shows an exemplary embodiment where the movable pin(s) stays integrated (e.g., physically coupled) with the microfluidic chip, and other elements of the dispensing module 5200. FIG. 32A shows an embodiments in which the movable pin 5600' is not disposed with the dispensing module 5200' but stays coupled to the actuator(s) 5500'.

[0298] In some embodiments, the one or more actuators may comprise a first number of actuators. The first number of actuators can be equal to or smaller than a second number of microfluidic pathways in the corresponding microfluidic chip. As a non-limiting example, the one or more actuators comprise one actuator, as shown in FIG 31C. Alternatively, the one or

more actuators may comprise 2, 3,4, or more actuators (e.g., as shown in FIG. 31A). The one or more actuators may be movable relative to the one or more microfluidic pathways to actuate corresponding movable pin(s), (e.g., syringe, plunger, etc.). For example, a single actuator can be configured to move relative to the microfluidic chip to actuate different movable pins of different microfluidic pathways. Alternatively, the one or more actuators comprise the same number of actuators as the number of movable pins, so that each actuator can be fixedly positioned relative to the corresponding moveable pin, e.g., immediately above it or beneath it to actuate the corresponding pin.

[0299] The one or more actuators may be fixedly coupled to a next generation sequencing (NGS) system, e.g., the sequencing system 100 herein. In some embodiments, the one or more actuators may be removably coupled to the sequencing system.

[0300] In some embodiments, the one or more actuators are configured to move to a corresponding spatial position to actuate the movable pin(s) to push a corresponding fluidic reagent (e.g., at a predetermined rate and/or volume) from the cartridge to the corresponding dispensing tip via a corresponding microfluidic pathway on the microfluidic chip.

[0301] The microfluidic chip, the one or more actuators, and the one or more movable pins, or their combinations may be comprised in a pump.

[0302] The fluid dispensing device may include one or more dispensing tips. The dispensing tips can be positioned below the reagent cartridge, the microfluidic chip, or both.

[0303] In some embodiments, the pump may include one or more syringe pumps. The syringe pumps can be configured to direct reagent(s) from the one or more compartments of the reagent cartridge to the one or more pathways in the microfluidic chip.

[0304] In some embodiments, the pump may comprise one or more plunger and barrel pairs. FIGS. 33A-33B show an embodiment with plunger and barrel pairs 6340. Each plunger and barrel pair may be in fluidic communication with a corresponding compartment 6210 of the reagent cartridge and a corresponding pathway 6320 of the microfluidic chip 6300. The plunger may be movable relative to the corresponding barrel to move a corresponding fluidic reagent therewithin in a predetermined direction. The barrel(s) may be immobilized relative the reagent cartridge 6200, the microfluidic chip 6300, or both. The plunger(s) may be actuated by the one or more actuators 6500. The plunger may move in two opposite directions to move the reagent(s) accordingly in two opposite directions.

[0305] FIGS. 34A-34C show an exemplary embodiment with a single plunger and barrel pair 6340'. The plunger and barrel pair may be in fluidic communication with a corresponding compartment (not shown, can be positioned above or underneath the

microfluidic chip 6300') and a corresponding pathway 6320'. The plunger may be movable relative to the corresponding barrel to move a corresponding fluidic reagent therewithin. The plunger may move in two opposite direction to move the reagent(s) accordingly in two opposite directions. The barrel(s) may be immobilized relative the reagent cartridge 6200', the microfluidic chip 6300', or both. The plunger(s) may be actuated by the one or more actuators 6500'. In some embodiments, one or more fluidic reservoirs are in communication with a single barrel and plunger pair. In some embodiments, the microfluidic chip may comprise one or more fluidic reservoirs or wells 6360' within the chip 6300'. In some embodiments, individual reservoirs 3360 are fluidically connected to the barrel and plunger pair 6340' via a connector 6361'. In some embodiments, one or more fluidic reservoirs 6360' are each in communication with a corresponding barrel and plunger pair. In some embodiments, the reservoirs 6360' are each fluidically coupled to a corresponding pathway 6320'.

[0306] In some embodiments, the connector 6361' can be of various designs or structures. Non-limiting examples of the connector include an O-ring connector, a luer lock connector, a luer slip connector. The connector structure can be molded directly or otherwise permanently fastened on the microfluidic chip 6300' to provide a fluidly sealed connection of the barrel and plunger pair 6340' to the microfluidic chip 6300'.

[0307] The one or more fluidic reservoirs 6360' can be of various sizes or volumes to fit within the microfluidic chip 6330'. In other words, the length, width, and height of the reservoirs 6360' may be no greater than the length, width, and height of the microfluidic chip 6330'. By way of example, the fluidic reservoirs 6360' can have identical sizes with a same cross section within the x-y plane, as shown in FIG. 34A. In some embodiments, different reservoirs 6360' may be of different sizes or shapes. The one or more fluidic reservoirs or wells 6360' may be fluidly sealed except at an inlet and outlet of the fluidic reservoirs 6360' that are in fluidic communication to one or more microfluidic pathways 6320'. The one or more fluidic reservoirs 6360' may be in fluidic communication with the one or more microfluidic pathways 6320'. The microfluidic chip 6300' may comprise a top film, a bottom film, or both bonded to a substrate positioned underneath the top film, above the bottom film, and/or between the top and bottom film, thereby forming the microfluidic pathways and/or the one or more reservoirs within the microfluidic chip (see. FIG. 34A, bottom panel). In some embodiments, the top film, bottom film, and/or the substrate in between the films may include polypropylene, cyclic olefin copolymer, cyclic olefin polymers, and/or various other materials.

[0308] For example, each reservoir 6360' can be positioned in a corresponding microfluidic pathway 6320' between the barrel and plunger pair 6340' and the dispensing tip 6400' (e.g., configured to be positioned under a respective outlet 6322'). More specifically, as shown in FIGS. 34A, each reservoir 6360' is positioned in a microfluidic pathway 6320' between the barrel and plunger pair 6340' and the three-way valve 6350'. In some embodiments, the one or more movable pins comprises the plunger in the barrel and plunger pair 6360'.

[0309] In some embodiments, the microfluidic chip 6300' may include one or more valves 6350' in the one or more microfluidic pathways 6320'. In some embodiments, the one or more valves can be 3-way valves that can switch between a first configuration (e.g., a position) and a second configuration (e.g., position). The valve(s) 6350' can be controlled by an electrical current or voltage, a mechanical motor, a pneumatic pressure, a magnetic force, or different actuating sources. Non-limiting examples of the valves include: a solenoid valve, a pneumatic valve, a rotary valve, or a membrane valve. Details of the membrane valve has been disclosed in U.S. Patent No. 10,830,362, titled, "Fluid handling device, fluid handling method, and flow path chip," filed August 3, 2017, there disclosure of which is incorporated herein by reference in its entirety. In some embodiments, the one or more valves 6350' may be replaced with other structures that can functionally enable (e.g., when switched between configurations): (1) fluidic communications from the reservoir via the inlets 6321' to the compartment(s) but not to the dispensing tip(s); and (2) fluidic communications from the reservoir to the dispensing tip(s) via the outlets 6322' but not to the compartment(s). The one or more valves 6350' may each be in fluidic communication with a corresponding one of the one or more dispensing tips 6400', one or more compartments (not shown), and one or more fluidic reservoirs 6360' via the one or more microfluidic pathways 6320'. As shown in FIG. 34A, there can be three different microfluidic pathways 6320': (i) from an individual valve 6350' to the reservoir 6360' and the barrel and plunger pair 6340', (ii) to the corresponding compartment of the cartridge (not shown), and (iii) to the dispensing tip 6400'. The valve can switch between the first and second configuration (e.g., position) to place the reservoir 6360' in fluid communication with either with the cartridge or the dispensing tip 6400'. The one or more compartments of the reagent cartridge and the one or more fluidic reservoirs 6360' may be in fluidic communication via the one or more microfluidic pathways 6320' when the corresponding valves are in a first configuration or position. The one or more dispensing tips 6400' and the one or more fluidic reservoirs 6600' may be in fluidic communication via the

one or more microfluidic pathways 6200' when the corresponding valves 6350' are in a second configuration or position.

[0310] The fluid dispensing device (e.g., fluid dispensing device 3000) may include one or more dispensing tips (e.g., dispensing tips 3400) to openly dispense one or more reagents to a flow cell device 300, 400, 500 (e.g., in FIG. 30A). The one or more dispensing tips are movable relative to an inlet of the flow cell device 300, 400, 500. For example, a first dispensing tip and a second dispensing tip may be moved above two different inlets leading to different microfluidic channels of the same flow cell device to simultaneously deliver two identical or different reagents. As another example, a first dispensing tip can move to a first inlet to deliver a washing buffer to a first channel of the flow cell device and then move to a second inlet to deliver the same washing buffer to a second channel of the same flow cell device.

[0311] In some embodiments, a various number of dispensing tips can be included, e.g., 1-100, 1-50, 1-40, 1-30, etc., inclusive of all ranges and subranges therebetween. In some embodiments, the number of dispensing tips can be identical to the number of compartments in the reagent cartridge. In some embodiments, the spatial arrangement of the dispensing tips can vary depending on different sequencing applications. For example, a first dispensing module may include 2, 3, or more rows of dispensing tips, while a second dispensing module may include a single row of dispensing tips spaced evenly from each other. The dispensing tips may be customized in various sizes and shapes, for example, a cone shape as shown in FIG. 30A.

[0312] Although in embodiments shown in figures herein include the cartridge, the cartridge compartments, and the actuator (e.g., motor) on top of the microfluidic chip while the dispensing tips are underneath the microfluidic chip, in other embodiments, the cartridge and its compartment and/or the actuator (e.g., motor) may be positioned in different locations relative to the microfluidic chip, e.g., underneath the microfluidic chip or on a side of the microfluidic chip. Similarly, the relative position of the barrel and plunger pair(s), the moveable pin, and the dispensing tips to the microfluidic chip may be but is not limited to the positions disclosed herein relative to the figures.

[0313] In some embodiments, the flow cell system may include a flow cell device comprising: a support comprising one or more substrates; one or more channels defined by the one or more substrates, wherein the one or more channels are configured to allow fluids and a gas gap between the fluids to flow therethrough; an inlet in the one or more substrates, the inlet in fluidic connection with the one or more channels; and an outlet in the one or more

substrates, wherein the one or more channels run from the inlet to the outlet. The flow cell system may further comprise a fluid dispensing device comprising a dispensing module comprising: a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein; a microfluidic chip in fluidic communication with each of the one or more compartments, wherein the microfluidic chip comprises one or more microfluidic pathways therewithin; and one or more dispensing tips; and one or more actuators that actuate a movable pin to push the fluidic reagents from the one or more compartments to the one or more dispensing tips through the one or more microfluidic pathways. In some embodiments, at least one of the one or more compartments, the microfluidic pathways, and the dispensing tips may be fluidically isolated from one another to prevent mixing of reagents.

Fluid dispensing device with transportation valves

[0314] In some embodiments, the fluid dispensing device comprises a dispensing module comprising: a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein; a microfluidic chip in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways 7320-3 therewithin; a transportation valve (e.g., as shown in FIGS. 35A-37D) having at least one open configuration (e.g., position) and at least one closed configuration (e.g., position). In some embodiments, the at least one closed position can be rotationally separated from the at least one open position. In some embodiments, the transportation valve can be positioned between the reagent cartridge and the microfluidic chip. The fluid dispensing device can include an actuator configured to actuate the transportation valve between the at least one open position and the at least one closed position. The fluid dispensing device can include one or more dispensing tips, wherein the transportation valve, in the at least one closed position, is configured to seal each of the one or more compartments and the microfluidic chip (e.g., prevent fluid from moving therebetween).

[0315] In some embodiments, the fluid dispensing device, can include a dispensing module comprising: a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein; a microfluidic chip in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways therewithin; a transportation valve having at least two open positions and at least one closed position that is rotationally separated from the at least two open positions; an actuator configured to actuate the transportation valve between the at least

two open positions and the at least one closed position; and one or more dispensing tips, wherein the transportation valve, in the at least one closed position, is configured to seal each of the one or more compartment and the microfluidic chip.

[0316] FIGS. 35A-35C, 36A-36B, and 37A-37D show an embodiment of the fluid dispensing device comprising the transportation valve 7700. FIGS. 35D-35E show an embodiment of the fluid dispensing device comprising the transportation valve 7700'. FIGS. 38A-38B, 39A-39C, and 40 show embodiments of the fluid dispensing device with the transportation valve 7700.

[0317] The transportation valve 7700 may be configured to seal the reagent(s) in the reagent cartridge 7200, and/or seal any fluid that may be contained within the microfluidic chip 7300. The fluidic dispensing module 7100 can be pre-assembled as a disposable and integrated device with reagent(s) contained therein. In some embodiments, the transportation valve 7700 may prevent the reagent(s) from leaking from the one or more compartment(s) (even if the in-chip valve(s) are closed), for example, during transportation of the dispensing module 7100, thereby preventing undesired damage and/or contamination to the fluid dispensing device. The transportation valve 7700 can be advantageously included in the fluid dispensing device to efficiently seal the one or more compartment(s) and prevent contamination or damage to the device. Further, transportation valve 7700 may also facilitate sealing of fluid, if any, that are contained within the microfluidic chip 7300.

[0318] FIGS. 35C and FIG. 35E show exemplary embodiments of the transportation valve 7700, 7700'. In some embodiments, the transportation valve (e.g. 7700 in FIGS. 35C and 7700' in FIG. 35E) comprises a valve substrate 7710, 7710'. The valve substrate 7710 may be a disk substrate or a ring substrate as shown in FIG. 35C and FIG. 38B. The valve substrate 7710 may comprise a plurality of open ports 7720. The plurality of open ports 7720 may be distributed on the valve substrate in various patterns. For example, the plurality of open ports 7720 may be distributed radially along a same circumference in the valve substrate, e.g., FIG. 35C. As another example, the plurality of open ports 10720 may be distributed radially along different circumferences in the valve substrate, e.g., FIG. 38B. In some embodiments, the open ports 10720are distributed along a same radius as in FIG. 38B or along different radius as in FIG. 35C. The angular separation, e.g., angle a in FIGS. 35C and 35E, between two radius where two open ports are positioned may be customized. In some embodiments, the angular separation or angle between two adjacent open ports 7720 may be based on the size, shape, orientation, and other possible aspects of the one or more compartments of the reagent cartridge 7200. In some embodiments, the angular separation or

angle between two adjacent open ports, e.g., angle a in FIG. 35C, can be in the range from 10 degrees to 180 degrees, inclusive of all ranges and subranges therebetween. In some embodiments, the angular separation or angle between two adjacent open ports may not be the same as the angular separation or angle between another two adjacent open ports, e.g., as shown in FIG. 35C. In some embodiments, the angular separation or angle, e.g., angle a in FIG. 35E, between two adjacent open ports may not be the same as the angular separation or angle between another two adjacent open ports.

[0319] The open ports 7720 may be of various shapes, e.g., circular, oval, diamond, rectangle, etc. In come embodiments, the open ports 7720 may be shaped to accommodate position errors or misalignment relative to the microfluidic chip. For example, the open ports 7720' in FIGS. 35E may be oval instead of circle to enable fluidic communication in the open position even if there is some positional error or misalignment, (e.g., of 0.01-0.3 degrees), in rotation of the transportation valve 7700'.

[0320] In some embodiments, a total number of open ports matches or is equivalent to the total number of compartments of the reagent cartridge, and each open port corresponds only to a corresponding compartment to allow fluidic communication from the corresponding compartment to the open port and then to the microfluidic chip, thereby preventing cross-contamination between reagents. A total number of open ports can be in the range from 1 to 20 or more. In some embodiments, two or compartments may share the same open port. For example, the compartment for holding washing solution may share a single open port with one or more other compartment containing reagents since the cross-contamination level may be controlled to satisfy a predetermined contamination threshold.

[0321] In some embodiments, the transportation valve 7700 can include a deformable member or a seal member (e.g., an over-mold or a gasket 7730) around each of the plurality of open ports 7720. The over-mold or gasket 7730 may be around each of the plurality of open ports 3720 on a first side facing the reagent cartridge 7200 and a second side facing the microfluidic chip 7300, e.g., a top side and a bottom side of the open ports 7720. In some embodiments, each over-mold or gasket, e.g., a first side thereof, is configured to compress the corresponding outlet of the reagent compartment 7210 thereby sealing the reagent within the compartments. In some embodiments, each over-mold or gasket, e.g., a second side thereof, is configured to block the corresponding pathway in the microfluidic chip thereby sealing any fluidic within the pathway of the microfluidic chip. The compression may be provided using various methods. For example, as shown in FIG. 35B, the microfluidic chip and the transportation valve can be mounted to the bottom of the reagent cartridge using

mounting hardware like screws. The size and thickness of the over-mold or gasket can be customized based on the size and/or shape of the compartments and the microfluidic chip. In some embodiments, the over-mold or gasket extends at least 5 to 45 degrees, inclusive of all range and subranges therebetween, along a circumference, e.g., angle b in FIG. 35C.

[0322] In some embodiments, the transportation valve 7700 comprises an actuation arm (e.g., 7740 in FIG. 35C) configured to be actuated by an actuator (e.g., different than the actuator 7500 of the fluid control device) thereby causing rotation of the transportation valve 7700. The actuation arm may extend within the x-y plane as shown in FIG. 35C. The transportation valve 7700 may be configured to rotate about an axis, e.g., z axis, that is orthogonal to the valve substrate 7710.

[0323] In some embodiments, instead of the actuation arm, the transportation valve 7700 may be actuated in various other ways to rotate. For example, the transportation valve 7700 may be actuated by an actuator. The actuator may include a rotor 7800 and a biasing element, 7810, e.g., a coil spring, and a motor(s) that drives the rotor and the biasing element. Instead of an actuation arm, the transportation valve 7700 may include one or more teeth 7740 that may mate with the teeth on the rotor 7800 to enable rotation of the transportation valve together with the rotor 7800. The biasing element may be configured to bias the rotor, e.g., upwards, to enable mating of the transportation valve and the motor, e.g., FIG. 36C. The rotator may be coupled to the motor or any other actuation means on the top, bottom, or both of the rotor. The number, size, shape of the one or more teeth may be customized to be in various ranges. In some embodiments, the transportation valve has 1 to 20 teeth, inclusive of all ranges and subranges therebetween, that are distributed evenly along the circumference of the transportation valve.

[0324] In some embodiments, the rotor 7800' may be pushed downward, e.g., via the biasing element 7810', to disengage the mated teeth of the transportation valve 7700' and the rotor 7800', and FIG. 35D shows an exploded view and the teeth that enable engagement. As a result, the rotor 7800' is not mated with the transportation valve, but engaged with the microfluidic chip, e.g., as shown in FIG. 37A. The rotor, when engaged with the microfluidic chip, may facilitate opening and/or closing of the in-chip valves, e.g., 7351, 7352 in FIG. 37B. For example, the rotor may rotate relative to the in-chip valves to provide pressure on the in-chip valve(s). The in-chip valve(s) are closed when the pressure is exerted on the corresponding valve, and open when the pressure is removed. The pressure exerted may be customized to be in various ranges.

[0325] In some embodiments, the transportation valve 7700 is configured to rotate 3 to 45 degrees, inclusive of all ranges and subranges therebetween, from the at least one open position to the at least one closed position in a first direction, e.g., clockwise. In some embodiments, the rotation of the transportation valve 7700 is relative to the microfluidic chip 7300. In some embodiments, the rotation of the transportation valve 7700 is relative to the reagent cartridge 7200. The transportation valve 7700 is configured to rotate 3 to 45 degrees, inclusive of all ranges and subranges therebetween, from the at least one closed position to the at least one open position in a second direction opposite to the first direction, e.g., counter-clockwise. FIG. 36A shows the at least one closed position of the transportation valve 7700. The at least one closed position may correspond to a first compartment of the reagent cartridge. In some embodiments, one or more compartments are simultaneously sealed when the transportation valve 7730 is in the at least one closed position. In some embodiments, the at least one closed position comprises a single closed position so that all the compartments are sealed in the single closed position. For example, when the dispensing module is in transportation, the transportation valve is in the at least one closed position, and all the compartments are sealed to prevent reagent leakage during transportation.

[0326] In some embodiments, the transportation valve 7700 may have one or more structural elements 7750 located on the substrate 7710 that limits the range of rotation between the open position and the close position. For example, the structural element 7750' may include one or more ribs that act as poke yoke, as shown in FIG. 35E. The structural element may include a cavity, e.g., oval hole 7750' in FIG. 35E, that functions to limit rotation of the transportation valve.

[0327] The transportation valve 7700 may comprise at least one open position. The at least one open position may include one or more open positions. FIG. 36B shows a first open position in which the corresponding compartment is open, and reagent can flow from the corresponding compartment through the open port into the microfluidic chip. In other words, at least one compartment is open when the transportation valve is in the first open position. In some embodiments, more than one compartment, e.g., all can be simultaneously open when the transportation valve is in the first open position, different reagent flows from different compartments are not blocked by the transportation valve, but may still be blocked by in-chip valves 7351, 7352 of the microfluidic chip 7300. FIG. 36A shows the closed position in which the corresponding compartment is closed.

[0328] In some embodiments, the first compartment is open when the transportation valve is in the first open position while the other compartments are closed, and a second

compartment is open when the transportation valve is in the second open position while the other compartments are closed. In some embodiments, the first compartment is open when the transportation valve is in the first open position while the other compartments are open or closed.

[0329] In some embodiments, the transportation valve 7700 may include a transportation configuration or position in which all the compartments of the cartridge are sealed, and one or more operational configurations in which one or more of the compartments of the cartridge are open and placed in fluidic communication with the fluid flow cell. In some embodiments, in each operational configuration, one compartment of the cartridge may be opened while all other compartments of the cartridge are sealed to flow a fluid contained therein to the fluid flow cell.

[0330] In some embodiments, the transportation valve 7700 is mounted to the bottom of the reagent cartridge 7200. In some embodiments, the microfluidic chip 7300 may be mounted to the bottom of the transportation valve 7700. In other words, the transportation valve 7700 may be disposed between the reagent cartridge 7200 and the microfluidic chip 7300.

[0331] In some embodiments, the microfluidic chip comprises a plurality of pairs of dispensing ports or outlets. Each dispensing port may be in fluidic communication with a corresponding dispending tip (shown in FIG. 34A). A distance between each pair of the dispensing ports is determined based on the distance between two open landing areas of a flow cell device so that the pair of dispensing ports may allow simultaneously dispensing to the different lanes of the flow cell device, e.g., FIG. 37A. In some embodiments, each pair of dispensing ports corresponds to only one corresponding in-chip well and one corresponding compartment of the reagent cartridge. In some embodiments, the number of dispensing ports in fluidic communication with a corresponding in-chip well and reagent compartment can increase when the flow cell device comprises more than 2 lanes or channels.

[0332] FIGS. 37A-37B show an embodiment of the microfluidic chip 7300 in relation to the transportation valve 7700. The microfluidic chip 7300 may comprise a first in-chip well or reservoir 7360 corresponding to a first pair of the dispensing ports 7322. The first in-chip well 7360 may be in fluidic communication with an off-chip pump 7340 via a first fluidic pathway 7320_1. The off-chip pump may pump air into the first in-chip well or pull air out from the first in-chip well to facilitate dispensing or aspiration of reagents. The off-chip pump may pump in or aspirate reagents and/or washing solutions.

[0333] FIGS. 37E-37F show an embodiment of the microfluidic chip 7300. The microfluidic chip 10300 may comprise multiple in-chip wells or reservoirs 7360a, 7360b,

each corresponding to one or more dispensing ports or outlets 7322. Each in-chip well 7360a, 7360b may be in fluidic communication with an off-chip pump 7340. The off-chip pump 7340 may be in fluidic communication with each in-chip well 7360a, 7360b via a separate fluidic pathway. In some embodiments, the off-chip pump 7340 may be in fluidic communication with each in-chip well 7360a, 7360b via a common fluidic pathway. The off-chip pump 7340 may facilitate aspirating reagent and/or washing solution, e.g., from reagent cartridges, when the corresponding first in-chip valve 7351 is open and the corresponding second in-chip valve is closed 7352. The off-chip pump may pump air into an in-chip well or pull air out from the in-chip well to facilitate dispensing or aspiration of reagents or washing solution, e.g., from the in-chip wells, when the corresponding second in-chip valve 7352 is open and the first in-chip valve 7351 is closed. Each in-chip well may be in fluidic communication with its corresponding first and second in-chip valves. The fluidic pathway between each in-chip well 7360aand its corresponding first and second in-chip valves may be independent to avoid cross contamination between different reagent(s) or washing solutions in different in-chip wells. For example, the first in-chip well can be in fluidic communication via the fluidic pathway 7320_1 to the pump 7340, and the second in-chip well (e.g., 7360b) can be in fluidic communication to the same pump 7340 or a second pump via the fluidic pathway 7320_1'. The first in-chip well can be in fluidic communication with a first compartment of the reagent cartridge via a second fluidic pathway 7320_2, and with a first pair of dispensing tips via a third fluidic pathway 7320_3. The second in-chip well can be in fluidic communication with a second compartment of the reagent cartridge via a fifth fluidic pathway 7320_2', and with a second pair of dispensing tips via a sixth fluidic pathway 7320_3'. FIGS. 37E-37F show an embodiment of a microfluidic chip with 6 in-chip wells and distribution of the in-chip wells. However, the number, size, shape, distribution of the in-chip wells may be customized and should not be limited to the exemplary embodiment in FIGS. 37E-37F.

[0334] The first in-chip well 7360a may be in fluidic communication with the reagent cartridge 7200 via a first in-chip valve 7351 and a second fluidic pathway 7320_2. The first in-chip well 7360 may be in fluidic communication with a first pair of the dispensing ports 7322 via a second in-chip valve 7352. The second in-chip valve 7352 may be in fluidic communication with the first pair of the dispensing ports 7322 via a third fluidic pathway 7320_3. In some embodiments, the first in-chip well 7360a may be in fluidic communication only with the first pair of the dispensing ports 7322 but no other dispensing ports to avoid cross contamination of reagents.

[0335] In some embodiments, the first fluidic pathway (7320_1), the second fluidic pathway (7320_2), the third fluidic pathway (7320_3), the first in-chip well 7360a, the first in-chip valve 7351, the second in-chip valve 7352, the first pair of dispensing ports 7322, or a combination thereof can correspond to only the first compartment 7210 of the reagent cartridge 7200, but not other compartments to avoid cross contamination of reagents.

[0336] In some embodiments, the first or second in-chip valve 7351, 7352 may have various shapes. Each of the first or second in-chip valve may comprise at least an open position and a closed position. The first or second in-chip valve may be configured to shift between the open position and the close position when a pressure exerted on the first or second in-chip valve is altered. Various mechanisms may be used to provide the pressure on the first and/or second in-chip valves. In some embodiments, the rotation of the rotor may be configured to provide the pressure when the rotor is disengaged with the transportation valve and coupled to the microfluidic chip. Therefore, the rotor may have a first configuration or set of configurations in which the rotor actuates the transportation valve and a second configuration or set of configurations in which the rotor actuates one of the in-chip valves.

[0337] For example, the first or second in-chip valve may be a three-way valve as shown in FIGS 34A-34C, so that the first or second in-chip valve may shift to connect two of the three different pathways to allow aspiration or dispensing. As another example, each in-chip valve may comprise a dimple dome between two non-connected in-chip fluidic pathways, e.g., FIG. 37B. When the dimple dome is depressed, the flow is blocked, when the dimple dome is released, the flow between two non-connected in-chip fluidic pathways can be connected.

[0338] In some embodiments, when the transport valve 7700 is in the at least one open position, the first in-chip valve 7351 is open, and the second in-chip valve 7352 is closed such that the dispensing module 7100 is configured to aspirate reagent from the first compartment 7210 to the in-chip well 7360. When the transport valve 7700 is in the at least one open position, the first in-chip valve 7351 is closed, and the second in-chip valve 7352 is open, the dispensing module is configured to dispense reagent from the in-chip well 7360 to only the first pair of the plurality of pairs of dispending ports 7322.

[0339] In some embodiments, each of the plurality of open ports 10720 of the transportation valve can comprise an elongated shape extending along a circumference of the valve substrate 10710 as shown in FIG. 38B. In some embodiments, the transportation valve 10700 may further comprise a central open port 10721 having the elongated shape extending along a radius of the valve substrate, e.g., in FIG. 38B. The central open port 10721 may be

in fluidic communication with the off-chip pump 10340 external to the transportation valve 10700.

[0340] In some embodiments, the microfluidic chip 10300 is positioned between the transportation valve 10700 and the reagent cartridge 10200, e.g., FIG. 39A-39C. The microfluidic chip 10300 may comprise one or more in-chip wells 10360. Each well may be configured to contain a different reagent to avoid cross contamination. The total number of wells may be based on the total number of reagents required in a sequencing application.

[0341] Each in-chip well may comprise a first well opening 10365 and a second well opening 10366. The microfluidic chip 10300 may comprise a first through-hole or outlet 10322 in fluidic communication with the first pair of dispensing tips 10400, optionally via a second fluidic pathway 10320_3. In some embodiments, the microfluidic chip can comprise the first through-hole that is in fluidic communication with only the first pair of dispensing tips but no other dispensing tips to avoid cross contamination of reagents.

[0342] In some embodiments, the fluidic pathways in the microfluidic chip, 10320, 10320-1, 10320_2, 10320_3 may include a lumen between a top and a bottom surface of the chip, e.g., the cross-sectional view at line AA' in FIG. 38A.

[0343] The microfluidic chip 10300 may comprise a second through-hole 10321 or inlet in fluidic communication with a first compartment 10210 of the reagent cartridge, optionally via a second fluidic pathway 10320_2. In some embodiments, the microfluidic chip can comprise the second through-hole or inlet that is in fluidic communication with only the first compartment of the reagent cartridge to avoid cross contamination of reagents, optionally via a third fluidic pathway 10320_3.

[0344] The transportation valve 10700 may be in a first open position of the at least two open positions when a first open port 10720 connects the first through-hole 10322 and a first well opening 10365 of a first in-chip well 10360, e.g., in FIG. 39C. The transportation valve may be in the first open position of the at least two open positions to allow reagent dispensing from the first in-chip well 10600 to the first pair of dispensing tips 10400. The transportation valve 10700 may be in the first open position when the first open port 10720 is positioned above the first through-hole 10322 and the first well opening 10365, e.g., in FIG. 39C.

[0345] The transportation valve 10700 may be in a second open position of the at least two open positions when a first open port 10720 connects the second through-hole 10321 and a first well opening 10365 of a first in-chip well 10360. The transportation valve 10700 may be in the second open position of the at least two open positions to allow reagent aspiration from the first compartment 10210 of the reagent cartridge 10200 to the first in-chip well 10360.

The transportation valve is in the second open position when the first open port 10720 is positioned above the second through-hole 10321 and the first well opening 10365, e.g., in FIG. 39B.

[0346] In some embodiments, when the transportation valve 10700 is in the first or second open position, the central open port 10721 connects a second well opening 10366 to the pump 10340 via a first fluidic pathway 10320_1. Correspondingly, the pump may be pulled to facilitate reagent aspiration from the compartment to the in-chip well. The pump may be pushed to facilitate reagent dispensing from the in-chip well to the dispensing tip(s).

[0347] In some embodiments, the microfluidic chip 10300 comprises a chip substrate 10710. The chip substrate may comprise a circular shape as shown in FIG. 39A with more than one circumferences shown as dotted lines. In some embodiments, the first and second through-holes 10322, 10321 correspond to the first in-chip well 10600, but not other in-chip wells to avoid cross contamination of reagents.

[0348] In some embodiments, the transportation valve 10700 further comprises a third and fourth through-holes corresponding to a second in-chip well. The first and second through-holes correspond only to a first open port, and the third and fourth through-hole correspond only to a second open port that is different from the first open port to avoid cross contamination.

[0349] In some embodiments, the first and second through-holes are positioned along a circumference of the chip substrate 10710. The third and fourth through-holes may be positioned along the same circumference or a second circumference of the chip substrate. The first and third through-holes may be 5 to 85 degrees, inclusive of all ranges and subranges therebetween, apart by rotation about an axis orthogonal to the valve substrate, e.g., angle c in FIG. 38A. The first and second through-holes may be 1 to 45 degrees, inclusive of all ranges and subranges therebetween, apart by rotation about an axis orthogonal to the valve substrate.

[0350] In some embodiments, the at least two open positions correspond to the first open port 10720 that correspond to a first compartment 10210. When the transportation valve is in the least two open positions, only the first in-chip well 10600 is in fluidic communication with the first pair of dispensing tips or the first compartment, the other in-chip well(s) is sealed from the reagent cartridge and the plurality of dispensing tips.

[0351] In other embodiments, when the transportation valve is in the least two open positions, the first in-chip well is in fluidic communication with the first pair of dispensing tips or the first compartment, and the second in-chip well may also be in fluidic communication with the reagent cartridge or the plurality of dispensing tips, so that reagent

aspiration from two different compartments may occur simultaneously and/or reagent dispensing from two different in-chip wells to different dispensing tips may occur simultaneously. Such simultaneous dispensing may be used for simultaneous aspiration of different reagent to save fluidic operation time during a sequencing run. Further, such simultaneous dispensing may be used for simultaneous dispensing to multiple flow cells, for example flow cells that are arranged along different radius and separated by a rotational angle from each other to further reduce fluidic operation time during a sequencing run and enable improved sequencing system throughput.

[0352] The transportation valve 10700 may be in a closed position when the first open port 10720 is positioned not directly above the first through-hole 10322, the second through-hole 10321, and the first well opening 10365, e.g., as shown in FIG. 39A. The transportation valve 10700 may be in the closed position to seal one or more (or all) of the compartments and prevent reagent leakage.

Methods of using

[0353] Disclosed herein are methods of using the flow cell devices 200, 300, 400, 500, 700 for performing, or facilitating, or combinations thereof, sequencing analysis using the sequencing system 110. Disclosed herein are also methods of manufacturing the flow cell devices 200, 300, 400, 500, 700 that can be used to perform, or to facilitate, or combinations thereof, sequencing analysis. The methods herein can include some or all of the operations disclosed herein. The operations may be performed in, but is not limited to, the order that is described herein.

[0354] The operations herein may be performed manually. The operations may be automatically performed by a robotic arm or the like (not shown). The robotic arm can be controlled by a computer system, e.g., 126 in FIG. 1, to automatically (or at least partly automatically) perform some or all of the operations disclosed herein. In some embodiments, the computer system 126, dedicated processors, 118, the FPGA(s) 120, or their combinations, may be programmed to control the robotic arm. The computer system 126 of the robotic arm can have installed on it software, firmware, hardware, or their combinations that in operation cause the computer system to perform the operations or actions disclosed herein.

[0355] The methods can be performed by one or more processors in the computer system, e.g., 126, disclosed herein. In some embodiments, the processor can include one or more of: a processing unit, an integrated circuit, or their combinations. For example, the processing unit can include a central processing unit (CPU), or a graphic processing unit (GPU), a microprocessor, a digital signal processor (DSP), a controller, a microcontroller, a state

machine, or combinations thereof. The integrated circuit can include a chip such as an application specific integrated circuit (ASIC), a programmable logic device (PLD), a field-programmable gate array (FPGA). In some embodiments, the processor can include the computing system.

[0356] In some embodiments, some or all operations in the methods can be performed by the FPGA(s) (e.g., FPGA(s) 120). In embodiments when some operations are performed by FPGA(s), the data after an operation performed by the FPGA(s) can be communicated by the FPGA(s)s to the computer system (e.g., CPU(s)) so that the CPU(s) can perform subsequent operation(s) in method using such data. Similarly, data can also be communicated from the computer system (e.g., CPU(s)) to the FPGA(s) for processing by the FPGA(s). In some embodiments, all the operations in methods disclosed herein can be performed by CPU(s). Alternatively, the operations performed by CPU(s) can be performed by other processors such as the dedicated processors, or GPU(s). In some embodiments, all the operations in method can be performed by FPGA(s).

[0357] The methods of manufacturing the flow cell devices disclosed herein can comprise an operation of obtaining the one or more substrates (e.g., substrates 320, 420, 520). The operation of obtaining the one or more substrates can comprises obtaining the one or more substrates separately so that the one or more substrates are not physically coupled to each other yet.

[0358] The methods disclosed herein can comprise an operation of generating one or more channels in the one or more substrates. In some embodiments, the channels are generated as holes or opening defined in the middle substrates. In some embodiments, generating a channel comprises generating a groove in the top and/or bottom substrates and generating a hole in the middle substrate, and the channel can be formed by stacking the groove and the hole together. In some embodiments, a location and shape of the groove(s) in the top and/or bottom substrate may correspond to the location and shape of the hole defined by the middle substrate such that when the substrates are stacked, the groove(s) and the hole align to form the channel. In some embodiments, generating a channel comprises generating a grove in each of the two adjacent substrates and combining the groves together to form the channel, via etching or any other mechanisms. The present disclosure does not limit the mechanisms by which the hole, groove, or cavity, can be formed in the substrates. The hole, groove, or cavity can form a lumen that allows fluids and a gas gap between the fluids to flow therethrough, when the substrates are fixedly coupled together, e.g., bonded, adhered, fastened, etc.

[0359] The methods disclosed herein can comprise an operation of forming an inlet. The operation of forming an inlet can comprise forming a hole or a void in at least one of the one or more substrates (e.g., the top, middle, and/or bottom substrate) and forming an open landing area. The hole or void can be at or near one end of the substrates, or the channels, or combinations thereof. For example, inlet may be formed near a first end of the substrate configured to be disposed near a dispensing tip of the fluid dispensing device. For example, forming the inlet can comprise forming a cylindrical hole in the top substrate and forming an open landing area in the middle substrate in a location that substantially matches the location of the cylindrical hole of the inlet, e.g., at the same location, so that when the two substrates are stacked together, the inlet is directly above the landing area or at least partly above the landing area. In other words, the openings formed in the top substrate and the middle substrate may at least partially align when the top substrate and the middle substrate are stacked to form a fluid flow path from the inlet to the open landing area.

[0360] The methods disclosed herein can comprise an operation of forming an outlet. The operation of forming an outlet can comprise forming a hole or a void in at least one of the one or more substrates. For example, forming the outlet can comprise forming a cylindrical hole in the bottom substrate at or near the opposite end of the substrates, or channels, or combinations thereof, from the inlet.

[0361] In some embodiments, the inlet and outlet are in fluidic connection or fluid communication with the one or more channels. In some embodiments, the inlet and the outlet are in fluid communication via the one or more channels defined in the substrates.

[0362] The methods disclosed herein can comprise an operation of fixedly coupling the substrates together. The coupling operation can be achieved via chemical, mechanical, or laser bonding, but is not limited to such bonding techniques.

[0363] The methods disclosed herein can comprise coating at least a portion of a surface of the one or more channels with a first coating, as disclosed herein. The surface can be interior surface defining the lumen(s) of the one or more channels. For example, the surface can include a top interior surface (e.g., the surface defined by the top substrate) or bottom interior surface (e.g., the surface defined by the bottom substrate).

[0364] The methods disclosed herein can comprise coating at least a portion of a surface of the one or more channels with an additional coating to the first coating, e.g., a coating of fluorescent beads.

[0365] The methods disclosed herein can comprise an operation of covering at least a portion of the open landing area with a second coating as disclosed herein. The second

coating can be different from or identical to the first coating in the channels. In some embodiments, the process of applying the second coating can be different from or identical to applying the first coating in the channels. In some embodiments, at least some actions in the entire process of applying the second coating can be different from or identical to applying the first coating in the channels.

[0366] In some embodiments, the second coating is disposed over the open landing area or at least a portion of the open landing area. In some embodiments, coating the open landing area comprises impregnating lubricants in one or more porous surfaces of the open landing area. In some embodiments, coating the open landing area comprises disposing acid-catalyzed graft polycondensation of one or more saline monomers on the open landing area.

[0367] In some embodiments, the methods of manufacturing the flow cell devices further comprises an operation of forming a cleaning outlet in the one or more substrates. The operation of forming the cleaning outlet can comprise forming the cleaning outlet in fluidic connection. In some embodiments, the operation of forming the cleaning outlet may include forming the cleaning outlet closer to the inlet (e.g., along the x-y plane) than to the outlet. The operation of forming the cleaning outlet can further comprise forming the cleaning outlet in a predetermined size and shape. For example, the size and shape of the cleaning outlet can be approximately the same as the outlet. The operation of forming the cleaning outlet can further comprise forming the cleaning outlet in the bottom substrate, the top substrate, the middle substrate, or their combinations. As an example, the cleaning outlet can be a side port formed by forming a half groove in the middle substrate and a half groove the bottom substrate such that when the middle and bottom substrate are stacked, the outlet is formed.

[0368] The methods of using the flow cells disclosed herein can comprise an operation of dispensing a first reagent openly to an open landing area of an inlet of the flow cell device. The dispensing operation can be performed manually or automatically by a robotic arm. The dispensing of the first reagent can be from a dispensing tip of a dispenser of the fluidic control device disclosed herein. In some embodiments, a dispensing tip is used for dispensing the first reagent and not any other reagents to avoid unintended mixing of reagents in the dispensing tip.

[0369] The methods can further comprise an operation of moving the dispensing tip to a specific location before dispensing. The specific location can be above the hole of the inlet of the flow cell. In some embodiments, the dispensing tip may be positioned such that the dispensing tip is at least partially inside the hole of the inlet. In some embodiments, at least a portion of the dispensing tip can be in contact with the wall of the hole or the open landing

area at the bottom of the hole. In some embodiments, the dispensing tip may comprise a shock absorbing portion such that when the dispensing tip contacts the open landing area, the dispensing tip does not exert a damaging force to the open landing area or the substrate(s). The dispensing operation can last for a predetermined period of time to ensure a predetermined amount of first reagent is dispensed into the outlet. The predetermined dispensing time can be on the scale of sub seconds to less than a minute. In some embodiments, the fluid may be dispensed at a predetermined rate for a predetermined period of time such that a predetermined volume of a first reagent is dispensed into the outlet.

[0370] The methods can further comprise an operation of retrieving (e.g., manually or automatically via a robotic arm and computer system) the dispensing tip from the specific dispensing location.

