IMMERSION MICROSCOPY ARRANGEMENT FOR SEQUENCING SYSTEM WITH RAPID SCANNING

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application S.N. 63/562,343, filed March 7, 2024, the contents of which is incorporated by reference herein in its entirety.

BACKGROUND

[0002] Aspects of the present disclosure relate generally to devices, systems, and methods providing biological or chemical analysis. Various protocols in biological or chemical research involve performing a large number of controlled reactions on local support surfaces or within predefined reaction chambers. The designated reactions may then be observed or detected, and subsequent analysis may help identify or reveal properties of chemicals involved in the reaction. For example, in some multiplex assays, an unknown analyte having an identifiable label (e.g., fluorescent label) may be exposed to thousands of known probes under controlled conditions. Each known probe may be deposited into a corresponding well of a flow cell channel. Observing any chemical reactions that occur between the known probes and the unknown analyte within the wells may help identify or reveal properties of the analyte. Other examples of such protocols include known DNA sequencing processes, such as sequencing-by-synthesis (SBS) or cyclic-array sequencing.

[0003] While a variety of devices, systems, and methods have been made and used to perform biological or chemical analysis, it is believed that no one prior to the inventor(s) has made or used the devices and techniques described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] FIG. 1 depicts a schematic view of an example of a system that may be used to provide biological or chemical analysis.

[0005] FIG. 2 depicts a schematic view of an example of a set of components that may cooperate to provide a fluid path in the system of FIG. 1.

[0006] FIG. 3 depicts a schematic view of another example of a system that may be used to provide biological or chemical analysis.

[0007] FIG. 4 depicts a cross-sectional view of an example of a flow cell that may be used in the system of FIG. 1.

[0008] FIG. 5 depicts a cross-sectional view of another example of a flow cell that may be used in the system of FIG. 1.

[0009] FIG. 6 depicts a top plan view of the flow cell of FIG. 5, with an upper wafer omitted to reveal a lower wafer.

[0010] FIG. 7 depicts a schematic view of another example of a system that may be used to provide biological or chemical analysis.

[0011] FIG. 8 depicts a schematic view of an example of imaging components that may be integrated into the system of FIG. 7.

[0012] FIG. 9 depicts a schematic view of another example of imaging components that may be integrated into the system of FIG. 7.

[0013] FIG. 10 depicts a schematic view of another example of imaging components that may be integrated into the system of FIG. 7.

[0014] FIG. 11 depicts a schematic view of another example of imaging components that may be integrated into the system of FIG. 7.

[0015] FIG. 12 depicts a schematic view of the imaging components of FIG. 11 with fluid lines coupled with an immersion fluid manifold.

[0016] FIG. 13 depicts a schematic view of the imaging components of FIG. 11 with a pressurized air assembly coupled with an air curtain manifold and further coupled with an air blade manifold.

[0017] FIG. 14 depicts a perspective view of the imaging components of FIG. 11.

[0018] FIG. 15 depicts a side elevation view of the imaging components of FIG. 11.

[0019] FIG. 16 depicts a cross-sectional view of the imaging components of FIG. 11, taken along line 16-16 of FIG. 14.

[0020] FIG. 17 depicts another side elevation view of the imaging components of FIG. 11.

[0021] FIG. 18 depicts a cross-sectional view of the imaging components of FIG. 11, taken along line 18-18 of FIG. 15.

[0022] FIG. 19 depicts a bottom plan view of the imaging components of FIG. 11.

[0023] FIG. 20 depicts an exploded perspective view of the imaging components of FIG. 11.

[0024] FIG. 21 depicts a cross-sectional side view of an objective lens assembly of the imaging components of FIG. 11.

[0025] FIG. 22 depicts a bottom plan view of the objective lens assembly of FIG. 22.

[0026] FIG. 23 depicts a cross-sectional side view of an example of an alternative objective lens element that may be incorporated into the objective lens assembly of FIG. 22.

[0027] FIG. 24 depicts a perspective view of the air curtain manifold of the imaging components of FIG. 11.

[0028] FIG. 25 depicts another perspective view of the air curtain manifold of FIG. 24.

[0029] FIG. 26 depicts a cross-sectional view of the air curtain manifold of FIG. 24, taken along line 26-26 of FIG. 24.

[0030] FIG. 27 depicts a cross-sectional view of the air curtain manifold of FIG. 24, taken along line 27-27 of FIG. 26.

[0031] FIG. 28 depicts an enlarged cross-sectional view of a portion of the air curtain manifold of FIG. 24.

[0032] FIG. 29 depicts a perspective view of an immersion fluid manifold of the imaging components of FIG. 11.

[0033] FIG. 30 depicts another perspective view of the immersion fluid manifold of FIG. 29.

[0034] FIG. 31 depicts a bottom plan view of the immersion fluid manifold of FIG. 29.

[0035] FIG. 32 depicts a cross-sectional view of the immersion fluid manifold of FIG. 29, taken along line 32-32 of FIG. 31.

[0036] FIG. 33 depicts a perspective view of the air blade manifold of the imaging components of FIG. 11.

[0037] FIG. 34 depicts another perspective view of the air blade manifold of FIG. 33.

[0038] FIG. 35 depicts a cross-sectional view of the air blade manifold of FIG. 33, taken along line 35-35 of FIG. 33.

[0039] FIG. 36A depicts a schematic view of a flow cell, with an immersion fluid footprint at a first position along the flow cell.

[0040] FIG. 36B depicts a schematic view of the flow cell of FIG. 36B, with the immersion fluid footprint at a second position along the flow cell.

[0041] FIG. 37 depicts a flow chart showing an example of a set of steps that may be carried out using the imaging components of FIG. 11.

[0042] FIG. 38A depicts a cross-sectional side view of the imaging components of FIG. 11 during an example of a first stage of operation.

[0043] FIG. 38B depicts a cross-sectional side view of the imaging components of FIG. 11 during an example of a second stage of operation.

[0044] FIG. 38C depicts a cross-sectional side view of the imaging components of FIG. 11 during an example of a third stage of operation.

[0045] FIG. 38D depicts a cross-sectional side view of the imaging components of FIG. 11 during an example of a fourth stage of operation.

[0046] FIG. 38E depicts a cross-sectional side view of the imaging components of FIG. 11 during an example of a fifth stage of operation.

[0047] FIG. 38F depicts a cross-sectional side view of the imaging components of FIG. 11 during an example of a sixth stage of operation.

[0048] FIG. 38G depicts a cross-sectional side view of the imaging components of FIG. 11 during an example of a seventh stage of operation.

DETAILED DESCRIPTION

[0049] The following detailed description of certain examples will be better understood when read in conjunction with the appended drawings. To the extent that the figures illustrate diagrams of the functional blocks of various examples, the functional blocks are not necessarily indicative of the division between hardware components. Thus, for example, one or more of the functional blocks (e.g., processors or memories) may be implemented in a single piece of hardware (e.g., a general purpose signal processor or random access memory, hard disk, or the like). Similarly, the programs may be stand-alone programs, may be incorporated as subroutines in an operating system, may be functions in an installed software package, and the like. It should be understood that the various examples are not limited to the arrangements and instrumentality shown in the drawings. It should be appreciated that all combinations of the foregoing aspects and additional concepts discussed in greater detail below (provided such concepts are not mutually inconsistent) are contemplated as being part of the inventive subject matter and to achieve the benefits and advantages disclosed herein.

[0050] I. Overview of System for Biological or Chemical Analysis

[0051] Examples described herein may be used in various biological or chemical processes and systems for academic analysis, commercial analysis, or other analysis. More specifically, examples described herein may be used in various processes and systems where it is desired to detect an event, property, quality, or characteristic that is indicative of a designated reaction. Bioassay systems such as those described herein may be configured to perform a plurality of designated reactions that may be detected individually or collectively. For example, bioassay systems may be used to sequence a dense array of nucleic acid features through iterative cycles of enzymatic manipulation and image acquisition. In some examples, nucleic acids can be attached to a surface and amplified. Examples of such amplification are described in U.S. Pat. No. 7,741,463, entitled "Method of Preparing Libraries of Template Polynucleotides," issued June 22, 2010, the disclosure of which is incorporated by reference herein, in its entirety; and/or U.S. Pat. No. 7,270,981, entitled "Recombinase Polymerase Amplification," issued September 18, 2007, the

disclosure of which is incorporated by reference herein, in its entirety.

[0052] Components that are used in the bioassay systems may include one or more microfluidic channels that deliver reagents or other reaction components to a reaction site. The reaction sites may be randomly distributed across a substantially planar surface; or may be patterned across a substantially planar surface. Each of the reaction sites may be imaged to detect light from the reaction site. The signals indicating photons emitted from the reaction sites and detected by image sensors may provide illumination values. These illumination values may be combined into an image indicating photons as detected from the reaction sites. These images may be further analyzed to identify compositions, reactions, conditions, etc., at each reaction site.