[0371] The methods can further comprise an operation of flowing at least part of the first reagent from the open landing area to one or more channels of the flow cell device. This operation of flowing the reagents can be driven passively without actively adding any mechanical force on the reagents. Alternatively, the operation can be facilitated by adding a mechanical force to transfer the reagent from one end of the channels in direct contact with the open landing pad to the opposite end of the channels that is in contact with the outlet. For example, the force can be applied by a pump or a vacuum at the inlet and/or outlet. The sequencing reactions can occur when the first reagent flows through the channels.

[0372] The methods can further comprise an operation of cleaning residuals of the first reagent from the one or more channels by driving an air gap before dispensing any second reagent to the flow cell device. The air gap can be driven by a mechanical force applied by a pump or a vacuum at the inlet and/or outlet. The air gap may clean (e.g., remove) some of the residuals on the landing pad. The mechanical force can be adjusted so that the air gap can occupy about 30% to about 80% volume, inclusive of all ranges and subranges therebetween, of each channel in a predetermined time window. Channels with a larger lumen may need a larger air gap for similar cleaning effect as compared to channels with smaller lumens. In some embodiments, the method can include driving an air gap of a predetermined volume through the channel. In some embodiments, the method may include flowing a gas (e.g., having a predetermined composition) through the channel for a predetermined amount of time. In some embodiments, the method may include moving the air gap through the channel until the channel is at least partially dry or fully dry.

[0373] The methods can further comprise an operation of washing the channels before dispensing any second reagents to achieve a cleaning effect.

[0374] The methods can further comprise an operation of dispensing a second reagent openly to the open landing area via a different dispensing tip from that of the first reagent when the second reagent is different from the first reagent.

[0375] Before dispensing the second reagent, the methods can further comprise an operation of confirming that the channels have been cleaned and a predetermined cleaning threshold (e.g., less than 0.001% contamination) has been met. For example, the predetermined cleaning threshold can be a contamination level that is required for the second reagent that is going to be administered. In some embodiments, the method may include increasing a homogeneity (e.g., reducing a concentration gradient) of the second reagent by moving the air gap through the channel before administration of the second reagent.

[0376] The methods can further comprise an operation of facilitating cleaning of residuals of the first reagent off the open landing area by using a coating on at least part of the open landing area. The residuals of the first reagent on the open landing pad may also contaminate the second reagent to be administered subsequent to the first reagent. Cleaning of such residuals can reduce contamination level of the second reagent and thus improve accuracy and reliability of the sequencing reactions based on the second reagent. The coating, e.g., liquid repelling or slippery, on the open landing pad can passively facilitate transfer of the first reagent to the channels and reduce residuals on the landing pad.

[0377] The methods can further comprise an operation of cleaning residuals of the first reagent from at least part of the open landing area by driving the residuals through the cleaning outlet. An active mechanical force can be applied via the cleaning outlet, e.g., by a pump or a vacuum, to suck the residuals from the open landing pad to the cleaning outlet. The active mechanical force can be combined with a passive second coating on the landing pad to facilitate cleaning of the open landing pad before administration of the second reagent.

[0378] Disclosed herein are methods of using a fluid dispensing device (e.g., fluid dispensing device 3000) for dispensing fluidic reagents to various nucleotide acid molecules on a support, e.g., the flow cell devices. The methods of using a fluid dispensing device herein may advantageously enable or facilitate sequencing analysis using the sequencing system 110. Methods of using a fluid dispensing device (e.g., fluid dispensing device 3000) for dispensing fluidic reagents can be advantageously more convenient, easy to use, efficient than existing methods with comparable or even reduced contamination. The methods herein can include some or all of the operations disclosed herein. The operations may be performed in, but is not limited to, the order that is described herein. While described with respect to fluid dispensing device 3000, it should be appreciated that the methods of using a fluid

dispensing device described herein can be applicable to other fluid dispensing devices (e.g., any fluid dispensing device embodiment described herein). All such variations should be considered within the scope of this application.

[0379] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise: providing a dispensing module comprising a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein; a microfluidic chip in fluidic connection with each of the one or more compartments, wherein the microfluidic chip comprises one or more microfluidic pathways therewithin; and one or more dispensing tips; and providing one or more actuators that actuate a movable pin to push the fluidic reagents from the one or more compartments to the one or more dispensing tips through the one or more microfluidic pathways.

[0380] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of fluidically connecting the one or more compartments of the reagent cartridge with the microfluidic chip. The fluidic connection can be permanently sealed to prevent liquid leakage from the connection, and the fluidic connection can be permanent, e.g., by welding, by molding as a single piece, etc., so that the user cannot removably separate the cartridge and the microfluidic chip and reconnect them. Instead, the user may move the cartridge and the microfluidic chip together when needed. The one or more compartments of the reagent cartridge may be configured for holding fluidic reagents therein. The microfluidic chip may include one or more microfluidic pathways therewithin. In some embodiments, the operation of fluidically connecting the one or more compartments of the reagent cartridge with the microfluidic chip comprises fluidically connecting each outlet of the compartment(s) to a corresponding inlet of a corresponding microfluidic pathway of the microfluidic chip. Each different reagent may have its own compartment and microfluidic pathway. Two or more compartments and microfluidic pathways may contain identical liquid reagent therewithin.

[0381] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip. In some embodiments, the operation of fluidically connecting the one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip comprises fluidically connecting each outlet of the microfluidic pathway to a corresponding dispensing tip.

[0382] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of removably coupling the dispensing module to the following: the one

or more actuators; the flow cell device, the sequencing system, or a combination thereof. The dispensing module may comprise: the reagent cartridge with the one or more compartments; the microfluidic chip in fluidic connection with the one or more compartments; and the one or more dispensing tips. Such operation of removably coupling the dispensing module to the device(s) disclosed herein can occur after removably uncoupling an old dispensing module for disposal.

[0383] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of applying, by the one or more actuators, a first force or pressure on the curved portion of a first microfluidic pathway for a first predetermined time to actuate a first reagent from a first compartment of the reagent cartridge to a first dispensing tip via a first microfluidic pathway. Such operation of applying the first force or pressure can occur during a sequencing run in a predetermined flow cycle. Such operation of applying the first force or pressure can be repeated for a number of times at a predetermined rate, e.g., washing the microfluidic channel in each flow cycle. Such operation of applying the first force or pressure can be for a single occurrence, e.g., for dispensing specific library molecules to the flow cell device. The one or more actuators may be controlled automatically by instructions executable on the computer system disclosed herein. For example, the computer system may store one or more parameters and/or instructions for applying the first force or pressure and may control application of the force or pressure (e.g., via an actuator) on the curved portion of the microfluidic pathway.

[0384] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of receiving the first reagent from the first dispensing tip openly (e.g., without tubing) at an inlet of the flow cell device. The first reagent may travel to and contact the nucleotide acid molecules immobilized on surface(s) of a microfluidic channel of the flow device.

[0385] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of applying a second force or pressure on the curved portion of second first microfluidic pathway for a second predetermined time to actuate a second reagent from a second compartment to a second dispensing tip via a second microfluidic pathway.

[0386] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of receiving the second reagent from the second dispensing tip openly (e.g., without tubing) to the inlet of the flow cell device.

[0387] In embodiments where a smaller number of actuators are included in comparison to the number of microfluidic pathways (e.g., a single actuator), the methods of using the fluid

dispensing device 3000 may comprise moving the actuator(s) relative to the flow cell device or the microfluidic pathways in order to exert force or pressure on a specific microfluidic pathway. For example, the single actuator may move from underneath the first microfluidic pathway to underneath a different microfluidic pathway (e.g., the microfluidic pathway corresponding to the next reagent to be delivered) in order to push fluid(s) therewithin.

[0388] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of switching one or more valves in the fluid dispensing device into a first configuration (e.g., position). When the one or more valves are in the first position, the method 3000 may further comprise applying a first force or pressure for a first period of time on the plunger to move fluids in one or more compartment(s) of the reagent cartridge through the one or more valves into a reservoir of the fluid dispensing device. In some embodiments, the first force or pressure and/or the first period of time can be predetermined so that the fluid does not contaminate any common line that is shared by flowing different reagents or otherwise fluids to the flow cell device. In some embodiments, the microfluidic chip may include one or more sensors. The fluid position, (e.g., along a y direction from inlet to outlet of the flow cell device, or a fluidic height along a z direction) during pulling and/or pushing may be detected by a sensor. The sensor may provide a feedback of the fluid position to the actuator that actuates the plunger so that the actuator may stop or continue to actuate the plunger based on the feedback. In some embodiments, the contamination in one or more of the microfluidic paths is controlled to be below a predetermined threshold hold level. In some embodiments, the methods of using the fluid dispensing device 3000 does not require any washing of the common line and/or the individual microfluidic pathways leading to the dispensing tips. For example, the reagents are kept in separate (e.g., fluidically isolated) fluid pathways, and therefore, no washing of the individual microfluidic pathways or common line may be needed. As shown in FIG. 34B, the common line 3323 is disposed between the connector 3361 and the barrel 3340. In some embodiments, the first force or pressure pulls the plunger away from the connector 3361.

[0389] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of switching one or more valves into a second configuration (e.g., position). When the one or more valves are in the second position, the method 3000 may further comprise applying a second force or pressure for a second period of time on the plunger to move fluids in the one or more valves to the one or more dispensing tips 3400. In some embodiments, the second force or pressure and/or the second period of time can be predetermined so that the fluid does not contaminate any common line that is shared and

possible air bubbles do not reach the dispensing tips. As shown in FIG. 34C, the common line is between the connector 3361 and the barrel. In some embodiments, the second force or pressure pushes the plunger toward the connector 3361. The second force or pressure may or may not empty the corresponding reservoir.

[0390] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise removing at least part of the first reagent or second reagent from the flow cell device. In some embodiments, such operation of removing the reagent(s) may be performed by some or all elements of the fluid operation device disclosed herein. In some embodiments, the removed first or second reagent may including recycling the reagent back into the reagent cartridge, in particular, the corresponding compartment of the reagent. Such removal and recycling operations may be repeated for various numbers of time, e.g., for each flow cycle in the sequencing run.

[0391] In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of providing a plurality of nucleic acid template molecules immobilized on the flow cell device, wherein each nucleic acid template molecule comprise: a first insert sequence ("sequence-of-interest") and a first sample index sequence (also referred to herein as an "index sequence"), wherein the first sample index sequence comprises a first universal sample index sequence, the first universal sample index identifying a sample source of the insert sequence.

[0392] In some embodiments, the methods of using the fluid dispensing device 3000 may further comprises an operation of conducting, by the sequencing system, one or more cycles of sequencing reactions of the first insert sequence before conducting one or more cycles of the sequencing reactions of the first sample index sequence to generate flow cell images comprising the first flow cell image and the second flow cell image in the first flow cycle. In some embodiments, the methods of using the fluid dispensing device 3000 may comprise an operation of conducting, by the sequencing system, one or more cycles of sequencing reactions of the first sample index sequence before conducting one or more cycles of the sequencing reactions of the first insert sequence to generate flow cell images.

[0393] In some embodiments, the operation of conducting the one or more cycles of sequencing reactions of the first insert sequence or the first sample index sequence comprises an operation of dispensing, by the fluid dispensing system, a first reagent comprising a first plurality of soluble sequencing primers that hybridize to a first plurality of nucleic acid template molecules to one or more inlets of a flow cell device. The operation of conducting the one or more cycles of sequencing reactions of the first insert sequence or the first sample

index sequence may further comprise an operation of allowing the first reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device.

[0394] After the operation of conducting the one or more cycles of sequencing reactions, the methods of using the fluid dispensing device 3000 may further comprise an operation of reversibly removing the dispensing module without removing the one or more actuators relative to the sequencing system. Subsequently, the methods of using the fluid dispensing device 3000 may further comprise an operation of removably coupling a second dispensing module to the one or more actuators and to the sequencing system.

[0395] In some embodiments, the operation of conducting the one or more cycles of sequencing reactions of the first insert sequence or the first sample index sequence may comprise an operation of dispensing, by at least a first dispensing tip of the fluid dispensing device, a first plurality of sequencing primers, a first plurality of polymerases and a first mixture of different types of multivalent molecules (sometimes referred to herein as "avidites") to one or more inlets of the flow cell device. In some embodiments, the first plurality of sequencing primers, the first plurality of polymerases and the first mixture of different types of multivalent molecules may be a mixture that can be dispensed from only a single dispensing tip (e.g., the first dispensing tip). In some embodiments, the first plurality of sequencing primers, the first plurality of polymerases and the first mixture of different types of multivalent molecules may be dispensed sequentially, in various orders, by different dispensing tips. Subsequently, the operation of conducting the one or more cycles of sequencing reactions may comprise: allowing first plurality of sequencing primers, the first plurality of polymerases and the first mixture of different types of multivalent molecules to travel from the one or more inlets to the surface(s) and contact the nucleotide acid template modules for sequencing reactions.

[0396] In some embodiments, the operation of conducting the one or more cycles of sequencing reactions of the first insert sequence or the first sample index sequence may comprise an operation of dispensing, by at least a second dispensing tip, a second plurality of sequencing primers, a second plurality of polymerases and a second mixture of different types of multivalent molecules . In some embodiments, the second plurality of sequencing primers, the second plurality of polymerases and the second mixture of different types of multivalent molecules may be a mixture that can be dispensed from only a single dispensing tip (e.g., the second dispensing tip). In some embodiments, the second plurality of sequencing primers, the second plurality of polymerases and the second mixture of different types of

multivalent molecules may be dispensed sequentially, in various orders, by different dispensing tips.

[0397] The first and second plurality of sequencing primers can be different or identical. The first and second plurality of polymerases can be different or identical. The first and second mixture of different types of multivalent molecules can be different or identical.

[0398] In some embodiments, the operation of conducting the one or more cycles of sequencing reactions of the first insert sequence or the first sample index sequence may comprise an operation of performing one or more operations of the two-stage methods for sequencing as disclosed herein. In some embodiments, the operation of conducting the one or more cycles of sequencing reactions of the first insert sequence or the first sample index sequence may comprise an operation of performing one or more operations of the sequencing-by-binding methods as disclosed herein. In some embodiments, the reagent(s) can be dispensed by one or more dispensing tips using the fluid dispensing device herein. In some embodiments, the reagent(s) can be removed using the fluid dispensing device herein.

[0399] In some embodiments, the methods of using the fluid dispensing device 3000 may further comprise one or more operation associated with a transportation valve (e.g., shown in FIGS. 35A and 38B) and a corresponding microfluidic chip.

[0400] In some embodiments, the methods of using the fluid dispensing device 3000 may further comprise an operation of switching the transportation valve into a first open configuration or position from a closed configuration or position. Such operation may comprise: rotating, by one or more actuators, the transportation valve about an axis orthogonal to the valve substrate for a predetermined rotational angle in a first direction, e.g., counter clockwise or clockwise. The predetermined rotational angle may be in a range from 3 degrees to 45 degrees, inclusive of all ranges and subranges therebetween. Such operation may open the transportation valve and its seal on one or more compartments of a reagent cartridge. As a result, such an operation may allow a first reagent from the first compartment through the transportation valve and flow to the microfluidic chip, e.g., into a first in-chip well via a fluidic pathway for a predetermined duration, e.g., FIG. 36B.

[0401] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching a first in-chip valve into an open configuration or position. The first in-chip valve corresponds to a first compartment of the reagent cartridge. Such operation may occur before or after opening the transportation valve. A second in-chip well may be closed while switching on (e.g., opening) the first in-chip valve.

[0402] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching the transportation valve into a second open configuration or position by rotating, by one or more actuators, the transportation valve about an axis orthogonal to the valve substrate for a predetermined rotational angle in a second direction or the first direction. The predetermined rotational angle may be in a range from 3 degrees to 45 degrees, inclusive of all ranges and subranges therebetween. Such operation may remove the seal of a second compartment thereby allowing a second reagent from the second compartment to flow to the microfluidic chip, e.g., into the second in-chip well via a fluidic pathway for a predetermined duration.

[0403] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching the transportation valve into the open configuration or position from a closed configuration or position. Such operation may comprise: rotating, by one or more actuators, the transportation valve about an axis orthogonal to the valve substrate for a predetermined rotational angle in a first direction, e.g., counter clockwise or clockwise. The predetermined rotational angle may be in a range from 3 degrees to 45 degrees, inclusive of all ranges and subranges therebetween. Such operation may open the transportation valve and its seal on all compartments of a reagent cartridge. As a result, such an operation may allow reagents to flow through the transportation valve and to the microfluidic chip, e.g., into corresponding in-chip wells via corresponding fluidic pathways for a predetermined duration, e.g., FIG. 36B. In some embodiments, the corresponding in-chip valves connecting the corresponding compartments to their in-chip wells are open to enable the flow to the in-chip wells.

[0404] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching transportation valve into a closed configuration or position, by rotating, by the one or more actuators, the transportation valve for the predetermined rotational angle in a second direction opposite the first direction, to seal the first compartment and/or the second compartment from the microfluidic chip.

[0405] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching a second in-chip valve into an open configuration or position to allow the first reagent to flow from the first in-chip well to a first pair of dispensing tips. The first in-chip well may be closed while switching on (e.g., opening) the second in-chip valve.

[0406] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching a third in-chip valve into an open configuration or

position. The third in-chip valve corresponds to a second compartment of the reagent cartridge. A fourth in-chip valve may be closed while switching on (e.g., opening) the third in-chip valve.

[0407] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching the fourth in-chip valve into an open configuration or position to allow the second reagent to flow from the second in-chip well to a second pair of dispensing tips. The third in-chip valve may be closed while switching on (e.g., opening) the fourth in-chip valve.

[0408] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching the transportation valve into a second open configuration or position from a closed configuration or position by rotating, by one or more actuators, the transportation valve for a first predetermined rotational angle in a first direction, to allow a first open port to connect a first compartment to a first in-chip well via a fluidic pathway, e.g., see FIG. 39A to FIG. 39B.

[0409] In some embodiments, the fluid dispensing device may be in a closed configuration or position during shipping, and it may be switched to the second open configuration or position for aspirating reagents from a first compartment to a first in-chip well. In some embodiments, such operation simultaneously connects the central open port to the first in-chip well. In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of operating the pump, e.g., pulling the plunger, to facilitate fluid aspiration into the first in-chip well. In some embodiments, the first predetermined rotational angle is in a range from 5 degrees to 60 degrees, inclusive of all ranges and subranges therebetween. In some embodiments, the first predetermined rotational angle is in a range from 15 degrees to 45 degrees, inclusive of all ranges and subranges therebetween. In some embodiments, the transportation valve may stay / remain in the first open configuration or position for a predetermined time, e.g., 0.1 second, 0.5 seconds or more to allow a predetermined amount (e.g., volume) of the first reagent to travel to the first in-chip well.

[0410] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching the transportation valve into a first open configuration or position by rotating, by the one or more actuators, the transportation valve for a second predetermined rotational angle in a second direction opposite the first direction, to allow the first open port to connect the first in-chip well to the first pair of dispensing tips, thereby enabling reagent dispensing from the first in-chip well to the first pair of dispensing tips, e.g., see FIG. 39B to FIG. 39C. In some embodiments, such operation simultaneously connects the

central open port to the first in-chip well. In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of operating the pump, e.g., pushing the plunger, to facilitate reagent dispensing. In some embodiments, the second predetermined rotational angle may be in a range from 5 degrees to 60 degrees, inclusive of all ranges and subranges therebetween. In some embodiments, the second predetermined rotational angle may be in a range from 15 degrees to 45 degrees, inclusive of all ranges and subranges therebetween. In some embodiments, the transportation valve may stay in the first open position for a predetermined time, e.g., 0.1 second, 0.5 seconds or more to allow a predetermined amount of the first reagent to be dispensed.

[0411] Subsequent to the dispensing of the first reagent, in some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching the transportation valve into a fourth open configuration or position by rotating, by one or more actuators, the transportation valve for a third predetermined rotational angle in the first direction or the second direction, to allow a second open port to connect a second compartment to a second in-chip well via a fluidic pathway. In some embodiments, the third predetermined rotational angle is in a range from 5 degrees to 90 degrees, inclusive of all ranges and subranges therebetween. In some embodiments, the transportation valve may stay / remain in the second open configuration or position for a predetermined time, e.g., 0.1 second, 0.5 seconds or more to allow a predetermined amount of the second reagent to travel to the second in-chip well.

[0412] In some embodiments, the methods of using the fluid dispensing device may further comprise an operation of switching the transportation valve into a third open configuration or position by rotating, by the one or more actuators, the transportation valve for a fourth predetermined rotational angle in a first or second direction, to allow the second open port to connect the second in-chip well to the second pair of dispensing tips thereby enabling reagent dispensing from the second in-chip well to the second pair of dispensing tips. In some embodiments, the fourth predetermined rotational angle may be in a range from 5 degrees to 80 degrees. In some embodiments, the transportation valve may stay in the second open position for a predetermined time, e.g., 0.1 second, 0.5 seconds or more to allow a predetermined amount of the second reagent to be dispensed.

Computer systems

[0413] Various embodiments of the methods may be implemented, for example, using one or more computer systems, such as computer system 800 shown in FIG. 8. One or more computer systems 800 may be used, for example, to implement any of the embodiments

discussed herein, as well as combinations and sub-combinations thereof. Computer system 800 may include one or more hardware processors 804. The hardware processor(s) 804 can be central processing unit (CPU), graphic processing units (GPU), or their combination. The hardware processor 804 may be connected to a bus or communication infrastructure 806.

[0414] Computer system 800 may also include user input/output device(s) 803, such as monitors, keyboards, pointing devices, etc., which may communicate with communication infrastructure 806 through user input/output interface(s) 802. The user input/output devices 803 may be coupled to the user interface124 in FIG. 1.

[0415] One or more of processors 804 may include a graphics processing unit (GPU). In an embodiment, a GPU may be a processor that is a specialized electronic circuit designed to process mathematically intensive applications. The GPU may have a parallel structure that is efficient for parallel processing of large blocks of data, such as mathematically intensive data common to computer graphics applications, images, videos, vector processing, array processing, etc., as well as cryptography (including brute-force cracking), generating cryptographic hashes or hash sequences, solving partial hash-inversion problems, or producing results of other proof-of-work computations for some blockchain-based applications, or combinations thereof, for example. With capabilities of general-purpose computing on graphics processing units (GPGPU), the GPU may be particularly useful in at least the image recognition and machine learning aspects described herein.

[0416] Additionally, one or more of processors 804 may include a coprocessor or other implementation of logic for accelerating cryptographic calculations or other specialized mathematical functions, including hardware-accelerated cryptographic coprocessors. Such accelerated processors may further include instruction set(s) for acceleration using coprocessors, or other logic, or combinations thereof, to facilitate such acceleration.

[0417] Computer system 800 may also include a data storage device such as a main or primary memory 808, e.g., random access memory (RAM). Main memory 808 may include one or more levels of cache. Main memory 808 may have stored therein control logic (e.g., computer software), or data, or combinations thereof.

[0418] Computer system 800 may also include one or more secondary data storage devices or secondary memory 810. Secondary memory 810 may include, for example, a main storage drive 812, or a removable storage device or drive 814, or combinations thereof. Main storage drive 812 may be a hard disk drive or solid-state drive, for example. Removable storage drive 814 may be a floppy disk drive, a magnetic tape drive, a compact disk drive, an optical

storage device, a tape backup device, or any other storage device/drive, or combinations thereof.

[0419] Removable storage drive 814 may interact with a removable storage unit 818.

[0420] Removable storage unit 818 may include a computer usable or readable storage device having stored thereon computer software, or data, or combinations thereof. The software can include control logic. The software may include instructions executable by the hardware processor(s) 804. Removable storage unit 818 may be a floppy disk, magnetic tape, compact disk, DVD, optical storage disk, and any other computer data storage device. Removable storage drive 814 may read from, or write to, or combinations thereof, removable storage unit 818.

[0421] Secondary memory 810 may include other methods, devices, components, instrumentalities or other approaches for allowing computer programs, or other instructions or data, or combinations thereof, to be accessed by computer system 800. Such methods, devices, components, instrumentalities or other approaches may include, for example, a removable storage unit 822 and an interface 820. Examples of the removable storage unit 822 and the interface 820 may include a program cartridge and cartridge interface (such as that found in video game devices), a removable memory chip (such as an EPROM or PROM) and associated socket, a memory stick and USB port, a memory card and associated memory card slot, or any other removable storage unit and associated interface, or combinations thereof.

[0422] Computer system 800 may further include a communication or network interface 824. The communication interface 824 may enable computer system 800 to communicate and interact with any combination of external devices, external networks, external entities, etc. (individually and collectively referenced by reference number 828). For example, communication interface 824 may allow computer system 800 to communicate with external or remote devices 828 over communication path 826, which may be wired, or wireless, or combinations thereof, and which may include any combination of LANs, WANs, the Internet, etc. Control logic, or data, or combinations thereof, may be transmitted to and from computer system 800 via communication path 826. In some embodiments, communication path 826 can provide the connection to the cloud 130, as depicted in FIG. 1. The external devices, etc. referred to by reference number 828 may be devices, networks, entities, etc. in the cloud 130.

[0423] Computer system 800 may also include any of a personal digital assistant (PDA), desktop workstation, laptop or notebook computer, netbook, tablet, smart phone, smart watch or other wearable, appliance, part of the Internet of Things (IoT), or embedded system, to name a few non-limiting examples, or any combination thereof.

[0424] It can be appreciated that the framework described herein may be implemented as a method, process, apparatus, system, or article of manufacture such as a non-transitory computer-readable medium or device. For illustration purposes, the present framework may be described in the context of distributed ledgers being publicly available, or at least available to untrusted third parties. One example as a modern use case is with blockchain-based systems. It can be appreciated, however, that the present framework may also be applied in other settings where sensitive or confidential information may need to pass by or through hands of untrusted third parties, and that this technology is in no way limited to distributed ledgers or blockchain uses.

[0425] Computer system 800 may be a client or server, accessing or hosting any applications, or data, or combinations thereof, through any delivery paradigm, including but not limited to: remote or distributed cloud computing solutions; local or on-premises software (e.g., "on-premise" cloud-based solutions); "as a service" models (e.g., content as a service (CaaS), digital content as a service (DcaaS), software as a service (SaaS), managed software as a service (MsaaS), platform as a service (PaaS), desktop as a service (DaaS), framework as a service (FaaS), backend as a service (BaaS), mobile backend as a service (MbaaS), infrastructure as a service (IaaS), database as a service (DbaaS), etc.); or a hybrid model including any combination of the foregoing examples or other services or delivery paradigms.

[0426] Any applicable data structures, file formats, and schemas may be derived from standards including but not limited to: JavaScript Object Notation (JSON), Extensible Markup Language (XML), Yet Another Markup Language (YAML), Extensible Hypertext Markup Language (XHTML), Wireless Markup Language (WML), MessagePack, XML User Interface Language (XUL), or any other functionally similar representations alone or in combination. Alternatively, proprietary data structures, formats or schemas may be used, either exclusively or in combination with existing or open standards.

[0427] Any pertinent data, files, or databases, or combinations thereof, may be stored, retrieved, accessed, or transmitted, or combinations thereof, in human-readable formats such as numeric, textual, graphic, or multimedia formats, further including various types of markup language, among other possible formats. Alternatively, or in combination with the above formats, the data, files, or databases, or combinations thereof, may be stored, retrieved, accessed, or transmitted, or combinations thereof, in binary, encoded, compressed, or encrypted, or combinations thereof, formats, or any other machine-readable formats.

[0428] Interfacing or interconnection among various systems and layers may employ any number of mechanisms, such as any number of protocols, programmatic frameworks,

floorplans, or application programming interfaces (API), including but not limited to Document Object Model (DOM), Discovery Service (DS), NSUserDefaults, Web Services Description Language (WSDL), Message Exchange Pattern (MEP), Web Distributed Data Exchange (WDDX), Web Hypertext Application Technology Working Group (WHATWG) HTML5 Web Messaging, Representational State Transfer (REST or RESTful web services), Extensible User Interface Protocol (XUP), Simple Object Access Protocol (SOAP), XML Schema Definition (XSD), XML Remote Procedure Call (XML-RPC), or any other mechanisms, open or proprietary, that may achieve similar functionality and results.

[0429] Such interfacing or interconnection may also make use of uniform resource identifiers (URI), which may further include uniform resource locators (URL) or uniform resource names (URN). Other forms of uniform, or unique, or combinations thereof, identifiers, locators, or names may be used, either exclusively or in combination with forms such as those set forth above.

[0430] Any of the above protocols or APIs may interface with or be implemented in any programming language, procedural, functional, or object-oriented, and may be compiled or interpreted. Non-limiting examples include C, C++, C#, Objective-C, Java, Scala, Clojure, Elixir, Swift, Go, Perl, PHP, Python, Ruby, JavaScript, WebAssembly, or virtually any other language, with any other libraries or schemas, in any kind of framework, runtime environment, virtual machine, interpreter, stack, engine, or similar mechanism, including but not limited to Node.js, V8, Knockout, jQuery, Dojo, Dijit, OpenUI5, AngularJS, Expressjs, Backbone.js, Ember.js, DHTMLX, Vue, React, Electron, and so on, among many other non-limiting examples.

[0431] In some embodiments, a tangible, non-transitory apparatus or article of manufacture comprising a tangible, non-transitory computer useable or readable medium having control logic (software) stored thereon may also be referred to herein as a computer program product or program storage device. This includes, but is not limited to, computer system 800, main memory 808, secondary memory 810, and removable storage units 818 and 822, as well as tangible articles of manufacture embodying any combination of the foregoing. Such control logic, when executed by one or more data processing devices (such as computer system 800), may cause such data processing devices to operate as described herein.

[0432] Based on the teachings contained in this disclosure, it may be apparent how to make and use embodiments of this disclosure using data processing devices, computer systems, or computer architectures, or combinations thereof, other than that shown in FIG. 8. In

particular, embodiments may operate with software, hardware, or operating system implementations, or combinations thereof, other than those described herein.

Optical systems

[0433] The imager 116 shown in FIG. 1 can include one or more optical systems. Further disclosed herein are optical system design guidelines and high-performance fluorescence imaging methods and systems that provide improved optical resolution and image quality for fluorescence imaging-based genomics applications. The disclosed optical imaging system designs provide at least one of larger fields-of-view, increased spatial resolution, improved modulation transfer, contrast-to-noise ratio, and image quality, higher spatial sampling frequency, faster transitions between image capture when repositioning the sample plane to capture a series of images (e.g., of different fields-of-view), and improved imaging system duty cycle, and thus, enable higher throughput image acquisition and analysis.

[0434] In some instances, improvements in imaging performance, e.g., for dual-side (flow cell) imaging applications, may be achieved by using an electro-optical phase plate in combination with an objective lens to compensate for the optical aberrations induced by the layer of fluid separating the upper (near) and lower (far) interior surfaces of a flow cell. In some instances, this design approach may also compensate for vibrations introduced by, e.g., a motion-actuated compensator that is moved in or out of the optical path depending on which surface of the flow cell is being imaged.

[0435] In some embodiments, improvements in imaging performance (e.g., for dual-side (flow cell) imaging applications comprising the use of thick flow cell walls (e.g., wall (or coverslip) thickness > 700 μm) and fluid channels (e.g., fluid channel height or thickness of 50 – 200 μm)), may be achieved even when using commercially-available, off-the-shelf objectives by using a tube lens design that corrects for the optical aberrations induced by the thick flow cell walls, or intervening fluid layer, or combinations thereof, in combination with the objective. In other words, the imager 116 may include a tube lens coupled to the objective in order, the tube lens configured to correct optical aberrations caused by thick flow cell walls.

[0436] In some instances, improvements in imaging performance, e.g., for multichannel (e.g., two-color or four-color) imaging applications, may be achieved by using multiple tube lenses, one for each imaging channel, where each tube lens design has been optimized for the specific wavelength range used in that imaging channel.

[0437] Embodiments disclosed herein may comprise fluorescence imaging systems, said systems comprising: a) at least one light source configured to provide excitation light within

one or more specified wavelength ranges; b) an objective lens configured to collect fluorescence arising from within a specified field-of-view of a sample plane upon exposure of the sample plane to the excitation light; and c) at least one image sensor, wherein the fluorescence collected by the objective lens is imaged onto the image sensor, and wherein a pixel dimension for the image sensor is chosen such that a spatial sampling frequency for the fluorescence imaging system is at least twice an optical resolution of the fluorescence imaging system. In some embodiments, a numerical aperture of the objective lens is at least 0.1, at least 0.2, at least 0.3, at least 0.4, at least 0.5, at least 0.6, at least 0.7, at least 0.8, or at least 0.9 or a numerical aperture value falling within a range defined by any two of the foregoing; wherein a working distance of the objective lens is at least 400 micron(μm), at least 500 μm, at least 600 μm, at least 700 μm, at least 800 μm, at least 900 μm, at least 1000 μm, or a working distance falling within a range defined by any two of the foregoing; and wherein the field-of-view has an area of at least 0.1 mm^2, at least 0.2 mm^2, at least 0.5 mm^2, at least 0.7 mm^2, at least 1 mm^2, at least 2 mm^2, at least 3 mm^2, at least 5 mm^2, or at least 10 mm^2, or a field of view falling within a range defined by any two of the foregoing.

[0438] In some embodiments, the numerical aperture may be at least 0.75. In some embodiments, the numerical aperture is at least 1.0. In some embodiments, the working distance is at least 850 μm. In some embodiments, the working distance is at least 1,000 μm. In some embodiments, the field-of-view may have an area of at least 2.5 mm^2. In some embodiments, the field-of-view may have an area of at least 3 mm^2. In some embodiments, the spatial sampling frequency may be at least 2.5 times the optical resolution of the fluorescence imaging system. In some embodiments, the spatial sampling frequency may be at least 3 times the optical resolution of the fluorescence imaging system.

[0439] In some embodiments, the system may further comprise an X-Y-Z translation stage such that the system is configured to acquire a series of two or more fluorescence images in an automated fashion, wherein each image of the series is or can be acquired for a different field-of-view. In some embodiments, a position of the sample plane may be simultaneously adjusted in an X direction, a Y direction, and/or a Z direction to match the position of an objective lens focal plane in between acquiring images for different fields-of-view. In some embodiments, the time required for the simultaneous adjustments in the X direction, Y direction, and Z direction may be less than 0.3 seconds, less than 0.4 seconds, less than 0.5 seconds, less than 0.7 seconds, or less than 1 second, or a time falling within a range defined by any two of the foregoing. In some embodiments, the system may further comprise an autofocus mechanism configured to adjust the focal plane position prior to acquiring an

image of a different field-of-view if an error signal indicates that a difference in the position of the focal plane and the sample plane in the Z direction is greater than a specified error threshold. In some embodiments, the specified error threshold is 100 nm or greater. In some embodiments, the specified error threshold is 50 nm or less. In some embodiments, the system comprises three or more image sensors, and wherein the system is configured to image fluorescence in each of three or more wavelength ranges onto a different image sensor. In some embodiments, a difference in the position of a focal plane for each of the three or more image sensors and the sample plane is less than 100 nm. In some embodiments, a difference in the position of a focal plane for each of the three or more image sensors and the sample plane is less than 50 nm. In some embodiments, the total time required to reposition the sample plane, adjust focus if necessary, and acquire an image is less than 0.4 seconds per field-of-view. In some embodiments, the total time required to reposition the sample plane, adjust focus if necessary, and acquire an image is less than 0.3 seconds per field-of-view.

[0440] Also disclosed herein are fluorescence imaging systems for dual-side imaging of a flow cell comprising: a) an objective lens configured to collect fluorescence arising from within a specified field-of-view of a sample plane within the flow cell; b) at least one tube lens positioned between the objective lens and at least one image sensor, wherein the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of the flow cell, and wherein the flow cell has a wall thickness of at least 700 μm and a gap between an upper interior surface and a lower interior surface of at least 50 μm; wherein the imaging performance metric is substantially the same for imaging the upper interior surface or the lower interior surface of the flow cell without moving compensation optics (e.g., an "optical compensator" or "compensator") into or out of an optical path between the flow cell and the at least one image sensor, without moving one or more optical elements of the tube lens along the optical path, and without moving one or more optical elements of the tube lens into or out of the optical path.

[0441] In some embodiments, the objective lens may be a commercially-available microscope objective. In some embodiments, the commercially-available microscope objective may have a numerical aperture of at least 0.3. In some embodiments, the objective lens may have a working distance of at least 700 μm. In some embodiments, the objective lens may be corrected to compensate for a cover slip thickness (or flow cell wall thickness) of 0.17 mm or of greater or lesser thickness than 0.17mm. In some embodiments, the optical system may be corrected to compensate for cover slip thickness, flow cell thickness, or

distance between focal planes. In some embodiments, said correction may be made by inserting a compensation optic, such as a lens or optical assembly into the light path of the optical system. In some embodiments, said correction may be made without inserting a compensation optic, such as a lens or optical assembly into the light path of the optical system. In some embodiments, the fluorescence imaging system may further comprise an electro-optical phase plate positioned adjacent to the objective lens and between the objective lens and the tube lens, wherein the electro-optical phase plate may provide correction for optical aberrations caused by a fluid filling the gap between the upper interior surface and the lower interior surface of the flow cell. In some embodiments, the at least one tube lens may be a compound lens comprising three or more optical components. In some embodiments, the at least one tube lens is a compound lens comprising four optical components, which may comprise one or more of a first asymmetric convex-convex lens, a second convex-plano lens, a third asymmetric concave-concave lens, and a fourth asymmetric convex-concave lens which may be present in the order as listed above, or in any alternate order. In some embodiments, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a wall thickness of at least 1 mm. In some embodiments, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a gap of at least 100 μm. In some embodiments, the at least one tube lens is configured to correct an imaging performance metric for a combination of the objective lens, the at least one tube lens, and the at least one image sensor when imaging an interior surface of a flow cell having a gap of at least 200 μm.

[0442] In some embodiments, the system comprises a single objective lens, two tube lenses, and two image sensors, and each of the two tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some embodiments, the system comprises a single objective lens, three tube lenses, and three image sensors, and each of the three tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some embodiments, the system comprises a single objective lens, four tube lenses, and four image sensors, and each of the four tube lenses is designed to provide optimal imaging performance at a different fluorescence wavelength. In some embodiments, the design of the objective lens or the at least one tube lens is configured to optimize the modulation transfer function in the mid to high spatial frequency range. In some

embodiments, the imaging performance metric comprises a measurement of modulation transfer function (MTF) at one or more specified spatial frequencies, defocus, spherical aberration, chromatic aberration, coma, astigmatism, field curvature, image distortion, contrast-to-noise ratio (CNR), or any combination thereof. In some embodiments, the difference in the imaging performance metric for imaging the upper interior surface and the lower interior surface of the flow cell Is less than 10%. In some embodiments, the difference in imaging performance metric for imaging the upper interior surface and the lower interior surface of the flow cell is less than 5%. In some embodiments, the use of the at least one tube lens provides for an at least equivalent or better improvement in the imaging performance metric for dual-side imaging compared to that for an existing system comprising an objective lens, a motion-actuated compensator, and an image sensor. In some embodiments, the use of the at least one tube lens provides for an at least 10% improvement in the imaging performance metric for dual-side imaging compared to that for an existing system comprising an objective lens, a motion-actuated compensator, and an image sensor.

[0443] Disclosed herein are illumination systems for use in imaging-based solid-phase genotyping and sequencing applications, the illumination system comprising: a) a light source; and b) a liquid light-guide configured to collect light emitted by the light source and deliver it to a specified field-of-illumination on a support surface comprising tethered biological macromolecules.

[0444] In some embodiments, the illumination system further comprises a condenser lens. In some embodiments, the specified field-of-illumination has an area of at least 2 mm^2, at least 3 mm^2, at least 4 mm^2, at least 5 mm^2, etc. In some embodiments, the light delivered to the specified field-of-illumination is of uniform intensity across a specified field-of-view for an imaging system used to acquire images of the support surface. In some embodiments, the specified field-of-view has an area of at least 2 mm^2, at least 3 mm^2, at least 4 mm^2, at least 5 mm^2, etc. In some embodiments, the light delivered to the specified field-of-illumination is of uniform intensity across the specified field-of-view when a coefficient of variation (CV) for light intensity is less than 10%. In some embodiments, the light delivered to the specified field-of-illumination is of uniform intensity across the specified field-of-view when a coefficient of variation (CV) for light intensity is less than 5%. In some embodiments, the light delivered to the specified field-of-illumination has a speckle contrast value of less than 0.1. In some embodiments, the light delivered to the specified field-of-illumination has a speckle contrast value of less than 0.05.

[0445] The disclosed optical systems, imaging systems, or modules may, in some instances, be stand-alone optical systems designed for imaging a sample or substrate surface. In some instances, they may comprise one or more processors or computers (e.g., separate from or the same as the processors 118, 120, and/or the computer system 126). In some instances, they may comprise one or more software packages that provide instrument control functionality, or image processing functionality, or combinations thereof. In some instances, in addition to optical components such as light sources (e.g., solid-state lasers, dye lasers, diode lasers, arc lamps, tungsten-halogen lamps, etc.), lenses, prisms, mirrors, dichroic reflectors, optical filters, optical bandpass filters, apertures, and image sensors (e.g., complementary metal oxide semiconductor (CMOS) image sensors and cameras, charge-coupled device (CCD) image sensors and cameras, etc.), they may also include mechanical, or optomechanical components, or combinations thereof, such as an X-Y translation stage, an X-Y-Z translation stage, a piezoelectric focusing mechanism, and the like. In some instances, they may function as modules, components, sub-assemblies, or sub-systems of larger systems designed for genomics applications (e.g., genetic testing, or nucleic acid sequencing applications, or combinations thereof). For example, in some instances, they may function as modules, components, sub-assemblies, or sub-systems of larger systems that further comprise light-tight, or other environmental control housings, temperature control modules, fluidics control modules, fluid dispensing robotics, pick-and-place robotics, one or more processors or computers, one or more local or cloud-based software packages (e.g., instrument / system control software packages, image processing software packages, data analysis software packages), data storage modules, data communication modules (e.g., Bluetooth, WiFi, intranet, or internet communication hardware and associated software), display modules, or any combination thereof.