[0053] II. Examples of Fluidics Devices and Fluid Flow Paths

[0054] A. Example of System with Higher Volume Throughput

[0055] FIG. 1 illustrates a schematic diagram of an example of a system (100) that may be used to perform an analysis on one or more samples of interest. In some implementations, the sample may include one or more clusters of nucleotides (e.g., DNA) that are linearized to form a single stranded DNA (sstDNA). In the implementation shown, system (100) is configured to receive a flow cell cartridge assembly (102) including a flow cell assembly (103) and a sample cartridge (104). System (100) includes a flow cell receptacle (122) that receives flow cell cartridge assembly (102), a vacuum chuck (124) that supports flow cell assembly (103), and a flow cell interface (126) that is used to establish a fluidic coupling between system (100) and flow cell assembly (103). Flow cell interface (126) may include one or more manifolds. System (100) further includes a sipper manifold assembly (106), a sample loading manifold assembly (108), and a pump manifold assembly (110). System (100) also includes a drive assembly (112), a controller (114), an imaging system (116), and a waste reservoir (118). Controller (114) is electrically and/or communicatively coupled to drive assembly (112) and to imaging system (116); and is configured to cause drive assembly (112) and/or the imaging system (116) to perform various functions as disclosed herein.

[0056] In the present example, flow cell assembly (103) includes a flow cell (128) having

a channel (130) and defining a plurality of first openings (132), which are fluidically coupled to the channel (130) and arranged on a first side (134) of the channel (130). Flow cell (128) further includes a plurality of second openings (136) fluidically coupled to the channel (130) and arranged on a second side (138) of the channel (130). Fluid may thus flow through flow cell (128) via channel. While the flow cell (128) is shown including one channel (130), flow cell (128) may include two or more channels (130). Flow cell assembly (103) also includes a flow cell manifold assembly (140) coupled to flow cell (128) and having a first manifold fluidic line (142) and a second manifold fluidic line (144). Flow cell manifold assembly (140) may be in the form of a laminate including a plurality of layers as discussed in more detail below.

[0057] In the implementation shown, first manifold fluidic line (142) has a first fluidic line opening (146) and is fluidically coupled to each of the first openings (132) of flow cell (128); and second manifold fluidic line (144) has a second fluidic line opening (148) and is fluidically coupled to each of the second openings (136). As shown, flow cell assembly (103) includes gaskets (150) coupled to flow cell manifold assembly (140) and fluidically coupled to fluidic line openings (146, 148). In some implementations where flow cell (128) includes a plurality of channels (130), flow cell manifold assembly (140) may include additional fluidic lines (152) that couple first fluidic line openings (146) to a single manifold port (154). In such implementations, a single gasket (150) may be coupled to flow cell manifold assembly (140) that surrounds the manifold port (154) and is in fluidic communication with a plurality of channels (130). In operation, flow cell interface (126) engages with corresponding gaskets (150) to establish a fluidic coupling between system (100) and flow cell (128). The engagement between flow cell interface (126) and gaskets (150) reduces or eliminates fluid leakage between flow cell interface (126) and flow cell (128).

[0058] In the implementation shown, first manifold fluidic line (142) has a portion (156) that is substantially parallel to a longitudinal axis (158) of channel (130); and second manifold fluidic line (144) has a portion (160) that is substantially parallel to longitudinal axis (158) of channel (130). Additionally, first manifold fluidic line (142) is shown being at least partially adjacent a first end (162) of flow cell (128) and spaced from a second end (164) of flow cell (128); and second manifold fluidic

line (144) is shown being at least partially adjacent second end (164) of flow cell (128) and spaced from first end (162). Other arrangements of manifold fluidic lines (142, 144) may prove suitable, however.

[0059] In the implementation shown, system (100) includes a sample cartridge receptacle (166) that receives sample cartridge (104) that carries one or more samples of interest (e.g., an analyte). System (100) also includes a sample cartridge interface (168) that establishes a fluidic connection with sample cartridge (104). Sample loading manifold assembly (108) includes one or more sample valves (170). Pump manifold assembly (110) includes one or more pumps (172), one or more pump valves (174), and a cache (176). Valves (170, 174) and pumps (172) may take any suitable form. Cache (176) may include a serpentine cache and may temporarily store one or more reaction components during, for example, bypass manipulations of the system (100). While cache (176) is shown being included in pump manifold assembly (110), cache (176) may alternatively be located elsewhere (e.g., in sipper manifold assembly (106) or in another manifold downstream of a bypass fluidic line (178), etc.).

[0060] Sample loading manifold assembly (108) and pump manifold assembly (110) flow one or more samples of interest from sample cartridge (104) through a fluidic line (180) toward flow cell cartridge assembly (102). In some implementations, sample loading manifold assembly (108) may individually load or address each channel (130) of flow cell (128) with a respective sample of interest. The process of loading channel (130) with a sample of interest may occur automatically using system (100). As shown in FIG. 1, sample cartridge (104) and sample loading manifold assembly (108) are positioned downstream of flow cell cartridge assembly (102). In the implementation shown, sample loading manifold assembly (108) is coupled between flow cell cartridge assembly (102) and pump manifold assembly (110). To draw a sample of interest from sample cartridge (104) and toward pump manifold assembly (110), sample valves (170), pump valves (174), and/or pumps (172) may be selectively actuated to urge the sample of interest toward pump manifold assembly (110). Sample cartridge (104) may include a plurality of sample reservoirs that are selectively fluidically accessible via the corresponding sample valves (170). To individually flow the sample of interest toward channel (130) of flow cell (128) and away from pump manifold assembly (110), sample valves (170), pump valves

(174), and/or pumps (172) may be selectively actuated to urge the sample of interest toward flow cell cartridge assembly (102) and into respective channels (130) of flow cell (128).

[0061] Drive assembly (112) interfaces with sipper manifold assembly (106) and pump manifold assembly (110) to flow one or more reagents that interact with the sample within flow cell (128). In some scenarios, a reversible terminator is attached to the reagent to allow a single nucleotide to be incorporated onto a growing DNA strand. In some such implementations, one or more of the nucleotides has a unique fluorescent label that emits a color when excited. The color (or absence thereof) is used to detect the corresponding nucleotide. In the implementation shown, imaging system (116) excites one or more of the identifiable labels (e.g., a fluorescent label) and thereafter obtains image data for the identifiable labels. The labels may be excited by incident light and/or a laser and the image data may include one or more colors emitted by the respective labels in response to the excitation. The image data (e.g., detection data) may be analyzed by system (100). Examples of features and functionalities that may be incorporated into imaging system (116) will be described in greater detail below.

[0062] After the image data is obtained, drive assembly (112) interfaces with sipper manifold assembly (106) and pump manifold assembly (110) to flow another reaction component (e.g., a reagent) through flow cell (128) that is thereafter received by waste reservoir (118) via a primary waste fluidic line (182) and/or otherwise exhausted by system (100). Some reaction components may perform a flushing operation that chemically cleaves the fluorescent label and the reversible terminator from the sstDNA. The sstDNA may then be ready for another cycle.

[0063] The primary waste fluidic line (182) is coupled between pump manifold assembly (110) and waste reservoir (118). In some implementations, pumps (172) and/or pump valves (174) of pump manifold assembly (110) selectively flow the reaction components from flow cell cartridge assembly (102), through fluidic line (180) and sample loading manifold assembly (108) to primary waste fluidic line (182). Flow cell cartridge assembly (102) is coupled to a central valve (184) via flow cell interface (126). Central valve (184) is coupled with flow cell interface (126) via a fluidic line (185). An auxiliary waste fluidic line (186) is coupled to central valve

(184) and to waste reservoir (118). In some implementations, auxiliary waste fluidic line (186) receives excess fluid of a sample of interest from flow cell cartridge assembly (102), via central valve (184), and flows the excess fluid of the sample of interest to waste reservoir (118) when back loading the sample of interest into flow cell (128), as described herein.

[0064] Sipper manifold assembly (106) includes a shared line valve (188) and a bypass valve (190). Shared line valve (188) may be referred to as a reagent selector valve. Central valve (184) and the valves (188, 190) of sipper manifold assembly (106) may be selectively actuated to control the flow of fluid through fluidic lines (192, 194, 196). Sipper manifold assembly (106) may be coupled to a corresponding number of reagent reservoirs (198) via reagent sippers (200). Reagent reservoirs (198) may contain fluid (e.g., reagent and/or another reaction component). In some implementations, sipper manifold assembly (106) includes a plurality of ports. Each port of sipper manifold assembly (106) may receive one of the reagent sippers (200). Reagent sippers (200) may be referred to as fluidic lines. Some forms of reagent sippers (200) may include an array of sipper tubes extending downwardly along the z-dimension from ports in the body of sipper manifold assembly (106). Reagent reservoirs (198) may be provided in a cartridge, and the tubes of reagent sippers (200) may be configured to be inserted into corresponding reagent reservoirs (198) in the reagent cartridge so that liquid reagent may be drawn from each reagent reservoir (198) into the sipper manifold assembly (106).

[0065] Shared line valve (188) of sipper manifold assembly (106) is coupled to central valve (184) via shared reagent fluidic line (196). Different reagents may flow through shared reagent fluidic line (196) at different times. In some versions, when performing a flushing operation before changing between one reagent and another, pump manifold assembly (110) may draw wash buffer through shared reagent fluidic line (196), central valve (184), and flow cell cartridge assembly (102).

[0066] Bypass valve (190) of sipper manifold assembly (106) is coupled to central valve (184) via dedicated reagent fluidic lines (194, 196). Each of the dedicated reagent fluidic lines (194, 196) may be associated with a single reagent. The fluids that may flow through dedicated reagent fluidic lines (194, 196) may be used during sequencing operations and may include a cleave reagent, an incorporation reagent,

a scan reagent, a cleave wash, and/or a wash buffer.

[0067] Bypass valve (190) is also coupled to cache (176) of pump manifold assembly (110) via bypass fluidic line (178). One or more reagent priming operations, hydration operations, mixing operations, and/or transfer operations may be performed using bypass fluidic line (178). The priming operations, the hydration operations, the mixing operations, and/or the transfer operations may be performed independent of flow cell cartridge assembly (102). Thus, the operations using bypass fluidic line (178) may occur during, for example, incubation of one or more samples of interest within flow cell cartridge assembly (102). That is, shared line valve (188) may be utilized independently of bypass valve (190) such that bypass valve (190) may utilize bypass fluidic line (178) and/or cache (176) to perform one or more operations while shared line valve (188) and/or central valve (184) simultaneously, substantially simultaneously, or offset synchronously perform other operations.

[0068] Drive assembly (112) includes a pump drive assembly (202) and a valve drive assembly (204). Pump drive assembly (202) may be adapted to interface with one or more pumps (172) to pump fluid through flow cell (128) and/or to load one or more samples of interest into flow cell (128). Valve drive assembly (204) may be adapted to interface with one or more of the valves (170, 174, 184, 188, 190) to control the position of the corresponding valves (170, 174, 184, 188, 190).

[0069] FIG. 2 shows an example of a fluidic arrangement (220) that may be incorporated into a variation of system (100). Fluidic arrangement (220) of this example includes a pump manifold assembly (222), which may operate similar to pump manifold assembly (110) described above; a sample loading manifold assembly (228), which may operate similar to sample loading manifold assembly (108) described above; a flow cell interface (240), which may operate similar to flow cell interface (126) described above; a sipper manifold assembly (250), which may operate similar to sipper manifold assembly (106) described above; and a waste reservoir (270), which may operate similar to waste reservoir (118) described above. Pump manifold assembly (222) is coupled with a port assembly (258) of sipper manifold assembly (250) via a fluidic line (224), which may be similar to fluidic line (178); and with sample loading manifold assembly (228) via a fluidic line (226). Sample loading manifold assembly (228) is coupled with flow cell interface (240) via fluidic line

(230), which may be similar to fluidic line (180); and with port assembly (258) via fluidic lines (232, 234). Flow cell interface (240) is coupled with sipper manifold assembly (250) via fluidic line (242), which may be similar to fluidic line (185). Sipper manifold assembly (250) includes a manifold body (252) and a common output port (256), which provides fluid communication via fluidic line (185). A valve assembly (254) controls fluid flow through common output port (256) and may operate similar to central valve (184). Port assembly (258) of sipper manifold assembly (250) is coupled with waste reservoir (270) via fluidic line (272), which may be similar to fluidic line (186).

[0070] A plurality of reagent sippers (260) extend from manifold body (252) and are fluidically coupled with valve assembly (254) via respective fluid channels (262) in manifold body (252). Reagent sippers (260) may operate similar to reagent sippers (200). Valve assembly (254) is operable to selectively couple fluid channels (262) with flow cell interface (240) via common output port (256) and fluidic line (230), to thereby selectively provide various reagents to flow cell interface (240). In other words, when each reagent sipper (260) is disposed in a different respective reagent (e.g., in a respective reagent reservoir (198)), a flow cell (e.g., like flow cell (128)) that is coupled with flow cell interface (240) may selectively receive those different reagents based on control of valve assembly (254).

[0071] Port assembly (258) may provide a fluidic interface between pump manifold assembly (222) and sipper manifold assembly (250), thereby allowing sipper manifold assembly (250) to receive pressurized fluid from pump manifold assembly (222). Port assembly (258) may also provide a fluidic interface between sample loading manifold assembly (228) and sipper manifold assembly (250), thereby allowing sipper manifold assembly (250) to receive sample fluid from sample loading manifold assembly (228). In addition, port assembly (258) may provide a fluidic interface between waste reservoir (270) and sipper manifold assembly (250), thereby allowing sipper manifold assembly (250) to communicate waste fluid to waste reservoir (270). Communication of fluids via port assembly (258) may be regulated, at least in part, by valve assembly (254).

[0072] Referring back to FIG. 1, controller (114) of the present example includes a user interface (206), a communication interface (208), one or more processors (210), and

a memory (212) storing instructions executable by the one or more processors (210) to perform various functions including the disclosed implementations. User interface (206), communication interface (133), and memory (212) are electrically and/or communicatively coupled to the one or more processors (210). User interface (206) may be adapted to receive input from a user and to provide information to the user associated with the operation of system (100) and/or an analysis taking place. User interface (206) may include a touch screen, a display, a keyboard, a speaker(s), a mouse, a track ball, and/or a voice recognition system.

[0073] Communication interface (208) is adapted to enable communication between system (100) and a remote system(s) (e.g., computers) via a network(s) (e.g., the Internet, an intranet, a local-area network (LAN), a wide-area network (WAN), a coaxial-cable network, a wireless network, a wired network, a satellite network, a digital subscriber line (DSL) network, a cellular network, a Bluetooth connection, a near field communication (NFC) connection, etc.). Some of the communications provided to the remote system may be associated with analysis results, imaging data, etc. generated or otherwise obtained by system (100). Some of the communications provided to system (100) may be associated with a fluidics analysis operation, patient records, and/or a protocol(s) to be executed by system (100).

[0074] The one or more processors (210) and/or system (100) may include one or more of a processor-based system(s) or a microprocessor-based system(s). In some implementations, the one or more processors (210) and/or system (100) includes one or more of a programmable processor, a programmable controller, a microprocessor, a microcontroller, a graphics processing unit (GPU), a digital signal processor (DSP), a reduced-instruction set computer (RISC), an application specific integrated circuit (ASIC), a field programmable gate array (FPGA), a field programmable logic device (FPLD), a logic circuit, and/or another logic-based device executing various functions including the ones described herein.

[0075] Memory (212) may include one or more of a semiconductor memory, a magnetically readable memory, an optical memory, a hard disk drive (HDD), an optical storage drive, a solid-state storage device, a solid-state drive (SSD), a flash memory, a read-only memory (ROM), erasable programmable read-only memory

(EPROM), electrically erasable programmable read-only memory (EEPROM), a random-access memory (RAM), a non-volatile RAM (NVRAM) memory, a compact disc (CD), a compact disc read-only memory (CD-ROM), a digital versatile disk (DVD), a Blu-ray disk, a redundant array of independent disks (RAID) system, a cache and/or any other storage device or storage disk in which information is stored for any duration (e.g., permanently, temporarily, for extended periods of time, for buffering, for caching).

[0076] B. Example of System with Lower Volume Throughput

[0077] FIG. 3 illustrates a schematic diagram of another example of a system (300) that may be used to perform an analysis on one or more samples of interest. Except as otherwise described below, system (300) of this example may be configured and operable like system (100) described above with reference to FIG. 1. In some instances, system (100) is used to provide a higher volume throughput; while system (300) is used to provide a lower volume throughput. Alternatively, systems (100, 300) may provide any other suitable amount or degree of throughput. System (300) of the present example receives a reagent cartridge (302) and includes, in part, a gas source (304), a drive assembly (306), a controller (308), an imaging system (310), and a waste reservoir (312). Reagent cartridge (302) may be referred to as a consumable, a reagent reservoir, or a reagent assembly. Controller (308) is electrically and/or communicatively coupled to drive assembly (306) and to imaging system (310) and causes drive assembly (306) and/or imaging system (310) to perform various functions as disclosed herein.

[0078] Reagent cartridge (302) in the implementation shown includes a well assembly (314) having a body (316). Body (316) has a first wall (318) defining a well (320) having a port (322). First wall (318) has a distal end (324) that defines an opening (326) having an opening perimeter (328). A second wall (330) surrounds first wall (318) and has a distal end (332). Distal end (332) may be referred to as an edge or an outer edge. A cover (334) is coupled to distal end (324) of first wall (318) and covers opening (326) along opening perimeter (328) at a connected portion (336); and is uncoupled from distal end (324) of first wall (318) at an unconnected portion (338). Connection portion (336) may be referred to as connection sections or connected segments and unconnected portion (338) may be referred to as

unconnected sections or unconnected segments. First wall (318) has a height and second wall (330) has a height that is greater than the height of first wall (318). First well (318) and second well (330) may alternatively be the same or similar heights. An impermeable barrier (340) is coupled to distal end (332) of second wall (330) and covers well (320). Impermeable barrier (340) may be foil, plastic, etc. and may prevent or inhibit moisture from infiltrating wells (320) of reagent cartridge (302).