Methods for Sequencing

[0446] The present disclosure provides methods for sequencing immobilized or non-immobilized nucleic acid template molecules. The methods can be operated using the sequencing systems disclosed herein. In some embodiments, the template molecules are immobilized. In some embodiments, the template molecules comprise a plurality of nucleic acid template molecules having one copy of a target sequence of interest. In some embodiments, nucleic acid template molecules having one copy of a target sequence of interest can be generated by conducting bridge amplification using linear library molecules. In some embodiments, the immobilized template molecules comprise a plurality of nucleic acid template molecules each having two or more tandem copies of a target sequence of

interest (e.g., concatemer template molecules). In some embodiments, nucleic acid template molecules comprising concatemer template molecules can be generated by conducting rolling circle amplification of circular library molecules. In some embodiments, the template molecule are non-immobilized and comprise circular molecules. In some embodiments, methods for sequencing employ soluble (e.g., non-immobilized) sequencing polymerases or sequencing polymerases that are immobilized to a support. Methods of generating circular library molecules and concatemer template molecules are known in the art, and described, for example in WO2022266470, WO2023168444, WO2023168443, WO2024159166, WO2023235865, WO2024011145 and WO2024059550, the contents of each of which are incorporated by reference in their entireties herein.

[0447] In some embodiments, the sequencing reactions employ detectably labeled nucleotide analogs. In some embodiments, the sequencing reactions employ a two-stage sequencing reaction comprising binding detectably labeled multivalent molecules and incorporating nucleotide analogs. In some embodiments, the sequencing reactions employ non-labeled nucleotide analogs. In some embodiments, the sequencing reactions employ phosphate chain labeled nucleotides.

[0448] In some embodiments, the immobilized concatemer template molecules each comprise tandem repeat units of the sequence-of-interest (e.g., insert region) and adaptor sequences. For example, as shown in FIG. 9, the tandem repeat unit comprises: (i) a left universal adaptor sequence having a surface pinning primer binding site (920) (e.g., surface pinning primer), (ii) a left universal adaptor sequence having a forward sequencing primer binding site (940) (e.g., first sequencing primer), (iii) a sequence-of-interest (910), (iv) a right universal adaptor sequence having a reverse sequencing primer binding site (950) (e.g., second sequencing primer), (v) a right universal adaptor sequence having a surface capture primer binding site (930) (e.g., surface capture primer), and (vii) a left index sequence (960), or a right index sequence (970), or combinations thereof. In some embodiments, the tandem repeat unit further comprises a left unique identification sequence (980), or a right unique identification sequence (990), or combinations thereof. In some embodiments, the tandem repeat unit further comprises at least one binding sequence for a compaction oligonucleotide. Exemplary compaction oligonucleotides are described in WO2024040058, the contents of which are incorporated by reference in their entirety herein. In some embodiments, FIG. 9 and FIG. 10 show linear library molecules or a unit of a concatemer molecule.

[0449] The immobilized concatemer template can self-collapse into a compact nucleic acid nanoball. Inclusion of one or more compaction oligonucleotides during the RCA reaction can

further compact the size, or shape, or combinations thereof, of the nanoball. Exemplary compaction oligonucleotides are described, for example, in WO2024040058, the contents of which are incorporated by reference in their entirety herein. An increase in the number of tandem repeat units in a given concatemer template molecule increases the number of sites along the concatemer template molecule for hybridizing to multiple sequencing primers (e.g., sequencing primers having a universal sequence) which serve as multiple initiation sites for polymerase-catalyzed sequencing reactions. When the sequencing reaction employs detectably labeled nucleotides or nucleotide analogs, or detectably labeled multivalent molecules (e.g., having nucleotide units), or combinations thereof, the signals emitted by the nucleotides or nucleotide units that participate in the parallel sequencing reactions along the concatemer template molecule yields an increased signal intensity for each concatemer template molecule. Multiple portions of a given concatemer template molecule can be simultaneously sequenced. Furthermore, a plurality of binding complexes can form along an individual concatemer molecule, each binding complex comprising a sequencing polymerase bound to a concatemer template molecule/primer duplex and bound to a multivalent molecule or labeled nucleotide or nucleotide analog, wherein the plurality of binding complexes remains stable without dissociation, resulting in increased persistence time which increases signal intensity and reduces imaging time.

Methods for Sequencing using Nucleotide Analogs

[0450] The present disclosure provides methods for sequencing any of the nucleic acid template molecules described herein, the methods comprising step (a): contacting a sequencing polymerase to (i) a nucleic acid template molecule and (ii) a nucleic acid sequencing primer, wherein the contacting is conducted under a condition suitable to bind the sequencing polymerase to the nucleic acid template molecule which is hybridized to the nucleic acid primer, wherein the nucleic acid template molecule hybridized to the nucleic acid primer forms the nucleic acid duplex. In some embodiments, the sequencing polymerase comprises a recombinant mutant sequencing polymerase that can bind and incorporate nucleotides or nucleotide analogs. In some embodiments, the nucleic acid template molecule is immobilized. In some embodiments, the nucleic acid template molecule is a concatemer template molecule as described herein.

[0451] In some embodiments, the sequencing primer comprises a 3’ extendible end or a 3’ non-extendible end. In some embodiments, the plurality of nucleic acid template molecules comprises amplified template molecules (e.g., clonally amplified template molecules). In some embodiments, the plurality of nucleic acid template molecules comprises one copy of a

target sequence of interest. In some embodiments, the plurality of nucleic acid molecules comprises two or more tandem copies of a target sequence of interest (e.g., concatemer template molecules). In some embodiments, the plurality of nucleic acid template molecules comprises the same target sequence of interest or different target sequences of interest. In some embodiments, the plurality of nucleic acid primers is in solution or is immobilized to the support, as described herein. In some embodiments, when the plurality of nucleic acid template molecules, or the plurality of nucleic acid primers, or combinations thereof, are immobilized to a support, the binding with the first sequencing polymerase generates a plurality of immobilized first complexed polymerases. In some embodiments, the plurality of nucleic acid template molecules, or nucleic acid primers, or combinations thereof, are immobilized to $10^2 – 10^{15}$ different sites on a support. In some embodiments, the binding of the plurality of nucleic acid template molecules and nucleic acid primers with the plurality of first sequencing polymerases generates a plurality of first complexed polymerases immobilized to $10^2 – 10^{15}$ different sites on the support. In some embodiments, the plurality of immobilized first complexed polymerases on the support are immobilized to pre-determined or to random sites on the support. In some embodiments, the plurality of immobilized first complexed polymerases are in fluid communication with each other to permit flowing a solution of reagents (e.g., enzymes including sequencing polymerases, multivalent molecules, nucleotides, or divalent cations, or combinations thereof) onto the support using the systems and methods described herein so that the plurality of immobilized complexed polymerases on the support are reacted with the solution of reagents in a massively parallel manner.

[0452] In some embodiments, the methods for sequencing further comprise step (b): contacting the sequencing polymerase with a plurality of nucleotides under a condition suitable for binding at least one nucleotide to the sequencing polymerase which is bound to the nucleic acid duplex and suitable for polymerase-catalyzed nucleotide incorporation which extends the sequencing primer by one nucleotide. In some embodiments, the sequencing polymerase is contacted with the plurality of nucleotides in the presence of at least one catalytic cation comprising magnesium, or manganese, or combinations thereof. In some embodiments, the plurality of nucleotides comprises at least one nucleotide analog having a chain terminating moiety at the sugar 2’ or 3’ position. In some embodiments, the chain terminating moiety is removable from the sugar 2’ or 3’ position to convert the chain terminating moiety to an OH or H group. In some embodiments, the plurality of nucleotides comprises at least one nucleotide that lacks a chain terminating moiety. In some

embodiments, at least on nucleotide is labeled with a detectable reporter moiety (e.g., fluorophore) that emits a detectable signal. In some embodiments, the detectable reporter moiety comprises a fluorophore. In some embodiments, the fluorophore is attached to the nucleo-base. In some embodiments, the fluorophore is attached to the nucleo-base with a linker which is cleavable/removable from the base. In some embodiments, at least one of the nucleotides in the plurality is not labeled with a detectable reporter moiety. In some embodiments, a particular detectable reporter moiety (e.g., fluorophore) that is attached to the nucleotide can correspond to the nucleotide base (e.g., dATP, dGTP, dCTP, dTTP or dUTP) to permit detection and identification of the nucleo-base. When the chain terminating nucleotide is detectably labeled, step (b) further comprises detecting the emitted signal from the chain terminating nucleotide which is incorporated into the 3' end of the sequencing primer. In some embodiments, step (b) further comprises identifying the nucleo-based of the chain terminating nucleotide.

[0453] In some embodiments, the methods for sequencing further comprise step (c): removing the chain terminating moiety from the chain terminating nucleotide incorporated into the 3' end of the sequencing primer to generate an extendible 3'OH group. In some embodiments, step (c) further comprises removing the detectable label from the chain terminating nucleotide. In some embodiments, the sequencing polymerase remains bound to the template molecule which is hybridized to the sequencing primer which is extended by one nucleo-base.

[0454] In some embodiments, the methods for sequencing further comprise step (d): repeating steps (b) and (c) at least once. In some embodiments, the methods for sequencing further comprise repeating steps (b) and (c), at least 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150 or 200 or more times.

Two-Stage Methods for Nucleic Acid Sequencing

[0455] The present disclosure provides a two-stage method for sequencing any of the nucleic acid template molecules described herein. In some embodiments, the nucleic acid template molecules are immobilized. In some embodiments, the first stage comprises binding multivalent molecules to complexed polymerases to form multivalent-complexed polymerases and detecting the multivalent-complexed polymerases.

[0456] In some embodiments, the first stage comprises step (a): contacting a plurality of a first sequencing polymerase to (i) a plurality of nucleic acid template molecules and (ii) a plurality of nucleic acid sequencing primers, wherein the contacting is conducted under a condition suitable to bind the plurality of first sequencing polymerases to the plurality of

nucleic acid template molecules and the plurality of nucleic acid primers thereby forming a plurality of first complexed polymerases each comprising a first sequencing polymerase bound to a nucleic acid duplex, wherein the nucleic acid duplex comprises a nucleic acid template molecule hybridized to a nucleic acid primer. In some embodiments, the first polymerase comprises a recombinant mutant sequencing polymerase.

[0457] In some embodiments, in the methods for sequencing nucleic acid template molecules, the sequencing primer comprises an oligonucleotide having a 3' extendible end or a 3' non-extendible end. In some embodiments, the plurality of nucleic acid template molecules comprises amplified template molecules (e.g., clonally amplified template molecules). In some embodiments, the plurality of nucleic acid template molecules comprises one copy of a target sequence of interest. In some embodiments, the plurality of nucleic acid molecules comprises two or more tandem copies of a target sequence of interest (e.g., concatemer template molecules). In some embodiments, the nucleic acid template molecules in the plurality of nucleic acid template molecules comprise the same target sequence of interest or different target sequences of interest. In some embodiments, the plurality of nucleic acid template molecules, or the plurality of nucleic acid primers, or combinations thereof, are in solution or are immobilized to a support. In some embodiments, when the plurality of nucleic acid template molecules, or the plurality of nucleic acid primers, or combinations thereof, are immobilized to a support, the binding with the first sequencing polymerase generates a plurality of immobilized first complexed polymerases. In some embodiments, the plurality of nucleic acid template molecules, or nucleic acid primers, or combinations thereof, are immobilized to $10^2 – 10^{15}$ different sites on a support. In some embodiments, the binding of the plurality of nucleic acid template molecules and nucleic acid primers with the plurality of first sequencing polymerases generates a plurality of first complexed polymerases immobilized to $10^2 – 10^{15}$ different sites on the support. In some embodiments, the plurality of immobilized first complexed polymerases on the support are immobilized to pre-determined or to random sites on the support. In some embodiments, the plurality of immobilized first complexed polymerases are in fluid communication with each other to permit flowing a solution of reagents (e.g., enzymes including sequencing polymerases, multivalent molecules, nucleotides, or divalent cations, or combinations thereof) onto the support so that the plurality of immobilized complexed polymerases on the support are reacted with the solution of reagents in a massively parallel manner.

[0458] In some embodiments, the methods for sequencing further comprise step (b): contacting the plurality of first complexed polymerases with a plurality of multivalent

molecules to form a plurality of multivalent-complexed polymerases (e.g., binding complexes). In some embodiments, individual multivalent molecules in the plurality of multivalent molecules comprise a core attached to multiple nucleotide arms and each nucleotide arm is attached to a nucleotide (e.g., nucleotide unit) (e.g., FIGS. 9-13). In some embodiments, the contacting of step (b) is conducted under a condition suitable for binding complementary nucleotide units of the multivalent molecules to at least two of the plurality of first complexed polymerases, thereby forming a plurality of multivalent-complexed polymerases. In some embodiments, the condition is suitable for inhibiting polymerase-catalyzed incorporation of the complementary nucleotide units into the primers of the plurality of multivalent-complexed polymerases. In some embodiments, the plurality of multivalent molecules comprises at least one multivalent molecule having multiple nucleotide arms (e.g., FIGS. 9-12) each attached with a nucleotide analog (e.g., nucleotide analog unit), where the nucleotide analog includes a chain terminating moiety at the sugar 2' position, or 3' position, or combinations thereof. In some embodiments, the plurality of multivalent molecules comprises at least one multivalent molecule comprising multiple nucleotide arms each attached with a nucleotide unit that lacks a chain terminating moiety. In some embodiments, at least one of the multivalent molecules in the plurality of multivalent molecules is labeled with a detectable reporter moiety that emits a signal. In some embodiments, the detectable reporter moiety comprises a fluorophore. In some embodiments, the contacting of step (b) is conducted in the presence of at least one non-catalytic cation comprising strontium, barium, or calcium, or combinations thereof.

[0459] In some embodiments, the methods for sequencing further comprises step (c): detecting the plurality of multivalent-complexed polymerases. In some embodiments, the detecting includes detecting the signals emitted by the multivalent molecules that are bound to the complexed polymerases, where the complementary nucleotide units of the multivalent molecules are bound to the primers but incorporation of the complementary nucleotide units is inhibited. In some embodiments, the multivalent molecules are labeled with a detectable reporter moiety (e.g., a fluorophore) to permit detection. In some embodiments, the labeled multivalent molecules comprise a fluorophore attached to the core, linker, or nucleotide unit, or combinations thereof, of the multivalent molecules.

[0460] In some embodiments, the methods for sequencing further comprise step (d): identifying the nucleo-base of the complementary nucleotide units that are bound to the plurality of first complexed polymerases, thereby determining the sequence of the template molecule. In some embodiments, the multivalent molecules are labeled with a detectable

reporter moiety that corresponds to the particular nucleotide units attached to the nucleotide arms to permit identification of the complementary nucleotide units (e.g., nucleotide base adenine, guanine, cytosine, thymine or uracil) that are bound to the plurality of first complexed polymerases.

[0461] In some embodiments, the methods for sequencing further comprises step (e): dissociating the plurality of multivalent-complexed polymerases and removing the plurality of first sequencing polymerases and their bound multivalent molecules, and retaining the plurality of nucleic acid duplexes.

[0462] In some embodiments, the second stage of the two-stage sequencing method may comprise nucleotide incorporation. In some embodiments, the methods for sequencing further comprises step (f): contacting the plurality of the retained nucleic acid duplexes of step (e) with a plurality of second sequencing polymerases, wherein the contacting is conducted under a condition suitable for binding the plurality of second sequencing polymerases to the plurality of the retained nucleic acid duplexes, thereby forming a plurality of second complexed polymerases each comprising a second sequencing polymerase bound to a nucleic acid duplex. In some embodiments, the second sequencing polymerase comprises a recombinant mutant sequencing polymerase.

[0463] In some embodiments, the plurality of first sequencing polymerases of step (a) has an amino acid sequence that is 100% identical to the amino acid sequence as the plurality of the second sequencing polymerases of step (f). In some embodiments, the plurality of first sequencing polymerases of step (a) has an amino acid sequence that differs from the amino acid sequence of the plurality of the second sequencing polymerases of step (f).

[0464] In some embodiments, the methods for sequencing further comprise step (g): contacting the plurality of second complexed polymerases with a plurality of nucleotides, wherein the contacting is conducted under a condition suitable for binding complementary nucleotides from the plurality of nucleotides to at least one of the second complexed polymerases thereby forming a plurality of nucleotide-complexed polymerases. In some embodiments, the contacting of step (g) is conducted under a condition that is suitable for promoting polymerase-catalyzed incorporation of the bound complementary nucleotides into the primers of the nucleotide-complexed polymerases thereby extending the sequencing primer by one nucleo-base. In some embodiments, the incorporating the nucleotide into the 3' end of the sequencing primer in step (g) comprises a primer extension reaction. In some embodiments, the contacting of step (g) is conducted in the presence of at least one catalytic cation comprising magnesium, or manganese, or combinations thereof. In some

embodiments, the plurality of nucleotides comprises native nucleotides (e.g., non-analog nucleotides) or nucleotide analogs. In some embodiments, the plurality of nucleotides comprises a 2', or 3', or combinations thereof, chain terminating moiety which is removable or is not removable. In some embodiments, at least one of the nucleotides in the plurality is not labeled with a detectable reporter moiety (e.g., a fluorophore). In some embodiments, the plurality of nucleotides is non-labeled. In some embodiments, the plurality of nucleotides comprises a plurality of nucleotides labeled with a detectable reporter moiety. In some embodiments, the detectable reporter moiety comprises a fluorophore. In some embodiments, the fluorophore is attached to the nucleotide base. In some embodiments, the fluorophore is attached to the nucleotide base with a linker which is cleavable/removable from the base or is not removable from the base. In some embodiments, a particular detectable reporter moiety (e.g., fluorophore) that is attached to the nucleotide can correspond to the nucleotide base (e.g., dATP, dGTP, dCTP, dTTP or dUTP) to permit detection and identification of the nucleotide base.

[0465] In some embodiments, when the plurality of nucleotides in step (g) is detectably labeled, the methods for sequencing further comprise step (h): detecting the complementary nucleotides which are incorporated into the primers of the nucleotide-complexed polymerases. In some embodiments, the plurality of nucleotides is labeled with a detectable reporter moiety to permit detection. In some embodiments, when the plurality of nucleotides in step (g) is non-labeled, the detecting of step (h) is omitted.

[0466] In some embodiments, when the plurality of nucleotides in step (g) is detectably labeled, the methods for sequencing further comprise step (i): identifying the bases of the complementary nucleotides which are incorporated into the primers of the nucleotide-complexed polymerases. In some embodiments, the identification of the incorporated complementary nucleotides in step (i) can be used to confirm the identity of the complementary nucleotides of the multivalent molecules that are bound to the plurality of first complexed polymerases in step (d). In some embodiments, the identifying of step (i) can be used to determine the sequence of the nucleic acid template molecules. In some embodiments, when the plurality of nucleotides in step (g) is non-labeled, the identifying of step (i) is omitted.

[0467] In some embodiments, the methods for sequencing further comprise step (j): removing the chain terminating moiety from the incorporated nucleotide when step (g) is conducted by contacting the plurality of second complexed polymerases with a plurality of

nucleotides that comprise at least one nucleotide having a 2', or 3', or combinations thereof, chain terminating moiety.

[0468] In some embodiments, the methods for sequencing further comprise step (k): repeating steps (b) – (j) or steps (a) –(j) at least once. In some embodiments, the sequence of the nucleic acid template molecules can be determined by detecting and identifying the multivalent molecules that bind the sequencing polymerases but do not incorporate into the 3' end of the primer at steps (c) and (d). In some embodiments, the sequence of the nucleic acid template molecules can be determined (or confirmed) by detecting and identifying the nucleotide that incorporates into the 3' end of the primer at steps (h) and (i).

[0469] In some embodiments, in any of the methods for sequencing nucleic acid molecules, the binding of the plurality of first complexed polymerases with the plurality of multivalent molecules forms at least one avidity complex, the method comprising the steps: (a) binding a first nucleic acid primer, a first sequencing polymerase, and a first multivalent molecule to a first portion of a concatemer template molecule thereby forming a first binding complex, wherein a first nucleotide unit of the first multivalent molecule binds to the first sequencing polymerase; and (b) binding a second nucleic acid primer, a second sequencing polymerase, and the first multivalent molecule to a second portion of the same concatemer template molecule thereby forming a second binding complex, wherein a second nucleotide unit of the first multivalent molecule binds to the second sequencing polymerase, wherein the first and second binding complexes which include the same multivalent molecule forms an avidity complex. In some embodiments, the first sequencing polymerase comprises any wild type or mutant polymerase described herein. In some embodiments, the second sequencing polymerase comprises any wild type or mutant polymerase described herein. In some embodiments, the concatemer template molecule comprises tandem repeat sequences of a sequence of interest and at least one universal sequencing primer binding site. In some embodiments, concatemer template molecule comprises tandem repeat sequences of a sequence of interest, at least one universal sequencing primer binding site, and an index sequence. The first and second nucleic acid primers can bind to a sequencing primer binding site along the concatemer template molecule. For example, multivalent molecules are shown in FIGS. 9-12.

[0470] In some embodiments, in any of the methods for sequencing nucleic acid molecules, wherein the method includes binding the plurality of first complexed polymerases with the plurality of multivalent molecules to form at least one avidity complex, the method comprising the steps: (a) contacting the plurality of sequencing polymerases and the plurality

of nucleic acid primers with different portions of a concatemer template molecule to form at least first and second complexed polymerases on the same concatemer template molecule; (b) contacting a plurality of multivalent molecules to the at least first and second complexed polymerases on the same concatemer template molecule, under conditions suitable to bind a single multivalent molecule from the plurality to the first and second complexed polymerases, wherein at least a first nucleotide unit of the single multivalent molecule is bound to the first complexed polymerase which includes a first primer hybridized to a first portion of the concatemer template molecule thereby forming a first binding complex (e.g., first ternary complex), and wherein at least a second nucleotide unit of the single multivalent molecule is bound to the second complexed polymerase which includes a second primer hybridized to a second portion of the concatemer template molecule thereby forming a second binding complex (e.g., second ternary complex), wherein the contacting is conducted under a condition suitable to inhibit polymerase-catalyzed incorporation of the bound first and second nucleotide units in the first and second binding complexes, and wherein the first and second binding complexes which are bound to the same multivalent molecule forms an avidity complex; and (c) detecting the first and second binding complexes on the same concatemer template molecule, and (d) identifying the first nucleotide unit in the first binding complex thereby determining the sequence of the first portion of the concatemer template molecule, and identifying the second nucleotide unit in the second binding complex thereby determining the sequence of the second portion of the concatemer template molecule. In some embodiments, the plurality of sequencing polymerases comprise any wild type or mutant sequencing polymerase described herein. In some embodiments, concatemer template molecule comprises tandem repeat sequences of a sequence of interest and at least one universal sequencing primer binding site. In some embodiments, concatemer template molecule comprises tandem repeat sequences of a sequence of interest, at least one universal sequencing primer binding site, and an index sequence. In some embodiments, plurality of nucleic acid primers can bind to a sequencing primer binding site along the concatemer template molecule. Exemplary multivalent molecules for use in the sequencing methods described herein are shown in FIGS. 9-12.

Sequencing-by-Binding

[0471] The present disclosure provides methods for sequencing any of the immobilized template molecules described herein, wherein the sequencing methods comprise a sequencing-by-binding (SBB) procedure which employs non-labeled chain-terminating nucleotides. In some embodiments, the sequencing-by-binding (SBB) method comprises the

steps of (a) sequentially contacting a primed template nucleic acid with at least two separate mixtures under ternary complex stabilizing conditions, wherein the at least two separate mixtures each include a polymerase and a nucleotide, whereby the sequentially contacting results in the primed template nucleic acid being contacted, under the ternary complex stabilizing conditions, with nucleotide cognates for first, second and third base types in the template; (b) examining the at least two separate mixtures to determine whether a ternary complex formed; and (c) identifying the next correct nucleotide for the primed template nucleic acid molecule, wherein the next correct nucleotide is identified as a cognate of the first, second or third base type if ternary complex is detected in step (b), and wherein the next correct nucleotide is imputed to be a nucleotide cognate of a fourth base type based on the absence of a ternary complex in step (b); (d) adding a next correct nucleotide to the primer of the primed template nucleic acid after step (b), thereby producing an extended primer; and (e) repeating steps (a) through (d) at least once on the primed template nucleic acid that comprises the extended primer. For example, sequencing-by-binding methods are described in U.S. Patent Nos. 10,246,744 and 10,731,141 (where the contents of both patents are hereby incorporated by reference in their entireties).

Methods for Sequencing using Phosphate-Chain Labeled Nucleotides

[0472] The present disclosure provides methods for sequencing using immobilized sequencing polymerases which bind non-immobilized template molecules, wherein the sequencing reactions are conducted with phosphate-chain labeled nucleotides. In some embodiments, the sequencing methods comprise step (a): providing a support having a plurality of sequencing polymerases immobilized thereon. In some embodiments, the sequencing polymerase comprises a processive DNA polymerase. In some embodiments, the sequencing polymerase comprises a wild type or mutant DNA polymerase, including, for example, a Phi29 DNA polymerase. In some embodiments, the support comprises a plurality of separate compartments and a sequencing polymerase that is immobilized to the bottom of a compartment. In some embodiments, the separate compartments comprise a silica bottom through which light can penetrate. In some embodiments, the separate compartments comprise a silica bottom configured with a nanophotonic confinement structure comprising a hole in a metal cladding film (e.g., aluminum cladding film). In some embodiments, the hole in the metal cladding has a small aperture, for example, approximately 70 nm. In some embodiments, the height of the nanophotonic confinement structure is approximately 100 nm. In some embodiments, the nanophotonic confinement structure comprises a zero mode

waveguide (ZMW). In some embodiments, the nanophotonic confinement structure contains a liquid.

[0473] In some embodiments, the sequencing method further comprises step (b): contacting the plurality of immobilized sequencing polymerases with a plurality of single stranded circular nucleic acid template molecules and a plurality of oligonucleotide sequencing primers, under a condition suitable for individual immobilized sequencing polymerases to bind a single stranded circular template molecule, and suitable for individual sequencing primers to hybridize to individual single stranded circular template molecules, thereby generating a plurality of polymerase/template/primer complexes. In some embodiments, the individual sequencing primers hybridize to a universal sequencing primer binding site on the single stranded circular template molecule.

[0474] In some embodiments, the sequencing method further comprises step (c): contacting the plurality of polymerase/template/primer complexes with a plurality of phosphate chain labeled nucleotides each comprising an aromatic base, a five carbon sugar (e.g., ribose or deoxyribose), and a phosphate chain comprising 3-20 phosphate groups, where the terminal phosphate group is linked to a detectable reporter moiety (e.g., a fluorophore). The first, second and third phosphate groups can be referred to as alpha, beta and gamma phosphate groups. In some embodiments, a particular detectable reporter moiety which is attached to the terminal phosphate group corresponds to the nucleotide base (e.g., dATP, dGTP, dCTP, dTTP or dUTP) to permit detection and identification of the nucleo-base. In some embodiments, the plurality of polymerase/template/primer complexes is contacted with the plurality of phosphate chain labeled nucleotides under a condition suitable for polymerase-catalyzed nucleotide incorporation. In some embodiments, the sequencing polymerases are capable of binding a complementary phosphate chain labeled nucleotide and incorporating the complementary nucleotide opposite a nucleotide in a template molecule. In some embodiment, the polymerase-catalyzed nucleotide incorporation reaction cleaves between the alpha and beta phosphate groups thereby releasing a multi-phosphate chain linked to a fluorophore.

[0475] In some embodiments, the sequencing method further comprises step (d): detecting the fluorescent signal emitted by the phosphate chain labeled nucleotide that is bound by the sequencing polymerase and incorporated into the terminal end of the sequencing primer. In some embodiments, step (d) further comprises identifying the phosphate chain labeled nucleotide that is bound by the sequencing polymerase and incorporated into the terminal end of the sequencing primer.

[0476] In some embodiments, the sequencing method further comprises step (d): repeating steps (c) – (d) at least once. In some embodiments, sequencing methods that employ phosphate chain labeled nucleotides can be conducted according to the methods described in U.S. Patent Nos. 7,170,050; 7,302,146; or 7,405,281, or combinations thereof, all of which are hereby incorporated by reference in their entireties.

Sequencing Polymerases

[0477] The present disclosure provides methods for sequencing nucleic acid molecules, where any of the sequencing methods described herein employ at least one type of sequencing polymerase and a plurality of nucleotides or employ at least one type of sequencing polymerase and a plurality of nucleotides and a plurality of multivalent molecules. In some embodiments, the sequencing polymerase(s) is/are capable of incorporating a complementary nucleotide opposite a nucleotide in a template molecule. In some embodiments, the sequencing polymerase(s) is/are capable of binding a complementary nucleotide unit of a multivalent molecule opposite a nucleotide in a template molecule. In some embodiments, the plurality of sequencing polymerases comprises recombinant mutant polymerases.

[0478] Examples of suitable polymerases for use in sequencing with nucleotides, or multivalent molecules, or combinations thereof, include but are not limited to: Klenow DNA polymerase; T*hermus aquaticus* DNA polymerase I (Taq polymerase); KlenTaq polymerase; *Candidatus altiarchaeales archaeon*; *Candidatus Hadarchaeum Yellowstonense*; *Hadesarchaea archaeon; Euryarchaeota archaeon*; T*hermoplasmata archaeon*; *Thermococcus* polymerases such as *Thermococcus litoralis*, bacteriophage T7 DNA polymerase; human alpha, delta and epsilon DNA polymerases; bacteriophage polymerases such as T4, RB69 and phi29 bacteriophage DNA polymerases; *Pyrococcus furiosus* DNA polymerase (Pfu polymerase); *Bacillus subtilis* DNA polymerase III; *E. coli* DNA polymerase III alpha and epsilon; 9 degree N polymerase; reverse transcriptases such as HIV type M or O reverse transcriptases; avian myeloblastosis virus reverse transcriptase; Moloney Murine Leukemia Virus (MMLV) reverse transcriptase; or telomerase. Further non-limiting examples of DNA polymerases include those from various Archaea genera, such as, *Aeropyrum*, *Archaeglobus*, *Desulfurococcus*, *Pyrobaculum*, *Pyrococcus*, *Pyrolobus*, *Pyrodictium*, *Staphylothermus*, *Stetteria*, *Sulfolobus*, *Thermococcus*, and *Vulcanisaeta* and the like or variants thereof, including existing polymerases such as 9 degrees N, VENT®, DEEP VENT®, THERMINATOR®, Pfu, KOD, Pfx, Tgo and RB69 polymerases. The phi29

DNA polymerase can be wild type phi29 DNA polymerase (*e.g.*, MagniPhi™ from Expedeon™), or variant EquiPhi29™ DNA polymerase (*e.g.*, from ThermoFisher Scientific®), or chimeric QualiPhi™ DNA polymerase (*e.g.*, from 4basebio™). Additional suitable polymerases are described, for example, in U.S. Patent No. 11,859,241, the contents of which are incorporated by reference in their entirety herein.

Nucleotides

[0479] The present disclosure provides methods for sequencing nucleic acid molecules, where any of the sequencing methods described herein employ at least one nucleotide. The nucleotides comprise a base, a sugar and at least one phosphate group. In some embodiments, at least one nucleotide in the plurality comprises an aromatic base, a five-carbon sugar (e.g., ribose or deoxyribose), and one or more phosphate groups (e.g., 1-10 phosphate groups). The plurality of nucleotides can comprise at least one type of nucleotide selected from a group consisting of dATP, dGTP, dCTP, dTTP and dUTP. The plurality of nucleotides can comprise at a mixture of any combination of two or more types of nucleotides selected from a group consisting of dATP, dGTP, dCTP, dTTP, or dUTP, or combinations thereof. In some embodiments, at least one nucleotide in the plurality is not a nucleotide analog. In some embodiments, at least one nucleotide in the plurality comprises a nucleotide analog.

[0480] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, at least one nucleotide in the plurality of nucleotides comprise a chain of one, two or three phosphorus atoms where the chain may be attached to the 5' carbon of the sugar moiety via an ester or phosphoramide linkage. In some embodiments, at least one nucleotide in the plurality is an analog having a phosphorus chain in which the phosphorus atoms are linked together with intervening O, S, NH, methylene or ethylene. In some embodiments, the phosphorus atoms in the chain include substituted side groups including O, S or BH_3. In some embodiments, the chain includes phosphate groups substituted with analogs including phosphoramidate, phosphorothioate, phosphorodithioate, and O-methylphosphoramidite groups.

[0481] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, at least one nucleotide in the plurality of nucleotides comprises a terminator nucleotide analog having a chain terminating moiety (e.g., blocking moiety) at the sugar 2' position, at the sugar 3' position, or at the sugar 2' and 3' position. In some embodiments, the chain terminating moiety can inhibit polymerase-catalyzed incorporation of a subsequent nucleotide unit or free nucleotide in a nascent strand during a primer extension reaction. In some embodiments, the chain terminating moiety is attached to the 3' sugar position where

the sugar comprises a ribose or deoxyribose sugar moiety. In some embodiments, the chain terminating moiety is removable/cleavable from the 3' sugar position to generate a nucleotide having a 3'OH sugar group which is extendible with a subsequent nucleotide in a polymerase-catalyzed nucleotide incorporation reaction. In some embodiments, the chain terminating moiety comprises an alkyl group, alkenyl group, alkynyl group, allyl group, aryl group, benzyl group, azide group, amine group, amide group, keto group, isocyanate group, phosphate group, thio group, disulfide group, carbonate group, urea group, silyl or acetal group. In some embodiments, the chain terminating moiety is cleavable/removable from the nucleotide, for example by reacting the chain terminating moiety with a chemical agent, pH change, light or heat. In some embodiments, the chain terminating moieties alkyl, alkenyl, alkynyl and allyl are cleavable with tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$), with piperidine, or with 2,3-Dichloro-5,6-dicyano-1,4-benzo-quinone (DDQ). In some embodiments, the chain terminating moieties aryl and benzyl are cleavable with H2 Pd/C. In some embodiments, the chain terminating moieties amine, amide, keto, isocyanate, phosphate, thio, or disulfide are cleavable with phosphine or with a thiol group including beta-mercaptoethanol or dithiothritol (DTT). In some embodiments, the chain terminating moiety carbonate is cleavable with potassium carbonate (K_2CO_3) in MeOH, with triethylamine in pyridine, or with Zn in acetic acid (AcOH). In some embodiments, the chain terminating moieties urea and silyl are cleavable with tetrabutylammonium fluoride, pyridine-HF, with ammonium fluoride, or with triethylamine trihydrofluoride. In some embodiments, the chain terminating moiety may be cleavable/removable with nitrous acid. In some embodiments, a chain terminating moiety may be cleavable/removable using a solution comprising nitrite, such as, for example, a combination of nitrite with an acid such as acetic acid, sulfuric acid, or nitric acid. In some further embodiments, said solution may comprise an organic acid.

[0482] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, at least one nucleotide in the plurality of nucleotides comprises a terminator nucleotide analog having a chain terminating moiety (e.g., blocking moiety) at the sugar 2' position, at the sugar 3' position, or at the sugar 2' and 3' position. In some embodiments, the chain terminating moiety comprises an azide, azido or azidomethyl group. In some embodiments, the chain terminating moiety comprises a 3'-O-azido or 3'-O-azidomethyl group. In some embodiments, the chain terminating moieties azide, azido and azidomethyl groups are cleavable/removable with a phosphine compound. In some embodiments, the phosphine compound comprises a derivatized tri-alkyl phosphine moiety or a derivatized tri-

aryl phosphine moiety. In some embodiments, the phosphine compound comprises Tris(2-carboxyethyl)phosphine (TCEP) or bis-sulfo triphenyl phosphine (BS-TPP) or Tri(hydroxyproyl)phosphine (THPP). In some embodiments, the cleaving agent comprises 4-dimethylaminopyridine (4-DMAP). In some embodiments, the chain terminating moiety comprising one or more of a 3'-O-amino group, a 3'-O-aminomethyl group, a 3'-O-methylamino group, or derivatives thereof may be cleaved with nitrous acid, through a mechanism utilizing nitrous acid, or using a solution comprising nitrous acid. In some embodiments, the chain terminating moiety comprising one or more of a 3'-O-amino group, a 3'-O-aminomethyl group, a 3'-O-methylamino group, or derivatives thereof may be cleaved using a solution comprising nitrite. In some embodiments, for example, nitrite may be combined with or contacted with an acid such as acetic acid, sulfuric acid, or nitric acid. In some further embodiments, for example, nitrite may be combined with or contacted with an organic acid such as, for example, formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, or the like. In some embodiments, the chain terminating moiety comprises a 3'-acetal moiety which can be cleaved with a palladium deblocking reagent (e.g., Pd(0)).

[0483] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, the nucleotide comprises a chain terminating moiety which is selected from a group consisting of 3'-deoxy nucleotides, 2',3'-dideoxynucleotides, 3'-methyl, 3'-azido, 3'-azidomethyl, 3'-O-azidoalkyl, 3'-O-ethynyl, 3'-O-aminoalkyl, 3'-O-fluoroalkyl, 3'-fluoromethyl, 3'-difluoromethyl, 3'-trifluoromethyl, 3'-sulfonyl, 3'-malonyl, 3'-amino, 3'-O-amino, 3'-sulfhydral, 3'-aminomethyl, 3'-ethyl, 3'butyl, 3'-*tert* butyl, 3'-Fluorenylmethyloxycarbonyl, 3' *tert*-Butyloxycarbonyl, 3'-O-alkyl hydroxylamino group, 3'-phosphorothioate, 3-O-benzyl, and 3'-O-benzyl, 3-acetal moiety, or derivatives thereof.

[0484] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, the plurality of nucleotides comprises a plurality of nucleotides labeled with a detectable reporter moiety. In some embodiments, the detectable reporter moiety comprises a fluorophore. In some embodiments, the fluorophore is attached to the nucleotide base. In some embodiments, the fluorophore is attached to the nucleotide base with a linker which is cleavable/removable from the base. In some embodiments, at least one of the nucleotides in the plurality is not labeled with a detectable reporter moiety. In some embodiments, a particular detectable reporter moiety (e.g., fluorophore) that is attached to the nucleotide can correspond to the nucleotide base (e.g., dATP, dGTP, dCTP, dTTP or dUTP) to permit detection and identification of the nucleotide base.

[0485] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, the nucleotide comprises a cleavable linker on the nucleotide base. In some embodiments, the cleavable linker on the nucleotide base comprises a cleavable moiety comprising an alkyl group, alkenyl group, alkynyl group, allyl group, aryl group, benzyl group, azide group, amine group, amide group, keto group, isocyanate group, phosphate group, thio group, disulfide group, carbonate group, urea group, or silyl group. In some embodiments, the cleavable linker on the base is cleavable/removable from the base by reacting the cleavable moiety with a chemical agent, pH change, light or heat. In some embodiments, the cleavable moieties alkyl, alkenyl, alkynyl and allyl are cleavable with tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$), with piperidine, or with 2,3-Dichloro-5,6-dicyano-1,4-benzo-quinone (DDQ). In some embodiments, the cleavable moieties aryl and benzyl are cleavable with H2 Pd/C. In some embodiments, the cleavable moieties amine, amide, keto, isocyanate, phosphate, thio, or disulfide are cleavable with phosphine or with a thiol group including beta-mercaptoethanol or dithiothritol (DTT). In some embodiments, the cleavable moiety carbonate is cleavable with potassium carbonate (K_2CO_3) in MeOH, with triethylamine in pyridine, or with Zn in acetic acid (AcOH). In some embodiments, the cleavable moieties urea and silyl are cleavable with tetrabutylammonium fluoride, pyridine-HF, with ammonium fluoride, or with triethylamine trihydrofluoride.

[0486] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, the cleavable linker on the nucleotide base comprises cleavable moiety including an azide, azido or azidomethyl group. In some embodiments, the cleavable moieties azide, azido and azidomethyl group are cleavable/removable with a phosphine compound. In some embodiments, the phosphine compound comprises a derivatized tri-alkyl phosphine moiety or a derivatized tri-aryl phosphine moiety. In some embodiments, the phosphine compound comprises Tris(2-carboxyethyl)phosphine (TCEP) or bis-sulfo triphenyl phosphine (BS-TPP) or Tri(hydroxyproyl)phosphine (THPP). In some embodiments, the cleaving agent comprises 4-dimethylaminopyridine (4-DMAP).

[0487] In some embodiments, in any of the methods for sequencing nucleic acid molecules described herein, the chain terminating moiety (e.g., at the sugar 2' position, or sugar 3' position, or combinations thereof) and the cleavable linker on the nucleotide base have the same or different cleavable moieties. In some embodiments, the chain terminating moiety (e.g., at the sugar 2' position, or sugar 3' position, or combinations thereof) and the detectable reporter moiety linked to the base are chemically cleavable/removable with the same chemical agent. In some embodiments, the chain terminating moiety (e.g., at the sugar 2'

position, or sugar 3' position, or combinations thereof) and the detectable reporter moiety linked to the base are chemically cleavable/removable with different chemical agents.

Multivalent Molecules

[0488] The present disclosure provides methods for sequencing nucleic acid molecules, where any of the sequencing methods described herein employ at least one multivalent molecule. In some embodiments, the multivalent molecule comprises a plurality of nucleotide arms attached to a core and having any configuration including a starburst, helter skelter, or bottle brush configuration (e.g., FIG. 11). The multivalent molecule comprises: (1) a core; and (2) a plurality of nucleotide arms which comprise (i) a core attachment moiety, (ii) a spacer comprising a PEG moiety, (iii) a linker, and (iv) a nucleotide unit, wherein the core is attached to the plurality of nucleotide arms, wherein the spacer is attached to the linker, wherein the linker is attached to the nucleotide unit. In some embodiments, the nucleotide unit comprises a base, sugar and at least one phosphate group, and the linker is attached to the nucleotide unit through the base. In some embodiments, the linker comprises an aliphatic chain or an oligo ethylene glycol chain where both linker chains having 2-6 subunits. In some embodiments, the linker also includes an aromatic moiety. An example of a nucleotide arm is shown in FIG. 15. Examples of multivalent molecules are shown in FIGS. 11-14. An example of a spacer is shown in FIG. 16 (top) and examples of linkers are shown in FIG. 16 (bottom) and FIG. 17. Examples of nucleotides attached to a linker are shown in FIGS. 18-21. An example of a biotinylated nucleotide arm is shown in FIG. 22.