[0079] Unconnected portion (338) of cover (334) forms a vent (342) that allows air flow out of well (320). Dried reagent (348) is contained within well (320), and vent (342) is sized to substantially retain dried reagent (348) within the well (320). Body (316) may include a plurality of wells (320) while one well (320) is shown in FIG. 3. Liquid (346) may flow into well (320) via port (322) in practice to rehydrate dried reagent (348). Vent (342) may vent gas from well (320) as liquid (346) flows into well (320); and cover (334) prevents or inhibits reagent (348) and/or liquid (346) from escaping from well (320). Put another way, vents (342) retain reagent (348) and/or liquid (346) within wells (320); and prevent or inhibit reagent (348) and/or liquid (346) from migrating out of wells (320). Vent (342) and cover (334) prevent or inhibit cross-contamination between reagents when reagent cartridge (302) includes more than one well (320). Liquid (346) and dried reagent (348) may be flowed into and out of well (320) to mix liquid (346) from liquid reservoir (362) and dried reagent (348). System (300) and/or reagent cartridge (302) may include a mixing chamber that is used to mix liquid (346) and dried reagent (348) in some implementations. Impermeable barrier (340) may be pierced prior to liquid (346) flowing into well (320).

[0080] Gas source (304) may be used to pressurize liquid reservoir (362) to flow liquid (346) into well (320); and/or a pump (350) may draw liquid (346) from liquid reservoir (362) and flow liquid (346) into well (320) to rehydrate reagent (348). Gas source (304) may be provided by system (300) and/or may be carried by reagent cartridge (302). Gas source (304) may alternatively be omitted. Pump (350) may be implemented by a syringe pump, a peristaltic pump, a diaphragm pump, etc. While pump (350) may be positioned downstream of flow cell (368) as shown, pump (350) may be positioned upstream of flow cell (368) or omitted entirely.

[0081] Reagent cartridge (302) and/or system (300) includes valves (352) that may be

selectively actuatable to control the flow of fluid through fluidic lines (356). Such valves (352) may be implemented by a valve manifold, a rotary valve, a selector valve, a pinch valve, a flat valve, a solenoid valve, a check valve, a piezo valve, etc. A regulator (354) may be positioned between gas source (304) and valve (352); and regulate the pressure of the gas provided to valve (352). Regulator (354) may include a valve that controls the flow of the gas from gas source (304).

[0082] Body (316) of well assembly (314) has an edge (364); and impermeable barrier (340) may be hermetically connected to body (316) along edge (364). Impermeable barrier (340) may include foil, plastic, and/or any other suitable material(s). System (300) may pierce impermeable barrier (340), impermeable barrier (340) may be pierced by an individual prior to use, or impermeable barrier (340) may be pierced by some other structure or methodology. System (300) includes an actuator assembly (360) in the implementation shown that interfaces with impermeable barrier (340) to pierce impermeable barrier (340). System (300) may include a protrusion such as a post having a blunt or sharp end that is movable by actuator assembly (360) to pierce impermeable barrier (340). Impermeable barrier (340) may alternatively be pierced by an operator prior to reagent cartridge (302) being positioned in system (300). System (300) also includes a liquid reservoir (362) containing liquid (346). Liquid (346) may comprise a rehydrating liquid, a wash buffer, and/or any other suitable kind(s) of liquid.

[0083] System (300) further includes a flow cell receptacle (366) that receives a flow cell (368). Flow cell (368) may be configured and operable like flow cell (128). In some variations, flow cell (368) is carried by and/or integrated into reagent cartridge (302). Flow cell (368) may carry the sample of interest. Gas source (304) and/or pump (350) may flow liquid (346) to rehydrate dry reagents (348) and to flow one or more liquid reagents through reagent cartridge (302) that interact with the sample. Imaging system (310) may be configured and operable like imaging system (116), such that imaging system (310) may be used to obtain image data from flow cell (368). After the image data is obtained, drive assembly (306) may interface with reagent cartridge (302) to flow another reaction component (e.g., a reagent) through flow cell (368) that is thereafter received by the waste reservoir (312) and/or otherwise exhausted by reagent cartridge (302). In the present example, drive assembly (306) includes a pump drive assembly (370), a valve drive assembly

(372), and actuator assembly (360). Pump drive assembly (370) interfaces with pump (350) to pump fluid through reagent cartridge (302) and/or flow cell (368); and valve drive assembly (372) interfaces with valve (352) to control the position of valve (352).

[0084] Controller (308) of this example includes a user interface (374), a communication interface (376), a processor (378), and a memory (380). User interface (374) may be configured and operable like user interface (206) of system (100). Communication interface (376) may be configured and operable like communication interface (208) of system (100). Processor (378) may be configured and operable like processor (210) of system (100). Memory (380) may be configured and operable like memory (212) of system (100).

[0085] Further examples and details of how various features of each system (100, 300) may be configured and operable will be described below. By way of further example only, the various features of system (100, 300) may be configured and operable in accordance with at least some of the teachings of International Pub. No. WO 2023/055873, entitled "Flow Cells and Related Flow Cell Manifold Assemblies and Methods," published April 6, 2023, the disclosure of which is incorporated by reference herein, in its entirety; U.S. Pat. No. 9,958,465, entitled "Detection Apparatus having a Microfluorometer, a Fluidic System, and a Flow Cell Latch Clamp Module," issued May 1, 2018, the disclosure of which is incorporated by reference herein, in its entirety; and/or U.S. Pat. App. No. 63/325,462, entitled "Well Assemblies and Related Systems and Methods," filed March 30, 2022, the disclosure of which is incorporated by reference herein, in its entirety.

[0086] III. Examples of Flow Cell Structures

[0087] As noted above, a system (100, 300) may execute reactions in a flow cell (128, 368) and/or perform analysis on one or more samples of interest in a flow cell (128, 368). The following describes examples of forms that such flow cells (128, 368) may take, it being understood that flow cells (128, 368) may take various other forms and have various other features in addition to or in lieu of the features described below.

[0088] A. Example of Single-Surface Patterned Flow Cell

[0089] FIG. 4 shows an example of a flow cell (400) that includes a patterned substrate (402), which includes depressions (404) separated by interstitial regions (406), and surface chemistry (410, 412) positioned in the depressions (404). Depressions (404) may be in the form of microwells or nanowells. Depressions (404) may be configured to contain nucleic acid strands or other oligonucleotides and thereby provide a reaction site for SBS and/or for other kinds of processes. In some versions, each depression (404) has a cylindraceous configuration, with a generally circular cross-sectional profile. In some other versions, each depression (404) has a polygonal (e.g., hexagonal, octagonal, square, rectangular, elliptical, etc.) cross-sectional profile. Alternatively, depressions (404) may have any other suitable configuration. It should also be understood that depressions (404) may be arranged in any suitable pattern, including but not limited to a grid pattern.

[0090] Surface chemistry (410, 412) of the present example includes functionalized coating layer (410) and primers (412). While not shown, it is to be understood that the depressions (404) may also have surface preparation or treatment chemistry (e.g., silane or a silane derivative) positioned between the substrate (402) and the functionalized coating layer (410). This same surface preparation or treatment chemistry may also be positioned on the interstitial regions (406). In the present example, a hydrogel (440) is applied before lid (420) is bonded to substrate (402). Hydrogel (440) covers surface chemistry (410, 412) in depressions (404), and at least a portion of the patterned substrate (402) (e.g., those interstitial regions (406) that are not also bonding regions (422)). By way of example only, hydrogel (440) may comprise PAZAM, crosslinked polyacrylamide, agarose gel, etc.

[0091] Flow cell (400) of this example further includes a lid (420) bonded to bonding region(s) (422) of patterned substrate (402). In the example shown in FIG. 4, lid (420) includes a top portion (424) that is connected to several sidewalls (426), and these components (424, 426) define a portion of each of the six flow channels (430A, 430B, 430C, 430D, 430E, 430F). The respective sidewalls (426) isolate one flow channel (430A, 430B, 430C, 430D, 430E, 430F) from each adjacent flow channel (430A, 430B, 430C, 430D, 430E, 430F). Each flow channel (430A, 430B, 430C, 430D, 430E, 430F) is in selective fluid communication with a respective set of depressions (404).

[0092] Lid (420) may be bonded to bonding region (422) of substrate (402) using any suitable technique, such as laser bonding, diffusion bonding, anodic bonding, eutectic bonding, plasma activation bonding, glass frit bonding, or other methods known in the art. In some versions, a spacer layer (428) may be used to bond lid (420) to bonding region (422). Spacer layer (428) may comprise any material that will seal at least some of interstitial regions (404) (e.g., bonding region (422)) of substrate (402) and lid (420) together. While not shown, lid (420) or the patterned substrate (402) may include inlet and outlet ports that are to fluidically engage other ports (not shown), such as those of sample cartridge interface (168), for directing fluid(s) into the respective flow channels (430A, 430B, 430C, 430D, 430E, 430F) (e.g., from a reagent cartridge or other fluid storage system) and out of the flow channel (e.g., to waste reservoir (118) or another waste removal system). Flow channels (430A, 430B, 430C, 430D, 430E, 430F) may serve to, for example, selectively introduce reaction components or reactants to hydrogel (440) and the underlying surface chemistry (410, 412) in order initiate designated reactions in/at depressions (404).

[0093] While flow cell (400) includes a pattern of depressions (404) to provide an array of reaction sites, other variations may provide reaction sites on or at various other kinds of structural features, including but not limited to continuously planer surfaces and/or protruding surfaces, etc. By way of further example only, flow cell (400) may be constructed and operable in accordance with at least some of the teachings of U.S. Pat. No. 10,919,033, entitled "Flow Cells with Hydrogel Coating," issued February 16, 2021, the disclosure of which is incorporated by reference herein, in its entirety.