[0489] In some embodiments, a multivalent molecule comprises a core attached to multiple nucleotide arms, wherein the multiple nucleotide arms have the same type of nucleotide unit which is selected from the group consisting of dATP, dGTP, dCTP, dTTP and dUTP.

[0490] In some embodiments, a multivalent molecule comprises a core attached to multiple nucleotide arms, where each arm includes a nucleotide unit. In some embodiments, the nucleotide unit comprises an aromatic base, a five carbon sugar (e.g., ribose or deoxyribose), and one or more phosphate groups (e.g., 1-10 phosphate groups). In some embodiments, the plurality of multivalent molecules can comprise one type of multivalent molecule having one type of nucleotide unit selected from a group consisting of dATP, dGTP, dCTP, dTTP and dUTP. The plurality of multivalent molecules can comprise a mixture of any combination of two or more types of multivalent molecules, where individual multivalent molecules in the mixture comprise nucleotide units selected from the group consisting of dATP, dGTP, dCTP, dTTP, or dUTP, or combinations thereof.

[0491] In some embodiments, the nucleotide unit comprises a chain of one, two or three phosphorus atoms where the chain may be attached to the 5' carbon of the sugar moiety via an ester or phosphoramide linkage. In some embodiments, at least one nucleotide unit is a nucleotide analog having a phosphorus chain in which the phosphorus atoms are linked together with intervening O, S, NH, methylene or ethylene. In some embodiments, the phosphorus atoms in the chain include substituted side groups including O, S or BH_3. In some embodiments, the chain includes phosphate groups substituted with analogs including phosphoramidate, phosphorothioate, phosphorodithioate, and O-methylphosphoramidite groups.

[0492] In some embodiments, the multivalent molecule comprises a core attached to multiple nucleotide arms, wherein individual nucleotide arms comprise a nucleotide unit which is a nucleotide analog having a chain terminating moiety (e.g., blocking moiety) at the sugar 2' position, at the sugar 3' position, or at the sugar 2' and 3' position. In some embodiments, the nucleotide unit comprises a chain terminating moiety (e.g., blocking moiety) at the sugar 2' position, at the sugar 3' position, or at the sugar 2' and 3' position. In some embodiments, the chain terminating moiety can inhibit polymerase-catalyzed incorporation of a subsequent nucleotide unit or free nucleotide in a nascent strand during a primer extension reaction. In some embodiments, the chain terminating moiety is attached to the 3' sugar position where the sugar comprises a ribose or deoxyribose sugar moiety. In some embodiments, the chain terminating moiety is removable/cleavable from the 3' sugar position to generate a nucleotide having a 3'OH sugar group which is extendible with a subsequent nucleotide in a polymerase-catalyzed nucleotide incorporation reaction. In some embodiments, the chain terminating moiety comprises an alkyl group, alkenyl group, alkynyl group, allyl group, aryl group, benzyl group, azide group, amine group, amide group, keto group, isocyanate group, phosphate group, thio group, disulfide group, carbonate group, urea group, or silyl group. In some embodiments, the chain terminating moiety is cleavable/removable from the nucleotide unit, for example by reacting the chain terminating moiety with a chemical agent, pH change, light or heat. In some embodiments, the chain terminating moieties alkyl, alkenyl, alkynyl and allyl are cleavable with tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$), with piperidine, or with 2,3-Dichloro-5,6-dicyano-1,4-benzo-quinone (DDQ). In some embodiments, the chain terminating moieties aryl and benzyl are cleavable with H2 Pd/C. In some embodiments, the chain terminating moieties amine, amide, keto, isocyanate, phosphate, thio, disulfide are cleavable with phosphine or with a thiol group including beta-mercaptoethanol or dithiothritol (DTT).

In some embodiments, the chain terminating moiety carbonate is cleavable with potassium carbonate (K_2CO_3) in MeOH, with triethylamine in pyridine, or with Zn in acetic acid (AcOH). In some embodiments, the chain terminating moieties urea and silyl are cleavable with tetrabutylammonium fluoride, pyridine-HF, with ammonium fluoride, or with triethylamine trihydrofluoride.

[0493] In some embodiments, the nucleotide unit comprises a chain terminating moiety (e.g., blocking moiety) at the sugar 2' position, at the sugar 3' position, or at the sugar 2' and 3' position. In some embodiments, the chain terminating moiety comprises an azide, azido or azidomethyl group. In some embodiments, the chain terminating moiety comprises a 3'-O-azido or 3'-O-azidomethyl group. In some embodiments, the chain terminating moieties azide, azido and azidomethyl group are cleavable/removable with a phosphine compound. In some embodiments, the phosphine compound comprises a derivatized tri-alkyl phosphine moiety or a derivatized tri-aryl phosphine moiety. In some embodiments, the phosphine compound comprises Tris(2-carboxyethyl)phosphine (TCEP), or bis-sulfo triphenyl phosphine (BS-TPP) or Tri(hydroxyproyl)phosphine (THPP). In some embodiments, the cleaving agent comprises 4-dimethylaminopyridine (4-DMAP).

[0494] In some embodiments, the nucleotide unit comprising a chain terminating moiety which is selected from a group consisting of 3'-deoxy nucleotides, 2',3'-dideoxynucleotides, 3'-methyl, 3'-azido, 3'-azidomethyl, 3'-O-azidoalkyl, 3'-O-ethynyl, 3'-O-aminoalkyl, 3'-O-fluoroalkyl, 3'-fluoromethyl, 3'-difluoromethyl, 3'-trifluoromethyl, 3'-sulfonyl, 3'-malonyl, 3'-amino, 3'-O-amino, 3'-sulfhydral, 3'-aminomethyl, 3'-ethyl, 3'butyl, 3'-*tert* butyl, 3'-Fluorenylmethyloxycarbonyl, 3' *tert*-Butyloxycarbonyl, 3'-O-alkyl hydroxylamino group, 3'-phosphorothioate, and 3-O-benzyl, or derivatives thereof.

[0495] In some embodiments, the multivalent molecule comprises a core attached to multiple nucleotide arms, wherein the nucleotide arms comprise a spacer, linker and nucleotide unit, and wherein the core, linker, or nucleotide unit, or combinations thereof, are labeled with a detectable reporter moiety. In some embodiments, the detectable reporter moiety comprises a fluorophore. In some embodiments, a particular detectable reporter moiety (e.g., fluorophore) that is attached to the multivalent molecule can correspond to the base (e.g., dATP, dGTP, dCTP, dTTP or dUTP) of the nucleotide unit to permit detection and identification of the nucleotide base.

[0496] In some embodiments, at least one nucleotide arm of a multivalent molecule has a nucleotide unit that is attached to a detectable reporter moiety. In some embodiments, the detectable reporter moiety is attached to the nucleotide base. In some embodiments, the

detectable reporter moiety comprises a fluorophore. In some embodiments, a particular detectable reporter moiety (e.g., fluorophore) that is attached to the multivalent molecule can correspond to the base (e.g., dATP, dGTP, dCTP, dTTP or dUTP) of the nucleotide unit to permit detection and identification of the nucleotide base.

[0497] In some embodiments, the core of a multivalent molecule comprises an avidin-like or streptavidin-like moiety and the core attachment moiety comprises biotin. In some embodiments, the core comprises a streptavidin-type or avidin-type moiety which includes an avidin protein, as well as any derivatives, analogs and other non-native forms of avidin that can bind to at least one biotin moiety. Other forms of avidin moieties include native and recombinant avidin and streptavidin as well as derivatized molecules, e.g. non-glycosylated avidin and truncated streptavidins. For example, avidin moiety includes de-glycosylated forms of avidin, bacterial streptavidin produced by *Streptomyces* (e.g., *Streptomyces avidinii*), as well as derivatized forms, for example, N-acyl avidins, e.g., N-acetyl, N-phthalyl and N-succinyl avidin, and the commercially-available products EXTRAVIDIN®, CAPTAVIDIN®, NEUTRAVIDIN® and NEUTRALITE AVIDIN®.

[0498] In some embodiments, any of the methods for sequencing nucleic acid molecules described herein can include forming a binding complex, where the binding complex comprises (i) a polymerase, a nucleic acid template molecule duplexed with a primer, and a nucleotide, or the binding complex comprises (ii) a polymerase, a nucleic acid template molecule duplexed with a primer, and a nucleotide unit of a multivalent molecule. In some embodiments, the binding complex has a persistence time of greater than about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1 second. The binding complex may have a persistence time of greater than about 0.1-0.25 seconds, or about 0.25-0.5 seconds, or about 0.5-0.75 seconds, or about 0.75-1 second, or about 1-2 seconds, or about 2-3 seconds, or about 3-4 second, or about 4-5 seconds, or wherein the method is or may be carried out at a temperature of at or above 15 °C, at or above 20 °C, at or above 25 °C, at or above 35 °C, at or above 37 °C, at or above 42 °C at or above 55 °C at or above 60 °C, or at or above 72 °C, or at or above 80 °C, or within a range defined by any of the foregoing, or combinations thereof. The binding complex (e.g., ternary complex) remains stable until subjected to a condition that causes dissociation of interactions between any of the polymerase, template molecule, primer, or the nucleotide unit or the nucleotide, or combinations thereof. For example, a dissociating condition comprises contacting the binding complex with any one or any combination of a detergent, EDTA, or water, or combinations thereof. In some embodiments, the present

disclosure provides said method wherein the binding complex is deposited on, attached to, or hybridized to, a surface showing a contrast to noise ratio in the detecting step of greater than 20. In some embodiments, the present disclosure provides said method wherein the contacting is performed under a condition that stabilizes the binding complex when the nucleotide or nucleotide unit is complementary to a next base of the template nucleic acid, and destabilizes the binding complex when the nucleotide or nucleotide unit is not complementary to the next base of the template nucleic acid.

Compaction Oligonucleotides

[0499] In some aspects, compaction oligonucleotide comprises a single-stranded linear oligonucleotide having a 5' region that can hybridize to a first portion of a concatemer template molecule and the compaction oligonucleotide having a 3' region that can hybridize to a second portion of the concatemer template molecule (e.g., the same concatemer template molecule). In some embodiments, hybridization of the compaction oligonucleotides to an individual concatemer template molecules causes the concatemer template molecule to collapse or fold into a DNA nanoball which is more compact in shape and size compared to a non-collapsed DNA molecule. A spot image of a DNA nanoball can be represented as a Gaussian spot and the size can be measured as a full width half maximum (FWHM). A smaller spot size as indicated by a smaller FWHM may correlate with an improved image of the spot. In some embodiments, the FWHM of a DNA nanoball spot can be about 10 μm or smaller. The DNA nanoball can be a compact nucleic acid structure having a full width half maximum (FWHM) that is smaller compared to a concatemer that is not collapsed/folded into a DNA nanoball.

[0500] In some embodiments, compaction oligonucleotides comprise single stranded oligonucleotides comprising DNA, RNA, or a combination of DNA and RNA. The compaction oligonucleotides can be any length, including 20-150 nucleotides, or 30-100 nucleotides, or 40-80 nucleotides in length.

[0501] In some embodiments, the compaction oligonucleotides comprise a 5' region and a 3' region, and optionally an intervening region between the 5' and 3' regions. The intervening region can be any length, for example about 2-20 nucleotides in length. In some embodiments, the intervening region comprises a homopolymer having consecutive identical bases (e.g., AAA, GGG, CCC, TTT or UUU). In some embodiments, the intervening region comprises a non-homopolymer sequence.

[0502] The 5' region of the compaction oligonucleotides can be wholly complementary or partially complementary along its length to a first portion of a concatemer template molecule. The 3' region of the compaction oligonucleotides can be wholly complementary or partially complementary along its length to a second portion of a concatemer template molecule. The 5' region of the compaction oligonucleotides can hybridize to a first universal sequence portion of a concatemer template molecule. The 3' region of the compaction oligonucleotides can hybridize to a second universal sequence portion of a concatemer template molecule. The 5' and 3' regions of the compaction oligonucleotide can hybridize to the concatemer to pull together distal portions of the concatemer causing compaction of the concatemer to form a DNA nanoball.

[0503] The 5' region of the compaction oligonucleotide can have the same sequence as the 3' region of the compaction oligonucleotide. The 5' region of the compaction oligonucleotide can have a sequence that is different from the 3' region of the compaction oligonucleotide. The 3' region of the compaction oligonucleotide can have a sequence that is a reverse sequence of the 5' region of the compaction oligonucleotide.

[0504] The skilled artisan will appreciate that the compaction oligonucleotides described herein are merely exemplary. Compaction oligonucleotides with other architectures (e.g., branched, forked, and/or comprising double stranded and single stranded regions) may be suitable for the methods described herein.

Supports and Low Non-Specific Coatings

[0505] In some embodiments, the flow cell devices described herein in can include a support, e.g., a solid support as disclosed herein. The present disclosure provides pairwise sequencing compositions and methods which employ a support comprising a plurality of oligonucleotide surface primers (e.g., surface capture primers) immobilized thereon. In some embodiments, the support is passivated with a low non-specific binding coating. The surface coatings described herein may exhibit very low non-specific binding to reagents that may be used for nucleic acid capture, amplification and sequencing workflows, such as dyes, nucleotides, enzymes, and nucleic acid primers. The surface coatings exhibit low background fluorescence signals or high contrast-to-noise (CNR) ratios compared to existing surface coatings.

[0506] The low non-specific binding coating may comprise one layer or multiple layers (FIG. 23). In some embodiments, the plurality of surface capture primers is immobilized to the low non-specific binding coating. In some embodiments, at least one surface capture

primer is embedded within the low non-specific binding coating. The low non-specific binding coating may enable improved nucleic acid hybridization and amplification performance. The supports may comprise a substrate (or support structure), one or more layers of a covalently or non-covalently attached low-binding, chemical modification layers, e.g., silane layers, polymer films, and one or more covalently or non-covalently attached surface capture primers that can be used for tethering single-stranded nucleic acid library molecules to the support. In some embodiments, the formulation of the coating, e.g., the chemical composition of one or more layers, the coupling chemistry used to cross-link the one or more layers to the support, or to each other, or combinations thereof, and the total number of layers, may be varied such that non-specific binding of proteins, nucleic acid molecules, and other hybridization and amplification reaction components to the coating are minimized or reduced relative to a comparable monolayer. The formulation of the coating described herein may be varied such that non-specific hybridization on the coating is minimized or reduced relative to a comparable monolayer. The formulation of the coating may be varied such that non-specific amplification on the coating is minimized or reduced relative to a comparable monolayer. The formulation of the coating may be varied such that specific amplification rates, or yields, or combinations thereof, on the coating are maximized. Amplification levels suitable for detection are achieved in no more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, or more than 30 amplification cycles in some cases disclosed herein.

[0507] In the support structure that comprises the one or more chemically-modified layers, e.g., layers of a low non-specific binding polymer, the layers may be independent or integrated into another structure or assembly. For example, in some embodiments, the support structure may comprise one or more surfaces within an integrated or assembled microfluidic flow cell. The support structure may comprise one or more surfaces within a microplate format, e.g., the bottom surface of the wells in a microplate. In some embodiments, the support structure comprises the interior surface (such as the lumen surface) of a capillary. In some embodiments, the support structure comprises the interior surface (such as the lumen surface) of a capillary etched into a planar chip.

[0508] The attachment chemistry used to graft a first chemically-modified layer to the surface of the support may be dependent on both the material from which the surface (e.g., the surface of the support structure, such as a flow cell) is fabricated and the chemical nature of the layer. In some embodiments, the first layer may be covalently attached to the surface. In some embodiments, the first layer may be non-covalently attached, e.g., adsorbed to the support through non-covalent interactions such as electrostatic interactions, hydrogen

bonding, or van der Waals interactions between the support and the molecular components of the first layer. In either case, the support may be treated prior to attachment or deposition of the first layer. Any of a variety of existing surface preparation techniques may be used to clean or treat the surface. For example, glass or silicon surfaces may be acid-washed using a Piranha solution (a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2)), base treatment in KOH and NaOH, or cleaned using an oxygen plasma treatment method, or combinations thereof.

[0509] Silane chemistries constitute non-limiting approaches for covalently modifying the silanol groups on glass or silicon surfaces to attach more reactive functional groups (e.g., amines or carboxyl groups), which may then be used in coupling linker molecules (e.g., linear hydrocarbon molecules of various lengths, such as C6, C12, C18 hydrocarbons, or linear polyethylene glycol (PEG) molecules) or layer molecules (e.g., branched PEG molecules or other polymers) to the surface. Examples of suitable silanes that may be used in creating any of the disclosed low binding coatings include, but are not limited to, (3-Aminopropyl) trimethoxysilane (APTMS), (3-Aminopropyl) triethoxysilane (APTES), any of a variety of PEG-silanes (e.g., comprising molecular weights of 1K, 2K, 5K, 10K, 20K, etc.), amino-PEG silane (e.g., comprising a free amino functional group), maleimide-PEG silane, biotin-PEG silane, and the like.

[0510] Any of a variety of existing molecules including, but not limited to, amino acids, peptides, nucleotides, oligonucleotides, other monomers or polymers, or combinations thereof may be used in creating the one or more chemically-modified layers on the support, where the choice of components used may be varied to alter one or more properties of the layers, e.g., the surface density of functional groups, or tethered oligonucleotide primers, or combinations thereof, the hydrophilicity/hydrophobicity of the layers, or the three three-dimensional nature (e.g., "thickness") of the layer. Examples of polymers that may be used to create one or more layers of low non-specific binding material in any of the disclosed coatings include, but are not limited to, polyethylene glycol (PEG) of various molecular weights and branching structures, streptavidin, polyacrylamide, polyester, dextran, poly-lysine, and poly-lysine copolymers, or any combination thereof. Examples of conjugation chemistries that may be used to graft one or more layers of material (e.g. polymer layers) to the surface, or to cross-link the layers to each other, or combinations thereof, include, but are not limited to, biotin-streptavidin interactions (or variations thereof), his tag – Ni/NTA conjugation chemistries, methoxy ether conjugation chemistries, carboxylate conjugation

chemistries, amine conjugation chemistries, NHS esters, maleimides, thiol, epoxy, azide, hydrazide, alkyne, isocyanate, and silane.

[0511] The low non-specific binding surface coating may be applied uniformly across the support. Alternatively, the surface coating may be patterned, such that the chemical modification layers are confined to one or more discrete regions of the support. For example, the coating may be patterned using photolithographic techniques to create an ordered array or random pattern of chemically-modified regions on the support. Alternately, or in combination, the coating may be patterned using, e.g., contact printing techniques, or ink-jet printing techniques, or combinations thereof. In some embodiments, an ordered array or random pattern of chemically-modified regions may comprise at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10,000 or more discrete regions.

[0512] In some embodiments, the low nonspecific binding coatings comprise hydrophilic polymers that are non-specifically adsorbed or covalently grafted to the support. Passivation may be performed utilizing poly(ethylene glycol) (PEG, also known as polyethylene oxide (PEO) or polyoxyethylene) or other hydrophilic polymers with different molecular weights and end groups that are linked to a support using, for example, silane chemistry. The end groups distal from the surface can include, but are not limited to, biotin, methoxy ether, carboxylate, amine, NHS ester, maleimide, and bis-silane. In some embodiments, two or more layers of a hydrophilic polymer, e.g., a linear polymer, branched polymer, or multi-branched polymer, may be deposited on the surface. In some embodiments, two or more layers may be covalently coupled to each other or internally cross-linked to improve the stability of the resulting coating. In some embodiments, surface primers with different nucleotide sequences, or base modifications (or other biomolecules, e.g., enzymes or antibodies), or combinations thereof, may be tethered to the resulting layer at various surface densities. In some embodiments, for example, both surface functional group density and surface primer concentration may be varied to attain a surface primer density range. Additionally, surface primer density can be controlled by diluting the surface primers with other molecules that carry the same functional group. For example, amine-labeled surface primers can be diluted with amine-labeled polyethylene glycol in a reaction with an NHS-ester coated surface to reduce the final primer density. Surface primers with different lengths of linker between the hybridization region and the surface attachment functional group can also be applied to control surface density. Example of suitable linkers include poly-T and poly-A strands at the 5' end of the primer (e.g., 0 to 20 bases), PEG linkers (e.g., 3 to 20

monomer units), and carbon-chain (e.g., C6, C12, C18, etc.). To measure the primer density, fluorescently-labeled primers may be tethered to the surface and a fluorescence reading then compared with that for a dye solution of an existing concentration.

[0513] In some embodiments, the low nonspecific binding coatings comprise a functionalized polymer coating layer covalently bound at least to a portion of the support via a chemical group on the support, a primer grafted to the functionalized polymer coating, and a water-soluble protective coating on the primer and the functionalized polymer coating. In some embodiments, the functionalized polymer coating comprises a poly(N-(5-azidoacetamidylpentyl)acrylamide-co-acrylamide (PAZAM).

[0514] In order to scale primer surface density and add additional dimensionality to hydrophilic or amphoteric coatings, supports comprising multi-layer coatings of PEG and other hydrophilic polymers have been developed. By using hydrophilic and amphoteric surface layering approaches that include, but are not limited to, the polymer/co-polymer materials described below, it is possible to increase primer loading density on the support significantly. Existing PEG coating approaches use monolayer primer deposition, which may be reported for single molecule applications, but do not yield high copy numbers for nucleic acid amplification applications. As described herein "layering" can be accomplished using existing crosslinking approaches with any compatible polymer or monomer subunits such that a surface comprising two or more highly crosslinked layers can be built sequentially. Examples of suitable polymers include, but are not limited to, streptavidin, poly acrylamide, polyester, dextran, poly-lysine, and copolymers of poly-lysine and PEG. In some embodiments, the different layers may be attached to each other through any of a variety of conjugation reactions including, but not limited to, biotin-streptavidin binding, azide-alkyne click reaction, amine-NHS ester reaction, thiol-maleimide reaction, and ionic interactions between positively charged polymers and negatively charged polymers. In some embodiments, high primer density materials may be constructed in solution and subsequently layered onto the surface in multiple steps.

[0515] Examples of materials from which the support structure may be fabricated include, but are not limited to, glass, fused-silica, silicon, a polymer (e.g. polystyrene (PS)), macroporous polystyrene (MPPS), polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyethylene terephthalate (PET)), or any combination thereof. Various compositions of both glass and plastic support structures are contemplated.

[0516] The support structure may be rendered in any of a variety of existing geometries and dimensions, and may comprise any of a variety of existing materials. For example, the support structure may be locally planar (e.g., comprising a microscope slide or the surface of a microscope slide) and may be of a rectangular shape, e.g., FIGS. 2, 4, 5A-5F, 24A-24E, 25A-25E, 26A-26C, 27A-27C, and 28A-28C. Globally, the support structure may be cylindrical (e.g., comprising a capillary or the interior surface of a capillary), spherical (e.g., comprising the outer surface of a non-porous bead), or irregular (e.g., comprising the outer surface of an irregularly-shaped, non-porous bead or particle). In some embodiments, the surface of the support structure used for nucleic acid hybridization and amplification may be a solid, non-porous surface. In some embodiments, the surface of the support structure used for nucleic acid hybridization and amplification may be porous, such that the coatings described herein penetrate the porous surface, and nucleic acid hybridization and amplification reactions performed thereon may occur within the pores.

[0517] The surface of the support can be coated with one or more compounds to produce a passivated layer on the support. In some embodiments, the support comprises a low non-specific binding surface that enable improved nucleic acid hybridization and amplification performance on the support. The support may comprise one or more layers of a covalently or non-covalently attached low-binding, chemical modification layers, e.g., silane layers, polymer films, and one or more covalently or non-covalently attached oligonucleotides that may be used for immobilizing a plurality of nucleic acid template molecules to the support.

[0518] The support structure that comprises the one or more chemically-modified layers, e.g., layers of a low non-specific binding polymer, may be independent or integrated into another structure or assembly. For example, the support structure may comprise one or more surfaces within an integrated or assembled microfluidic flow cell. The support structure may comprise one or more surfaces within a microplate format, e.g., the bottom surface of the wells in a microplate. In some embodiments, the support structure comprises the interior surface (such as the lumen surface) of a capillary. In some embodiments the support structure comprises the interior surface (such as the lumen surface) of a capillary etched into a planar chip.

[0519] As noted, the low non-specific binding supports of the present disclosure exhibit reduced non-specific binding of proteins, nucleic acids, and other components of the hybridization formulation, or amplification formulation, or combinations thereof, used for solid-phase nucleic acid amplification. The degree of non-specific binding exhibited by a given support surface may be assessed either qualitatively or quantitatively. For example,

exposure of the surface to fluorescent dyes (e.g., cyanins such as Cy3, or Cy5, etc., fluoresceins, coumarins, rhodamines, etc. or other dyes disclosed herein), fluorescently-labeled nucleotides, fluorescently-labeled oligonucleotides, or fluorescently-labeled proteins (e.g. polymerases), or combinations thereof, under a standardized set of conditions, followed by a specified rinse protocol and fluorescence imaging, may be used as a qualitative tool for comparison of non-specific binding on supports comprising different surface formulations. In some embodiments, exposure of the surface to fluorescent dyes, fluorescently-labeled nucleotides, fluorescently-labeled oligonucleotides, or fluorescently-labeled proteins (e.g. polymerases), or combinations thereof, under a standardized set of conditions, followed by a specified rinse protocol and fluorescence imaging, may be used as a quantitative tool for comparison of non-specific binding on supports comprising different surface formulations—provided that care has been taken to ensure that the fluorescence imaging is performed under conditions where fluorescence signal is linearly related (or related in a predictable manner) to the number of fluorophores on the support surface (e.g., under conditions where signal saturation, or self-quenching, or combinations thereof, of the fluorophore is not an issue) and suitable calibration standards are used. In some embodiments, other existing techniques, for example, radioisotope labeling and counting methods, may be used for quantitative assessment of the degree to which non-specific binding is exhibited by the different support surface formulations of the present disclosure.

[0520] Some surfaces disclosed herein exhibit a ratio of specific to nonspecific binding of a fluorophore such as Cy3 of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100, or greater than 100, or any intermediate value spanned by the range herein. Some surfaces disclosed herein exhibit a ratio of specific to nonspecific fluorescence of a fluorophore such as Cy3 of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100, or greater than 100, or any intermediate value spanned by the range herein.

[0521] The degree of non-specific binding exhibited by the disclosed low-binding supports may be assessed using a standardized protocol for contacting the surface with a labeled protein (e.g., bovine serum albumin (BSA), streptavidin, a DNA polymerase, a reverse transcriptase, a helicase, a single-stranded binding protein (SSB), etc., or any combination thereof), a labeled nucleotide, a labeled oligonucleotide, etc., under a standardized set of incubation and rinse conditions, followed by detection of the amount of label remaining on the surface and comparison of the signal resulting therefrom to an appropriate calibration standard. In some embodiments, the label may comprise a fluorescent label. In some

embodiments, the label may comprise a radioisotope. In some embodiments, the label may comprise any other existing detectable label. In some embodiments, the degree of non-specific binding exhibited by a given support surface formulation may thus be assessed in terms of the number of non-specifically bound protein molecules (or nucleic acid molecules or other molecules) per unit area. In some embodiments, the low-binding supports of the present disclosure may exhibit non-specific protein binding (or non-specific binding of other specified molecules, (e.g., cyanins such as Cy3, or Cy5, etc., fluoresceins, coumarins, rhodamines, etc. or other dyes disclosed herein)) of less than 0.001 molecule per μm^2, less than 0.01 molecule per μm^2, less than 0.1 molecule per μm^2, less than 0.25 molecule per μm^2, less than 0.5 molecule per μm^2, less than 1 molecule per μm^2, less than 10 molecules per μm^2, less than 100 molecules per μm^2, or less than 1,000 molecules per μm^2. A given support surface of the present disclosure may exhibit non-specific binding falling anywhere within this range, for example, of less than 86 molecules per μm^2. For example, some modified surfaces disclosed herein exhibit nonspecific protein binding of less than 0.5 molecule/μm^2 following contact with a 1 μM solution of Cy3 labeled streptavidin (GE® Amersham) in phosphate buffered saline (PBS) buffer for 15 minutes, followed by 3 rinses with deionized water. Some modified surfaces disclosed herein exhibit nonspecific binding of Cy3 dye molecules of less than 0.25 molecules per μm^2. As an example, in independent nonspecific binding assays, 1 μM labeled Cy3 SA (Thermo Fisher Scientific®), 1 μM Cy5 SA dye (Thermo Fisher Scientific), 10 μM Aminoallyl-dUTP-ATTO-647N (Jena Biosciences), 10 μM Aminoallyl-dUTP-ATTO-Rhol 1 (Jena Biosciences), 10 μM Aminoallyl-dUTP-ATTO-Rhol 1 (Jena Biosciences), 10 μM 7-Propargylamino-7-deaza-dGTP-Cy5 (Jena Biosciences, and 10 μM 7-Propargylamino-7-deaza-dGTP-Cy3 (Jena Biosciences) were incubated on the low binding coated supports at 37° C for 15 minutes in a 384 well plate format. Each well was rinsed 2-3× with 50 μl of deionized RNase/DNase Free water and 2-3× with 25 mM ACES buffer pH 7.4. The 384 well plates were imaged on a GE Typhoon™ instrument using the Cy3, Alexa Fluor® AF555, or Cy5 filter sets (according to dye test performed) as specified by the manufacturer at a PMT gain setting of 800 and resolution of 50-100 μm. For higher resolution imaging, images were collected on an Olympus® IX83 microscope (e.g., inverted fluorescence microscope) (Olympus Corp., Center Valley, Pa.) with a total internal reflectance fluorescence (TIRF) objective (100×, 1.5 NA, Olympus), a CCD camera (e.g., an Olympus EM-CCD monochrome camera, Olympus XM-10 monochrome camera, or an Olympus DP80 color and monochrome camera), an illumination source (e.g., an Olympus 100W Hg lamp, an Olympus 75W Xe lamp, or an

Olympus U-HGLGPS fluorescence light source), and excitation wavelengths of 532 nm or 635 nm. Dichroic mirrors were purchased from Semrock (IDEX Health & Science, LLC, Rochester, N.Y.), e.g., 405, 488, 532, or 633 nm dichroic reflectors/beamsplitters, and band pass filters were chosen as 532 LP or 645 LP concordant with the appropriate excitation wavelength. Some modified surfaces disclosed herein exhibit nonspecific binding of dye molecules of less than 0.25 molecules per μm^2. In some embodiments, the coated support was immersed in a buffer (e.g., 25 mM ACES, pH 7.4) while the image was acquired.

[0522] In some embodiments, the surfaces disclosed herein exhibit a ratio of specific to nonspecific binding of a fluorophore such as Cy3 of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100, or greater than 100, or any intermediate value spanned by the range herein, using the assays described herein. In some embodiments, the surfaces disclosed herein exhibit a ratio of specific to nonspecific fluorescence signals for a fluorophore such as Cy3 of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100, or greater than 100, or any intermediate value spanned by the range herein.

[0523] The low-background surfaces consistent with the disclosure herein may exhibit specific dye attachment (e.g., Cy3 attachment) to non-specific dye adsorption (e.g., Cy3 dye adsorption) ratios of at least 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 30:1, 40:1, 50:1, or more than 50 specific dye molecules attached per molecule nonspecifically adsorbed. Similarly, when subjected to an excitation energy, low-background surfaces consistent with the disclosure herein to which fluorophores, e.g., Cy3, have been attached may exhibit ratios of specific fluorescence signal (e.g., arising from Cy3-labeled oligonucleotides attached to the surface) to non-specific adsorbed dye fluorescence signals of at least 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 30:1, 40:1, 50:1, or more than 50:1.

[0524] In some embodiments, the degree of hydrophilicity (or "wettability" with aqueous solutions) of the disclosed support surfaces may be assessed, for example, through the measurement of water contact angles in which a small droplet of water is placed on the surface and its angle of contact with the surface is measured using, e.g., an optical tensiometer. In some embodiments, a static contact angle may be determined. In some embodiments, an advancing or receding contact angle may be determined. In some embodiments, the water contact angle for the hydrophilic, low-binding support surface disclosed herein may range from about 0 degrees to about 30 degrees. In some embodiments, the water contact angle for the hydrophilic, low-binding support surfaced disclosed herein may be no more than 50 degrees, 40 degrees, 30 degrees, 25 degrees, 20 degrees, 18 degrees,

16 degrees, 14 degrees, 12 degrees, 10 degrees, 8 degrees, 6 degrees, 4 degrees, 2 degrees, or 1 degree. In many cases, the contact angle may not be more than 40 degrees. A given hydrophilic, low-binding support surface of the present disclosure may exhibit a water contact angle having a value of anywhere within this range.

[0525] In some embodiments, the hydrophilic surfaces disclosed herein facilitate reduced wash times for bioassays, often due to reduced nonspecific binding of biomolecules to the low-binding surfaces. In some embodiments, adequate wash steps may be performed in less than 60, 50, 40, 30, 20, 15, 10, or less than 10 seconds. For example, adequate wash steps may be performed in less than 30 seconds.

[0526] Some low-binding surfaces of the present disclosure exhibit significant improvement in stability or durability to prolonged exposure to solvents and elevated temperatures, or to repeated cycles of solvent exposure or changes in temperature. For example, the stability of the disclosed surfaces may be tested by fluorescently labeling a functional group on the surface, or a tethered biomolecule (e.g., an oligonucleotide primer) on the surface, and monitoring fluorescence signal before, during, and after prolonged exposure to solvents and elevated temperatures, or to repeated cycles of solvent exposure or changes in temperature. In some embodiments, the degree of change in the fluorescence used to assess the quality of the surface may be less than 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, or 25% over a time period of 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 60 minutes, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 15 hours, 20 hours, 25 hours, 30 hours, 35 hours, 40 hours, 45 hours, 50 hours, or 100 hours of exposure to solvents, or elevated temperatures, or combinations thereof (or any combination of these percentages as measured over these time periods). In some embodiments, the degree of change in the fluorescence used to assess the quality of the surface may be less than 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, or 25% over 5 cycles, 10 cycles, 20 cycles, 30 cycles, 40 cycles, 50 cycles, 60 cycles, 70 cycles, 80 cycles, 90 cycles, 100 cycles, 200 cycles, 300 cycles, 400 cycles, 500 cycles, 600 cycles, 700 cycles, 800 cycles, 900 cycles, or 1,000 cycles of repeated exposure to solvent changes, or changes in temperature, or combinations thereof (or any combination of these percentages as measured over this range of cycles).

[0527] In some embodiments, the surfaces disclosed herein may exhibit a high ratio of specific signal to nonspecific signal or other background. For example, when used for nucleic acid amplification, some surfaces may exhibit an amplification signal that is at least 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 75, 100, or greater than 100-fold greater than a signal of an

adjacent unpopulated region of the surface. Similarly, some surfaces exhibit an amplification signal that is at least 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 75, 100, or greater than 100-fold greater than a signal of an adjacent amplified nucleic acid population region of the surface.

[0528] In some embodiments, fluorescence images of the disclosed low background surfaces when used in nucleic acid hybridization or amplification applications to create polonies of hybridized or clonally-amplified nucleic acid molecules (e.g., that have been directly or indirectly labeled with a fluorophore) exhibit contrast-to-noise ratios (CNRs) of at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 20, 210, 220, 230, 240, 250, or greater than 250.

[0529] One or more types of primer may be attached or tethered to the support surface. In some embodiments, the one or more type of adaptors or primers may comprise spacer sequences, adaptor sequences for hybridization to adaptor-ligated target library nucleic acid sequences, surface capture primers, surface pinning primers (e.g., used to "pin" a second portion of a template molecule to the support surface), forward amplification primers, reverse amplification primers, sequencing primers, or molecular barcoding sequences, or any combination thereof. In some embodiments, 1 primer or adaptor sequence may be tethered to at least one layer of the surface. In some embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 different primer or adaptor sequences may be tethered to at least one layer of the surface.

[0530] In some embodiments, the tethered adaptor, or primer sequences, or combinations thereof, may range in length from about 10 nucleotides to about 100 nucleotides. In some embodiments, the tethered adaptor, or primer sequences, or combinations thereof, may be at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 nucleotides in length. In some embodiments, the tethered adaptor, or primer sequences, or combinations thereof, may be at most 100, at most 90, at most 80, at most 70, at most 60, at most 50, at most 40, at most 30, at most 20, or at most 10 nucleotides in length. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some embodiments the length of the tethered adaptor, or primer sequences, or combinations thereof, may range from about 20 nucleotides to about 80 nucleotides. The length of the tethered adaptor, or primer sequences, or combinations thereof, may have any value within this range, e.g., about 24 nucleotides.

[0531] In some embodiments, the resultant surface density of primers (e.g., capture primers) on the low binding support surfaces of the present disclosure may range from about

100 primer molecules per μm^2 to about 100,000 primer molecules per μm^2. In some embodiments, the resultant surface density of primers on the low binding support surfaces of the present disclosure may range from about 1,000 primer molecules per μm^2 to about 1,000,000 primer molecules per μm^2. In some embodiments, the surface density of primers may be at least 1,000, at least 10,000, at least 100,000, or at least 1,000,000 molecules per μm^2. In some embodiments, the surface density of primers may be at most 1,000,000, at most 100,000, at most 10,000, or at most 1,000 molecules per μm^2. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some embodiments the surface density of primers may range from about 10,000 molecules per μm^2 to about 100,000 molecules per μm^2. The surface density of primer molecules may have any value within this range, e.g., about 455,000 molecules per μm^2. In some embodiments, the surface density of target library nucleic acid sequences initially hybridized to adaptor or primer sequences on the support surface may be less than or equal to that indicated for the surface density of tethered primers. In some embodiments, the surface density of clonally-amplified target library nucleic acid sequences hybridized to adaptor or primer sequences on the support surface may span the same range as that indicated for the surface density of tethered primers.

[0532] Local densities as listed above do not preclude variation in density across a surface, such that a surface may comprise a region having an oligo density of, for example, 500,000/μm^2, while also comprising at least a second region having a substantially different local density.

[0533] In some embodiments, the performance of nucleic acid hybridization, or amplification reactions, or combinations thereof, using the disclosed reaction formulations and low-binding supports may be assessed using fluorescence imaging techniques, where the contrast-to-noise ratio (CNR) of the images provides a key metric in assessing amplification specificity and non-specific binding on the support. CNR is commonly defined as: CNR=(Signal-Background)/Noise. The background term is commonly taken to be the signal measured for the interstitial regions surrounding a particular feature (diffraction limited spot, DLS) in a specified region of interest (ROI). While signal-to-noise ratio (SNR) is often considered to be a benchmark of overall signal quality, it can be shown that improved CNR can provide a significant advantage over SNR as a benchmark for signal quality in applications that require rapid image capture (e.g., sequencing applications for which cycle times may be minimized). At high CNR, the imaging time required to reach accurate discrimination (and thus accurate base-calling in the case of sequencing applications) can be

drastically reduced even with moderate improvements in CNR. Improved CNR in imaging data on the imaging integration time provides a method for more accurately detecting features such as clonally-amplified nucleic acid colonies on the support surface.

[0534] In most ensemble-based sequencing approaches, the background term may be measured as the signal associated with "interstitial" regions, i.e. the regions between polonies. In addition to "interstitial" background(B_{inter}), "intrastitial" background (B_{intra}) exists within the region occupied by an amplified DNA colony. The combination of these two background signals dictates the achievable CNR, and subsequently directly impacts the optical instrument requirements, architecture costs, reagent costs, run-times, cost/genome, and ultimately the accuracy and data quality for cyclic array-based sequencing applications. The B_{inter} background signal arises from a variety of sources; a few examples include auto-fluorescence from consumable flow cells, non-specific adsorption of detection molecules that yield spurious fluorescence signals that may obscure the signal from the ROI, and the presence of non-specific DNA amplification products (e.g., those arising from primer dimers). In existing next generation sequencing (NGS) applications, this background signal in the current field-of-view (FOV) may be averaged over time and subtracted. The signal arising from individual DNA colonies (e.g., (Signal)-B(interstitial) in the FOV) yields a discernable feature that can be classified. In some embodiments, the intrastitial background (B(intrastitial)) can contribute a confounding fluorescence signal that is not specific to the target of interest, but is present in the same ROI, thus making it far more difficult to average and subtract.

[0535] Nucleic acid amplification on the low-binding coated supports described herein may decrease the B(interstitial) background signal by reducing non-specific binding, may lead to improvements in specific nucleic acid amplification, and may lead to a decrease in non-specific amplification that can impact the background signal arising from both the interstitial and intrastitial regions. In some embodiments, the disclosed low-binding coated supports, optionally used in combination with the disclosed hybridization, or amplification reaction formulations, or combinations thereof, may lead to improvements in CNR by a factor of 2, 5, 10, 100, 250, 500 or 1000-fold over those achieved using existing supports and hybridization, amplification, or sequencing protocols, or combinations thereof. Although described here in the context of using fluorescence imaging as the read-out or detection mode, the same principles apply to the use of the disclosed low-binding coated supports and nucleic acid hybridization and amplification formulations for other detection modes as well, including both optical and non-optical detection modes.

[0536] The headings provided herein are not limitations of the various aspects of the disclosure, which aspects can be understood by reference to the specification as a whole.

Definitions:

[0537] Unless defined otherwise, technical and scientific terms used herein have meanings that are commonly understood by those of ordinary skill in the art unless defined otherwise. Generally, terminologies pertaining to techniques of molecular biology, nucleic acid chemistry, protein chemistry, genetics, microbiology, transgenic cell production, and hybridization described herein are those well-known and commonly used in the art. Techniques and procedures described herein are generally performed according to existing methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the instant specification. For example, see Sambrook et al., Molecular Cloning: A Laboratory Manual (Third ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 2000), which is hereby incorporated by reference. See also Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992), which is hereby incorporated by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques described herein are those well-known and commonly used in the art.

[0538] Unless otherwise required by context herein, singular terms shall include pluralities and plural terms shall include the singular. Singular forms “a”, “an” and “the”, and singular use of any word, include plural referents unless expressly and unequivocally limited on one referent.

[0539] It is understood the use of the alternative term (e.g., “or”) is taken to mean either one or both or any combination thereof of the alternatives.

[0540] The term “and/or” used herein is to be taken to mean specific disclosure of each of the specified features or components with or without the other. For example, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include: “A and B”; “A or B”; “A” (A alone); and “B” (B alone). In a similar manner, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects: “A, B, and C”; “A, B, or C”; “A or C”; “A or B”; “B or C”; “A and B”; “B and C”; “A and C”; “A” (A alone); “B” (B alone); and “C” (C alone).