[0094] B. Example of Dual-Surface Patterned Flow Cell

[0095] While FIG. 4 shows an example of a flow cell (400) that has a single surface patterned with reaction sites (i.e., depressions (404) formed in substrate (402)), it may be desirable in some instances to provide a variation of flow cell (400) that provide two surfaces patterned with reaction sites. An example of a dual-surface patterned flow cell (450) is shown in FIGS. 5-6. In this example, flow cell (450) includes a pair of wafers (452, 454) that are bonded together, with a spacer layer

(456) interposed between wafers (452, 454). Each wafer (452, 454) is patterned to provide a respective plurality of depressions (462, 464), such that depressions (462) of wafer (452) align with depressions (464) of wafer (454) when flow cell (450) is assembled. Depressions (462) are separated from each other by interstitial regions (466); and depressions (464) are separated from each other by interstitial regions (468). Spacer layer (456) does not contact interstitial regions (466, 468) in this example.

[0096] Depressions (462, 464) of flow cell (450) may be configured and operable like depressions (404) of flow cell (400) described above. Each depression (462, 464) of the present example includes a grafted coating (470), which may be similar to functionalized coating layer (410); and primers (472), which may be similar to primers (412) described above. Each depression (462, 464) may further include hydrogel, like hydrogel (440), and/or any other suitable feature(s). As shown in FIGS. 5-6, depressions (462, 464) are provided within a plurality of flow channels (480A, 480B, 480C, 480D). Flow channels (480A, 480B, 480C, 480D) are separated from each other by walls (458) and ends (459) formed by spacer layer (456). In the example shown in FIG. 6, flow cell (450) provides four flow channels (480A, 480B, 480C, 480D), with each flow channel (480A, 480B, 480C, 480D) containing several rows and columns of depressions (462, 464). When flow cell (450) is used in system (100, 300), flow channels (480A, 480B, 480C, 480D) may serve to, for example, selectively introduce reaction components or reactants surface chemistry (470, 472) in order initiate designated reactions in/at depressions (462, 464). In some instances, since each wafer (452, 454) has its own set of depressions (462, 464) providing corresponding reaction sites, flow cell (450) may provide twice as many reactions as a similarly sized flow cell (400) during a given time period.

[0097] The broken lines in FIG. 6 indicate how flow cell (450) may be diced to effectively form smaller flow cells (450A, 450A), with each smaller flow cell (450A, 450A) having its own respective pair of flow channels (480A, 480B, 480C, 480D). In the present example, however, a single flow cell (450) has more than two flow channels (480A, 480B, 480C, 480D). While flow cell (450) includes a pattern of depressions (462, 464) to provide an array of reaction sites, other variations may provide reaction sites on or at various other kinds of structural features, including but not

limited to continuously planer surfaces and/or protruding surfaces, etc. By way of further example only, flow cell (450) may be constructed and operable in accordance with at least some of the teachings of U.S. Pat. No. 10,955,332, entitled "Flow Cell Package and Method for Making the Same," issued March 23, 2021, the disclosure of which is incorporated by reference herein, in its entirety.

[0098] IV. Examples of Imaging System Features

[0099] As noted above, system (100, 300) includes an imaging system (116, 310) that excites one or more identifiable labels (e.g., a fluorescent label) in samples in reaction sites provided by depressions (404, 462, 464) of a flow cell (128, 368, 400, 450); and thereafter obtains image data for the identifiable labels. This image data is used to identify nucleotides as part of a nucleic acid sequencing process. Alternatively, the image data may be used for various other purposes. The following description provides details on how some versions of imaging system (116, 310) may be configured and operable.

[00100] FIG. 7 illustrates a schematic diagram of another example of a system (500) that may be used to perform an analysis on one or more samples of interest. Except as otherwise described below, system (500) of this example may be configured and operable like systems (100, 300) described above. System (500) is configured to perform a large number of parallel reactions within a flow cell (510). Flow cell (510) may be configured and operable like flow cells (400, 450) described above or may have any other suitable configuration. Flow cell (510) may thus include one or more flow channels that receive a solution from system (500) and direct the solution toward reaction sites of flow cell (510).

[00101] System (500) includes a system controller (520) that may communicate with the various components, assemblies, and sub-systems of the system (500). Controller (520) may be configured and operable like controllers (114, 308) described above. An imaging assembly (522) of system (500) includes a light emitting assembly (550) that emits light that reaches reaction sites on flow cell (510). Light emitting assembly (550) may include an incoherent light emitter (e.g., emit light beams output by one or more excitation diodes), or a coherent light emitter such as emitter of light output by one or more lasers or laser diodes. In some implementations, light emitting assembly (550) may include a plurality of different light sources (not

shown), each light source emitting light of a different wavelength range. Some versions of light emitting assembly (550) may also include one or more collimating lenses (not shown), a light structuring optical assembly (not shown), a projection lens (not shown) that is operable to adjust a structured beam shape and path, epifluorescence microscopy components, and/or other components. Although system (500) is illustrated as having a single light emitting assembly (550), multiple light emitting assemblies (550) may be included in some other implementations.

[00102] In the present example, the light from light emitting assembly (550) is directed by dichroic mirror assembly (546) through an objective lens assembly (542) onto a sample of a flow cell (510), which is positioned on a motion stage (570). In the case of fluorescent microscopy of a sample, a fluorescent element associated with the sample of interest fluoresces in response to the excitation light, and the resultant light is collected by objective lens assembly (542) and is directed to an image sensor of camera system (540) to detect the emitted fluorescence. In some implementations, a tube lens assembly may be positioned between the objective lens assembly (542) and the dichroic mirror assembly (546) or between the dichroic mirror (546) and the image sensor of the camera system (540). A moveable lens element may be translatable along a longitudinal axis of the tube lens assembly to account for focusing on an upper interior surface or lower interior surface of the flow cell (510) and/or spherical aberration introduced by movement of the objective lens assembly (542).

[00103] In the present example, a filter switching assembly (544) is interposed between dichroic mirror assembly (546) and camera system (540). Filter switching assembly (544) includes one or more emission filters that may be used to pass through particular ranges of emission wavelengths and block (or reflect) other ranges of emission wavelengths. For example, emission filters may be used to direct different wavelength ranges of emitted light to different image sensors of the camera system (540) of imaging assembly (522). For instance, the emission filters may be implemented as dichroic mirrors that direct emission light of different wavelengths from flow cell (510) to different image sensors of camera system (540). In some variations, a projection lens is interposed between filter switching assembly (544) and camera system (540). Filter switching assembly (544) may be omitted in some versions.

[00104] System (500) further includes a fluid delivery assembly (590) that may direct the flow of reagents (e.g., fluorescently labeled nucleotides, buffers, enzymes, cleavage reagents, etc.) to (and through) flow cell (510) and waste valve (580). Fluid delivery assembly (590) may be configured and operable like the various fluid delivery components described above in the context of FIGS. 1-3. System (500) of the present example also includes a temperature station actuator (530) and heater/cooler (532) that may optionally regulate the temperature of conditions of the fluids within the flow cell (510). In some implementations, the heater/cooler (532) may be fixed to sample stage (570), upon which the flow cell (510) is placed, and/or may be integrated into sample stage (570).

[00105] Flow cell (510) may be removably mounted on sample stage (570), which may provide movement and alignment of flow cell (510) relative to objective lens assembly (542). Sample stage (570) may have one or more actuators to allow sample stage (570) to move in any of three dimensions. For example, actuators may be provided to allow sample stage (570) to move in the x, y, and z directions relative to objective lens assembly (542), tilt relative to objective lens assembly (542), and/or otherwise move relative to objective lens assembly (542). Movement of sample stage (570) may allow one or more sample locations on flow cell (510) to be positioned in optical alignment with objective lens assembly (542). Movement of sample stage (570) relative to objective lens assembly (542) may be achieved by moving sample stage (570) itself, by moving objective lens assembly (542), by moving some other component of imaging assembly (522), by moving some other component of system (500), or any combination of the foregoing. For instance, in some implementations, the sample stage (570) may be actuatable in the x and y directions relative to the objective lens assembly (542) while a focus component (562) or z-stage may move the objective lens assembly (542) along the z direction relative to the sample stage (570).

[00106] In some implementations, a focus component (562) may be included to control positioning of one or more elements of objective lens assembly (542) relative to the flow cell (510) in the focus direction (e.g., along the z-axis or z-dimension). Focus component (562) may include one or more actuators physically coupled to the objective lens assembly (542), the optical stage, the sample stage (570), or a combination thereof, to move flow cell (510) on sample stage (570) relative to the

objective lens assembly (542) to provide proper focusing for the imaging operation. In the present example, the focus component (562) utilizes a focus tracking module (560) that is configured to detect a displacement of the objective lens assembly (542) relative to a portion of the flow cell (510) and output data indicative of an in-focus position to the focus component (562) or a component thereof or operable to control the focus component (562), such as controller (520), to move the objective lens assembly (542) to position the corresponding portion of the flow cell (510) in focus of the objective lens assembly (542).