[0541] As used herein and in the appended claims, the terms “comprising”, “including”, “having” and “containing”, and their grammatical variants, as used herein are intended to be non-limiting so that one item or multiple items in a list do not exclude other items that can be substituted or added to the listed items. It is understood that wherever aspects are described

herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of", or "consisting essentially of", or combinations thereof, are also provided.

[0542] As used herein, the terms "about," "approximately," and "substantially" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, e.g., the limitations of the measurement system. For example, "about," "approximately," or "substantially" can mean within one or more than one standard deviation per the practice in the art. Alternatively, "about" or "approximately" can mean a range of up to 10% (e.g., ±10%) or more depending on the limitations of the measurement system. For example, about 5 mg can include any number between 4.5 mg and 5.5 mg. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the instant disclosure, unless otherwise stated, the meaning of "about," "approximately," and "substantially" can be assumed to be within an acceptable error range for that particular value or composition. Also, where ranges, or subranges, or combinations thereof, of values are provided, the ranges, or subranges, or combinations thereof, can include the endpoints of the ranges, or subranges, or combinations thereof.

[0543] As disclosed herein, a "reagent" can be a substance or compound that facilitate or otherwise alter the likelihood of a reaction or test, e.g., a chemical reaction or a sequencing reaction. A reagent can be a mixture of substances or compounds. Different reagents herein can be different compounds or different mixtures of compounds. Different reagents herein can also be different solutions, e.g., with different concentrations, or different level of contaminations, of the same compound or same mixture of compounds.

[0544] The term "glass" refers to silica-based material, including silicate, borosilicate, fused silica, fused quartz, glass, quartz, or lead glass.

[0545] The term "polony" used herein refers to a nucleic acid library molecule that can be clonally amplified in-solution or on-support to generate an amplicon that can serve as a template molecule for sequencing. In some embodiments, a linear library molecule can be circularized to generate a circularized library molecule, and the circularized library molecule can be clonally amplified in-solution or on-support to generate a concatemer molecule. In some embodiments, the concatemer molecule can serve as a nucleic acid template molecule which can be sequenced. The concatemer molecule is sometimes referred to as a polony.

Alternatively, a polony can include a plurality of template molecules. In some embodiments, a polony includes nucleotide strands.

[0546] The terms "peptide", "polypeptide" and "protein" and other related terms used herein are used interchangeably and refer to a polymer of amino acids and are not limited to any particular length. Polypeptides may comprise natural and non-natural amino acids. Polypeptides include recombinant or chemically-synthesized forms. Polypeptides also include precursor molecules that have not yet been subjected to post-translation modification such as proteolytic cleavage, cleavage due to ribosomal skipping, hydroxylation, methylation, lipidation, acetylation, SUMOylation, ubiquitination, glycosylation, phosphorylation, or disulfide bond formation, or combinations thereof. These terms encompass native and artificial proteins, protein fragments and polypeptide analogs (such as muteins, variants, chimeric proteins and fusion proteins) of a protein sequence as well as post-translationally, or otherwise covalently or non-covalently, modified proteins.

[0547] The term “polymerase” and its variants, as used herein, comprises any enzyme that can catalyze polymerization of nucleotides (including analogs thereof) into a nucleic acid strand. Such nucleotide polymerization may occur in a template-dependent fashion. A polymerase may comprise one or more active sites at which nucleotide binding, or catalysis of nucleotide polymerization, or combinations thereof, can occur. In some embodiments, a polymerase includes other enzymatic activities, such as for example, 3′ to 5′ exonuclease activity or 5′ to 3′ exonuclease activity. In some embodiments, a polymerase has strand displacing activity. A polymerase can include without limitation naturally occurring polymerases and any subunits and truncations thereof, mutant polymerases, variant polymerases, recombinant, fusion or otherwise engineered polymerases, chemically modified polymerases, synthetic molecules or assemblies, and any analogs, derivatives or fragments thereof that retain the ability to catalyze nucleotide polymerization (e.g., catalytically active fragment). In some embodiments, a polymerase can be isolated from a cell, or generated using recombinant DNA technology or chemical synthesis methods. In some embodiments, a polymerase can be expressed in prokaryote, eukaryote, viral, or phage organisms. In some embodiments, a polymerase can be post-translationally modified proteins or fragments thereof. A polymerase can be derived from a prokaryote, eukaryote, virus or phage. A polymerase may comprise DNA-directed DNA polymerase and RNA-directed DNA polymerase.

[0548] As used herein, the term “fidelity” refers to the accuracy of DNA polymerization by template-dependent DNA polymerase. The fidelity of a DNA polymerase may be measured

by the error rate (the frequency of incorporating an inaccurate nucleotide, e.g., a nucleotide that is not complementary to the template nucleotide). The accuracy or fidelity of DNA polymerization is maintained by both the polymerase activity and the 3′-5′ exonuclease activity of a DNA polymerase.

[0549] As used herein, the term "binding complex" refers to a complex formed by binding together a nucleic acid duplex, a polymerase, and a free nucleotide or a nucleotide unit of a multivalent molecule, where the nucleic acid duplex comprises a nucleic acid template molecule hybridized to a nucleic acid primer. In the binding complex, the free nucleotide or nucleotide unit may or may not be bound to the 3' end of the nucleic acid primer at a position that is opposite a complementary nucleotide in the nucleic acid template molecule. A "ternary complex" is an example of a binding complex which is formed by binding together a nucleic acid duplex, a polymerase, and a free nucleotide or nucleotide unit of a multivalent molecule, where the free nucleotide or nucleotide unit is bound to the 3' end of the nucleic acid primer (as part of the nucleic acid duplex) at a position that is opposite a complementary nucleotide in the nucleic acid template molecule.

[0550] The term "persistence time" and related terms refers to the length of time that a binding complex remains stable without dissociation of any of the components, where the components of the binding complex include a nucleic acid template and nucleic acid primer, a polymerase, or a nucleotide unit of a multivalent molecule or a free (e.g., unconjugated) nucleotide, or combinations thereof. The nucleotide unit or the free nucleotide can be complementary or non-complementary to a nucleotide residue in the template molecule. The nucleotide unit or the free nucleotide can bind to the 3' end of the nucleic acid primer at a position that is opposite a complementary nucleotide residue in the nucleic acid template molecule. The persistence time is indicative of the stability of the binding complex and strength of the binding interactions. Persistence time can be measured by observing the onset, or duration, or combinations thereof, of a binding complex, such as by observing a signal from a labeled component of the binding complex. For example, a labeled nucleotide or a labeled reagent comprising one or more nucleotides may be present in a binding complex, thus allowing the signal from the label to be detected during the persistence time of the binding complex. One label, for example, is a fluorescent label. The binding complex (e.g., ternary complex) remains stable until subjected to a condition that causes dissociation of interactions between any of the polymerase, template molecule, primer, or the nucleotide unit or the nucleotide, or combinations thereof. For example, a dissociating condition comprises

contacting the binding complex with any one or any combination of a detergent, EDTA, or water, or combinations thereof.

[0551] The terms “nucleic acid”, "polynucleotide" and "oligonucleotide" and other related terms used herein are used interchangeably and refer to polymers of nucleotides and are not limited to any particular length. Nucleic acids include recombinant and chemically-synthesized forms. Nucleic acids include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs (e.g., peptide nucleic acids and non-naturally occurring nucleotide analogs), and chimeric forms containing DNA and RNA. Nucleic acids can be single-stranded or double-stranded. Nucleic acids comprise polymers of nucleotides, where the nucleotides include natural or non-natural bases, or sugars, or combinations thereof. Nucleic acids comprise naturally-occurring internucleosidic linkages, for example phosphodiester linkages. Nucleic acids comprise non-natural internucleoside linkages, including phosphorothioate, phosphorothiolate, or peptide nucleic acid (PNA) linkages. In some embodiments, nucleic acids comprise a one type of polynucleotides or a mixture of two or more different types of polynucleotides.

[0552] The term “primer” and related terms used herein refers to an oligonucleotide, either natural or synthetic, that is capable of hybridizing with a DNA, or RNA, or combinations thereof, polynucleotide template to form a duplex molecule. Primers may have any length and may range from 4-50 nucleotides. A primer may comprise a 5’ end and a 3’ end. The 3’ end of the primer can include a 3’ OH moiety which serves as a nucleotide polymerization initiation site in a polymerase-mediated primer extension reaction. Alternatively, the 3’ end of the primer can lack a 3’ OH moiety, or can include a terminal 3’ blocking group that inhibits nucleotide polymerization in a polymerase-mediated reaction. Any one nucleotide, or more than one nucleotide, along the length of the primer can be labeled with a detectable reporter moiety. A primer can be in solution (e.g., a soluble primer) or can be immobilized to a support (e.g., a capture primer).

[0553] The term “template nucleic acid”, “template polynucleotide”, “target nucleic acid” “target polynucleotide”, “template strand” and other variations refer to a nucleic acid strand that serves as the base nucleic acid molecule for generating a complementary nucleic acid strand. The template nucleic acid can be single-stranded or double-stranded, or the template nucleic acid can have single-stranded or double-stranded portions. The sequence of the template nucleic acid can be partially or wholly complementary to the sequence of the complementary strand. The template nucleic acid can be obtained from a naturally-occurring

source, recombinant form, or chemically synthesized to include any type of nucleic acid analog. The template nucleic acid can be linear, circular, or can come in other forms. The template nucleic acids can include an insert region having an insert sequence which is also known as a sequence of interest. The template nucleic acids can also include at least one adaptor sequence. The template nucleic acid can be a concatemer template molecule having two or tandem copies of a sequence of interest and at least one adaptor sequence. The insert region (sometimes termed "sequence-of-interest") can be isolated from any suitable source and in any form, including chromosomal, genomic, organellar (e.g., mitochondrial, chloroplast or ribosomal), recombinant molecules, cloned, amplified, cDNA, RNA such as precursor mRNA or mRNA, oligonucleotides, whole genomic DNA, obtained from fresh frozen paraffin embedded tissue, needle biopsies, cell free circulating DNA, or any type of nucleic acid library. The insert region can be isolated from any source including from organisms such as prokaryotes, eukaryotes (e.g., humans, plants and animals), fungus, viruses cells, tissues, normal or diseased cells or tissues, transfected cells, displaced cells, mammalian cells, bacteria, yeast, body fluids including blood, urine, serum, lymph, tumor, saliva, anal and vaginal secretions, amniotic samples, perspiration, semen, environmental samples, culture samples, or synthesized nucleic acid molecules prepared using recombinant molecular biology or chemical synthesis methods. The insert region can be isolated from any organ, including head, neck, brain, breast, ovary, cervix, colon, rectum, endometrium, gallbladder, intestines, bladder, prostate, testicles, liver, lung, kidney, esophagus, pancreas, thyroid, pituitary, thymus, skin, heart, larynx, or other organs. The template nucleic acid can be subjected to nucleic acid analysis, including sequencing and composition analysis.

[0554] When used in reference to nucleic acid molecules, the terms "hybridize" or "hybridizing" or "hybridization" or other related terms refers to hydrogen bonding between two different nucleic acids to form a duplex nucleic acid. Hybridization also includes hydrogen bonding between two different regions of a single nucleic acid molecule to form a self-hybridizing molecule having a duplex region. Hybridization can comprise Watson-Crick or Hoogstein binding to form a duplex double-stranded nucleic acid, or a double-stranded region within a nucleic acid molecule. The double-stranded nucleic acid, or the two different regions of a single nucleic acid, may be wholly complementary, or partially complementary. Complementary nucleic acid strands may not need to hybridize with each other across their entire length. The complementary base pairing can be the standard A-T or C-G base pairing or can be other forms of base-pairing interactions. Duplex nucleic acids can include mismatched base-paired nucleotides.

[0555] The term "nucleotides" and related terms refers to a molecule comprising an aromatic base, a five carbon sugar (e.g., ribose or deoxyribose), and at least one phosphate group. Canonical or non-canonical nucleotides are consistent with use of the term. The phosphate in some embodiments comprises a monophosphate, diphosphate, or triphosphate, or corresponding phosphate analog. In some embodiments, the nucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 phosphate groups. The term "nucleoside" refers to a molecule comprising an aromatic base and a sugar.

[0556] Nucleotides (and nucleosides) may comprise a hetero cyclic base including substituted or unsubstituted nitrogen-containing parent heteroaromatic ring which are commonly found in nucleic acids, including naturally-occurring, substituted, modified, or engineered variants, or analogs of the same. The base of a nucleotide (or nucleoside) is capable of forming Watson-Crick, or Hoogstein hydrogen bonds, or combinations thereof, with an appropriate complementary base. Examples of bases include, but are not limited to, purines and pyrimidines such as: 2-aminopurine, 2,6-diaminopurine, adenine (A), ethenoadenine, N^6-Δ^2-isopentenyladenine (6iA), N^6-Δ^2-isopentenyl-2-methylthioadenine (2ms6iA), N^6-methyladenine, guanine (G), isoguanine, N^2-dimethylguanine (dmG), 7-methylguanine (7mG), 2-thiopyrimidine, 6-thioguanine (6sG), hypoxanthine and O^6-methylguanine; 7-deaza-purines such as 7-deazaadenine (7-deaza-A) and 7-deazaguanine (7-deaza-G); pyrimidines such as cytosine (C), 5-propynylcytosine, isocytosine, thymine (T), 4-thiothymine (4sT), 5,6-dihydrothymine, O^4-methylthymine, uracil (U), 4-thiouracil (4sU) and 5,6-dihydrouracil (dihydrouracil; D); indoles such as nitroindole and 4-methylindole; pyrroles such as nitropyrrole; nebularine; inosines; hydroxymethylcytosines; 5-methycytosines; base (Y); as well as methylated, glycosylated, and acylated base moieties; and the like. Additional bases can be found in Fasman, 1989, in "Practical Handbook of Biochemistry and Molecular Biology", pp. 385-394, CRC Press, Boca Raton, Fla., which is hereby incorporated by reference.

[0557] Nucleotides (and nucleosides) may comprise a sugar moiety, such as carbocyclic moiety (Ferraro and Gotor 2000 Chem. Rev. 100: 4319-48, which is hereby incorporated by reference), acyclic moieties (Martinez, et al., 1999 Nucleic Acids Research 27: 1271-1274; Martinez, et al., 1997 Bioorganic & Medicinal Chemistry Letters vol. 7: 3013-3016, which is hereby incorporated by reference), and other sugar moieties (Joeng, et al., 1993 J. Med. Chem. 36: 2627-2638; Kim, et al., 1993 J. Med. Chem. 36: 30-7; Eschenmosser 1999 Science 284:2118-2124; and U.S. Pat. No. 5,558,991, which is hereby incorporated by reference). The sugar moiety comprises: ribosyl; 2′-deoxyribosyl; 3′-deoxyribosyl; 2′,3′-dideoxyribosyl; 2′,3′-

didehydrodideoxyribosyl; 2′-alkoxyribosyl; 2′-azidoribosyl; 2′-aminoribosyl; 2′-fluororibosyl; 2′-mercaptoriboxyl; 2′-alkylthioribosyl; 3′-alkoxyribosyl; 3′-azidoribosyl; 3′-aminoribosyl; 3′-fluororibosyl; 3′-mercaptoriboxyl; 3′-alkylthioribosyl carbocyclic; acyclic or other modified sugars.

[0558] In some embodiments, nucleotides comprise a chain of one, two or three phosphorus atoms where the chain may be attached to the 5' carbon of the sugar moiety via an ester or phosphoramide linkage. In some embodiments, the nucleotide is an analog having a phosphorus chain in which the phosphorus atoms are linked together with intervening O, S, NH, methylene or ethylene. In some embodiments, the phosphorus atoms in the chain include substituted side groups including O, S or BH_3. In some embodiments, the chain includes phosphate groups substituted with analogs including phosphoramidate, phosphorothioate, phosphorodithioate, and O-methylphosphoramidite groups.

[0559] When used in reference to nucleic acids, the terms "extend", "extending", "extension" and other variants, refers to incorporation of one or more nucleotides into a nucleic acid molecule. Nucleotide incorporation comprises polymerization of one or more nucleotides into the terminal 3' OH end of a nucleic acid strand, resulting in extension of the nucleic acid strand. Nucleotide incorporation can be conducted with natural nucleotides, or nucleotide analogs, or combinations thereof. Nucleotide incorporation may occur in a template-dependent fashion. Any suitable method of extending a nucleic acid molecule may be used, including primer extension catalyzed by a DNA polymerase or RNA polymerase.

[0560] The term "reporter moiety", "reporter moieties" or related terms refers to a compound that generates, or causes to generate, a detectable signal. A reporter moiety is sometimes called a "label". Any suitable reporter moiety may be used, including luminescent, photoluminescent, electroluminescent, bioluminescent, chemiluminescent, fluorescent, phosphorescent, chromophore, radioisotope, electrochemical, mass spectrometry, Raman, hapten, affinity tag, atom, or an enzyme. A reporter moiety generates a detectable signal resulting from a chemical or physical change (e.g., heat, light, electrical, pH, salt concentration, enzymatic activity, or proximity events). A proximity event includes two reporter moieties approaching each other, or associating with each other, or binding each other. Reporter moieties may be selected so that each absorbs excitation radiation, or emits fluorescence, or combinations thereof, at a wavelength distinguishable from the other reporter moieties to permit monitoring the presence of different reporter moieties in the same reaction or in different reactions. Two or more different reporter moieties can be selected having spectrally distinct emission profiles or having minimal overlapping spectral emission profiles.

Reporter moieties can be linked (e.g., operably linked) to nucleotides, nucleosides, nucleic acids, enzymes (e.g., polymerases or reverse transcriptases), or support (e.g., surfaces).

[0561] A reporter moiety (or label) comprises a fluorescent label or a fluorophore. Examples of fluorescent moieties which may serve as fluorescent labels or fluorophores include, but are not limited to fluorescein and fluorescein derivatives such as carboxyfluorescein, tetrachlorofluorescein, hexachlorofluorescein, carboxynapthofluorescein, fluorescein isothiocyanate, NHS-fluorescein, iodoacetamidofluorescein, fluorescein maleimide, SAMSA-fluorescein, fluorescein thiosemicarbazide, carbohydrazinomethylthioacetyl-amino fluorescein, rhodamine and rhodamine derivatives such as TRITC, TMR, lissamine rhodamine, Texas Red, rhodamine B, rhodamine 6G, rhodamine 10, NHS-rhodamine, TMR-iodoacetamide, lissamine rhodamine B sulfonyl chloride, lissamine rhodamine B sulfonyl hydrazine, Texas Red sulfonyl chloride, Texas Red hydrazide, coumarin and coumarin derivatives such as AMCA, AMCA-NHS, AMCA-sulfo-NHS, AMCA-HPDP, DCIA, AMCE-hydrazide, BODIPY® and derivatives such as BODIPY FL C3-SE, BODIPY 530/550 C3, BODIPY 530/550 C3-SE, BODIPY 530/550 C3 hydrazide, BODIPY 493/503 C3 hydrazide, BODIPY FL C3 hydrazide, BODIPY FL IA, BODIPY 530/551 IA, Br-BODIPY 493/503, Cascade Blue and derivatives such as Cascade Blue acetyl azide, Cascade Blue cadaverine, Cascade Blue ethylenediamine, Cascade Blue hydrazide, Lucifer Yellow and derivatives such as Lucifer Yellow iodoacetamide, Lucifer Yellow CH, cyanine and derivatives such as indolium based cyanine dyes, benzo-indolium based cyanine dyes, pyridium based cyanine dyes, thiozolium based cyanine dyes, quinolinium based cyanine dyes, imidazolium based cyanine dyes, Cy 3, Cy5, lanthanide chelates and derivatives such as BCPDA, TBP, TMT, BHHCT, BCOT, Europium chelates, Terbium chelates, Alexa Fluor® dyes, DyLight® dyes, Atto^{TM} dyes, LightCycler® Red dyes, CAL Flour dyes, JOE and derivatives thereof, Oregon Green^{TM} dyes, WellRED dyes, IRD dyes, phycoerythrin and phycobilin dyes, Malachite green, stilbene, DEG dyes, NR dyes, near-infrared dyes and others such as those described in Haugland, Molecular Probes Handbook, (Eugene, Oreg.) 6th Edition; Lakowicz, Principles of Fluo$_r$escence Spectroscopy, 2nd Ed., Plenum Press New York (1999), or Hermanson, B^{io}conjugate Techniques, 2nd Edition, all of which are hereby incorporated by reference in their entireties, or derivatives thereof, or any combination thereof. Cyanine dyes may exist in either sulfonated or non-sulfonated forms and consist of two indolenin, benzo-indolium, pyridium, thiozolium, or quinolinium groups separated by a polymethine bridge between two nitrogen atoms. Commercially available cyanine fluorophores include, for example, Cy3, (which may comprise 1-[6-(2,5-

dioxopyrrolidin-1-yloxy)-6-oxohexyl]-2-(3-{1-[6-(2,5-dioxopyrrolidin-1-yloxy)-6-oxohexyl]-3,3-dimethyl-1,3-dihydro-2H-indol-2-ylidene}prop-1-en-1-yl)-3,3-dimethyl-3H-indolium or 1-[6-(2,5-dioxopyrrolidin-1-yloxy)-6-oxohexyl]-2-(3-{1-[6-(2,5-dioxopyrrolidin-1-yloxy)-6-oxohexyl]-3,3-dimethyl-5-sulfo-1,3-dihydro-2H-indol-2-ylidene}prop-1-en-1-yl)-3,3-dimethyl-3H-indolium-5-sulfonate), Cy5 (which may comprise 1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6Iohexyl)-2-((1E,3E)-5-((E)-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethyl-5-indolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3H-indol-1-ium or 1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-2-((1E,3E)-5-((E)-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethyl-5-sulfoindolin-2-ylidene)penta-1,3-dien-1-yl)-3,3-dimethyl-3H-indol-1-ium-5-sulfonate), and Cy7 (which may comprise 1-(5-carboxypentyl)-2-[(1E,3E,5E,7Z)-7-(1-ethyl-1,3-dihydro-2H-indol-2-ylidene)hepta-1,3,5-trien-1-yl]-3H-indolium or 1-(5-carboxypentyl)-2-[(1E,3E,5E,7Z)-7-(1-ethyl-5-sulfo-1,3-dihydro-2H-indol-2-ylidene)hepta-1,3,5-trien-1-yl]-3H-indolium-5-sulfonate), where “Cy” stands for 'cyanine', and the first digit identifies the number of carbon atoms between two indolenine groups. Cy2, which is an oxazole derivative rather than indolenin, and the benzo-derivatized Cy3.5, Cy5.5 and Cy7.5 are exceptions to this rule. Additional fluorophores are described in WO 2024/124008, the contents of which are incorporated by reference in their entirety herein.

[0562] In some embodiments, the reporter moiety can be a fluorescence resonance energy transfer (FRET) pair, such that multiple classifications can be performed under a single excitation and imaging step. As used herein, FRET may comprise excitation exchange (Forster) transfers, or electron-exchange (Dexter) transfers.

[0563] The terms “linked”, “joined”, “attached”, and variants thereof comprise any type of fusion, bond, adherence or association between any combination of compounds or molecules that is of sufficient stability to withstand use in the particular procedure. The procedure can include but is not limited to: nucleotide transient-binding; nucleotide incorporation; de-blocking; washing; removing; flowing; detecting; imaging, or identifying, or combinations thereof. Such linkage can comprise, for example, covalent, ionic, hydrogen, dipole-dipole, hydrophilic, hydrophobic, or affinity bonding, bonds or associations involving van der Waals forces, mechanical bonding, and the like. In some embodiments, such linkage occurs intramolecularly, for example linking together the ends of a single-stranded or double-stranded linear nucleic acid molecule to form a circular molecule. In some embodiments, such linkage can occur between a combination of different molecules, or between a molecule and a non-molecule, including but not limited to: linkage between a nucleic acid molecule

and a solid surface; linkage between a protein and a detectable reporter moiety; linkage between a nucleotide and detectable reporter moiety; and the like. Some examples of linkages can be found, for example, in Hermanson, G., "Bioconjugate Techniques", Second Edition (2008); Aslam, M., Dent, A., "Bioconjugation: Protein Coupling Techniques for the Biomedical Sciences", London: Macmillan (1998); Aslam, M., Dent, A., "Bioconjugation: Protein Coupling Techniques for the Biomedical Sciences", London: Macmillan (1998), all of which are hereby incorporated by reference in their entireties.

[0564] The term "operably linked" and "operably joined" or related terms as used herein refers to juxtaposition of components. The juxtapositioned components can be linked together covalently, or non-covalently. For example, two nucleic acid components can be enzymatically ligated together where the linkage that joins together the two components comprises phosphodiester linkage. A first and second nucleic acid component can be linked together, where the first nucleic acid component can confer a function on a second nucleic acid component. For example, linkage between a primer binding sequence and a sequence of interest forms a nucleic acid library molecule having a portion that can bind to a primer. In another example, a transgene (e.g., a nucleic acid encoding a polypeptide or a nucleic acid sequence of interest) can be ligated to a vector where the linkage permits expression or functioning of the transgene sequence contained in the vector. As an example, a transgene is operably linked to a host cell regulatory sequence (e.g., a promoter sequence) that affects expression of the transgene.

[0565] The term "adaptor" and related terms refers to oligonucleotides that can be operably linked (appended) to a target polynucleotide, where the adaptor confers a function to the co-joined adaptor-target molecule. Adaptors comprise DNA, RNA, chimeric DNA/RNA, or analogs thereof. Adaptors can include at least one ribonucleoside residue. Adaptors can be single-stranded, double-stranded, or have single-stranded portions, or double-stranded portions, or combinations thereof. Adaptors can be configured to be linear, stem-looped, hairpin, or Y-shaped forms. Adaptors can be any length, including 4-100 nucleotides or longer. Adaptors can have blunt ends, overhang ends, or a combination of both. Overhang ends include 5' overhang and 3' overhang ends. The 5' end of a single-stranded adaptor, or one strand of a double-stranded adaptor, can have a 5' phosphate group or lack a 5' phosphate group. Adaptors can include a 5' tail that does not hybridize to a target polynucleotide (e.g., tailed adaptor), or adaptors can be non-tailed. An adaptor can include a sequence that is complementary to at least a portion of a primer, such as an amplification primer, a sequencing primer, or a capture primer (e.g., soluble or immobilized capture

primers). Adaptors can include a random sequence or degenerate sequence. Adaptors can include an index sequence, such as a sample index sequence, which can be used to identify the sample of origin of a sequence. Adaptors can include at least one inosine residue. Adaptors can include at least one phosphorothioate, phosphorothiolate, or phosphoramidate, or combinations thereof, linkage. Adaptors can include a barcode sequence which can be used to distinguish polynucleotides (e.g., insert sequences) from different sample sources in a multiplex assay. Adaptors can include a unique identification sequence (e.g., unique molecular index (UMI); or a unique molecular tag) that can be used to uniquely identify a nucleic acid molecule to which the adaptor is appended. In some embodiments, a unique identification sequence can be used to increase error correction and accuracy, reduce the rate of false-positive variant calls, or increase sensitivity of variant detection, or combinations thereof. Adaptors can include at least one restriction enzyme recognition sequence, including any one or any combination of two or more selected from a group consisting of type I, type II, type III, type IV, type Hs or type IIB.

[0566] The term “universal sequence”, “universal adaptor sequences” and related terms refers to a sequence in a nucleic acid molecule that is common among two or more polynucleotide molecules. For example, adaptors having the same universal sequence can be joined to a plurality of polynucleotides so that the population of co-joined molecules carry the same universal adaptor sequence. Examples of universal adaptor sequences include an amplification primer sequence, a sequencing primer sequence or a capture primer sequence (e.g., soluble or support-immobilized capture primers).

[0567] The supports of the disclosure can be solid, semi-solid, or a combination of both. In some embodiments, the support is porous, semi-porous, non-porous, or any combination of porosity. In some embodiments, the support can be substantially planar, concave, convex, or any combination thereof. In some embodiments, the support can be cylindrical, for example comprising a capillary or interior surface of a capillary.

[0568] The surface of the support can be substantially smooth. In some embodiments, the support can be regularly or irregularly textured, including bumps, etched, pores, three-dimensional scaffolds, or any combination thereof.

[0569] In some embodiments, the support comprises a bead having any shape, including spherical, hemi-spherical, cylindrical, barrel-shaped, toroidal, disc-shaped, rod-like, conical, triangular, cubical, polygonal, tubular or wire-like.

[0570] The support can be fabricated from any material, including but not limited to glass, fused-silica, silicon, a polymer (e.g., polystyrene (PS), macroporous polystyrene (MPPS),

polymethylmethacrylate (PMMA), polycarbonate (PC), polypropylene (PP), polyethylene (PE), high density polyethylene (HDPE), cyclic olefin polymers (COP), cyclic olefin copolymers (COC), polyethylene terephthalate (PET)), or any combination thereof. Various compositions of both glass and plastic substrates are contemplated.

[0571] The present disclosure provides a plurality (e.g., two or more) of nucleic acid template molecules immobilized to any of the supports described herein. In some embodiments, the plurality of nucleic acid template molecules has the same sequence or has different sequences. In some embodiments, individual nucleic acid template molecules in the plurality of nucleic acid template molecules are immobilized to a different site on the support. In some embodiments, two or more individual nucleic acid template molecules in the plurality of nucleic acid template molecules are immobilized to a site on the support. In some embodiments, the support comprises a plurality of sites arranged in an array. The term "array" refers to a support comprising a plurality of sites located at pre-determined locations on the support to form an array of sites. The sites can be discrete and separated by interstitial regions. In some embodiments, the pre-determined sites on the support can be arranged in one dimension in a row or a column, or arranged in two dimensions in rows and columns. In some embodiments, the plurality of pre-determined sites is arranged on the support in an organized fashion. In some embodiments, the plurality of pre-determined sites is arranged in any organized pattern, including rectilinear, hexagonal patterns, grid patterns, patterns having reflective symmetry, patterns having rotational symmetry, or the like. The pitch between different pairs of sites can be that same or can vary. In some embodiments, the support can have nucleic acid template molecules immobilized at a plurality of sites at a surface density of about $10^2 – 10^{15}$ sites per mm^2, or more, to form a nucleic acid template array. In some embodiments, the support comprises at least 10^2 sites, at least 10^3 sites, at least 10^4 sites, at least 10^5 sites, at least 10^6 sites, at least 10^7 sites, at least 10^8 sites, at least 10^9 sites, at least 10^{10} sites, at least 10^{11} sites, at least 10^{12} sites, at least 10^{13} sites, at least 10^{14} sites, at least 10^{15} sites, or more, where the sites are located at pre-determined locations on the support. In some embodiments, a plurality of pre-determined sites on the support (e.g., $10^2 – 10^{15}$ sites or more) are immobilized with nucleic acid template molecules to form a nucleic acid template molecule array. In some embodiments, the nucleic acid template molecules that are immobilized at a plurality of pre-determined sites by hybridization to immobilized surface capture primers, or the nucleic acid template molecules are covalently attached to the surface capture primers. In some embodiments, the nucleic acid template molecules that are immobilized at a plurality of pre-determined sites are, for example, immobilized at $10^2 – 10^{15}$

sites or more. In some embodiments, the nucleic acid template molecules that are immobilized at a plurality of sites on the support comprise linear or circular nucleic acid template molecules or a mixture of both linear and circular molecules. In some embodiments, the immobilized nucleic acid template molecules are clonally-amplified to generate immobilized nucleic acid polonies at the plurality of pre-determined sites. In some embodiments, individual immobilized nucleic acid template molecules comprise one copy of a target sequence of interest, or comprise concatemer template molecules having two or more tandem copies of a target sequence of interest.

[0572] A support comprising a plurality of sites located at random locations on the support is referred to herein as a support having randomly located sites thereon. In some embodiments, the location of the randomly located sites on the support are not pre-determined. In some embodiments, the plurality of randomly-located sites is arranged on the support in a disordered fashion, or unpredictable fashion, or combinations thereof. In some embodiments, the support comprises at least 10^2 sites, at least 10^3 sites, at least 10^4 sites, at least 10^5 sites, at least 10^6 sites, at least 10^7 sites, at least 10^8 sites, at least 10^9 sites, at least 10^{10} sites, at least 10^{11} sites, at least 10^{12} sites, at least 10^{13} sites, at least 10^{14} sites, at least 10^{15} sites, or more, where the sites are randomly located on the support. In some embodiments, a plurality of randomly located sites on the support (e.g., $10^2 – 10^{15}$ sites or more) is immobilized with nucleic acid template molecules to form a support immobilized with nucleic acid template molecules. In some embodiments, the nucleic acid template molecules that are immobilized at a plurality of randomly located sites by hybridization to immobilized surface capture primers, or the nucleic acid template molecules, are covalently attached to the surface capture primer. In some embodiments, the nucleic acid template molecules that are immobilized at a plurality of randomly located sites are, for example, immobilized at $10^2 – 10^{15}$ sites or more. In some embodiments, the nucleic acid template molecules that are immobilized at a plurality of sites on the support comprise linear or circular nucleic acid template molecules or a mixture of both linear and circular molecules. In some embodiments, the immobilized nucleic acid template molecules are clonally-amplified to generate immobilized nucleic acid polonies at the plurality of randomly located sites. In some embodiments, individual immobilized nucleic acid template molecules comprise one copy of a target sequence of interest or comprise concatemer template molecules having two or more tandem copies of a target sequence of interest.

[0573] In some embodiments, with respect to nucleic acid template molecules immobilized to pre-determined or random sites on the support, the plurality of immobilized nucleic acid

template molecules on the support are in fluid communication with each other to permit flowing a solution of reagents (e.g., enzymes including polymerases, multivalent molecules, nucleotides, divalent cations, or buffers and the like, or combinations thereof) onto the support so that the plurality of immobilized nucleic acid template molecules on the support can be reacted with the reagents in a massively parallel manner. In some embodiments, the fluid communication of the plurality of immobilized nucleic acid template molecules can be used to conduct nucleotide binding assays, or conduct nucleotide polymerization reactions (e.g., primer extension or sequencing), or combinations thereof, on the plurality of immobilized nucleic acid template molecules, and to conduct detection and imaging for massively parallel sequencing.

[0574] As used herein, the term "immobilized" and related terms refer to nucleic acid molecules or enzymes (e.g., polymerases) that are attached to the support at pre-determined or random locations, where the nucleic acid molecules or enzymes are attached directly to a support through covalent bonds or non-covalent interactions, or the nucleic acid molecules or enzymes are attached to a coating on the support.

[0575] When used in reference to a low binding surface coating, one or more layers of a multi-layered surface coating may comprise a branched polymer or may be linear. Examples of suitable branched polymers include, but are not limited to, branched PEG, branched poly(vinyl alcohol) (branched PVA), branched poly(vinyl pyridine), branched poly(vinyl pyrrolidone) (branched PVP), branched), poly(acrylic acid) (branched PAA), branched polyacrylamide, branched poly(N-isopropylacrylamide) (branched PNIPAM), branched poly(methyl methacrylate) (branched PMA), branched poly(2-hydroxylethyl methacrylate) (branched PHEMA), branched poly(oligo(ethylene glycol) methyl ether methacrylate) (branched POEGMA), branched polyglutamic acid (branched PGA), branched poly-lysine, branched poly-glucoside, and dextran.

[0576] In some embodiments, the branched polymers used to create one or more layers of any of the multi-layered surfaces disclosed herein may comprise at least 4 branches, at least 5 branches, at least 6 branches, at least 7 branches, at least 8 branches, at least 9 branches, at least 10 branches, at least 12 branches, at least 14 branches, at least 16 branches, at least 18 branches, at least 20 branches, at least 22 branches, at least 24 branches, at least 26 branches, at least 28 branches, at least 30 branches, at least 32 branches, at least 34 branches, at least 36 branches, at least 38 branches, or at least 40 branched.

[0577] Linear, branched, or multi-branched polymers used to create one or more layers of any of the multi-layered surfaces disclosed herein may have a molecular weight of at least

500, at least 1,000, at least 2,000, at least 3,000, at least 4,000, at least 5,000, at least 10,000, at least 15,000, at least 20,000, at least 25,000, at least 30,000, at least 35,000, at least 40,000, at least 45,000, or at least 50,000 daltons.

[0578] In some embodiments, e.g., wherein at least one layer of a multi-layered surface comprises a branched polymer, the number of covalent bonds between a branched polymer molecule of the layer being deposited and molecules of the previous layer may range from about one covalent linkage per molecule and about 32 covalent linkages per molecule. In some embodiments, the number of covalent bonds between a branched polymer molecule of the new layer and molecules of the previous layer may be at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 12, at least 14, at least 16, at least 18, at least 20, at least 22, at least 24, at least 26, at least 28, at least 30, or at least 32 covalent linkages per molecule.

[0579] Any reactive functional groups that remain following the coupling of a material layer to the surface may optionally be blocked by coupling a small, inert molecule using a high yield coupling chemistry. For example, in the case that amine coupling chemistry is used to attach a new material layer to the previous one, any residual amine groups may subsequently be acetylated or deactivated by coupling with a small amino acid such as glycine.

[0580] The number of layers of low non-specific binding material, e.g., a hydrophilic polymer material, deposited on the surface, may range from 1 to about 10. In some embodiments, the number of layers is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10. In some embodiments, the number of layers may be at most 10, at most 9, at most 8, at most 7, at most 6, at most 5, at most 4, at most 3, at most 2, or at most 1. Any of the lower and upper values described in this paragraph may be combined to form a range included within the present disclosure, for example, in some embodiments the number of layers may range from about 2 to about 4. In some embodiments, all of the layers may comprise the same material. In some embodiments, each layer may comprise a different material. In some embodiments, the plurality of layers may comprise a plurality of materials. In some embodiments at least one layer may comprise a branched polymer. In some embodiment, all of the layers may comprise a branched polymer.

[0581] One or more layers of low non-specific binding material may in some cases be deposited on, or conjugated to, or combinations thereof, the substrate surface using a polar protic solvent, a polar or polar aprotic solvent, a nonpolar solvent, or any combination

thereof. In some embodiments, the solvent used for layer deposition, or coupling, or combinations thereof, may comprise an alcohol (e.g., methanol, ethanol, propanol, etc.), another organic solvent (e.g., acetonitrile, dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), etc.), water, an aqueous buffer solution (e.g., phosphate buffer, phosphate buffered saline, 3-(N-morpholino)propanesulfonic acid (MOPS), etc.), or any combination thereof. In some embodiments, an organic component of the solvent mixture used may comprise at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the total, with the balance made up of water or an aqueous buffer solution. In some embodiments, an aqueous component of the solvent mixture used may comprise at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of the total, with the balance made up of an organic solvent. The pH of the solvent mixture used may be less than 6, about 6, 6.5, 7, 7.5, 8, 8.5, 9, or greater than pH 9.

[0582] The term "branched polymer" and related terms refers to a polymer having a plurality of functional groups that help conjugate a biologically active molecule such as a nucleotide, and the functional group can be either on the side chain of the polymer or directly attached to a central core or central backbone of the polymer. The branched polymer can have a linear backbone with one or more functional groups coming off the backbone for conjugation. The branched polymer can also be a polymer having one or more sidechains, wherein the side chain has a site suitable for conjugation. Examples of the functional group include but are limited to hydroxyl, ester, amine, carbonate, acetal, aldehyde, aldehyde hydrate, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, hydrazide, thiol, alkanoic acid, acid halide, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxal, dione, mesylate, tosylate, and tresylate.

[0583] As used herein, the term "clonally amplified" and its variants refers to a nucleic acid template molecule that has been subjected to one or more amplification reactions either in-solution or on-support. In the case of in-solution amplified template molecules, the resulting amplicons are distributed onto the support. Prior to amplification, the template molecule may comprise a sequence of interest and at least one universal adaptor sequence. In some embodiments, clonal amplification comprises the use of a polymerase chain reaction (PCR), multiple displacement amplification (MDA), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), real-time SDA, bridge amplification, isothermal bridge amplification, rolling circle amplification (RCA), circle-to-circle amplification, helicase-dependent amplification,

recombinase-dependent amplification, single-stranded binding (SSB) protein-dependent amplification, or any combination thereof.

[0584] As used herein, the term "sequencing" and its variants comprise obtaining sequence information from a nucleic acid strand, which may be by determining the identity of at least some nucleotides (including their nucleobase components) within the nucleic acid template molecule. While in some embodiments, "sequencing" a given region of a nucleic acid molecule includes identifying each and every nucleotide within the region that is sequenced, in some embodiments, "sequencing" comprises methods whereby the identity of some of the nucleotides in the region is determined, while the identity of some nucleotides remains undetermined or incorrectly determined. Any suitable method of sequencing may be used. In an embodiment, sequencing can include label-free or ion based sequencing methods. In some embodiments, sequencing can include labeled or dye-containing nucleotide or fluorescent based nucleotide sequencing methods. In some embodiments, sequencing can include polony-based sequencing or bridge sequencing methods. In some embodiments, sequencing includes massively parallel sequencing platforms that employ sequence-by-synthesis, sequence-by-hybridization or sequence-by-binding procedures. Examples of massively parallel sequence-by-synthesis procedures include polony sequencing, pyrosequencing (e.g., from 454 Life Sciences; U.S. Patent Nos. 7,211,390, 7,244,559 and 7,264,929, all of which are hereby incorporated by reference), chain-terminator sequencing (e.g., from Illumina; U.S. Patent No. 7,566,537; Bentley 2006 Current Opinion Genetics and Development 16:545-552; and Bentley, et al., 2008 Nature 456:53-59, all of which are hereby incorporated by reference), ion-sensitive sequencing (e.g., from Ion Torrent), probe-anchor ligation sequencing (e.g., Complete Genomics), DNA nanoball sequencing, nanopore DNA sequencing. Examples of single molecule sequencing include Heliscope single molecule sequencing, and single molecule real time (SMRT) sequencing from Pacific Biosciences (Levene, et al., 2003 Science 299(5607):682-686; Eid, et al., 2009 Science 323(5910): 133-138; U.S. Patent Nos. 7,170,050; 7,302,146; and 7,405,281, all of which are hereby incorporated by reference). An example of sequence-by-hybridization includes SOLiD sequencing (e.g., from Life Technologies; WO 2006/084132, which is hereby incorporated by reference). An example of sequence-by-binding includes Omniome sequencing (e.g., U.S Patent No. 10,246,744, which is hereby incorporated by reference).