[00107] In some implementations, an actuator of focus component (562) or for sample stage (570) may be physically coupled to objective lens assembly (542), the optical stage, sample stage (570), or a combination thereof, such as, for example, by mechanical, magnetic, fluidic, or other attachment or contact directly or indirectly to or with the stage or a component thereof. The actuator of focus component (562) may be configured to move objective lens assembly (542) in the z-direction while maintaining sample stage (570) in the same plane (e.g., maintaining a level or horizontal attitude, perpendicular to the optical axis). In some implementations, sample stage (570) includes an x direction actuator and a y direction actuator to form an x-y stage. Sample stage (570) may also be configured to include one or more tip or tilt actuators to tip or tilt sample stage (570) and/or a portion thereof, to account for any slope in its surfaces.

[00108] Camera system (540) may include one or more image sensors to monitor and track the imaging (e.g., sequencing) of flow cell (510). Camera system (540) may be implemented, for example, as a CCD or CMOS image sensor camera, but other image sensor technologies (e.g., active pixel sensor) may be used. By way of further example only, camera system (540) may include a dual-sensor time delay integration (TDI) camera, a single-sensor camera, a camera with one or more two-dimensional image sensors, and/or other kinds of camera technologies. While camera system (540) and associated optical components are shown as being positioned above flow cell (510) in FIG. 7, one or more image sensors or other camera components may be incorporated into system (500) in numerous other ways as will be apparent to those skilled in the art in view of the teachings herein. For instance, one or more image sensors may be positioned under flow cell (510), such as within the sample stage (570) or below the sample stage (570); or may even be

integrated into flow cell (510).

[00109] FIG. 8 shows an example of various components that may be integrated into imaging assembly (522) of system (500). In particular, the arrangement shown in FIG. 8 may represent a variation of light emitting assembly (550). The arrangement shown in FIG. 8 may be particularly useful in scenarios where camera system (540) includes a TDI camera. In the arrangement shown in FIG. 8, a line generation module (LGM) (602) and emission optics module (EOM) (604) are aligned and mechanically coupled to precision mounting plate (610), as well as to each other. EOM (604) includes an objective lens assembly (606) that is aligned, via a mirror (608) with a tube lens (620), which in turn is optically coupled to LGM (602). LGM (602) may include one or more light sources (e.g., coherent light sources such as laser diodes). In some examples, LGM (602) may include a first light source configured to emit light in red wavelengths, and a second light source configured to emit light in green wavelengths. LGM (602) may further include optical components, such as focusing surfaces, lenses, reflective surfaces, or mirrors. The optical components may be positioned within an enclosure of LGM (602) to direct and focus the light emitted from the one or more light sources into an adjacent modular subassembly. One or more of the optical components of LGM (602) may also be configured to shape the light emitted from the one or more light sources into desired patterns. For example, in some implementations, the optical components may shape the light into line patterns (e.g., by using one or more Powell lenses, or other beam shaping lenses, diffractive or scattering components). In some variations, LGM (602) may include one or more laser modules which may be individually removed from LGM (602) and replaced.

[00110] Light beams generated by LGM (602) transmit through an interface baffle between LGM (602) and EOM (604), pass through objective lens assembly (606), and strike an optical target (e.g., flow cell (510)). In some versions, the interface baffle includes an aperture shaped to enable light to pass through its center, while obscuring interference from external light sources. Responsive light radiation from the target may pass back through objective lens assembly (606) and into tube lens (622). A lens element (622), which may form part of tube lens (620), is configured to articulate along an axis (e.g., a z-axis) to correct for spherical aberration artifacts introduced by objective lens assembly (606) imaging through varied thickness of

flow cell (510) components. As illustrated, lens element (622) may be articulated closer to or further away from objective lens assembly (606) to adjust the beam shape and path. Objective lens assembly (606) may emit excitation light toward the optical target (e.g., flow cell (510)) and receive fluorescence emission from the optical target. An actuator may be configured to position objective lens assembly (606) to a region of interest proximate to the optical target. The processor of controller (520) may then execute program instructions for detecting fluorescence emission from the optical target.

[00111] FIG. 9 shows an example of another configuration that may be provided in imaging assembly (522). In particular, FIG. 9 shows an imaging assembly (650) positioned in relation to a flow cell (670). Flow cell (670) may be representative of any of the variations of flow cells (128, 368, 400, 450, 510) described herein. Flow cell (670) has an upper layer (671) and a lower layer (673) that are separated by a fluid filled channel (675). In the configuration shown, upper layer (671) is optically transparent and imaging assembly (650) is focused to an area (676) on inner surface (672) of upper layer (671). In other variations, imaging assembly (650) may be focused on inner surface (674) of lower layer (673). One or both of surfaces (672, 674) may include array features that are to be detected by imaging assembly (650).

[00112] Imaging assembly (650) includes an objective lens assembly (666) that is configured to direct excitation radiation from a light emitting assembly (652) to flow cell (670); and to direct emission from flow cell (670) to a detector (664). In the arrangement shown, excitation radiation from light emitting assembly (652) passes through a lens (658), though a beam splitter (660), and through objective lens assembly (666) on to reach flow cell (670). In the present example, light emitting assembly (652) includes two light emitting diodes (LEDs) (656, 654), which produce radiation at different wavelengths from each other. The emission radiation from flow cell (670) is captured by objective lens assembly (666) and is reflected by beam splitter (660) through conditioning optics (662) and to detector (664) (e.g., a CMOS sensor). Beam splitter (660) functions to direct the emission radiation in a direction that is orthogonal to the path of the excitation radiation. The position of objective lens assembly (666) may be moved in the z dimension to alter focus of imaging assembly (650). The imaging assembly (650) may be moved back and forth in the y direction to capture images of several areas of at least one inner

surface (672, 674) of flow cell (670).

[00113] In the present example, a single imaging assembly (650) includes two LEDs (656, 654) that emit light at two different respective wavelengths, with a single detector (664) detecting light emitted from fluorophore labels in flow cell (670) in response to irradiation at these two different wavelengths. In some other versions, there are two or more imaging assemblies (650), with each imaging assembly (650) including a single LED (656, 654) and a single detector (664), such that each imaging assembly (650) provides irradiation at only one single respective wavelength. As another variation, two or more detectors (664) may receive excitation radiation from a common light emitting assembly (652).

[00114] FIG. 10 shows an example of another configuration that may be provided in imaging assembly (522). In particular, FIG. 10 shows an imaging assembly (700) positioned in relation to a flow cell (774). Flow cell (774) may be representative of any of the variations of flow cells (128, 368, 400, 450, 510) described herein. Flow cell (770) has a translucent cover plate (772), a substrate (774), and a liquid layer (776) that is interposed between cover plate (772) and substrate (774). A biological sample may be located on an inside surface of cover plate (772) (above liquid layer (776)) and/or on an inside surface of substrate (774) (below liquid layer (776)).

[00115] Imaging assembly (700) of this example includes an LGM (710) with two light sources (712, 714), disposed therein. Light sources (712, 714) may include laser diodes, diode pumped solid state lasers, or other light sources as known in the art, which output laser beams at different wavelengths (e.g., red or green light). The light beams output from light sources (712, 714) are directed through a beam shaping lens or lenses (716). In some implementations, one or more light shaping lenses may be used to shape the light beams output from each or both light sources. LGM (710) may use one or more Powell lenses to spread and/or shape the laser beams from single or near-single mode laser light sources. Other beam shaping optics may be used to control uniformity and increase tolerance such as an active beam expander, an attenuator, one relay lenses, cylindrical lenses, actuated mirrors, diffractive elements, and scattering components. Laser beams may intersect at the back focal point of the objective lens to provide better tolerance on surfaces of flow cell (770).

[00116] LGM (710) of this example further includes mirrors (718, 720). A light beam generated by light source (712) reflects off mirror (718), as to be directed through an aperture or semi-reflective surface of mirror (720), and into EOM (740) through a single interface port. Similarly, a light beam generated by light source (714) reflects off of mirror (720) as to be directed into EOM (740) through a single interface port. In some examples, an additional set of articulating mirrors may be incorporated adjacent to mirrors (718, 720) to provide additional tuning surfaces. Both light beams may be combined using dichroic mirror (720). Mirrors (718, 720) may each be configured to articulate using manual or automated controls to align the light beams from light sources (712, 714). The light beams also pass through a shutter element (722) in the present example.

[00117] EOM (740) includes an objective lens assembly (756) and a z-stage (758), which moves objective lens assembly (756) longitudinally closer to or further away from flow cell (770). LGM (710) is configured to generate a uniform line illumination through objective lens assembly (756). Z-stage (758) may then move objective lens assembly (756) as to focus the light beams onto either of the inside surfaces of flow cell (770) (e.g., focused on a biological sample). In some implementations, the objective lens assembly (756) may be configured to focus the light beams at a focal point beyond flow cell (770), such as to increase the line width of the light beams at the surfaces of flow cell (770).

[00118] EOM (740) of the present example also include a semi-reflective mirror (754) to direct light through objective lens assembly (756), while allowing light returned from flow cell (774) to pass through. EOM (740) further includes a tube lens (744) and a corrective lens (748). Corrective lens (748) may be articulated longitudinally by a z-stage (746), either closer to or further away from objective lens assembly (756), to ensure accurate imaging (e.g., to correct spherical aberration caused by moving objective lens assembly (756); and/or from imaging through a thicker substrate, etc.). Light transmitted through corrective lens (748) and tube lens (744) passes through filter element (742) and into camera system (730). Camera system (730) includes one or more optical sensors (732) to detect light emitted from the biological sample in response to the incident light beams.