Enumerated Embodiments

[0585] The disclosure can be further understood with reference to the following enumerated embodiments, which are for illustrative purposes only.

[0586] 1. A flow cell system comprising:

a flow cell device comprising:

a support comprising one or more substrates;

one or more channels defined by the one or more substrates, wherein the one or more channels are configured to allow fluids to flow therethrough and a gas gap to flow therethrough between one or more of the fluids;

an inlet in the one or more substrates, the inlet in fluidic connection with the one or more channels, wherein the inlet is coupled to or includes an open landing area disposed on a substrate of the one or more substrates; and

an outlet in the one or more substrates,

wherein the one or more channels run from the inlet to the outlet.

[0587] 2. A flow cell system comprising:

a flow cell device comprising:

a support comprising one or more substrates;

one or more channels defined by the one or more substrates;

an inlet in the one or more substrates, the inlet in fluidic connection with the one or more channels, the inlet coupled to or including an open landing area disposed on a substrate of the one or more substrates, wherein the open landing area is at least partly covered with a coating; and

an outlet in the one or more substrates,

wherein the one or more channels run from the inlet to the outlet.

[0588] 3. A flow cell system comprising:

a flow cell device comprising:

a support comprising one or more substrates;

one or more channels defined between the one or more substrates;

an inlet in the one or more substrates, the inlet in fluidic connection with the one or more channels, wherein the inlet is coupled to or includes an open landing area disposed on a substrate of the one or more substrates;

a cleaning outlet in the one or more substrates, wherein the cleaning outlet is configured to be in fluidic connection with the inlet; and

an outlet in the one or more substrates,

wherein the one or more channels run from the inlet to the outlet.

[0589] 4. The flow cell system of any one of the preceding embodiments, wherein the fluids include one or more reagents including a first reagent and a second reagent, the one or

more channels are configured to allow the gas gap to flow therethrough between allowing the first reagent and the second reagent to flow therethrough.

[0590] 5. The flow cell system of any one of the preceding embodiments, wherein the one or more channels are configured to allow the gas gap to flow therethrough during DNA sequencing.

[0591] 6. The flow cell system of any one of the preceding embodiments, wherein the one or more channels are configured to allow the gas gap to flow therethrough from the inlet.

[0592] 7. The flow cell system of embodiment 4, wherein the one or more channels are configured to allow the gas gap to flow therethrough to reduce contamination of the second reagent by the first reagent to DNA sequencing.

[0593] 8. The flow cell system of embodiment 4, wherein the one or more channels are configured to allow the gas gap to flow therethrough to reduce a total volume of the first reagent, the second reagent, or a washing reagent required for DNA sequencing.

[0594] 9. The flow cell system of any one of the preceding embodiments, wherein each of the one or more channels comprises a surface.

[0595] 10. The flow cell system of any one of the preceding embodiments, wherein the surface is an inner surface.

[0596] 11. The flow cell system of any one of the preceding embodiments, wherein the surface is an exterior surface.

[0597] 12. The flow cell system of any one of the preceding embodiments, wherein the surface comprises an interior top surface, an interior bottom surface, or both.

[0598] 13. The flow cell system of any one of the preceding embodiments, wherein the surface comprises an exterior top surface, an exterior bottom surface, or both.

[0599] 14. The flow cell system of any one of the preceding embodiments, wherein the surface is a planar surface.

[0600] 15. The flow cell system of any one of the preceding embodiments, wherein the surface is passivated.

[0601] 16. The flow cell system of any one of the preceding embodiments, wherein the surface is passivated with a coating that immobilize surface capture primers, nucleic acid template molecules, or both for capturing polynucleotides.

[0602] 17. The flow cell system of any one of the preceding embodiments, wherein the surface comprises polynucleotides captured thereon.

[0603] 18. The flow cell system of any one of the preceding embodiments, wherein the gas gap is configured to dry at least part of the surface of the one or more channels.

[0604] 19. The flow cell system of any one of the preceding embodiments, wherein the gas gap does not impair chemical functions of the surface of the one or more channels.

[0605] 20. The flow cell system of any one of the preceding embodiments, wherein the coating of the surface comprises at least one hydrophilic polymer coating layer.

[0606] 21. The flow cell system of any one of the preceding embodiments, wherein the coating of the surface comprises a plurality of oligonucleotide molecules attached to at least one hydrophilic polymer coating layer.

[0607] 22. The flow cell system of any one of the preceding embodiments, wherein the surface of the one or more channels comprises at least one discrete region that comprises a plurality of clonally-amplified sample nucleic acid molecules that have been annealed to a plurality of attached oligonucleotide molecules.

[0608] 23. The flow cell system of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer has a water contact angle of no more than about 50 degrees.

[0609] 24. The flow cell system of any one of the preceding embodiments, wherein at least one of the plurality of clonally-amplified sample nucleic acid molecules comprises a concatemer annealed to at least one of the plurality of attached oligonucleotide.

[0610] 25. The flow cell system of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer comprises PEG.

[0611] 26. The flow cell system of any one of the preceding embodiments, wherein the surface further comprises a second hydrophilic polymer coating layer.

[0612] 27. The flow cell system of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer comprises a branched hydrophilic polymer.

[0613] 28. The flow cell system of any one of the preceding embodiments, wherein the branched hydrophilic polymer comprises at least 8 branches.

[0614] 29. The flow cell system of any one of the preceding embodiments, wherein the at least one of the plurality of the clonally-amplified sample nucleic acid molecules comprises a single-stranded multimeric nucleic acid molecule comprising repeats of a regularly occurring monomer unit.

[0615] 30. The flow cell system of any one of the preceding embodiments, wherein the single-stranded multimeric nucleic acid molecule is at least 10 kilobases in length.

[0616] 31. The flow cell system of any one of the preceding embodiments, wherein the at least one of the plurality of the clonally-amplified sample nucleic acid molecules further comprises a double-stranded monomeric copy of the regularly occurring monomer unit.

[0617] 32. The flow cell system of any one of the preceding embodiments, wherein the plurality of oligonucleotide molecules is present at about a uniform surface density across the surface.

[0618] 33. The flow cell system of any one of the preceding embodiments, wherein the plurality of oligonucleotide molecules is present at a local surface density of at least 100,000 molecules/μm2 at a first position on the surface, and at a second local surface density at a second position on the surface.

[0619] 34. The flow cell system of any one of the preceding embodiments, wherein the coating comprises:

a first layer comprising a monolayer of polymer molecules tethered to the surface of the substrate;

a second layer comprising a second monolayer of polymer molecules tethered to the polymer molecules of the first layer; and

a third layer comprising a third monolayer of polymer molecules tethered to the polymer molecules of the second layer, wherein at least one of the first layer, the second layer, or the third layer comprises branched polymer molecules.

[0620] 35. The flow cell system of any one of the preceding embodiments, wherein the third layer further comprises oligonucleotides tethered to the polymer molecules of the third layer.

[0621] 36. The flow cell system of any one of the preceding embodiments, wherein the oligonucleotides tethered to the polymer molecules of the third layer are distributed at a plurality of depths throughout the third layer.

[0622] 37. The flow cell system of any one of the preceding embodiments, wherein the coating further comprises:

a fourth layer comprising branched polymer molecules tethered to the polymer molecules of the third layer, and

a fifth layer comprising polymer molecules tethered to the branched polymer molecules of the fourth layer.

[0623] 38. The flow cell system of any one of the preceding embodiments, wherein the polymer molecules of the fifth layer further comprise oligonucleotides tethered to the polymer molecules of the fifth layer.

[0624] 39. The flow cell system of any one of the preceding embodiments, wherein the oligonucleotides tethered to the polymer molecules of the fifth layer are distributed at a plurality of depths throughout the fifth layer.

[0625] 40. The flow cell system of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer comprises a molecule selected from the group consisting of: polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), poly(vinyl pyridine), poly(vinyl pyrrolidone) (PVP), poly(acrylic acid) (PAA), polyacrylamide, poly(N-isopropylacrylamide) (PNIPAM), poly(methyl methacrylate) (PMA), poly(2-hydroxylethyl methacrylate) (PHEMA), poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA), polyglutamic acid (PGA), poly-lysine, poly-glucoside, streptavidin, and dextran.

[0626] 41. The flow cell system of any one of the preceding embodiments, wherein when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, an image of the surface exhibits a ratio of fluorescence intensities for the clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and nonspecific Cyanine dye-3 dye adsorption background (Binter) of at least 3:1.

[0627] 42. The flow cell system of any one of the preceding embodiments, wherein the image of the surface exhibits a ratio of fluorescence intensities for clonally amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and a combination of nonspecific Cyanine dye-3 dye adsorption background and nonspecific amplification background (B_{inter}+Bintra) of at least 3:1.

[0628] 43. The flow cell system of any one of the preceding embodiments, wherein when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, the image of the surface exhibits a ratio of fluorescence intensities for clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and nonspecific dye adsorption background (Binter) of at least 5:1.

[0629] 44. The flow cell system of any one of the preceding embodiments, wherein the image of the surface exhibits a ratio of fluorescence intensities for clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and a combination of nonspecific Cyanine dye-3 dye adsorption background and nonspecific amplification background (Binter+Bintra) of at least 5:1.

[0630] 45. The flow cell system of any one of the preceding embodiments, wherein when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, the fluorescence image of the surface exhibits a contrast-to-noise ratio (CNR) of at least 20 when the fluorescence image is acquired using an inverted microscope equipped with a 20× objective, NA=0.75, dichroic mirror optimized for 532 nm

light, a bandpass filter optimized for Cyanine dye-3 emission, and a camera under non-signal saturating conditions, while the surface is immersed in a buffer.

[0631] 46. The flow cell system of any one of the preceding embodiments, wherein the plurality of oligonucleotide molecules is present at a surface density of at least 1,000 molecules/m^2.

[0632] 47. The flow cell system of any one of the preceding embodiments, wherein the first reagent is configured to wet the surface of the one or more channels.

[0633] 48. The flow cell system of any one of the preceding embodiments, wherein the second reagent is configured to rewet the surface of the one or more channels after at the gas gap at least partly dries the surface of the one or more channels when flowed through the one or more channels.

[0634] 49. The flow cell system of any one of the preceding embodiments, wherein the flow cell system further comprises:

a fluidic operation device comprising:

a first pump coupled to the outlet; and

a dispenser that is configured to openly dispense one or more reagents to the inlet.

[0635] 50. The flow cell system of any one of the preceding embodiments, wherein the first pump or a second pump is configured to introduce the gas gap via the inlet and flow the gas gap at least partly through the one or more channels.

[0636] 51. The flow cell system of any one of the preceding embodiments, wherein the gas gap comprises air.

[0637] 52. The flow cell system of any one of the preceding embodiments, wherein the gas gap comprises ambient air.

[0638] 53. The flow cell system of any one of the preceding embodiments, wherein the gas gap comprises one or more inert gases.

[0639] 54. The flow cell system of any one of the preceding embodiments, wherein the gas gap comprises one or more active gases.

[0640] 55. The flow cell system of any one of the preceding embodiments, wherein the first or the second reagent comprise liquid.

[0641] 56. The flow cell system of any one of the preceding embodiments, wherein the first or the second reagent is deprived of air bubbles that are greater than a predetermined size.

[0642] 57. The flow cell system of any one of the preceding embodiments, wherein the coating comprises a liquid-repelling coating.

[0643] 58. The flow cell system of any one of the preceding embodiments, wherein the coating comprises an omniphobic coating.

[0644] 59. The flow cell system of any one of the preceding embodiments, wherein the coating comprises a slippery liquid-infused porous surface (SLIPS).

[0645] 60. The flow cell system of any one of the preceding embodiments, wherein the coating comprises a slippery omniphobic covalently attached liquid (SOCAL) coating.

[0646] 61. The flow cell system of any one of the preceding embodiments, wherein the coating comprises a liquid-like polymer brush surface that is covalently attached to the one or more substrates.

[0647] 62. The flow cell system of any one of the preceding embodiments, wherein the coating is formed by impregnating lubricants in one or more porous surfaces.

[0648] 63. The flow cell system of any one of the preceding embodiments, wherein the lubricants comprise a liquid with a surface energy below about 20 mJ/m^2.

[0649] 64. The flow cell system of any one of the preceding embodiments, wherein the lubricants comprise a silicone oil.

[0650] 65. The flow cell system of any one of the preceding embodiments, wherein the coating comprises a surface energy that is below about 20 mJ/m^2.

[0651] 66. The flow cell system of any one of the preceding embodiments, wherein the coating is formed by acid-catalyzed graft polycondensation of one or more saline monomers.

[0652] 67. The flow cell system of any one of the preceding embodiments, wherein the one or more saline monomers comprises dimethyldimethoxysilane.

[0653] 68. The flow cell system of any one of the preceding embodiments, wherein the open landing area is in fluidic connection with the one or more channels.

[0654] 69. The flow cell system of any one of the preceding embodiments, wherein the open landing area is in fluidic connection with one corresponding channel of the one or more channels.

[0655] 70. The flow cell system of any one of the preceding embodiments, wherein the one or more channels includes a plurality of channels, and the open landing area is in fluidic connection with two or more of the plurality of channels.

[0656] 71. The flow cell system of any one of the preceding embodiments, wherein the open landing area is disposed on a bottom substrate of the one or more substrates.

[0657] 72. The flow cell system of any one of the preceding embodiments, wherein the inlet comprises a hole defined by a top substrate of the one or more substrates.

[0658] 73. The flow cell system of any one of the preceding embodiments, wherein the hole defined by the top substrate is positioned to vertically align with at least a portion of the open landing area.

[0659] 74. The flow cell system of any one of the preceding embodiments, wherein the dispenser is configured to openly dispense the one or more reagents through the hole defined by the top substrate to the open landing area.

[0660] 75. The flow cell system of any one of the preceding embodiments, wherein the dispenser is configured to openly dispense the one or more reagents from a tip of the dispenser to the open landing area.

[0661] 76. The flow cell system of any one of the preceding embodiments, wherein the dispenser is configured to openly dispense the one or more reagents from the tip of the dispenser to the open landing area, without any tubing in between.

[0662] 77. The flow cell system of any one of the preceding embodiments, wherein at least part of the tip of the dispenser is in contact with the open landing area.

[0663] 78. The flow cell system of any one of the preceding embodiments, wherein the tip of the dispenser is not in contact with the open landing area.

[0664] 79. The flow cell system of any one of the preceding embodiments, wherein the flow cell device further comprises a cleaning outlet in the one or more substrates.

[0665] 80. The flow cell system of any one of the preceding embodiments, wherein the cleaning outlet is in fluidic connection with the inlet.

[0666] 81. The flow cell system of any one of the preceding embodiments, wherein the cleaning outlet is in fluidic connection with the open landing area.

[0667] 82. The flow cell system of any one of the preceding embodiments, wherein the cleaning outlet is positioned underneath the open landing area.

[0668] 83. The flow cell system of any one of the preceding embodiments, wherein the cleaning outlet is defined by in the top or bottom substrate of the one or more substrates.

[0669] 84. The flow cell system of any one of the preceding embodiments, wherein the cleaning outlet includes a side port on the one or more substrates.

[0670] 85. The flow cell system of any one of the preceding embodiments, wherein the cleaning outlet is configured to be coupled to the first pump or the second pump.

[0671] 86. The flow cell system of any one of the preceding embodiments, wherein the one or more channels comprises microfluidic channels.

[0672] 87. The flow cell system of any one of the preceding embodiments, wherein the surface of the one or more channels is coated with fluorescent beads that are chemically immobilized to the surface.

[0673] 88. The flow cell system of any one of the preceding embodiments, wherein the fluorescent beads are covalently attached to the surface of the one or more channels.

[0674] 89. The flow cell system of any one of the preceding embodiments, wherein a gap between the interior top surface and the interior bottom surface is about 150 μm, 130 μm, 120 μm, 110 μm, 100 μm, 90 μm, 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm.

[0675] 90. The flow cell system of any one of the preceding embodiments, wherein a height of the one or more channels is about 150 μm, 130 μm, 120 μm, 110 μm, 100 μm, 90 μm, 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm.

[0676] 91. The flow cell system of any one of the preceding embodiments, wherein the polynucleotides captured thereon are configured to be imaged in a sequencing cycle.

[0677] 92. The flow cell system of any one of the preceding embodiments, wherein the one or more substrates comprises a top substrate and a bottom substrate.

[0678] 93. The flow cell system of any one of the preceding embodiments, wherein the one or more channels are defined between the top substrate and the bottom substrate.

[0679] 94. The flow cell system of any one of the preceding embodiments, wherein the one or more channels are at least partially defined by a top surface of the bottom substrate.

[0680] 95. The flow cell system of any one of the preceding embodiments, wherein the one or more channels are at least partially defined by a bottom surface of the top substrate.

[0681] 96. The flow cell system of any one of the preceding embodiments, wherein the one or more substrates further comprises a middle substrate.

[0682] 97. The flow cell system of any one of the preceding embodiments, wherein the one or more channels are at least partially defined by the middle substrate.

[0683] 98. The flow cell system of any one of the preceding embodiments, wherein the one or more substrates comprise glass or plastic.

[0684] 99. The flow cell system of any one of the preceding embodiments, wherein at least a portion of the support of the flow cell device is transparent.

[0685] 100. The flow cell system of any one of the preceding embodiments, wherein at least a portion of the one or more substrates is transparent.

[0686] 101. The flow cell system of any one of the preceding embodiments, wherein the support at least a portion of the support is opaque.

[0687] 102. The flow cell system of any one of the preceding embodiments, wherein the one or more channels comprises 1, 2, 3, 4, 5, 6, 7, or 8 channels.

[0688] 103. The flow cell system of any one of the preceding embodiments, wherein the one or more channels comprises 2, 4, 6, 8, or 10 channels.

[0689] 104. The flow cell system of any one of the preceding embodiments, wherein each channel of the one or more channels comprises a lane length of less than about 70 mm, 75 mm, 80 mm, or 90 mm.

[0690] 105. The flow cell system of any one of the preceding embodiments, wherein each channel of the one or more channels comprises a lane width of less than about 10 mm, 8 mm, 7 mm, 6 mm, 5 mm, 4 mm, or 3 mm.

[0691] 106. The flow cell system of any one of the preceding embodiments, wherein the coating configured to cover at least a portion of the open landing area includes a slippery coating.

[0692] 107. The flow cell system of any one of the preceding embodiments, the coating configured to cover at least a portion of the open landing area includes a liquid-repelling coating.

[0693] 108. The flow cell system of any one of the preceding embodiments, wherein the coating configured to cover at least a portion of the open landing area includes an omniphobic coating.

[0694] 109. The flow cell system of any one of the preceding embodiments, wherein the coating configured to cover at least a portion of the open landing area includes a slippery liquid-infused porous surface (SLIPS).

[0695] 110. The flow cell system of any one of the preceding embodiments, wherein the coating configured to cover at least a portion of the open landing area includes a slippery omniphobic covalently attached liquid (SOCAL) coating.

[0696] 111. The flow cell system of any one of the preceding embodiments, wherein the coating configured to cover at least a portion of the open landing area includes a liquid-like polymer brush surface that is covalently attached to the one or more substrates.

[0697] 112. The flow cell system of any one of the preceding embodiments, wherein the open landing area includes a porous surface, and the coating configured to cover at least a portion of the open landing area includes lubricants impregnated in the porous surface to generate the coating with a surface energy below about 20 mJ/m^2.

[0698] 113. The flow cell system of any one of the preceding embodiments, wherein the coating configured to cover at least a portion of the open landing area includes acid-catalyzed graft polycondensation of one or more saline monomers.

[0699] 114. The flow cell system of any one of the preceding embodiments, wherein the one or more saline monomers comprises dimethyldimethoxysilane.

[0700] 115. The flow cell system of any one of the preceding embodiments, wherein of the flow cell system is configured to clean, via at least one of the fluids or the gas gap, the first reagent from at least part of the one or more channels during DNA sequencing.

[0701] 116. The flow cell system of any one of the preceding embodiments, wherein the fluid cell system is configured to maintain at least part of the first reagent in the one or more channels during DNA sequencing.

[0702] 117. The flow cell system of any one of the preceding embodiments, wherein the first reagent and the second reagent are different.

[0703] 118. The flow cell system of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 40% of a total volume or length of the corresponding channel .

[0704] 119. The flow cell system of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 50 % of a total volume or length of the corresponding channel.

[0705] 120. The flow cell system of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 60% of a total volume or length of the corresponding channel.

[0706] 121. The flow cell system of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 70% of a total volume or length of the corresponding channel.

[0707] 122. The flow cell system of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 40% of a total volume or length of the corresponding channel .

[0708] 123. The flow cell system of any one of the preceding embodiments, wherein the cleaning outlet is configured to allow residuals of a first reagent on the open landing area to flow therethrough.

[0709] 124. The flow cell system of any one of the preceding embodiments, wherein the flow cell system further comprises:

a fluidic operation device comprising:

a first pump in fluidic connection with the cleaning outlet;

the first pump or a second pump in fluidic connection with the outlet; and

a dispenser that is configured to openly dispense the one or more reagents to the inlet.

[0710] 125. The flow cell system of any one of the preceding embodiments, wherein the first pump is configured to clean the open landing area by causing residuals of a first reagent to flow off the open landing area and through the cleaning outlet out of the fluid cell device.

[0711] 126. The flow cell system of any one of the preceding embodiments, wherein the residuals of the first reagent on the open landing area comprises meniscus of the first reagent.

[0712] 127. A method for manufacturing flow cell devices, comprising:

obtaining one or more substrates;

generating one or more channels in the one or more substrates, wherein the one or more channels are configured to allow fluids and a gas gap to flow therethrough, the gas gap configured to flow between one or more of the fluids;

forming an inlet in one of the one or more substrates and an open landing area, wherein the inlet is in fluidic connection with the one or more channels;

forming an outlet that is in fluidic connection with the one or more channels;

coating at least a portion of a surface of the one or more channels with a first coating, wherein the surface is configured to be dried and rewet during DNA sequencing; and

fixedly coupling the one of one or more substrates together.

[0713] 128. A method for manufacturing flow cell devices, comprising:

obtaining one or more substrates;

generating one or more channels in the one or more substrates;

forming an inlet in one of the one or more substrates and an open landing area, wherein the inlet is in fluidic connection with the one or more channels;

coating at least a portion of a surface of the one or more channels with a first coating;

covering at least a portion of the open landing area with a second coating; and

fixedly coupling the one of one or more substrates together.

[0714] 129. A method for sequencing with flow cell devices, comprising:

dispensing a first reagent openly to an open landing area of an inlet of the flow cell device;

flowing at least part of the first reagent from the open landing area through one or more channels of the flow cell device;

cleaning, after flowing at least part of the first reagent, residuals of the first reagent from the one or more channels by driving a gas gap from the inlet and through at least part of the one or more channels; and

dispensing a second reagent openly to the open landing area.

[0715] 130. A method for sequencing with flow cell devices, comprising:

dispensing a first reagent openly to an open landing area of an inlet of the flow cell device;

flowing at least part of the first reagent from the open landing area to one or more channels of the flow cell device;

facilitating cleaning of residuals of the first reagent off the open landing area by using a coating on at least part of the open landing area; and

dispensing a second reagent openly to the open landing area.

[0716] 131. A method for manufacturing flow cell devices, comprising:

obtaining one or more substrates;

forming an inlet in one of the one or more substrates and an open landing area;

generating one or more channels in the one or more substrates;

forming an outlet in the one or more substrates, wherein the inlet and outlet are in fluidic connection with the one or more channels;

forming a cleaning outlet in the one or more substrates, wherein the cleaning outlet is in fluidic connection with the inlet, and wherein the cleaning outlet is closer to the inlet than to the outlet; and

fixedly coupling the one of one or more substrates together.

[0717] 132. A method for sequencing with flow cell devices, comprising:

dispensing a first reagent openly to an open landing area of an inlet of the flow cell device;

flowing at least part of the first reagent from the open landing area to one or more channels of the flow cell device;

cleaning residuals of the first reagent from at least part of the open landing area by driving the residuals through a cleaning outlet; and

dispensing a second reagent openly to the open landing area.

[0718] 133. The method of any one of the preceding embodiments, wherein the one or more channels are configured to allow the gas gap to flow therethrough between allowing the first reagent and the second reagent to flow therethrough.

[0719] 134. The method of any one of the preceding embodiments, wherein the one or more channels are configured to allow the gas gap to flow therethrough during DNA sequencing.

[0720] 135. The method of any one of the preceding embodiments, wherein the one or more channels are configured to allow the gas gap to flow therethrough from the inlet.

[0721] 136. The method of any one of the preceding embodiments, wherein the one or more channels are configured to allow the gas gap to flow therethrough to reduce contamination of the second reagent by the first reagent in DNA sequencing.

[0722] 137. The method of any one of the preceding embodiments, wherein the one or more channels are configured to allow the gas gap to flow therethrough to reduce a total volume of the first reagent, the second reagent, or a washing reagent for DNA sequencing.

[0723] 138. The method of any one of the preceding embodiments, wherein each of the one of the one or more channels comprises a surface.

[0724] 139. The method of any one of the preceding embodiments, wherein the surface is an inner surface.

[0725] 140. The method of any one of the preceding embodiments, wherein the surface is an exterior surface.

[0726] 141. The method of any one of the preceding embodiments, wherein the surface comprises an interior top surface, an interior bottom surface, or both.

[0727] 142. The method of any one of the preceding embodiments, wherein the surface comprises an exterior top surface, an exterior bottom surface, or both.

[0728] 143. The method of any one of the preceding embodiments, wherein the surface is a planar surface.

[0729] 144. The method of any one of the preceding embodiments, wherein the surface is passivated.

[0730] 145. The method of any one of the preceding embodiments, wherein the surface is passivated with a coating that immobilizes surface capture primers, nucleic acid template molecules, or both for capturing polynucleotides.

[0731] 146. The method of any one of the preceding embodiments, wherein the surface comprises polynucleotides captured thereon.

[0732] 147. The method of any one of the preceding embodiments, wherein the gas gap is configured to dry at least part of the surface of the one or more channels.

[0733] 148. The method of any one of the preceding embodiments, wherein the gas gap does not impair chemical functions of the surface of the one or more channels.

[0734] 149. The method of any one of the preceding embodiments, wherein the coating of the surface of the one or more channels comprises at least one hydrophilic polymer coating layer.

[0735] 150. The method of any one of the preceding embodiments, wherein the coating of the surface of the one or more channels comprises a plurality of oligonucleotide molecules attached to at least one hydrophilic polymer coating layer.

[0736] 151. The method of any one of the preceding embodiments, wherein the surface of the one or more channels comprises at least one discrete region that comprises a plurality of clonally-amplified sample nucleic acid molecules that have been annealed to a plurality of attached oligonucleotide molecules.

[0737] 152. The method of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer has a water contact angle of no more than about 50 degrees.

[0738] 153. The method of any one of the preceding embodiments, wherein at least one of the plurality of clonally-amplified sample nucleic acid molecules comprises a concatemer annealed to at least one of the plurality of attached oligonucleotide.

[0739] 154. The method of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer comprises PEG.

[0740] 155. The method of any one of the preceding embodiments, wherein the surface further comprises a second hydrophilic polymer coating layer.

[0741] 156. The method of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer comprises a branched hydrophilic polymer.

[0742] 157. The method of any one of the preceding embodiments, wherein the branched hydrophilic polymer comprises at least 8 branches.

[0743] 158. The method of any one of the preceding embodiments, wherein the at least one of the plurality of the clonally-amplified sample nucleic acid molecules comprises a single-stranded multimeric nucleic acid molecule comprising repeats of a regularly occurring monomer unit.

[0744] 159. The method of any one of the preceding embodiments, wherein the single-stranded multimeric nucleic acid molecule is at least 10 kilobases in length.

[0745] 160. The method of any one of the preceding embodiments, wherein at least one of the plurality of the clonally-amplified sample nucleic acid molecules further comprises a double-stranded monomeric copy of the regularly occurring monomer unit.

[0746] 161. The method of any one of the preceding embodiments, wherein the plurality of oligonucleotide molecules is present at about a uniform surface density across the surface.

[0747] 162. The method of any one of the preceding embodiments, wherein the plurality of oligonucleotide molecules is present at a local surface density of at least 100,000 molecules/μm^2 at a first position on the surface, and at a second local surface density at a second position on the surface.

[0748] 163. The method of any one of the preceding embodiments, wherein the coating comprises:

a first layer comprising a monolayer of polymer molecules tethered to the surface of the substrate;

a second layer comprising a second monolayer of polymer molecules tethered to the polymer molecules of the first layer; and

a third layer comprising a third monolayer of polymer molecules tethered to the polymer molecules of the second layer, wherein at least one of the first layer, the second layer, or the third layer comprises branched polymer molecules.

[0749] 164. The method of any one of the preceding embodiments, wherein the third layer further comprises oligonucleotides tethered to the polymer molecules of the third layer.

[0750] 165. The method of any one of the preceding embodiments, wherein the oligonucleotides tethered to the polymer molecules of the third layer are distributed at a plurality of depths throughout the third layer.

[0751] 166. The method of any one of the preceding embodiments, wherein the coating further comprises:

a fourth layer comprising branched polymer molecules tethered to the polymer molecules of the third layer, and

a fifth layer comprising polymer molecules tethered to the branched polymer molecules of the fourth layer.

[0752] 167. The method of any one of the preceding embodiments, wherein the polymer molecules of the fifth layer further comprise oligonucleotides tethered to the polymer molecules of the fifth layer.

[0753] 168. The method of any one of the preceding embodiments, wherein the oligonucleotides tethered to the polymer molecules of the fifth layer are distributed at a plurality of depths throughout the fifth layer.

[0754] 169. The method of any one of the preceding embodiments, wherein the at least one hydrophilic polymer coating layer comprises a molecule selected from the group consisting

of: polyethylene glycol (PEG), poly(vinyl alcohol) (PVA), poly(vinyl pyridine), poly(vinyl pyrrolidone) (PVP), poly(acrylic acid) (PAA), polyacrylamide, poly(N-isopropylacrylamide) (PNIPAM), poly(methyl methacrylate) (PMA), poly(2-hydroxylethyl methacrylate) (PHEMA), poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA), polyglutamic acid (PGA), poly-lysine, poly-glucoside, streptavidin, and dextran.

[0755] 170. The method of any one of the preceding embodiments, wherein when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, an image of the surface exhibits a ratio of fluorescence intensities for the clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and nonspecific Cyanine dye-3 dye adsorption background (Binter) of at least 3:1.

[0756] 171. The method of any one of the preceding embodiments, wherein the image of the surface exhibits a ratio of fluorescence intensities for clonally amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and a combination of nonspecific Cyanine dye-3 dye adsorption background and nonspecific amplification background (B_{inter}+B_{intra}) of at least 3:1.

[0757] 172. The method of any one of the preceding embodiments, wherein when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, the image of the surface exhibits a ratio of fluorescence intensities for clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and nonspecific dye adsorption background (Binter) of at least 5:1.

[0758] 173. The method of any one of the preceding embodiments, wherein the image of the surface exhibits a ratio of fluorescence intensities for clonally-amplified, Cyanine dye-3-labeled sample nucleic acid molecules, or complementary sequences thereof, and a combination of nonspecific Cyanine dye-3 dye adsorption background and nonspecific amplification background (B_{inter}+B_{intra}) of at least 5:1.

[0759] 174. The method of any one of the preceding embodiments, wherein when the clonally-amplified sample nucleic acid molecules or complementary sequences thereof are labeled with Cyanine dye-3, the fluorescence image of the surface exhibits a contrast-to-noise ratio (CNR) of at least 20 when the fluorescence image is acquired using an inverted microscope equipped with a 20× objective, NA=0.75, dichroic mirror optimized for 532 nm light, a bandpass filter optimized for Cyanine dye-3 emission, and a camera under non-signal saturating conditions, while the surface is immersed in a buffer.

[0760] 175. The method of any one of the preceding embodiments, wherein the plurality of oligonucleotide molecules is present at a surface density of at least 1,000 molecules/m2.

[0761] 176. The method of any one of the preceding embodiments, wherein the first reagent is configured to wet the surface of the one or more channels.

[0762] 177. The method of any one of the preceding embodiments, wherein the second reagent is configured to rewet the surface of the one or more channels after at least partly drying the surface by the gas gap.

[0763] 178. The method of any one of the preceding embodiments, wherein the flow cell system further comprises:

a fluidic operation device comprising:

a first pump coupled with the outlet; and

a dispenser that is configured to openly dispense one or more reagents to the inlet.

[0764] 179. The method of any one of the preceding embodiments, wherein the first pump or a second pump is configured to introduce the gas gap via the inlet and flow the gas gap at least partly through the one or more channels.

[0765] 180. The method of any one of the preceding embodiments, wherein the gas gap comprises air.

[0766] 181. The method of any one of the preceding embodiments, wherein the gas gap comprises dry air.

[0767] 182. The method of any one of the preceding embodiments, wherein the gas gap comprises one or more inert gases.

[0768] 183. The method of any one of the preceding embodiments, wherein the gas gap comprises one or more active gases.

[0769] 184. The method of any one of the preceding embodiments, wherein the first or second reagent comprise liquid.

[0770] 185. The method of any one of the preceding embodiments, wherein the first or the second reagent is deprived of air bubbles that are greater than a predetermined size.

[0771] 186. The method of any one of the preceding embodiments, wherein the coating comprises a liquid-repelling coating.

[0772] 187. The method of any one of the preceding embodiments, wherein the coating comprises an omniphobic coating.

[0773] 188. The method of any one of the preceding embodiments, wherein the coating comprises a slippery liquid-infused porous surface (SLIPS).

[0774] 189. The method of any one of the preceding embodiments, wherein the coating comprises a slippery omniphobic covalently attached liquid (SOCAL) coating.

[0775] 190. The method of any one of the preceding embodiments, wherein the coating comprises a liquid-like polymer brush surface that is covalently attached to the one or more substrates.

[0776] 191. The method of any one of the preceding embodiments, wherein the coating is formed by impregnating lubricants in one or more porous surfaces.

[0777] 192. The method of any one of the preceding embodiments, wherein the lubricants comprise a liquid with a surface energy below about 20 mJ/m^2.

[0778] 193. The method of any one of the preceding embodiments, wherein the lubricants comprise a silicone oil.

[0779] 194. The method of any one of the preceding embodiments, wherein the coating comprises a surface energy that is below about 20 mJ/m^2.

[0780] 195. The method of any one of the preceding embodiments, wherein the coating is formed by acid-catalyzed graft polycondensation of one or more saline monomers.

[0781] 196. The method of any one of the preceding embodiments, wherein the one or more saline monomers comprises dimethyldimethoxysilane.

[0782] 197. The method of any one of the preceding embodiments, wherein the open landing area is in fluidic connection with the one or more channels.

[0783] 198. The method of any one of the preceding embodiments, wherein the open landing area is in fluidic connection with one of the one or more channels.

[0784] 199. The method of any one of the preceding embodiments, wherein the open landing area is disposed on a bottom substrate of the one or more substrates.

[0785] 200. The method of any one of the preceding embodiments, wherein the inlet comprises a hole defined by a top substrate of the one or more substrates.

[0786] 201. The method of any one of the preceding embodiments, wherein the hole defined by the top substrate is vertically align with at least a portion of the open landing area.

[0787] 202. The method of any one of the preceding embodiments, wherein the dispenser is configured to openly dispense the one or more reagents through the hole to the open landing area.

[0788] 203. The method of any one of the preceding embodiments, wherein the dispenser is configured to openly dispense the one or more reagents from a tip of the dispenser to the open landing area.

[0789] 204. The method of any one of the preceding embodiments, wherein the dispenser is configured to openly dispense the one or more reagents from the tip of the dispenser to the open landing area, without any tubing in between.

[0790] 205. The method of any one of the preceding embodiments, wherein at least part of the tip of the dispenser is in contact with the open landing area.

[0791] 206. The method of any one of the preceding embodiments, wherein the tip of the dispenser is not in contact with the open landing area.

[0792] 207. The method of any one of the preceding embodiments, wherein the flow cell device further comprises a cleaning outlet in the one or more substrates.

[0793] 208. The method of any one of the preceding embodiments, wherein the cleaning outlet is in fluidic connection with the inlet.

[0794] 209. The method of any one of the preceding embodiments, wherein the cleaning outlet is defined by a top or a bottom substrate of the one or more substrates.

[0795] 210. The method of any one of the preceding embodiments, wherein the cleaning outlet includes a side port on the one or more substrates.

[0796] 211. The method of any one of the preceding embodiments, wherein the cleaning outlet is configured to be coupled to the first pump or the second pump.

[0797] 212. The method of any one of the preceding embodiments, wherein the one or more channels comprises microfluidic channels.

[0798] 213. The method of any one of the preceding embodiments, wherein the surface of the one or more channels is coated with fluorescent beads that are chemically immobilized to the surface.

[0799] 214. The method of any one of the preceding embodiments, wherein the fluorescent beads are covalently attached to the surface of the one or more channels.

[0800] 215. The method of any one of the preceding embodiments, wherein a gap between the interior top surface and the interior bottom surface is about 150 μm, 130 μm, 120 μm, 110 μm, 100 μm, 90 μm, 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm.

[0801] 216. The method of any one of the preceding embodiments, wherein a height of the one or more channels is about 150 μm, 130 μm, 120 μm, 110 μm, 100 μm, 90 μm, 80 μm, 70 μm, 60 μm, 50 μm, or 40 μm.

[0802] 217. The method of any one of the preceding embodiments, wherein the polynucleotides captured thereon are configured to be imaged in a sequencing cycle.

[0803] 218. The method of any one of the preceding embodiments, wherein the one or more substrates comprises a top substrate and a bottom substrate.

[0804] 219. The method of any one of the preceding embodiments, wherein the one or more channels are defined between the top substrate and the bottom substrate.

[0805] 220. The method of any one of the preceding embodiments, wherein the one or more channels are defined at least partially defined by a top surface of the bottom substrate.

[0806] 221. The method of any one of the preceding embodiments, wherein the one or more channels are defined at least partially defined by a bottom surface of the top substrate.

[0807] 222. The method of any one of the preceding embodiments, wherein the one or more substrates further comprises a middle substrate.

[0808] 223. The method of any one of the preceding embodiments, wherein the one or more channels are defined at least partially defined by in the middle substrate.

[0809] 224. The method of any one of the preceding embodiments, wherein the one or more substrates comprises glass or plastic.

[0810] 225. The method of any one of the preceding embodiments, wherein at least a portion of the support is transparent.

[0811] 226. The method of any one of the preceding embodiments, wherein at least a portion of the one or more substrates is transparent.

[0812] 227. The method of any one of the preceding embodiments, wherein at least a portion the support is opaque.

[0813] 228. The method of any one of the preceding embodiments, wherein the one or more channels comprises 1, 2, 3, 4, 5, 6, 7, or 8 channels.

[0814] 229. The method of any one of the preceding embodiments, wherein the one or more channels comprises 2, 4, 6, 8, or 10 channels.

[0815] 230. The method of any one of the preceding embodiments, wherein each channel of the one or more channels comprises a lane length of less than about 70 mm, 75 mm, 80 mm, or 90 mm.

[0816] 231. The method of any one of the preceding embodiments, wherein each channel of the one or more channels comprises a lane width of less than about 10 mm, 8 mm, 7 mm, 6 mm, 5 mm, 4 mm, or 3 mm.

[0817] 232. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises covering at least a portion of the open landing area with a slippery coating.

[0818] 233. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises covering at least a portion of the open landing area with a liquid-repelling coating.

[0819] 234. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises covering at least a portion of the open landing area with an omniphobic coating.

[0820] 235. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises covering at least a portion of the open landing area with a slippery liquid-infused porous surface (SLIPS).

[0821] 236. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises covering at least a portion of the open landing area with a slippery omniphobic covalently attached liquid (SOCAL) coating.

[0822] 237. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises covering at least a portion of the open landing area with a liquid-like polymer brush surface that is covalently attached to the one or more substrates.

[0823] 238. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises impregnating lubricants in porous surfaces to generate the coating with a surface energy below about 20 mJ/m^2.

[0824] 239. The method of any one of the preceding embodiments, wherein covering at least a portion of the open landing area with the second coating comprises acid-catalyzed graft polycondensation of one or more saline monomers.

[0825] 240. The method of any one of the preceding embodiments, wherein the one or more saline monomers comprises dimethyldimethoxysilane.

[0826] 241. The method of any one of the preceding embodiments, wherein cleaning at least part of the first reagent from at least part of the one or more channels is during DNA sequencing.

[0827] 242. The method of any one of the preceding embodiments, wherein the at least part of the first reagent remain2 in the one or more channels.

[0828] 243. The method of any one of the preceding embodiments, wherein the first reagent and the second reagent are different.

[0829] 244. The method of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 40% of a total volume or length of the corresponding channel comprises more than about 40% of a corresponding volume or length of each of the one or more channels.

[0830] 245. The method of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 50% of a total volume or length of the corresponding channel.

[0831] 246. The method of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 60% of a total volume or length of the corresponding channel.

[0832] 247. The method of any one of the preceding embodiments, wherein a body portion of a channel of the one or more channels comprises more than 70% of a total volume or length of the corresponding channel.

[0833] 248. The method of any one of the preceding embodiments, wherein at least part of the one or more channels comprises more than about 80% of a corresponding volume or length of each of the one or more channels.

[0834] 249. The method of any one of the preceding embodiments, further comprising:

driving residuals of the first reagent or the second reagent off the open landing area via a cleaning outlet of the flow cell device.

[0835] 250. The method of any one of the preceding embodiments, wherein the cleaning outlet is configured to allow residuals of the first reagent on the open landing area to flow therethrough out of a fluid cell device.

[0836] 251. The method of any one of the preceding embodiments, wherein the flow cell system further comprises:

a fluidic operation device comprising:

a first pump in fluidic connection with the cleaning outlet;

the first pump or the second pump in fluidic connection with the outlet; and

a dispenser that is configured to openly dispense the one or more reagents to the inlet.

[0837] 252. The method of any one of the preceding embodiments, wherein the first pump is configured to clean the open landing area by driving residuals of the first reagent off the open landing area to flow through the cleaning outlet.

[0838] 253. The method of any one of the preceding embodiments, further comprising:

cleaning at least part of the first reagent from at least part of the one or more channels by driving the gas gap between fluids from the inlet and through at least part of the one or more channels.

[0839] 254. The method of any one of the preceding embodiments, wherein the residuals of the first reagent on the open landing area comprises meniscus of the first reagent.

[0840] 255. A fluid dispensing device, comprising:

a dispensing module comprising:

a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein;

a microfluidic chip in fluidic communication with each of the one or more compartments, the microfluidic chip having one or more microfluidic pathways therewithin; and

one or more dispensing tips; and

one or more actuators that actuate one or more movable pins for pushing the fluidic reagents from the one or more compartments to the one or more dispensing tips via the one or more microfluidic pathways.

[0841] 256. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip, the one or more actuators, and the one or more movable pins are comprised in a pump.

[0842] 257. The fluid dispensing device of any one of the preceding embodiments, wherein the fluid dispensing device is coupled to a flow cell system.

[0843] 258. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more compartments are not in fluidic communication with each other.

[0844] 259. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more compartments includes two or more compartments having different volumes.

[0845] 260. The fluid dispensing device of any one of the preceding embodiments, wherein each of the one or more dispensing tips is in fluidic communication with a corresponding compartment of the one or more compartments.

[0846] 261. The fluid dispensing device of any one of the preceding embodiments, wherein each of the one or more compartments comprises a corresponding compartment outlet.

[0847] 262. The fluid dispensing device of any one of the preceding embodiments, wherein the dispensing module is disposable.

[0848] 263. The fluid dispensing device of any one of the preceding embodiments, wherein the dispensing module is removable when it is mechanically coupled to a sequencing system.

[0849] 264. The fluid dispensing device of any one of the preceding embodiments, wherein the dispensing module is configured to be removably coupled to a flow cell device and a sequencing system for sequencing analysis of a sample immobilized on the flow cell device.

[0850] 265. The fluid dispensing device of any one of the preceding embodiments, wherein the sample comprises nucleotide acid template molecules.

[0851] 266. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators are configured to be removeable from the dispensing module.

[0852] 267. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators comprise only one actuator.

[0853] 268. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators comprise only 2, 3, 4, 5, 6, 7, 8, 9, or 10 actuators.

[0854] 269. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators are fixedly coupled to a next generation sequencing (NGS) system.

[0855] 270. The fluid dispensing device of any one of the preceding embodiments, wherein the dispensing module is configured to be removably coupled to the NGS system.

[0856] 271. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators are removably coupled to the NGS system, and wherein the one or more actuators are movable relative to the one or more dispensing tips.

[0857] 272. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators are configured to move to a corresponding spatial position to actuate a corresponding movable pin for pushing a corresponding fluidic reagent from the reagent cartridge to the corresponding dispensing tip via a corresponding microfluidic pathway on the microfluidic chip.

[0858] 273. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators are configured to move to a corresponding spatial position to actuate a corresponding movable pin for pulling a corresponding fluidic reagent to the reagent cartridge via a corresponding microfluidic pathway on the microfluidic chip.

[0859] 274. The fluid dispensing device of any one of the preceding embodiments, wherein each of the microfluidic pathways is in fluidic communication with a corresponding pathway inlet and a corresponding pathway outlet in the microfluidic chip.

[0860] 275. The fluid dispensing device of any one of the preceding embodiments, wherein the corresponding compartment outlet of one of the one or more compartments is fluidically connected with a corresponding pathway in a fluidically sealed fashion to prevent leakage from the connection.

[0861] 276. The fluid dispensing device of any one of the preceding embodiments, wherein the corresponding compartment outlet of one of the one or more compartments is fluidically connected with a corresponding pathway inlet in a fluidically sealed fashion to prevent leakage from the connection.

[0862] 277. The fluid dispensing device of any one of the preceding embodiments, wherein the corresponding microfluidic pathway is fluidically connected with a corresponding dispensing tip in a fluidically sealed fashion to prevent leakage from the connection.

[0863] 278. The fluid dispensing device of any one of the preceding embodiments, wherein each of the microfluidic pathways may include a curved portion between the corresponding pathway inlet and outlet.

[0864] 279. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip is fixedly coupled to the reagent cartridge.

[0865] 280. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip is positioned underneath the reagent cartridge.

[0866] 281. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more dispensing tips are positioned below the reagent cartridge, the microfluidic chip, or both.

[0867] 282. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more movable pins are configured to apply pressure or force on the curved portion of the corresponding pathway to push or pull reagents therein in a predetermined direction.

[0868] 283. The fluid dispensing device of any one of the preceding embodiments, wherein each movable pin is configured to rotate about a rotational axis that is orthogonal to a plane of the microfluidic chip to move fluid in the curved portion in one or both directions.

[0869] 284. The fluid dispensing device of any one of the preceding embodiments, wherein the curved portion is curved within the plane of the microfluidic chip.

[0870] 285. The fluid dispensing device of any one of the preceding embodiments, wherein the curved portion extends in a direction that is orthogonal to the plane of the microfluidic chip.

[0871] 286. The fluid dispensing device of any one of the preceding embodiments, wherein each movable pin is configured to translate toward the microfluidic chip to apply pressure to the curved portion to move fluid in the curved portion in one or both directions.

[0872] 287. The fluid dispensing device of any one of the preceding embodiments, wherein each movable pin is configured to translate in a direction that is parallel to the plane of the microfluidic chip to move fluid in the curved portion in one or both direction.

[0873] 288. The fluid dispensing device of any one of the preceding embodiments, wherein each movable pin is coupled to the microfluidic chip to seal the corresponding fluid reagent in a corresponding microfluidic pathway within the microfluidic chip to prevent leakage of the dispensing module.

[0874] 289. The fluid dispensing device of any one of the preceding embodiments, wherein each movable pin is fixedly coupled to the one or more actuator.

[0875] 290. The fluid dispensing device of any one of the preceding embodiments, wherein the pump comprises one or more plunger and barrel pairs, and wherein each plunger and barrel pair is in fluidic communication with a corresponding compartment and a corresponding pathway.

[0876] 291. The fluid dispensing device of any one of the preceding embodiments, wherein each plunger is movable relative to the corresponding barrel to move a corresponding fluidic reagent therewithin in a predetermined direction.

[0877] 292. The fluid dispensing device of any one of the preceding embodiments, wherein each moveable pin is a plunger movable relative to the corresponding barrel to move a corresponding fluidic reagent therewithin in a predetermined direction.

[0878] 293. The fluid dispensing device of any one of the preceding embodiments, wherein each barrel of the one or more plunger barrel pairs is immobilized relative to the reagent cartridge, the microfluidic chip, or both.

[0879] 294. The fluid dispensing device of any one of the preceding embodiments, wherein each plunger of the one or more plunger barrel pairs is actuated by the one or more actuators.

[0880] 295. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more actuators comprises a single actuator movable relative to the one or more microfluidic pathways to actuate a corresponding movable pin, syringe, or plunger.

[0881] 296. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more dispensing tips are configured to openly dispense one or more reagents to a flow cell device.

[0882] 297. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more dispensing tips are movable relative to an inlet of the flow cell device.

[0883] 298. The fluid dispensing device of any one of the preceding embodiments, wherein each fluidic reagent is configured to travel from a corresponding compartment to a corresponding dispensing tip via a corresponding pathway to avoid cross contamination with other fluidic reagents.

[0884] 299. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip may comprise one or more fluidic reservoirs within the chip.

[0885] 300. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more fluidic reservoirs are in fluidic communication with the one or more microfluidic pathways.

[0886] 301. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more movable pins comprises the plunger in the barrel and plunger pair.

[0887] 302. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip includes one or more valves that are 3-way valves.

[0888] 303. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more valves are in fluidic communication with the one or more dispensing tips, one or more compartments, and one or more fluidic reservoirs via the one or more microfluidic pathways.

[0889] 304. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more compartments of the reagent cartridge and the one or more fluidic reservoirs of the microfluidic chip are in fluidic communication via the one or more microfluidic pathways when the corresponding valves are in a first position.

[0890] 305. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more dispensing tips and the one or more fluidic reservoirs are in fluidic communication via the one or more microfluidic pathways when the corresponding valves are in a second position.

[0891] 306. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more valves are configured to switch between the first and second positions.

[0892] 307. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip may comprise a top film, a bottom film, or both bonded to a substrate, thereby forming the microfluidic pathways and/or the one or more reservoirs within the microfluidic chip.

[0893] 308. The fluid dispensing device of any one of the preceding embodiments, wherein individual reservoirs are fluidically connected to the barrel and plunger pair via a same connector.

[0894] 309. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more valves comprise: a solenoid valve, a pneumatic valve, a rotary valve, a membrane valve, or a combination thereof.

[0895] 310. The fluid dispensing device of any one of the preceding embodiments, wherein one or more fluidic reservoirs are each in communication with a corresponding barrel and plunger pair.

[0896] 311. The fluid dispensing device of any one of the preceding embodiments, wherein one or more fluidic reservoirs are in communication with a single barrel and plunger pair.

[0897] 312. A flow cell system comprising:

a flow cell device comprising:

a support comprising one or more substrates;

one or more channels defined by the one or more substrates, wherein the one or more channels are configured to allow fluids to flow therethrough and a gas gap to flow therethrough between the fluids;

an inlet in the one or more substrates, the inlet in fluidic connection with the one or more channels; and

an outlet in the one or more substrates, wherein the one or more channels run from the inlet to the outlet; and

a fluid dispensing device comprising a dispensing module comprising:

a reagent cartridge with one or more compartments, wherein the one or more compartments are configured to contain fluidic reagents therein;

a microfluidic chip in fluidic communication with each of the one or more compartments, wherein the microfluidic chip comprises one or more microfluidic pathways therewithin;

one or more dispensing tips; and

one or more actuators configured to actuate one or more movable pins for pushing the fluidic reagents from the one or more compartments to the one or more dispensing tips through the one or more microfluidic pathways.

[0898] 313. The flow cell system of any one of the preceding embodiments, wherein the one or more compartments are not in fluidic communication with each other.

[0899] 314. The flow cell system of any one of the preceding embodiments, wherein the one or more compartment includes two or more compartments having different volumes.

[0900] 315. The flow cell system of any one of the preceding embodiments, wherein each of the one or more dispensing tips is in fluidic communication with a corresponding compartment of the one or more compartments.

[0901] 316. The flow cell system of any one of the preceding embodiments, wherein each of the one or more compartments comprises a corresponding compartment outlet.

[0902] 317. The flow cell system of any one of the preceding embodiments, wherein the dispensing module is disposable.

[0903] 318. The flow cell system of any one of the preceding embodiments, wherein the dispensing module is configured to be removably coupled to a flow cell device for sequencing analysis of a sample immobilized on the flow cell device.

[0904] 319. The flow cell system of any one of the preceding embodiments, wherein the the actuators are configured to be resvirsibly coupled to the dispensing module.

[0905] 320. The flow cell system of any one of the preceding embodiments, wherein the one or more actuators comprise only one actuator.

[0906] 321. The flow cell system of any one of the preceding embodiments, wherein the one or more actuators comprise only two or more actuators.

[0907] 322. The flow cell system of any one of the preceding embodiments, wherein the one or more actuators are fixedly coupled to a next generation sequencing (NGS) system.

[0908] 323. The flow cell system of any one of the preceding embodiments, wherein the dispensing module is configured to be removably coupled to the NGS system.

[0909] 324. The flow cell system of any one of the preceding embodiments, wherein the one or more actuators are removably coupled to the NGS system, and wherein the one or more actuators are movable relative to the one or more dispensing tips.

[0910] 325. The flow cell system of any one of the preceding embodiments, wherein the one or more actuators are configured to move to a corresponding spatial position to actuate the pump for pumping a corresponding fluidic reagent from the reagent cartridge to the corresponding dispensing tip via a corresponding microfluidic pathway on the microfluidic chip.

[0911] 326. The flow cell system of any one of the preceding embodiments, wherein each of the microfluidic pathway is in fluidic communication with a corresponding pathway inlet and a corresponding pathway outlet in the microfluidic chip.

[0912] 327. The flow cell system of any one of the preceding embodiments, wherein the corresponding compartment outlet is connected with a corresponding pathway in a fluidically sealed fashion to prevent leakage from the connection.

[0913] 328. The flow cell system of any one of the preceding embodiments, wherein the corresponding compartment outlet is connected with a corresponding pathway inlet in a fluidically sealed fashion to prevent leakage from the connection.

[0914] 329. The flow cell system of any one of the preceding embodiments, wherein the corresponding microfluidic pathway is connected with a corresponding dispensing tip in a fluidically sealed fashion to prevent leakage from the connection.

[0915] 330. The flow cell system of any one of the preceding embodiments, wherein each of the microfluidic pathway may include a curved portion between the corresponding pathway inlet and outlet.

[0916] 331. The flow cell system of any one of the preceding embodiments, wherein the microfluidic chip is fixedly coupled to the reagent cartridge.

[0917] 332. The flow cell system of any one of the preceding embodiments, wherein the microfluidic chip is positioned underneath the reagent cartridge.

[0918] 333. The flow cell system of any one of the preceding embodiments, wherein the one or more dispensing tips are positioned below the reagent cartridge, the microfluidic chip, or both.

[0919] 334. The flow cell system of any one of the preceding embodiments, wherein the one or more movable pins are configured to apply pressure or force on the curved portion of the corresponding pathway to move reagent therein in a predetermined direction.

[0920] 335. The flow cell system of any one of the preceding embodiments, wherein each movable pin is configured to rotate about a rotational axis that is orthogonal to a plane of the microfluidic chip to move fluid in the curved portion in one or both directions.

[0921] 336. The flow cell system of any one of the preceding embodiments, wherein the curved portion is curved within the plane of the microfluidic chip.

[0922] 337. The flow cell system of any one of the preceding embodiments, wherein the curved portion extends in a direction that is orthogonal to the plane of the microfluidic chip.

[0923] 338. The flow cell system of any one of the preceding embodiments, wherein each movable pin is configured to translate toward the microfluidic chip to apply pressure to the curved portion move fluid in the curved portion in one or both directions.

[0924] 339. The flow cell system of any one of the preceding embodiments, wherein each movable pin is configured to translate in a direction that is parallel to the plane of the microfluidic chip to move fluid in the curved portion in one or both direction.

[0925] 340. The flow cell system of any one of the preceding embodiments, wherein each movable pin is coupled to the microfluidic chip to seal the corresponding fluid reagent in the one or more microfluidic pathways within the microfluidic chip to prevent leakage of the dispensing module.

[0926] 341. The flow cell system of any one of the preceding embodiments, wherein each movable pin is fixedly coupled to the one or more actuators.

[0927] 342. The flow cell system of any one of the preceding embodiments, wherein the pump further comprises one or more plunger and barrel pairs, and wherein each plunger and barrel pair is in fluidic communication with a corresponding compartment and a corresponding pathway.

[0928] 343. The flow cell system of any one of the preceding embodiments, wherein each plunger is movable relative to the corresponding barrel to move a corresponding fluidic reagent therewithin in a predetermined direction.

[0929] 344 The flow cell system of any one of the preceding embodiments, wherein each barrel of the one or more plunger barrel pairs is immobilized relative to the reagent cartridge, the microfluidic chip, or both.

[0930] 345. The flow cell system of any one of the preceding embodiments, wherein each plunger of the one or more plunger barrel pairs is actuated by the one or more actuators.

[0931] 346. The flow cell system of any one of the preceding embodiments, wherein the pump further comprises one or more syringes that are configured to direct reagent from the one or more compartments to the one or more pathways.

[0932] 347. The flow cell system of any one of the preceding embodiments, wherein the one or more actuators comprises a single actuator movable relative to the one or more microfluidic pathways to actuate a corresponding movable pin, syringe, or plunger.

[0933] 348. The flow cell system of any one of the preceding embodiments, wherein the one or more dispensing tips are configured to openly dispense one or more reagents to a flow cell device.

[0934] 349. The flow cell system of any one of the preceding embodiments, wherein the one or more dispensing tips are movable relative to an inlet of the flow cell device.

[0935] 350. The flow cell system of any one of the preceding embodiments, wherein each fluidic reagent is configured to travel from a corresponding compartment to a corresponding dispensing tip via a corresponding pathway to avoid cross contamination with other fluidic reagents.

[0936] 351. The flow cell system of any one of the preceding embodiments, wherein the microfluidic chip may comprises one or more fluidic reservoirs within the chip.

[0937] 352. The flow cell system of any one of the preceding embodiments, wherein the one or more fluidic reservoirs are in fluidic communication with the one or more microfluidic pathways.

[0938] 353. The flow cell system of any one of the preceding embodiments, wherein the one or more movable pins comprises the plunger in the barrel and plunger pair.

[0939] 354. The flow cell system of any one of the preceding embodiments, wherein the microfluidic chip includes one or more valves that are 3-way valves.

[0940] 355. The flow cell system of any one of the preceding embodiments, wherein the one or more valves are in fluidic communication with the one or more dispensing tips, one or

more compartments, and one or more fluidic reservoirs via the one or more microfluidic pathways.

[0941] 356. The flow cell system of any one of the preceding embodiments, wherein the one or more compartments of the reagent cartridge and the one or more fluidic reservoirs of the microfluidic chip are in fluidic communication via the one or more microfluidic pathways when the corresponding valves are in a first position.

[0942] 357. The flow cell system of any one of the preceding embodiments, wherein the one or more dispensing tips and the one or more fluidic reservoirs are in fluidic communication via the one or more microfluidic pathways when the corresponding valves are in a second position.

[0943] 358. The flow cell system of any one of the preceding embodiments, wherein the one or more valves are configured to switch between the first and second positions.

[0944] 359. The flow cell system of any one of the preceding embodiments, wherein the microfluidic chip may comprise a top film, a bottom film, or both bonded to a substrate, thereby forming the microfluidic pathways and/or the one or more reservoirs within the microfluidic chip.

[0945] 360. The flow cell system of any one of the preceding embodiments, wherein individual reservoirs are fluidically connected to the barrel and plunger pair via a same connector.

[0946] 361. The flow cell system of any one of the preceding embodiments, wherein the one or more valves comprise: a solenoid valve, a pneumatic valve, a rotary valve, a membrane valve, or a combination thereof.

[0947] 362. The flow cell system of any one of the preceding embodiments, wherein one or more fluidic reservoirs are each in communication with a corresponding barrel and plunger pair.

[0948] 363. The flow cell system of any one of the preceding embodiments, wherein one or more fluidic reservoirs are in communication with a single barrel and plunger pair.

[0949] 364. A method of using a fluid dispensing device, comprising:

providing a dispensing module comprising:

a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein;

a microfluidic chip in fluidic connection with each of the one or more compartments, wherein the microfluidic chip comprises one or more microfluidic pathways therewithin; and

one or more dispensing tips; and

providing one or more actuators that actuate one or more movable pins for pushing the fluidic reagents from the one or more compartments to the one or more dispensing tips through the one or more microfluidic pathways.

[0950] 365. A method of using fluid dispensing device, comprising:

fluidically connecting one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured to hold fluidic reagents therein, and wherein the microfluidic chip comprises one or more microfluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip;

removably coupling a dispensing module to one or more actuators, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments; and

the one or more dispensing tips; and

actuating, by one or more actuators, one or more movable pins to apply a first force or pressure on a curved portion of a first microfluidic pathway for a first predetermined time to actuate a first reagent from a first compartment of the one or more compartments to a first dispensing tip of the one or more dispensing tips via a first microfluidic pathway of the one or more microfluidic pathways; and

receiving the first reagent from the first dispensing tip openly at an inlet of a flow cell device.

[0951] 366. A method of using fluid dispensing device, comprising:

fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured for holding fluidic reagents therein, and wherein the microfluidic chip comprises one or more microfluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip;

removably coupling a dispensing module to one or more actuators, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments; and

the one or more dispensing tips; and

switching one or more valves into a first position;

actuating, by one or more actuators, one or more movable pins to apply a first force or pressure on a first microfluidic pathway for a first predetermined time to actuate a first reagent from a first compartment of the one or more compartments to a first reservoir via a first microfluidic pathway of the one or more microfluidic pathways;

switching one or more valves into a second position;

actuating, by one or more actuators, one or more movable pins to apply a second force or pressure on the first microfluidic pathway of the one or more microfluidic pathways for a second predetermined time to actuate the first reagent from the first reservoir to a first dispensing tip via the first microfluidic pathway of the one or more microfluidic pathways; and

receiving the first reagent from the first dispensing tip openly at an inlet of a flow cell device.

[0952] 367. The method of any one of the preceding embodiments further comprising:

applying a second force or pressure on a curved portion of a second microfluidic pathway for a second predetermined time to actuate a second reagent from a second compartment to a second dispensing tip via the second microfluidic pathway; and

receiving the second reagent from the second dispensing tip openly to the inlet of the flow cell device.

[0953] 368. The method of any one of the preceding embodiments further comprising:

moving the one or more actuators relative to the flow cell device.

[0954] 369. The method of any one of the preceding embodiments further comprising:

removing at least part of the first or second reagent from the flow cell device.

[0955] 370. The method of any one of the preceding embodiments further comprising:

recycling, the at least part of the first or second reagent removed from the flow cell device back to the one or more compartments of the reagent cartridge.

[0956] 371. The method of any one of the preceding embodiments further comprising:

providing a plurality of nucleic acid template molecules immobilized on the flow cell device, wherein each nucleic acid template molecule comprises: a first insert

sequence and a first sample index sequence, wherein the first sample index sequence comprises a first universal sample index sequence, the first universal sample index identifying a sample source of the insert sequence.

[0957] 372. The method of any one of the preceding embodiments further comprising:

conducting, by the sequencing system, one or more cycles of sequencing reactions of the first insert sequence before conducting one or more cycles of the sequencing reactions of the first sample index sequence to generate flow cell images comprising a first flow cell image and a second flow cell image from a first flow cycle.

[0958] 373. The method of any one of the preceding embodiments further comprising:

conducting, by the sequencing system, one or more cycles of sequencing reactions of the first sample index sequence before conducting one or more cycles of the sequencing reactions of the first insert sequence to generate flow cell images.

[0959] 374. The method of any one of the preceding embodiments, wherein conducting, by the sequencing system, one or more cycles of sequencing reactions of the first insert sequence comprises:

dispensing, by at least a first dispensing tip, a first reagent comprising a first plurality of soluble sequencing primers that hybridize to the plurality of nucleic acid template molecules to one or more inlets of a flow cell device;

allowing the first reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device;

dispensing, by at least a second dispensing tip, a second reagent comprising with a second plurality of soluble sequencing primers that hybridize to the plurality of nucleic acid template molecules; and

allowing the second reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device.

[0960] 375. The method of any one of the preceding embodiments further comprising:

reversibly removing the dispensing module without removing the one or more actuators relative to the sequencing system; and

removably coupling a second dispensing module to the one or more actuators and to the sequencing system.

[0961] 376. The method of any one of the preceding embodiments further comprising:

dispensing, by at least a first dispensing tip, a first plurality of sequencing primers, a first plurality of polymerases and a first mixture of different types of multivalent molecules to one or more inlets of the flow cell device; and

allowing the first reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device.

[0962] 377. The method of any one of the preceding embodiments further comprising:

dispensing, by at least a first dispensing tip, a first plurality of sequencing primers, a first plurality of polymerases and a first mixture of different types of multivalent molecules to one or more inlets of the flow cell device;

allowing the first reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device;

removing the plurality of first sequencing polymerases; and

dispensing, by at least a second dispensing tip, a second plurality of polymerases to one or more inlets of the flow cell device.

[0963] 378. A fluid dispensing device, comprising:

a dispensing module comprising:

a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein;

a microfluidic chip in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways therewithin;

a transportation valve having at least one open position and at least one closed position rotationally separated from the at least one open position, and wherein the transportation valve is positioned between the reagent cartridge and the microfluidic chip;

an actuator configured to actuate the transportation valve between the at least one open position and the at least one closed position; and

one or more dispensing tips,

wherein the transportation valve, in the at least one closed position, is configured to seal each of the one or more compartment and the microfluidic chip.

[0964] 379. A fluid dispensing device, comprising:

a dispensing module comprising:

a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein;

a microfluidic chip in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways therewithin;

a transportation valve having at least two open positions and at least one closed position that is rotationally separated from the at least two open positions;

an actuator configured to actuate the transportation valve between the at least two open positions and the at least one closed position; and

one or more dispensing tips,

wherein the transportation valve, in the at least one closed position, is configured to seal each of the one or more compartment and the microfluidic chip.

[0965] 380. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve comprises a valve substrate.

[0966] 381. The fluid dispensing device of any one of the preceding embodiments, wherein the valve substrate is a disk substrate or a ring substrate.

[0967] 382. The fluid dispensing device of any one of the preceding embodiments, wherein the valve substrate comprises a plurality of open ports.

[0968] 383. The fluid dispensing device of any one of the preceding embodiments, wherein the plurality of open ports are distributed radially along a circumference in the valve substrate.

[0969] 384. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve comprises an over-mold or a gasket around each of the plurality of open ports.

[0970] 385. The fluid dispensing device of any one of the preceding embodiments, wherein the over-mold or gasket is around each of the plurality of open ports on a first side facing the reagent cartridge and a second side facing the microfluidic chip.

[0971] 386. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve comprises an actuation arm configured to be actuated by an actuator thereby causing rotation of the transportation valve.

[0972] 387. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve comprises one or more teeth that are configured to couple to a rotor, and wherein the rotor is configured to be actuated by an actuator to rotate the transportation valve.

[0973] 388. The fluid dispensing device of any one of the preceding embodiments further comprising: a biasing spring that is configured to bias the rotor along a z axis.

[0974] 389. The fluid dispensing device of any one of the preceding embodiments, wherein the biasing spring is configured to bias the rotor along the z axis in a first direction to mate with the transportation valve.

[0975] 390. The fluid dispensing device of any one of the preceding embodiments, wherein the biasing spring is configured to bias the transportation valve along the z axis in a second direction opposite the first direction to disengage the rotor from the transportation valve and couple the rotor with the microfluidic chip.

[0976] 391. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is configured to rotate about an axis that is orthogonal to the valve substrate.

[0977] 392. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is configured to rotate 3 to 45 degrees from the at least one open position to the at least one closed position in a first direction.

[0978] 393. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is configured to rotate 3 to 45 degrees from the at least one closed position to the at least one open position in a second direction opposite to the first direction.

[0979] 394. The fluid dispensing device of any one of the preceding embodiments, wherein a total number of open ports matches the total number of compartments of the reagent cartridge, and each open port corresponds to a corresponding compartment for allowing fluidic communication from the corresponding compartment to the open port.

[0980] 395. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is mounted to a bottom of the reagent cartridge.

[0981] 396. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a plurality of pairs of dispensing ports.

[0982] 397. The fluid dispensing device of any one of the preceding embodiments, wherein each dispensing port is in fluidic communication with a corresponding dispensing tip.

[0983] 398. The fluid dispensing device of any one of the preceding embodiments, wherein each dispensing port is configured to mount a corresponding dispensing tip.

[0984] 399. The fluid dispensing device of any one of the preceding embodiments, wherein a distance between each pair of dispensing ports is determined based on a distance between two open landing areas of a flow cell device.

[0985] 400. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a first in-chip well corresponding to a first pair of dispensing tips from the one or more dispensing tips.

[0986] 401. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is configured to placed in fluidic communication with an off-chip pump via a first fluidic pathway.

[0987] 402. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well comprises one or more cut-outs of a wall of the first in-chip well at or near a first end thereof, wherein the first end of the first in-chip well is configured to placed in fluidic communication with the off-chip pump via the first fluidic pathway.

[0988] 403. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is configured to placed in fluidic communication with the reagent cartridge via a first in-chip valve and a second fluidic pathway.

[0989] 404. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is configured to placed in fluidic communication with a first pair of the dispensing ports via a second in-chip valve.

[0990] 405. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is configured to placed in fluidic communication with only the first pair of the dispensing ports via the second in-chip valve.

[0991] 406. The fluid dispensing device of any one of the preceding embodiments, wherein the second in-chip valve is configured to placed in fluidic communication with the first pair of the dispensing ports via a third fluidic pathway.

[0992] 407. The fluid dispensing device of any one of the preceding embodiments, wherein the first fluidic pathway, the second fluidic pathway, the third fluidic pathway, the first in-chip well, the first in-chip valve, the second in-chip valve, or a combination thereof correspond to only a first compartment of the reagent cartridge.

[0993] 408. The fluid dispensing device of any one of the preceding embodiments, wherein each of the first or second in-chip valve comprises an open position and a closed position.

[0994] 409. The fluid dispensing device of any one of the preceding embodiments, wherein the first or second in-chip valve is configured to shift between the open position and the close position when a pressure exerted on the first or second in-chip valve is altered.

[0995] 410. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a second in-chip well corresponding to a second pair of the dispensing tips.

[0996] 411. The fluid dispensing device of any one of the preceding embodiments, wherein the second in-chip well is configured to placed in fluidic communication with the off-chip pump or a second pump via a fourth fluidic pathway.

[0997] 412. The fluid dispensing device of any one of the preceding embodiments, wherein the second in-chip well comprises one or more cut-outs of a wall of the second in-chip well at or near a first end thereof, wherein the first end of the second in-chip well is configured to placed in fluidic communication with the off-chip pump via the fourth fluidic pathway.

[0998] 413. The fluid dispensing device of any one of the preceding embodiments, wherein the second in-chip well is configured to placed in fluidic communication with the reagent cartridge via a third in-chip valve and a fifth fluidic pathway.

[0999] 414. The fluid dispensing device of any one of the preceding embodiments, wherein the second in-chip well is configured to placed in fluidic communication with a second pair of the dispensing ports via a fourth in-chip valve.

[01000] 415. The fluid dispensing device of any one of the preceding embodiments, wherein the second in-chip well is configured to placed in fluidic communication with only a second pair of the dispensing ports via the fourth in-chip valve.

[01001] 416. The fluid dispensing device of any one of the preceding embodiments, wherein the fourth in-chip valve is configured to placed in fluidic communication with the second pair of the dispensing ports via a sixth fluidic pathway.

[01002] 417. The fluid dispensing device of any one of the preceding embodiments, wherein the fourth fluidic pathway, the fifth fluidic pathway, the sixth fluidic pathway, the second in-chip well, the third in-chip valve, the fourth in-chip valve, or a combination thereof correspond to only a second compartment of the reagent cartridge.

[01003] 418. The fluid dispensing device of any one of the preceding embodiments, wherein each of the third or fourth in-chip valve comprises an open position and a closed position.

[01004] 419. The fluid dispensing device of any one of the preceding embodiments, wherein the third or fourth in-chip valve is configured to shift between the open position and the close position when a pressure exerted on the third or fourth in-chip valve is altered.

[01005] 420. The fluid dispensing device of any one of the preceding embodiments, wherein when the transport valve is in the at least one open position, the first in-chip valve is open, and the second in-chip valve is closed, the dispensing module is configured to aspirate reagent from the first compartment to the in-chip well.

[01006] 421. The fluid dispensing device of any one of the preceding embodiments, wherein when the transport valve is in the at least one open position, the first in-chip valve is closed, and the second in-chip valve is open, the dispensing module is configured to dispense reagent from the in-chip well to only the first pair of the plurality of pairs of dispending ports.

[01007] 422. The fluid dispensing device of any one of the preceding embodiments, wherein each of the plurality of open ports comprises an elongated shape extending along a circumference of the valve substrate.

[01008] 423. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve comprises a central open port having the elongated shape extending along a radius of the valve substrate.

[01009] 424. The fluid dispensing device of any one of the preceding embodiments, wherein at least two of the plurality of open ports are at two different circumferences of the valve substrate.

[01010] 425. The fluid dispensing device of any one of the preceding embodiments, wherein at least two of the plurality of open ports are located along a same radius of the valve substrate.

[01011] 426. The fluid dispensing device of any of the preceding embodiments, wherein the central open port is in fluidic communication with a pump.

[01012] 427. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip is positioned between the transportation valve and the reagent cartridge.

[01013] 428. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises one or more in-chip wells.

[01014] 429. The fluid dispensing device of any one of the preceding embodiments, wherein each in-chip well is in fluidic communication with a first well opening and a second well opening.

[01015] 430. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a first through-hole in fluidic communication with a first pair of dispensing ports via a third fluidic pathway.

[01016] 431. The fluid dispensing device of any one of the preceding embodiments. wherein the microfluidic chip comprises a second through-hole in fluidic communication with a first compartment of the reagent cartridge via a second fluidic pathway.

[01017] 432. The fluid dispensing device of any one of the preceding embodiments ,wherein the microfluidic chip comprises a first through-hole in fluidic communication with only a first pair of dispensing ports via a third fluidic pathway.

[01018] 433. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a second through-hole in fluidic communication with only a first compartment of the reagent cartridge via a second fluidic pathway.

[01019] 434. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is in a first open position of the at least two open positions when a first open port connects the first through-hole and a first well opening of a first in-chip well.

[01020] 435. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is in a first open position of the at least two open positions to allow reagent dispensing from the first in-chip well to the first pair of dispensing tips.

[01021] 436. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is in a first open position when the first open port is positioned above the first through-hole and the first well opening.

[01022] 437. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is in a second open position of the at least two open positions when a first open port connects the second through-hole and a first well opening of a first in-chip well.

[01023] 438. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is in a second open position of the at least two open positions to allow reagent aspiration from the first compartment of the reagent cartridge to the first in-chip well.

[01024] 439. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is in a second open position when the first open port is positioned above the second through-hole and the first well opening.

[01025] 440. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve is a closed position when the first open port is positioned not directly above the first through-hole, the second through-hole, and the first well opening.

[01026] 441. The fluid dispensing device of any one of the preceding embodiments, wherein when the transportation valve is in the first or second open position, the central open port connects a second well opening to the pump via a first fluidic pathway.

[01027] 442. The fluid dispensing device of any one of the preceding embodiments, wherein the first and second through-holes correspond to the first in-chip well.

[01028] 443. The fluid dispensing device of any one of the preceding embodiments, wherein the transportation valve further comprises third and fourth through-holes corresponding to a second in-chip well.

[01029] 444. The fluid dispensing device of any one of the preceding embodiments, wherein the first and second through-holes correspond only to a first open port, and the third and

fourth through-hole correspond only to a second open port that is different from the first open port to avoid cross contamination.

[01030] 445. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a chip substrate.

[01031] 446. The fluid dispensing device of any one of the preceding embodiments, wherein the first and second through-holes are positioned along a circumference of the chip substrate and a first radius of the chip substrate.

[01032] 447. The fluid dispensing device of any one of the preceding embodiments, wherein the third and fourth through-holes are positioned along a second circumference of the chip substrate and a second radius of the chip substrate.

[01033] 448. The fluid dispensing device of any one of the preceding embodiments, wherein the second radius is 5 to 75 degrees apart from the first radius by rotation about an axis at the center of the chip substate.

[01034] 449. The fluid dispensing device of any one of the preceding embodiments, wherein the chip substrate comprises a circular shape.

[01035] 450. The fluid dispensing device of any one of the preceding embodiments, wherein a first and second open position of at least two open positions correspond to the first open port.

[01036] 451. The fluid dispensing device of any one of the preceding embodiments, wherein a third and fourth open position of at least two open positions correspond to the second open port.

[01037] 452. The fluid dispensing device of any one of the preceding embodiments, wherein when the transportation valve is in a first or second open position of the least two open positions, only the first in-chip well is in fluidic communication with the first pair of dispensing tips or the first compartment, the second in-chip well is sealed from the reagent cartridge and the plurality of dispensing tips.

[01038] 453. The fluid dispensing device of any one of the preceding embodiments, wherein when the transportation valve is in a first or second open position of the least two open positions, the first in-chip well is in fluidic communication with the first pair of dispensing tips or the first compartment, and the second in-chip well is in fluidic communication with the reagent cartridge or the plurality of dispensing tips.

[01039] 454. The fluid dispensing device of any one of the preceding embodiments, wherein when the transportation valve is in a third or fourth open position of the least two open positions, only the second in-chip well is in fluidic communication with the second pair of

dispensing tips or the second compartment, the first in-chip well is sealed from the reagent cartridge and the plurality of dispensing tips.

[01040] 455. The fluid dispensing device of any one of the preceding embodiments, wherein when the transportation valve is in a third or fourth open position of the least two open positions, the second in-chip well is in fluidic communication with the second pair of dispensing tips or the second compartment, and the first in-chip well is in fluidic communication with the reagent cartridge or the plurality of dispensing tips.

[01041] 456. The fluid dispensing device of any one of the preceding embodiments, wherein the at least two open positions comprises 2, 4, 6, 8, 10, 12, 14, 16, 18, or 20 open positions, and each pair of open positions correspond to a different individual open port and a different individual compartment of the reagent cartridge.

[01042] 457. A method of using a fluid dispensing device, comprising:

fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured to hold fluidic reagents therein, and wherein the microfluidic chip comprises one or more fluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip;

removably coupling a dispensing module to one or more actuators, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments;

a transportation valve; and

the one or more dispensing tips; and

switching a transportation valve into an open position by rotating, by one or more actuators, the transportation valve for a predetermined rotational angle in a first direction;

switching a first in-chip valve into an open position to allow a first reagent from a first compartment to flow to a first in-chip well via a second fluidic pathway for a first predetermined duration; and

switching a second in-chip valve into an open position to allow the first reagent to flow from the first in-chip well to a first pair of dispensing tips for a second predetermined duration.

[01043] 458. A method of using a fluid dispensing device, comprising:

fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured for holding fluidic reagents therein, and wherein the microfluidic chip comprises one or more fluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip;

removably coupling a dispensing module to one or more actuators, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments;

a transportation valve; and

the one or more dispensing tips; and

switching the transportation valve into a first open position from a closed position by rotating, by one or more actuators, the transportation valve for a first predetermined rotational angle in a first direction to allow a first open port to connect a first compartment to a first in-chip well via a fluidic pathway; and

switching the transportation valve into a second open position by rotating, by the one or more actuators, the transportation valve for a second predetermined rotational angle in a second direction opposite the first direction to allow the first open port to connect the first in-chip well to a first pair of dispensing tips.

[01044] 459. The method of any one of the preceding embodiments, wherein fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip comprises:

moving the microfluidic chip from a washing position to a sequencing position;

connecting one or more sippers that are in fluidic communication with the microfluidic chip to the one or more compartments to enable fluidic communication between the microfluidic chip and the one or more compartment.

[01045] 460. The method of any one of the preceding embodiments, wherein moving the microfluidic chip from a washing position to a sequencing position is before starting a new sequencing run.

[01046] 461. The method of any one of the preceding embodiments further comprising:

moving the microfluidic chip from the sequencing position to the washing position; and

connecting the one or more sippers that are in fluidic communication with the microfluidic chip to one or more washing compartments in the reagent cartridge; and

washing the one or more sippers and one or more fluidic pathways in the microfluidic chip using a washing buffer in the one or more washing compartments; and

disposing washing waste to a waste collector.

[01047] 462. The method of any one of the preceding embodiments further comprising disposing the reagent cartridge and installing a new reagent cartridge subsequent to washing the one or more sippers and one or more fluidic pathways in the microfluidic chip.

[01048] 463. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the sequencing position in a x-y plane.

[01049] 464. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the sequencing position for at least 1mm, 3mm, 5mm, 8mm, 10 mm, 20mm, or 30mm.

[01050] 465. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the sequencing position for no more than 1 mm, 3mm, 5mm, 8mm, 10 mm, 20mm, 30mm, or 50mm.

[01051] 466. The method of any one of the preceding embodiments further comprising:

moving the microfluidic chip from the sequencing position to the washing position; and

connecting the one or more sippers that are in fluidic communication with the microfluidic chip to one or more washing compartments in the reagent cartridge; and

washing the one or more sippers and one or more fluidic pathways in the microfluidic chip using a washing buffer in the one or more washing compartments; and

moving the microfluidic chip from the washing position to a second washing position.

[01052] 467. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the second position in a x-y plane.

[01053] 468. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the second position for at least 1mm, 3mm, 5mm, 8mm, 10 mm, 20mm, or 30mm.

[01054] 469. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the second position for no more than 1 mm, 3mm, 5mm, 8mm, 10 mm, 20mm, 30mm, or 50mm.

[01055] 470. The method of any one of the preceding embodiments, wherein connecting the one or more sippers that are in fluidic communication with the microfluidic chip to one or more washing compartments in the reagent cartridge comprises:

inserting each sipper of the one or more sippers into a corresponding compartment of the reagent cartridge.

[01056] 471. The method of any one of the preceding embodiments further comprising:

switching the transportation valve into a third open position by rotating, by the one or more actuators, the transportation valve for a third predetermined rotational angle, to allow a second open port to connect a second compartment to a second in-chip well; and

switching the transportation valve into a fourth open position by rotating, by the one or more actuators, the transportation valve for a fourth predetermined rotational angle, to allow the second open port to connect the second in-chip well to a second pair of dispensing tips.

[01057] 472. The method of any one of the preceding embodiments further comprising:

switching the transportation valve into a closed position by rotating, by the one or more actuators, the transportation valve for the predetermined rotational angle in a second direction opposite the first direction, to seal the first compartment from the microfluidic chip.

[01058] 473. The method of any one of the preceding embodiments, the method further comprises:

switching the first in-chip valve into an open position while switching or maintaining the second in-chip valve in a close position to allow a first reagent from a first compartment to flow to a first in-chip well via a second fluidic pathway for a first predetermined duration.

[01059] 474. The method of any one of the preceding embodiments, wherein switching a second in-chip valve into an open position to allow the first reagent to flow from the first in-chip well to a first pair of dispensing tips for a second predetermined duration comprises:

switching the second in-chip valve into the open position while switching or maintaining the first in-chip valve in the closed position to allow the first reagent to flow from the first in-chip well to a first pair of dispensing tips for a second predetermined duration.