[00119] In the present example, EOM (740) further includes semi-reflective mirror (752) to

reflect a focus tracking light beam emitted from a focus tracking module (FTM) (760) onto flow cell (774), and then to reflect light returned from flow cell (774) back into FTM (760). FTM (760) may include a focus tracking optical sensor to detect characteristics of the returned focus tracking light beam and generate a feedback signal to optimize focus of objective lens assembly (756) on flow cell (774).

[00120] The direction, size, and/or polarization of the laser beams may be adjusted by using lenses, mirrors, and/or polarizers. Optical lenses (e.g., cylindrical, spherical, or aspheric) may be used to actively adjust the illumination focus on dual surfaces of the flow cell (770) target. LGM (710) may also include multiple units, with each unit being designed for particular/different wavelengths and polarization. Stacking multiple units may be used to increase the laser power and wavelength options. Two or more laser wavelengths may be combined with dichroics and polarizers.

[00121] By way of example only, focus tracking module (560) and/or other components of imaging assembly (522) may be constructed and operable in accordance with at least some of the teachings of U.S. Pat. No. 10,416,428, entitled "Systems and Methods for Improved Focus Tracking Using a Light Source Configuration," issued September 17, 2019, the disclosure of which is incorporated by reference herein, in its entirety; U.S. Pub. No. 2023/0228984, entitled "Dynamic Detilt Focus Tracking," published July 20, 2023, the disclosure of which is incorporated by reference herein, in its entirety; and/or U.S. Pat. App. No. 63/410,961, entitled "Spot Error Handling for Focus Tracking," filed September 28, 2022, the disclosure of which is incorporated by reference herein, in its entirety. By way of further example only, components of imaging assembly (522) may be configured and operable in accordance with at least some of the teachings of U.S. Pat. No. 10,774,371, entitled "Laser Line Illuminator for High Throughput Sequencing," issued September 15, 2020, the disclosure of which is incorporated by reference herein, in its entirety; and/ U.S. Pat. No. 9,958,465, entitled "Detection Apparatus having a Microfluorometer, a Fluidic System, and a Flow Cell Latch Clamp Module," issued May 1, 2018, the disclosure of which is incorporated by reference herein, in its entirety.

[00122] VI. Examples of Immersion Microscopy Features for Sequencing System

[00123] As noted above, some versions of an imaging assembly (522, 650, 700) in a sequencing system (500) may include an objective lens assembly (542, 606, 666, 756) positioned over a flow cell (128, 368, 400, 450, 450A, 510, 670, 770) to capture images with fluorescent emissions from fluorophore labels of nucleotides in flow cell (128, 368, 400, 450, 450A, 510, 670, 770). In some instances, it may be desirable to provide an immersion fluid (e.g., water, oil, etc.) between objective lens assembly (542, 606, 666, 756) and the upper surface of flow cell (128, 368, 400, 450, 450A, 510, 670, 770), to thereby provide an immersion microscopy arrangement. Such an immersion fluid may enhance the resolution and clarity of images captured via objective lens assembly (542, 606, 666, 756), effectively increasing the numerical aperture of objective lens assembly by increasing the refractive index that would otherwise be provided in an air gap between objective lens assembly (542, 606, 666, 756) positioned over a flow cell (128, 368, 400, 450, 450A, 510, 670, 770). The following provides examples of how immersion fluid features may be integrated into an imaging assembly of a sequencing system, such as any of the imaging assemblies (522, 650, 700) described herein.

[00124] A. Overview

[00125] FIG. 11 shows an example of an arrangement (1000) that may be incorporated into any of the various sequencing systems described herein. Arrangement (1000) of this example includes a frame (1010), a sample stage (1020), a flow cell (1030), an imaging assembly (1040), an air curtain manifold (2020), and an air blade manifold (2060). Frame (1010) is shown schematically and take various suitable forms. For instance, frame (1010) may comprise a stationary superstructure within a sequencing instrument or system enclosure, providing a mechanical ground for other features of arrangement (1000).

[00126] Sample stage (1020) may be configured and operable like sample stage (570) described above, such that sample stage (1020) may provide structural support to flow cell (1030); and further provide movement and alignment of flow cell (1030) relative to objective lens assembly (2000). Sample stage (1020) may have one or more actuators to allow sample stage (1020) to move in any of three dimensions. For example, actuators may be provided to allow sample stage (1020) to move in the x, y, and z directions relative to objective lens assembly (2000), tilt relative to

objective lens assembly (2000), and/or otherwise move relative to objective lens assembly (2000). Flow cell (1030) may be configured and operable like any of the other flow cells (128, 368, 400, 450, 450A, 510, 670, 770) described herein.

[00127] Imaging assembly (1040) of this example comprises an objective lens assembly (2000), an immersion fluid manifold (2040), and a set of additional imaging components (1044) that are shown schematically in FIG. 11. These additional imaging components (1044) may include any of the various imaging components of any of the various imaging assemblies (522, 650, 700) described herein. Specific features and functionalities of objective lens assembly (2000) and immersion fluid manifold (2040) will be described in greater detail below. Imaging assembly (1040) of this example is coupled with frame (1010) via a mount (1042) in this example. In some versions, mount (1042) provides a static, fixed relationship between imaging assembly (1040) and frame (1010), such that imaging assembly (1040) does not move relative to frame (1010). In some other versions, at least a portion of imaging assembly (1040) moves relative to frame (1010). For instance, mount (1042) may include one or more motors, servos, or other actuators, etc. that provide(s) controlled movement of imaging assembly (1040) in one or more of the x-dimension, the y-dimension, or the z-dimension relative to frame (1010). In addition, or in the alternative, one or more features within imaging assembly may provide movement of objective lens assembly (2000) and/or one or more focusing lens elements relative to frame (1010), such as to provide controlled focus of an imaging region on or in flow cell (1030), etc.

[00128] As shown in FIGS. 11-20, air curtain manifold (2020) of this example comprises an annular body (2022) that is positioned to encircle a distal portion of objective lens assembly (2000). Specific features and functionalities of air curtain manifold (2020) will be described in greater detail below. Air curtain manifold (2020) is coupled with frame (1010) via mounts (1012) and corresponding beams (1014) in this example. Mounts (1012) and beams (1014) thus support air curtain manifold (2020) relative to objective lens assembly (2000) in this example, with a gap (G) being maintained between air curtain manifold (2020) and objective lens assembly (2000) as best seen in FIGS. 16, 18, and 38A-38G. In some versions, mounts (1012) and beams (1014) provide a static, fixed relationship between air curtain manifold (2020) and frame (1010). In some other versions, each mount (1012) may include

one or more motors, servos, or other actuators, etc. that provide(s) controlled movement of air curtain manifold (2020) in one or more of the x-dimension, the y-dimension, or the z-dimension relative to frame (1010).

[00129] Each mount (1012) and/or frame (1010) may also provide substantial mass to absorb any vibrations that may be communicated along beams (1014) by air curtain manifold (2020) during operation of air curtain manifold (2020) as described below. This may effectively isolate objective lens assembly (2000) relative to such vibrations, thereby preventing such vibrations from otherwise having an adverse impact on the quality of images captured via objective lens assembly (2000). In addition, or in the alternative, a foam, rubber, or other elastomeric material, etc., may be interposed between air curtain manifold (2020) and objective lens assembly (2000) to prevent vibrations of air curtain manifold (2020) from reaching objective lens assembly (2000).

[00130] As shown in FIGS. 11-20, air blade manifold (2060) of this example comprises a body (2062) that is positioned at a location laterally offset from the field of view of objective lens assembly (2000). Specific features and functionalities of air blade manifold (2060) will be described in greater detail below. Air blade manifold (2060) is coupled with frame (1010) via a mount (1016) and corresponding beam (1018) in this example. Mount (1016) and beam (1016) thus support air blade manifold (2060) relative to objective lens assembly (2000) in this example. In some versions, mount (1016) and beam (1018) provide a static, fixed relationship between air blade manifold (2060) and frame (1010). In some other versions, mount (1016) may include one or more motors, servos, or other actuators, etc. that provide(s) controlled movement of air blade manifold (2060) in one or more of the x-dimension, the y-dimension, or the z-dimension relative to frame (1010). Mount (1016) and/or frame (1010) may also provide substantial mass to absorb any vibrations that may be communicated along beam (1018) by air blade manifold (2060) during operation of air blade manifold (2060) as described below.

[00131] As shown in FIG. 12, an immersion fluid source (3000) is fluidically coupled with immersion fluid manifold (2040) via a fluid conduit (3002). Immersion fluid source (3000) may include any suitable kind of immersion fluid, including but not limited to water (e.g., HPLC double distilled water, etc.), oil, etc. A suction source (3010)

is also fluidically coupled with immersion fluid manifold (2040) via a fluid conduit (3012). By way of example only, each fluid conduit (3002, 3012) may include a flexible tube and/or any other suitable component(s). In some versions, suction source (3010) is also fluidically coupled with immersion fluid source (3000) via a third conduit (not shown) such that fluid communicated from immersion fluid source (3000) to immersion fluid manifold (2040) may be recirculated back to immersion fluid source (3000) via suction source (3010) and the third conduit. In some other versions, suction source (3010) is fluidically coupled with a tank or other fluid container, such that fluid drawn from immersion fluid manifold (2040) by suction source (3010) is deposited in the tank or other fluid container for disposal or other handling.