[01060] 475. A method of using a fluid dispensing device in connection with a DNA sequencings system, comprising:

fluidically connecting one or more compartments of a reagent cartridge of the DNA sequencing system with a microfluidic chip of the fluidic dispensing device, the one or more compartments configured for containing fluidic reagents therein, and wherein the microfluidic chip comprises one or more microfluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip;

removably coupling a dispensing module to one or more actuators, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments; and

the one or more dispensing tips; and

applying, by the one or more actuators, a first force or pressure on a first valve of a first microfluidic pathway for a first predetermined time to allow a first reagent from a first compartment to flow to an in-chip well in the microfluidic chip via a first microfluidic pathway (3320_2); and

applying, by the one or more actuators, a second force or pressure on a second valve of a second microfluidic pathway for a second predetermined time to keep the second valve open while applying, by the one or more actuators, a dispensing force or pressure to the in-chip well in the microfluidic chip to flow the first reagent from the in-chip well to the one or more dispensing tips via the second valve and the third fluidic pathway (3320_3) of the microfluidic chip.

[01061] 476. The method of any one of the preceding embodiments, wherein the second fluidic pathway comprises a first portion that is between a top film and a chip substrate, and a second portion that is between the chip substrate and a bottom film of the microfluidic chip.

[01062] 477. The method of any one of the preceding embodiments, wherein the first fluidic pathway comprises a first portion that is between a top film and a chip substrate, and a second portion that is between the chip substrate and a bottom film of the microfluidic chip.

[01063] 478. The method of any one of the preceding embodiments, wherein the in-chip well extends vertically between a top and a bottom film of the microfluidic chip.

[01064] 479. The method of any one of the preceding embodiments, wherein the first or second fluidic pathway comprises a third portion connecting the first and second portion of the fluidic pathway, and wherein the third portion extends in the microfluidic substrate in a direction that is orthogonal to the microfluidic substrate.

[01065] 480. The method of any one of the preceding embodiments further comprising:

applying, by the one or more actuators, a third force or pressure on a third valve of a third microfluidic pathway for a third predetermined time to allow a second reagent from a second compartment to flow to a second in-chip well in the microfluidic chip via a third microfluidic pathway;

applying, by the one or more actuators, a fourth force or pressure on a fourth valve of a second microfluidic pathway for a fourth predetermined time to keep the fourth valve open while applying, by the one or more actuators, a second dispensing force or pressure to the second in-chip well in the microfluidic chip to flow the second reagent from the second in-chip well to the one or more dispensing tips via the fourth valve and the fourth fluidic pathway of the microfluidic chip; and

receiving the second reagent from the one or more dispensing tip openly at an inlet of a flow cell device.

[01066] 481. The method of any one of the preceding embodiments, wherein applying the first force or pressure on the first valve of the first microfluidic pathway is during when the second valve of the second microfluidic pathway is closed.

[01067] 482. The method of any one of the preceding embodiments, wherein applying the second force or pressure on the second valve of the second microfluidic pathway is during when the first valve of the microfluidic chip is closed.

[01068] 483. The method of any one of the preceding embodiments, wherein applying the dispensing force or pressure to the in-chip well in the microfluidic chip comprises pushing air through a fluidic pathway connected to the in-chip well at a top portion of the in-chip well.

[01069] 484. The method of any one of the preceding embodiments, wherein applying the dispensing force or pressure to the in-chip well in the microfluidic chip comprises driving the first reagent in the in-chip well downward into the second portion of the second fluidic

pathway, then upward in the third portion of the second fluidic pathway, and then in the first portion of the second fluidic pathway.

[01070] 485. The method of any one of the preceding embodiments further comprising:

moving the one or more actuators relative to the flow cell device.

[01071] 486. The method of any one of the preceding embodiments further comprising:

removing at least part of the first or second reagent from the flow cell device.

[01072] 487. The method of any one of the preceding embodiments further comprising:

recycling, the at least part of the first or second reagent removed from the flow cell device back to the one or more compartments of the reagent cartridge.

[01073] 488. The method of any one of the preceding embodiments further comprising:

providing a plurality of nucleic acid template molecules immobilized on the flow cell device, wherein each nucleic acid template molecule comprises: a first insert sequence and a first sample index sequence, wherein the first sample index sequence comprises a first universal sample index sequence, the first universal sample index identifying a sample source of the insert sequence.

[01074] 489. The method of any one of the preceding embodiments further comprising:

conducting, by the sequencing system, one or more cycles of sequencing reactions of the first insert sequence before conducting one or more cycles of the sequencing reactions of the first sample index sequence to generate flow cell images comprising the first flow cell image and the second flow cell image in the first flow cycle.

[01075] 490. The method of any one of the preceding embodiments further comprising:

conducting, by the sequencing system, one or more cycles of sequencing reactions of the first sample index sequence before conducting one or more cycles of the sequencing reactions of the first insert sequence to generate flow cell images.

[01076] 491. The method of any one of the preceding embodiments, wherein conducting, by the sequencing system, one or more cycles of sequencing reactions of the first insert sequence comprises:

dispensing, by at least a first dispensing tip, a first reagent comprising a first plurality of soluble sequencing primers that hybridize to the plurality of nucleic acid template molecules to one or more inlets of a flow cell device;

allowing the first reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device;

dispensing, by at least a second dispensing tip, a second reagent comprising with a second plurality of soluble sequencing primers that hybridize to the plurality of nucleic acid template molecules; and

allowing the second reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device.

[01077] 492. The method of any one of the preceding embodiments further comprising:

reversibly removing the dispensing module without removing the one or more actuators relative to the sequencing system; and

removably coupling a second dispensing module to the one or more actuators and to the sequencing system.

[01078] 493. The method of any one of the preceding embodiments further comprising:

dispensing, by at least a first dispensing tip, a first plurality of sequencing primers, a first plurality of polymerases and a first mixture of different types of multivalent molecules to one or more inlets of the flow cell device; and

allowing the first reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device.

[01079] 494. The method of any one of the preceding embodiments further comprising:

dispensing, by at least a first dispensing tip, a first plurality of sequencing primers, a first plurality of polymerases and a first mixture of different types of multivalent molecules to one or more inlets of the flow cell device;

allowing the first reagent to travel to and contact the plurality of nucleic acid template molecules at the flow cell device;

removing the plurality of first sequencing polymerases; and

dispensing, by at least a second dispensing tip, a second plurality of polymerases to one or more inlets of the flow cell device.

[01080] 495. A fluid dispensing device, comprising:

a dispensing module comprising:

a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein;

a microfluidic chip in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways and one more in-chip wells therewithin, wherein at least one of the fluidic pathways comprises a first portion and a second portion displaced from each other along a direction orthogonal to a chip substrate of the microfluidic chip;

a transportation valve positioned between the reagent cartridge and the microfluidic chip;

an actuator configured to actuate the transportation valve between the at least one open position and the at least one closed position; and

one or more dispensing tips.

[01081] 496. A fluid dispensing device, comprising:

a dispensing module comprising:

a reagent cartridge with one or more compartments, wherein the one or more compartments are configured for holding fluidic reagents therein;

a microfluidic chip in fluidic communication with the one or more compartments, the microfluidic chip having a first valve, a second valve, one or more fluidic pathways and one more in-chip wells therewithin, wherein at least one of the fluidic pathways comprises a first portion and a second portion displaced from each other along a direction orthogonal to a chip substrate of the microfluidic chip;

a transportation valve positioned between the reagent cartridge and the microfluidic chip;

an actuator configured to actuate the first and second valves of the microfluidic chip; and

one or more dispensing tips.

[01082] 497. The fluid dispensing device of any one of the preceding embodiments, wherein the at least one of the fluidic pathways comprises a third portion connecting the first and second portion, and wherein the third portion extends at least in a direction that is orthogonal to the microfluidic substrate.

[01083] 498. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises: a top film, a microfluidic chip substrate, and a bottom film.

[01084] 499. The fluid dispensing device of any one of the preceding embodiments, wherein the top film covers and seals at least a portion of a top surface of the microfluidic substrate.

[01085] 500. The fluid dispensing device of any one of the preceding embodiments, wherein the bottom film covers and seals at least a portion of a bottom surface of the microfluidic substrate.

[01086] 501. The fluid dispensing device of any one of the preceding embodiments, wherein the bottom film comprises an area that is smaller than the top film.

[01087] 502. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic substrate comprises a pump port configured to couple to an off-chip pump.

[01088] 503. The fluid dispensing device of any one of the preceding embodiments, wherein the pump port extends downward from the microfluidic substrate.

[01089] 504. The fluid dispensing device of any one of the preceding embodiments, wherein the fluidic pathway is configured for gas passage only.

[01090] 505. The fluid dispensing device of any one of the preceding embodiments, wherein the fluidic pathway comprises a filter that blocks fluids disposed in the in-chip well from entering the fluidic pathway.

[01091] 506. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a plurality of pairs of dispensing ports.

[01092] 507. The fluid dispensing device of any one of the preceding embodiments, wherein each dispensing port is in fluidic communication with a corresponding dispending tip.

[01093] 508. The fluid dispensing device of any one of the preceding embodiments, wherein each dispensing port is configured to mount a corresponding dispending tip to the microfluidic chip.

[01094] 509. The fluid dispensing device of any one of the preceding embodiments, wherein a distance between each pair of the dispensing ports is determined based on the distance between two open landing areas of a flow cell device.

[01095] 510. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip comprises a first in-chip well corresponding to a first pair of the dispensing tips.

[01096] 511. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is in fluidic communication with an off-chip pump via a first fluidic pathway.

[01097] 512. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is in fluidic communication with the reagent cartridge via a first in-chip valve and a second fluidic pathway.

[01098] 513. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is in fluidic communication with a first pair of the dispensing ports via a second in-chip valve.

[01099] 514. The fluid dispensing device of any one of the preceding embodiments, wherein the first in-chip well is in fluidic communication with only the first pair of the dispensing ports via the second in-chip valve.

[01100] 515. The fluid dispensing device of any one of the preceding embodiments, wherein the second in-chip valve is in fluidic communication with the first pair of the dispensing ports via a third fluidic pathway.

[01101] 516. The fluid dispensing device of any one of the preceding embodiments, wherein the first fluidic pathway, the second fluidic pathway, the third fluidic pathway, the first in-chip well, the second in-chip valve, or a combination thereof correspond to only a first compartment of the reagent cartridge.

[01102] 517. The fluid dispensing device of any one of the preceding embodiments, wherein each of the first or second in-chip valve comprises an open position and a closed position.

[01103] 518. The fluid dispensing device of any one of the preceding embodiments, wherein the first or second in-chip valve is configured to shift between the open position and the close position when a pressure or force exerted on the first or second in-chip valve is altered.

[01104] 519. The fluid dispensing device of any one of the preceding embodiments, wherein when a transport valve of the fluid dispensing device is in the at least one open position, the first in-chip valve is open, and the second in-chip valve is closed, the dispensing module is configured to aspirate reagent from the first compartment to the first in-chip well.

[01105] 522. The fluid dispensing device of any one of the preceding embodiments, wherein when the transport valve is in the at least one open position, the first in-chip valve is closed, and the second in-chip valve is open, the dispensing module is configured to dispense reagent from the first in-chip well to only a first pair of the plurality of pairs of dispensing ports.

[01106] 523. A fluid dispensing device, comprising:

a dispensing module comprising:

a reagent cartridge with one or more compartments for containing fluidic reagents therein;

a microfluidic chip configured to be in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways and one or more in-chip wells therewithin;

a first, second, and third valve;

a press plate configured to press on the microfluidic chip thereby forming a first, second, and third seals of the one or more fluidic pathways and the one or more in-chip wells; and

one or more dispensing tips,

wherein the microfluidic chip is disposed in a first position and the second seal is formed to allow reagent aspiration to the one or more in-chip wells, and

wherein the microfluidic chip is disposed in a second position and the first and the third seals are formed to allow reagent dispensing to the one or more dispensing tips.

[01107] 524. The fluid dispensing device of any one of the preceding embodiments, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips are removably connected to the press plate and the first, second, and third valves.

[01108] 525. The fluid dispensing device of any one of the preceding embodiments, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips lack liquid communication with the press plate or the first, second, and third valves.

[01109] 526. The fluid dispensing device of any one of the preceding embodiments, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips are configured to be disconnected from the press plate and the first, second, and third valves for disposal after completion of a sequencing run.

[01110] 527. The fluid dispensing device of any one of the preceding embodiments, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips are permanently attached to each other.

[01111] 528. The fluid dispensing device of any one of the preceding embodiments, wherein the press plate comprises one or more geometrical shapes, and wherein each of the one or more geometrical shapes is configured to surround an opening in the one or more of the fluidic pathways or an in-chip well.

[01112] 529. The fluid dispensing device of any one of the preceding embodiments, wherein each of the one or more geometrical shapes protrudes toward the microfluidic chip from a bottom of the press plate.

[01113] 530. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip further comprises a film covering at least a portion of the one or more fluidic pathways.

[01114] 531. The fluid dispensing device of any one of the preceding embodiments, wherein the film is a top film positioned on top of the microfluidic chip and sealed to thereto at a sealed contour.

[01115] 532. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more in-chip wells are within the sealed contour in a x-y plane.

[01116] 533. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more fluidic pathways are only partly contained within the sealed contour in a x-y plane.

[01117] 534. The fluid dispensing device of any one of the preceding embodiments, wherein all openings of the one or more fluidic pathways are contained within the sealed contour in a x-y plane.

[01118] 535. The fluid dispensing device of any one of the preceding embodiments, wherein the first, second, or third seal is formed within the sealed contour in a x-y plane.

[01119] 536. The fluid dispensing device of any one of the preceding embodiments, wherein the first, second, or third seal is formed using the press plate which presses the top film into contact with the microfluidic chip, thereby blocking fluidic flow in at least a portion of the one or more fluidic pathways.

[01120] 537. The fluid dispensing device of any one of the preceding embodiments, wherein each of the first, second, or third seal is configured to seal around an opening in the one or more fluidic pathways, a connection in the one or more fluidic pathways, or an in-well chip.

[01121] 538. The fluid dispensing device of any one of the preceding embodiments, wherein each of the first, second, or third seal is configured to seal around an opening in the one or more fluidic pathways, a connection in the one or more fluidic pathways, or an in-well chip thereby blocking fluidic flow from entering or existing the opening in the one or more fluidic pathways, the connection in the one or more fluidic pathways, or the in-well chip.

[01122] 539. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip further comprises a second film covering at least a portion of the one or more fluidic pathways of the microfluidic chip.

[01123] 540. The fluid dispensing device of any one of the preceding embodiments, wherein the second film is a bottom film sealed to the chip substrate at a second sealed contour.

[01124] 541. The fluid dispensing device of any one of the preceding embodiments, wherein the first, second, and third valves are actuated by pneumatic pressure or force.

[01125] 542. The fluid dispensing device of any one of the preceding embodiments, wherein the one or more fluidic pathways comprise at a portion of a fluidic pathway that comprises gaseous flow and lacks liquid flow.

[01126] 543. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip is configured to be removably connected to the reagent cartridge and the one or more dispensing tips in a sequencing position.

[01127] 544. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip is disconnected from the reagent cartridge and the one or more dispensing tips in a washing position.

[01128] 545. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip is configured to spatially move relative to the reagent cartridge and the one or more dispensing tips between the sequencing position and the washing position.

[01129] 546. The fluid dispensing device of any one of the preceding embodiments, wherein the first, second, and third valves are configured to spatially move at least within an x-y plane relative to the one or more fluidic pathways and the in-chip wells.

[01130] 547. The fluid dispensing device of any one of the preceding embodiments, wherein the first, second, and third valves are spatially fixed within the x-y plane relative to the one or more fluidic pathways and the in-chip wells.

[01131] 548. The fluid dispensing device of any one of the preceding embodiments, wherein the first, second, and third valves corresponds to the first, second, and third seals of a first fluidic pathway and a first in-chip well.

[01132] 549. The fluid dispensing device of any one of the preceding embodiments further comprising a fourth, fifth, and sixth valves corresponding to a fourth, fifth, and sixth seal of a second fluidic pathway and a second fluidic chip.

[01133] 550. The fluid dispensing device of any one of the preceding embodiments, wherein the microfluidic chip is in the first position for reagent aspiration with only the second seal, and wherein the microfluidic chip is in the second position for reagent dispensing to the one or more dispensing tips with only the first and third seals.

[01134] 551. A flow cell system comprising:

a flow cell device comprising:

a support comprising one or more substrates;

one or more channels defined by the one or more substrates, wherein the one or more channels are configured to allow fluids to flow therethrough and a gas gap to flow therethrough between the fluids;

an inlet in the one or more substrates, the inlet in fluidic connection with the one or more channels; and

an outlet in the one or more substrates, wherein the one or more channels run from the inlet to the outlet; and

a fluid dispensing device comprising a dispensing module comprising:

a reagent cartridge with one or more compartments for holding fluidic reagents therein;

a microfluidic chip configured to be in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways and one or more in-chip wells therewithin;

a first, second, and third valve;

a press plate configured to press on the microfluidic chip thereby forming a first, second, and third seals of the one or more fluidic pathways and the one or more in-chip wells; and

one or more dispensing tips,

wherein the microfluidic chip is in a first position for reagent aspiration to the one or more in-chip wells with the second seal, and wherein the microfluidic chip is in a second position for reagent dispending to the one or more dispensing tips with the first and third seals.

[01135] 552. The flow cell system of any one of the preceding embodiments, wherein each fluidic reagent is configured to travel from a corresponding compartment to a corresponding dispensing tip of the one or more dispensing tips via a corresponding fluidic pathway and a corresponding in-chip well to avoid cross contamination with other fluidic reagents.

[01136] 553. The flow cell system of any one of the preceding embodiments, wherein two or more fluidic reagents are configured to from a corresponding compartment to a same in-chip well and to a same dispensing tip of the one or more dispensing tips to allow premixing of the two or more fluidic reagents before they reach the flow cell device.

[01137] 554. The flow cell system of any one of the preceding embodiments, wherein the inlet is coupled to or includes an open landing area disposed on a substrate of the one or more substrates.

[01138] 555. The flow cell system of any one of the preceding embodiments, wherein the open landing area is in fluidic connection with the one or more channels, and wherein the fluid dispenser device is configured to openly dispense the fluidic reagents from the one or more dispensing tips to the open landing area, without using any tubing in between.

[01139] 556. The flow cell system of any one of the preceding embodiments, wherein flow cell device further comprises a coating configured to cover at least a portion of the open landing area.

[01140] 557. The flow cell system of any one of the preceding embodiments, wherein the coating comprises at least one of a slippery liquid-infused porous surface (SLIPS) or a slippery omniphobic covalently attached liquid (SOCAL) coating.

[01141] 558. The flow cell system of any one of the preceding embodiments, wherein the coating is hydrophobic.

[01142] 559. The flow cell system of any one of the preceding embodiments, wherein the flow cell device further comprises a cleaning outlet in the one or more substrates, wherein the cleaning outlet is in fluidic connection with the inlet.

[01143] 560. The flow cell system of any one of the preceding embodiments, wherein the flow cell device is configured for holding one or more in situ samples of cells or tissue to be sequenced.

[01144] 570. A method of using a fluid dispensing device, comprising:

fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured to contain fluidic reagents therein, and wherein the microfluidic chip comprises one or more fluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more fluidic pathways of the microfluidic chip;

removably coupling a dispensing module to a first, second, and third valve, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments, the microfluidic chip comprising:

a chip substrate; and

a film sealed to the chip substrate;

a press plate that presses the film to regionally forms a first seal, a second seal, a third seal, or a combination thereof with the chip substrate;

the one or more dispensing tips; and

switching the first valve or the first valve and the third valve into a closed position and switching the second valve into an open position to move the press plate thereby forming the second seal to allow a first reagent from a first compartment to flow into a first in-chip well; and

switching the second valve into a closed position and switch the first and third valve into an open position to move the press plate thereby forming the first and third seals to allow the first reagent to flow from the first in-chip well to one or more of dispensing tips.

[01145] 580. A method of using a fluid dispensing device, comprising:

fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured to hold fluidic reagents therein, and wherein the microfluidic chip comprises one or more fluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip;

removably coupling a dispensing module to one or more valves, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments; and

the one or more dispensing tips; and

switching a first valve, second valve, or third valve of the one or more valves into an open position to block fluidic communication between the one or more compartments and the microfluidic chip or fluidic communication between the microfluidic chip and the one or more dispensing tips; and

moving the dispensing module away from a sequencing system without moving the one or more valves relative to the sequencing system.

[01146] 590. The method of any one of the preceding embodiments, wherein one or more actuators actuate the one or more valves.

[01147] 591. The method of any one of the preceding embodiments, wherein the press plate is attached to the one or more valves.

[01148] 592. The method of any one of the preceding embodiments, wherein the press plate comprises one or more of: a first geometric shape, a second geometric shape, and a third geometric shape.

[01149] 593. The method of any one of the preceding embodiments, wherein the press plate comprises a plurality of geometrical shapes protruding toward the microfluidic chip, and wherein each of the geometrical shape corresponds to one of: an opening of the one or more fluidic pathways, a connection of the one or more fluidic pathways, and an in-chip well for forming a corresponding seal.

[01150] 594. The method of any one of the preceding embodiments, wherein switching a first valve, second valve, or third valve of the one or more valves into an open position to block fluidic communication between the one or more compartments and the microfluidic

chip or fluidic communication between the microfluidic chip and the one or more dispensing tips comprises:

pressing, by the press plate, the film sealed to the microfluidic chip to be in contact with the chip substrate of the microfluidic chip and form one or more of the first, second, and third seals.

[01151] 595. The method of any one of the preceding embodiments, wherein switching the first valve or the first valve and the third valve into a closed position and switching the second valve into an open position to move the press plate thereby forming the second seal to allow a first reagent from a first compartment to flow into a first in-chip well comprises:

pressing, by the press plate, the film sealed to the chip substrate of the microfluidic chip to form the second seal.

[01152] 596. The method of any one of the preceding embodiments, wherein switching the second valve into a closed position and switch the first and third valve into an open position to move the press plate thereby forming the first and third seals to allow the first reagent to flow from the first in-chip well to one or more of dispensing tips comprises:

pressing, by the press plate, the film sealed to the chip substrate of the microfluidic chip to be in contact with the chip substrate and form the first and third seals.

[01153] 597. The method of any one of the preceding embodiments, wherein pressing, by the press plate, the membrane sealed to the chip substrate of the microfluidic chip to form one or more of the first, second, and third seals is generating by actuation of the one or more valves by the one or more actuators.

[01154] 598. The method of any one of the preceding embodiments, wherein pressing, by the press plate, the membrane sealed to the chip substrate of the microfluidic chip to form one or more of the first, second, and third seals is caused by pneumatic pressure on the press plate caused by opening of the one or more of the first, second, and third valves.

[01155] 599. The method of any one of the preceding embodiments, wherein the first seal is configured to seal fluidic communication from the one or more compartment to the first in-chip well.

[01156] 600. The method of any one of the preceding embodiments, wherein the third seal is configured to seal fluid flow from entering or exiting the first in-chip well.

[01157] 601. The method of any one of the preceding embodiments, wherein the third seal is configured to seal fluidic communication from the first in-chip well to the one or more dispensing tips.

[01158] 602. The method of any one of the preceding embodiments, wherein the film is sealed to the chip substrate at a sealed contour, and wherein the first, second, and third seals are spatially contained within the sealed contour.

[01159] 603. The method of any one of the preceding embodiments, wherein fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip comprises:

connecting one or more sippers that are in fluidic communication with the microfluidic chip to the one or more compartments to enable fluidic communication between the microfluidic chip and the one or more compartments.

[01160] 604. The method of any one of the preceding embodiments, wherein fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip comprises:

moving the microfluidic chip from a washing position to a sequencing position before starting a new sequencing run.

[01161] 605. The method of any one of the preceding embodiments further comprising:

moving the microfluidic chip from the sequencing position to the washing position; and

connecting the one or more sippers that are in fluidic communication with the microfluidic chip to one or more washing compartments in the reagent cartridge; and

washing the one or more sippers and one or more fluidic pathways in the microfluidic chip using a washing buffer in the one or more washing compartments; and

disposing washing waste to a waste collector.

[01162] 606. The method of any one of the preceding embodiments further comprising disposing the reagent cartridge and installing a new reagent cartridge subsequent to washing the one or more sippers and one or more fluidic pathways in the microfluidic chip.

[01163] 607. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the sequencing position in a x-y plane.

[01164] 608. The method of any one of the preceding embodiments further comprising:

moving the microfluidic chip from the sequencing position to the washing position; and

connecting the one or more sippers that are in fluidic communication with the microfluidic chip to one or more washing compartments in the reagent cartridge; and

washing the one or more sippers and one or more fluidic pathways in the microfluidic chip using a washing buffer in the one or more washing compartments; and

moving the microfluidic chip from the washing position to a second washing position.

[01165] 609. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the second position in a x-y plane.

[01166] 610. The method of any one of the preceding embodiments, wherein the washing position is spatially displaced from the second position for no more than 1 mm, 3mm, 5mm, 8mm, 10 mm, 20mm, 30mm, or 50mm.

[01167] 611. The fluid dispensing device of any one of the preceding claims is configured to dispense a volume with less than ±1%, ±2%, ±3%, ±4%, ±5%, ±6%, ±7%, ±8%, ±9%, or ±10% of volume difference from a predetermined dispensing volume in more than 1000, 10000, or 20000 dispenses.

[01168] 612. The fluid dispensing device of any one of the preceding claims is configured to dispense a volume with less than ±1%, ±2%, ±3%, ±4%, ±5%, ±6%, ±7%, ±8%, ±9%, or ±10% of volume difference from a predetermined dispensing volume in all dispenses required in more than 50, 100, 200 cycles.

[01169] It is to be appreciated that the Detailed Description section, and not any other section, is intended to be used to interpret the claims. Other sections may set forth one or more but not all embodiments as contemplated by the inventor(s), and thus, are not intended to limit this disclosure or the appended claims in any way.

[01170] While this disclosure describes embodiments for fields and applications, it can be understood that the disclosure is not limited thereto. Other embodiments and modifications thereto are possible and are within the scope and spirit of this disclosure. For example, and without limiting the generality of this paragraph, embodiments are not limited to the software, hardware, firmware, or entities illustrated in the figures or described herein, or any combination thereof. Further, embodiments (whether or not explicitly described herein) have significant utility to fields and applications beyond the examples described herein.

[01171] Embodiments have been described herein with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The

boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries may be defined as long as the specified functions and relationships (or equivalents thereof) are appropriately performed. Also, alternative embodiments may perform functional blocks, steps, operations, methods, etc. using orderings different from those described herein.

[01172] References herein to "one embodiment," "an embodiment," "an example embodiment," "some embodiments," or similar phrases, indicate that the embodiment described may include a particular feature, structure, or characteristic, but every embodiment may not necessarily include the particular feature, structure, or characteristic. Moreover, such phrases are not necessarily referring to the same embodiment. Further, when a particular feature, structure, or characteristic is described in connection with an embodiment, it may be within the knowledge of persons skilled in the relevant art(s) to incorporate such feature, structure, or characteristic into other embodiments whether or not explicitly mentioned or described herein.

[01173] Additionally, some embodiments may be described using the expression "coupled" and "connected" along with their derivatives. These terms are not necessarily intended as synonyms for each other. For example, some embodiments may be described using the terms "connected", or "coupled", or combinations thereof, to indicate that two or more elements are in direct physical or electrical contact with each other. The term "coupled," however, may also mean that two or more elements are not in direct contact with each other, but yet still co-operate or interact with each other.

[01174] While embodiments of the present inventive concepts have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. The breadth and scope of this disclosure may not be limited by any of the above-described embodiments. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the inventive concepts. It may be understood that various alternatives to the embodiments of the inventive concepts described herein may be employed in practicing the inventive concepts. It is intended that the following claims define the scope of the inventive concepts and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

What is claimed is:

1. A fluid dispensing device, comprising:

a dispensing module comprising:

a reagent cartridge with one or more compartments for containing fluidic reagents therein;

a microfluidic chip configured to be in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways and one or more in-chip wells therewithin;

a first, a second, and a third valve;

a press plate configured to press on the microfluidic chip thereby forming a first, a second, and a third seal in a respective portion of the one or more fluidic pathways and the one or more in-chip wells; and

one or more dispensing tips,

wherein the microfluidic chip is disposed in a first position and the second seal is formed to allow reagent aspiration to the one or more in-chip wells, and wherein the microfluidic chip is disposed in a second position and the first and the third seals are formed to allow reagent dispensing to the one or more dispensing tips.

2. The fluid dispensing device of claim 1, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips are removably coupleable to the press plate and the first, second, and third valves.
3. The fluid dispensing device of claim 1, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips are not in liquid communication with the press plate or the first, second, and third valves.
4. The fluid dispensing device of any one of the preceding claims, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips are configured to be disconnected from the press plate and the first, second, and third valves for disposal after completion of a sequencing run.
5. The fluid dispensing device of any one of the preceding claims, wherein the reagent cartridge, the microfluidic chip, and the one or more dispensing tips are permanently attached to each other.
6. The fluid dispensing device of any one of the preceding claims, wherein the press plate comprises one or more geometrical shapes, and wherein each of the one or more

geometrical shapes is configured to surround an opening defined by the one or more fluidic pathways or the one or more in-chip wells.

7. The fluid dispensing device of claim 5, wherein each of the one or more geometrical shapes protrudes toward the microfluidic chip from a bottom of the press plate.
8. The fluid dispensing device of any one of the preceding claims, wherein the microfluidic chip further comprises a film covering at least a portion of the one or more fluidic pathways.
9. The fluid dispensing device of claim 8, wherein the film is a top film disposed on a top side of the microfluidic chip and sealed thereto at a sealed contour.
10. The fluid dispensing device of claim 9, wherein the one or more in-chip wells are contained within the sealed contour in an x-y plane.
11. The fluid dispensing device of claim 9, wherein a portion of the one or more fluidic pathways are contained within the sealed contour in an x-y plane.
12. The fluid dispensing device of claim 9, wherein all openings of the one or more fluidic pathways are contained within the sealed contour in an x-y plane.
13. The fluid dispensing device of claim 9, wherein the first, the second, or the third seal is formed within the sealed contour in an x-y plane.
14. The fluid dispensing device of claim 9, wherein the first, the second, or the third seal is formed when the press plate presses the top film into contact with the microfluidic chip, thereby blocking fluidic flow in at least a portion of the one or more fluidic pathways.
15. The fluid dispensing device of any one of the preceding claims, wherein each of the first, the second, or the third seal is configured to seal around an opening in the one or more fluidic pathways, a connection in the one or more fluidic pathways, or an in-well chip.
16. The fluid dispensing device of any one of the preceding claims, wherein each of the first, the second, or the third seal is configured to seal around an opening in the one or more fluidic pathways, a connection in the one or more fluidic pathways, or an in-well chip, thereby blocking fluidic flow from entering or exiting the opening in the one or more fluidic pathways, the connection in the one or more fluidic pathways, or the in-well chip.
17. The fluid dispensing device of any one of the preceding claims, wherein the microfluidic chip further comprises a second film covering at least a portion of the one or more fluidic pathways of the microfluidic chip.

18. The fluid dispensing device of claim 17, wherein the second film is a bottom film sealed to the chip substrate at a second sealed contour.
19. The fluid dispensing device of any one of the preceding claims, wherein the first, the second, and the third valve are actuated by pneumatic pressure or force.
20. The fluid dispensing device of any one of the preceding claims, wherein the one or more fluidic pathways comprise at least a portion of a fluidic pathway that comprises gaseous flow and lacks liquid flow.
21. The fluid dispensing device of any one of the preceding claims, wherein the microfluidic chip is configured to be removably connected to the reagent cartridge and the one or more dispensing tips in a sequencing position.
22. The fluid dispensing device of any one of the preceding claims, wherein the microfluidic chip is configured to be disconnected from the reagent cartridge and the one or more dispensing tips in a washing position.
23. The fluid dispensing device of any one of the preceding claims, wherein the microfluidic chip is configured to move relative to the reagent cartridge and the one or more dispensing tips between the sequencing position and the washing position.
24. The fluid dispensing device of any one of the preceding claims, wherein the first, the second, and the third valve are configured to move at least within an x-y plane relative to the one or more fluidic pathways and the in-chip wells.
25. The fluid dispensing device of any one of claims 1-23, wherein the first, the second, and the third valve are spatially fixed within the x-y plane relative to the one or more fluidic pathways and the in-chip wells.
26. The fluid dispensing device of claim 25, wherein the first, the second, and the third valve corresponds to the first, the second, and the third seal of a first fluidic pathway and a first in-chip well.
27. The fluid dispensing device of claim 26 further comprising a fourth, a fifth, and a sixth valve corresponding to a fourth, a fifth, and a sixth seal of a second fluidic pathway and a second fluidic chip.
28. The fluid dispensing device of any one of the preceding claims, wherein the microfluidic chip is in the first position for reagent aspiration with only the second seal, and wherein the microfluidic chip is in the second position for reagent dispensing to the one or more dispensing tips with only the first and third seals.
29. A flow cell system comprising:
    a flow cell device comprising:

a support comprising one or more substrates;

one or more channels defined by the one or more substrates, wherein the one or more channels are configured to allow fluids to flow therethrough and a gas gap to flow therethrough between the fluids;

an inlet in defined by the one or more substrates, the inlet in fluid communication with the one or more channels; and

an outlet defined by the one or more substrates, wherein the one or more channels run from the inlet to the outlet; and

a fluid dispensing device comprising a dispensing module comprising:

a reagent cartridge with one or more compartments for holding fluidic reagents therein;

a microfluidic chip configured to be in fluidic communication with the one or more compartments, the microfluidic chip having one or more fluidic pathways and one or more in-chip wells therewithin;

a first, a second, and a third valve;

a press plate configured to press on the microfluidic chip, thereby forming a first, a second, and a third seal in a respective portion of the one or more fluidic pathways and the one or more in-chip wells; and

one or more dispensing tips,

wherein the microfluidic chip is disposed in a first position and the second seal is formed to allow reagent aspiration to the one or more in-chip wells, and wherein the microfluidic chip is disposed in a second position and the first and third seals are formed for reagent dispensing to the one or more dispensing tips.

30. The flow cell system of claim 29, wherein each fluidic reagent is configured to travel from a corresponding compartment to a corresponding dispensing tip of the one or more dispensing tips via a corresponding fluidic pathway and a corresponding in-chip well to avoid cross contamination with other fluidic reagents.

31. The flow cell system of any one of claims 29-30, wherein two or more fluidic reagents are configured to flow from a corresponding compartment to a same in-chip well and to a same dispensing tip of the one or more dispensing tips to allow premixing of the two or more fluidic reagents before they reach the flow cell device.

32. The flow cell system of any one of claims 29-31, wherein the inlet is coupled to or includes an open landing area disposed on a substrate of the one or more substrates.

33. The flow cell system of claim 32, wherein the open landing area is in fluidic connection with the one or more channels, and wherein the fluid dispenser device is configured to openly dispense the fluidic reagents from the one or more dispensing tips to the open landing area, without using any tubing in between.
34. The flow cell system of any one of claims 29-33, wherein the flow cell device further comprises a coating configured to cover at least a portion of the open landing area.
35. The flow cell system of any one of claims 29-34, wherein the coating comprises at least one of a slippery liquid-infused porous surface (SLIPS) or a slippery omniphobic covalently attached liquid (SOCAL) coating.
36. The flow cell system of any one of claims 29-35, wherein the coating is hydrophobic.
37. The flow cell system of any one of claims 29-36, wherein the flow cell device further comprises a cleaning outlet in the one or more substrates, wherein the cleaning outlet is in fluidic connection with the inlet.
38. The flow cell system of any one of claims 29-37, wherein the flow cell device is configured for holding one or more in situ samples of cells or tissue to be sequenced.
39. A method of using a fluid dispensing device, comprising:

fluidically connecting one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured to contain fluidic reagents therein, and wherein the microfluidic chip comprises one or more fluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more fluidic pathways of the microfluidic chip;

removably coupling a dispensing module to a first, a second, and a third valve, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments, the microfluidic chip comprising:

a chip substrate; and

a film sealed to the chip substrate;

a press plate that presses the film to form a first seal, a second seal, a third seal, or a combination thereof with the chip substrate;

the one or more dispensing tips; and

switching at least one of the first valve or the third valve into a closed position and switching the second valve into an open position, thereby forming the second seal to allow a first reagent from a first compartment to flow into a first in-chip well; and

switching the second valve into a closed position and switching the first and third valve into an open position, thereby forming the first and third seals to allow the first reagent to flow from the first in-chip well to one or more of dispensing tips.

40. A method of using a fluid dispensing device, comprising:

fluidically connecting one or more compartments of a reagent cartridge with a microfluidic chip, wherein the one or more compartments are configured to hold fluidic reagents therein, and wherein the microfluidic chip comprises one or more fluidic pathways therewithin;

fluidically connecting one or more dispensing tips with the one or more microfluidic pathways of the microfluidic chip;

removably coupling a dispensing module to one or more valves, the dispensing module comprising:

the reagent cartridge with the one or more compartments;

the microfluidic chip in fluidic connection with the one or more compartments; and

the one or more dispensing tips; and

switching a first valve, a second valve, or a third valve of the one or more valves into an open position to block fluidic communication between the one or more compartments and the microfluidic chip or to block fluidic communication between the microfluidic chip and the one or more dispensing tips; and

moving the dispensing module away from a sequencing system without moving the one or more valves relative to the sequencing system.

41. The method of any one of the preceding claims, wherein one or more actuators actuate the one or more valves.

42. The method of any one of the preceding claims, wherein a press plate is attached to the one or more valves.

43. The method of any one of the preceding claims, wherein the press plate comprises at least one of a first geometric shape, a second geometric shape, or a third geometric shape.

44. The method of any one of the preceding claims, wherein the press plate comprises a plurality of geometrical shapes protruding toward the microfluidic chip, and wherein

each of the geometrical shape corresponds to one of: an opening of the one or more fluidic pathways, a connection of the one or more fluidic pathways, and an in-chip well for forming a corresponding seal.

45. The method of any one of the preceding claims, wherein switching the first valve, the second valve, or the third valve of the one or more valves into an open position to block fluidic communication between the one or more compartments and the microfluidic chip or to block fluidic communication between the microfluidic chip and the one or more dispensing tips comprises:

pressing, using the press plate, a film sealed to the microfluidic chip to make contact with the chip substrate of the microfluidic chip and to form one or more of the first, the second, and the third seals.

46. The method of any one of the preceding claims, wherein switching atleast one of the first valve or the third valve into a closed position and switching the second valve into an open position comprises:

pressing, by the press plate, the film sealed to the chip substrate of the microfluidic chip to form the second seal.

47. The method of any one of the preceding claims, wherein switching the second valve into a closed position and switching the first and third valve into an open position comprises:

pressing, using the press plate, the film sealed to the chip substrate of the microfluidic chip to make contact with the chip substrate and form the first and third seals.

48. The method of any one of the preceding claims, further comprising: pressing, using the press plate, a membrane sealed to the chip substrate of the microfluidic chip to form one or more of the first, second, and third seals, the pressing being the membrane caused by actuation of the one or more valves by the one or more actuators.

49. The method of any one of the preceding claims, wherein pressing, using the press plate, the membrane sealed to the chip substrate of the microfluidic chip to form one or more of the first, second, and third seals is caused by pneumatic pressure on the press plate, the pneumatic pressure caused by opening of the one or more of the first, second, and third valves.

50. The method of any one of the preceding claims, wherein the first seal is configured to seal fluidic communication from the one or more compartment to the first in-chip well.
51. The method of any one of the preceding claims, wherein the third seal is configured to seal fluid from entering or exiting the first in-chip well.
52. The method of any one of the preceding claims, wherein the third seal is configured to seal fluidic communication from the first in-chip well to the one or more dispensing tips.
53. The method of any one of the preceding claims, wherein the film is sealed to the chip substrate at a sealed contour, and wherein the first, second, and third seals are spatially contained within the sealed contour.
54. The method of any one of the preceding claims, wherein fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip comprises:

connecting one or more sippers that are in fluidic communication with the microfluidic chip to the one or more compartments to enable fluidic communication between the microfluidic chip and the one or more compartments.
55. The method of any one of the preceding claims, wherein fluidically connecting the one or more compartments of a reagent cartridge with a microfluidic chip comprises:

moving the microfluidic chip from a washing position to a sequencing position before starting a new sequencing run.
56. The method of any one of the preceding claims further comprising:

moving the microfluidic chip from the sequencing position to the washing position; and

connecting the one or more sippers that are in fluidic communication with the microfluidic chip to one or more washing compartments in the reagent cartridge; and

washing the one or more sippers and one or more fluidic pathways in the microfluidic chip using a washing buffer contained in the one or more washing compartments; and

disposing washing waste generated from the washing to a waste collector.
57. The method of any one of the preceding claims, wherein the reagent cartridge is a first reagent cartridge, the method further comprising disposing of the first reagent cartridge and installing a second reagent cartridge subsequent to washing the one or more sippers and one or more fluidic pathways in the microfluidic chip.

58. The method of any one of the preceding claims, wherein the washing position is spatially displaced from the sequencing position in a x-y plane.
59. The method of any one of the preceding claims further comprising:

moving the microfluidic chip from the sequencing position to the washing position, wherein the washing position is a first washing position; and

connecting the one or more sippers that are in fluidic communication with the microfluidic chip to one or more washing compartments in the reagent cartridge; and

washing the one or more sippers and one or more fluidic pathways in the microfluidic chip using a washing buffer in the one or more washing compartments; and

moving the microfluidic chip from the first washing position to a second washing position.

60. The method of any one of the preceding claims, wherein the first washing position is spatially displaced from the second washing position in a x-y plane.
61. The method of any one of the preceding claims, wherein the first washing position is spatially displaced from the second washing position for no more than 1 mm, 3mm, 5mm, 8mm, 10 mm, 20mm, 30mm, or 50mm.
62. The method of any one of the preceding claims wherein the fluid dispensing device is configured to dispense a volume with less than ±1%, ±2%, ±3%, ±4%, ±5%, ±6%, ±7%, ±8%, ±9%, or ±10% of volume difference from a predetermined dispensing volume in more than 1000, 10000, or 20000 dispenses.
63. The method of any one of the preceding claims wherein the wherein the fluid dispensing device is configured to dispense a volume with less than ±1%, ±2%, ±3%, ±4%, ±5%, ±6%, ±7%, ±8%, ±9%, or ±10% of volume difference from a predetermined dispensing volume in dispenses in more than 50, 100, 200 cycles.