[00132] As shown in FIG. 13, a pressurized air source (3020) is fluidically coupled with a valve (3030) via a fluid conduit (3022). By way of example only, pressurized air source (3020) may comprise a pump or a cartridge containing pressurized air. Valve (3030) is fluidically coupled with air curtain manifold (2020) via a fluid conduit (3032); and with air blade manifold (2060) via another fluid conduit (3034). Valve (3030) is operable to toggle between at least two different states, including a first state where pressurized air from pressurized air source (3020) is communicated to air curtain manifold (2020) and a second state where pressurized air from pressurized air source (3020) is communicated to air blade manifold (2020). Valve (3030) may take any suitable form as may be apparent to those skilled in the art in view of the teachings herein. Operation of pressurized air source (3020) and valve (3030) may be automated under control of a feature like controller (114, 308) in accordance with the teachings herein. By way of further example only, each fluid conduit (3022, 3032, 3034) may include a flexible tube and/or any other suitable component(s).

[00133] B. Example of Objective Lens Assembly

[00134] FIGS. 21-22 show features of objective lens assembly (2000) in greater detail. As shown, objective lens assembly (2000) of this example includes an objective lens element (2002) that is supported by a housing (2006). Housing (2006) includes a tapered distal portion (2008) that terminates in an annular distal face (2010). By way of example only, annular distal face (2010) may include

polytetrafluoroethylene, nickel, and/or any other suitable material(s). Annular distal face (2010) surrounds distal face (2004) of objective lens element (2002). In some versions, distal face (2004) of objective lens element (2002) is substantially flush with annular distal face (2010). In some other versions, objective lens element (2002) is recessed relative to annular distal face (2010). In some such versions, objective lens element (2002) is recessed relative to annular distal face (2010) at a depth ranging from approximately 100 μm to approximately 50 μm. Alternatively, objective lens element (2002) may be recessed relative to annular distal face (2010) at any other suitable depth.

[00135] In the present example, an annular recess (2012) is formed in annular distal face (2010). Annular recess (2012) of has a cross-sectional profile resembling a pyramidal frustum in this example. Alternatively, annular recess (2012) may have any other suitable kind of cross-sectional profile, including but not limited to rounded/concave, triangular, rectangular, etc. While only one annular recess (2012) is provided in this example, other versions may include two or more annular recesses (2012) (e.g., in a concentric arrangement along annular distal face (2010)). Annular recess (2012) of the present example is configured to assist in retention of a volume of immersion fluid (IF) between objective lens assembly (2000) and an upper surface (1032) of flow cell (1030), even during movement of flow cell (1030) relative to objective lens assembly (2000), as described in greater detail below. By way of example only, annular recess (2012) may have a depth ranging from between approximately 12.5 μm to approximately 50 μm. Alternatively, annular recess (2012) may have any other suitable depth.

[00136] FIG. 23 shows an example of an alternative objective lens element (2003) that may be incorporated into objective lens assembly (2000) in lieu of objective lens element (2002). While objective lens element (2002) has a flat distal face (2004), objective lens element (2003) of this example has a concave distal face (2005). In some scenarios, the concave configuration of distal face (2005) may assist in retention of a volume of immersion fluid (IF) between objective lens assembly (2000) and an upper surface (1032) of flow cell (1030), even during movement of flow cell (1030) relative to objective lens assembly (2000), as described in greater detail below. By way of example only, some versions of objective lens assembly (2000) may include a combination of concave distal face (2005) and one or more annular recesses

(2012) to assist in retention of a volume of immersion fluid (IF) between objective lens assembly (2000) and an upper surface (1032) of flow cell (1030). In addition, or in the alternative, distal face (2004, 2005) of objective lens element (2002, 2003) and/or distal face (2010) of housing (2006) may include a hydrophilic material (e.g., polyetherimide, acrylic, Inconel, etc.) and/or other treatment to assist in retention of a volume of immersion fluid (IF) between objective lens assembly (2000) and an upper surface (1032) of flow cell (1030).

[00137] C. Example of Air Curtain Manifold

[00138] FIGS. 24-28 show features of air curtain manifold (2020) in greater detail. As shown, air curtain manifold (2020) of this example includes annular body (2022) with an outer surface (2024) that is angled to taper inwardly toward the bottom of body (2022). A plurality of openings (2026) is formed through outer surface (2024). Openings (2026) are spaced equidistantly from each other about the entire circumference of annular body (2022) in this example.

[00139] As best seen in FIGS. 26-28, an annular channel (2032) is formed in body (2022) and is in fluid communication with openings (2026). Air curtain manifold (2020) further includes a pair of ports (2028) extending upwardly from body (2022). Each port (2028) defines a passageway (2034) that is in fluid communication with channel (2032). Each port (2028) is configured to fluidically couple with a respective fluid conduit (3032). As described above with reference to FIG. 13, fluid conduits (3032) are configured to receive pressurized air from pressurized air source (3020) via fluid conduit (3022) and valve (3030).

[00140] Ports (2028) thus allow air curtain manifold (2020) to receive this pressurized air from pressurized air source (3020). The pressurized air passes through passageways (2034) and channel (2032) to reach openings (2026), through which the pressurized air is expelled from air curtain manifold (2020). As described in greater detail below, with openings (2026) being positioned on the obliquely angled surface (2026) of air curtain manifold (2020), and with air curtain manifold (2020) being positioned coaxially about objective lens assembly (2000), the pressurized air creates a frustoconical air curtain oriented downwardly and outwardly relative to the central longitudinal axis (LA) of imaging assembly (1040) as the pressurized air exits openings (2026). As will also be described in greater detail below, this air

curtain provided by air curtain manifold (2020) may substantially prevent any fluid, debris, etc. on upper surface (1032) of flow cell (1030) from reaching the field of view under objective lens assembly (2000). In other words, the air curtain may tend to blow or sweep away such fluid, debris, etc. from upper surface (1032) before the fluid, debris, etc. would otherwise reach the field of view under objective lens assembly (2000).

[00141] Air curtain manifold (2020) of the present example further includes a pair of openings (2030) on a region of body (2022) above surface (2024). Openings (2030) are configured to receive fasteners (e.g., pins, screws, etc.) to thereby secure air curtain manifold (2020) to beams (1014). As noted above with reference to FIG. 11, beams (1014) are secured to frame (1010) via respective mounts (1012). Openings (2030) are not in fluid communication with channel (2032), passageways (2034), or openings (2026) in this example. In other versions, air curtain manifold (2020) is secured to frame (1010) (or is otherwise secured relative to objective lens assembly (2000)) using other structures or arrangements. In the present example, air curtain manifold (2020) is secured at a position where air curtain manifold (2020) is slightly higher than immersion fluid manifold (2040) along the central longitudinal axis (LA). In some other versions, air curtain manifold (2020) may be positioned at or slightly lower than the position of immersion fluid manifold (2040) along the central longitudinal axis (LA).

[00142] D. Example of Immersion Fluid Manifold

[00143] FIGS. 29-32 show features of immersion fluid manifold (2040) in greater detail. As shown, immersion fluid manifold (2040) of this example includes an annular body (2042) with an inner surface (2043) that is angled to taper inwardly toward the bottom of body (2022). The angle of inner surface (2043) is configured to complement the angle of tapered distal portion (2008) of housing (2006) of objective lens assembly (2000). Inner surface (2043) thus fits together in apposition with tapered distal portion (2008) when immersion fluid manifold (2040) is secured at the bottom of objective lens assembly (2000) as best seen in FIGS. 16 and 18. In some versions, the fit between inner surface (2043) and tapered distal portion (2008) is substantially fluid tight, thereby preventing undesired migration of immersion fluid, into a space that may otherwise be defined between inner surface (2043) and

tapered distal portion (2008), during operation as described in greater detail below.

[00144] A central opening (2045) of immersion fluid manifold (2040) is sized to accommodate annular distal face (2010) of housing (2006) of objective lens assembly (2000), such that immersion fluid manifold (2040) does not obstruct the field of view through objective lens element (2002). Similarly, immersion fluid manifold (2040) does not cover any region of annular recess (2012), such that immersion fluid manifold (2040) does not impede the fluid retention capabilities of annular recess (2012) as described herein.

[00145] In some versions, immersion fluid manifold (2040) is secured to housing (2006) of objective lens assembly (2000) via an adhesive or epoxy. In some other versions, immersion fluid manifold (2040) is secured to housing (2006) of objective lens assembly (2000) via complementary threading and/or some other mechanical feature(s). Alternatively, immersion fluid manifold (2040) may be secured to housing (2006) of objective lens assembly (2000) in any other suitable fashion. As yet another example of an alternative arrangement, immersion fluid manifold (2040) may be secured relative to housing (2006) without being secured directly to housing (2006). For instance, immersion fluid manifold may be secured relative to housing (2006) via structures like beams (1014) and mounts (1012), etc.

[00146] As also shown in FIGS. 29-32, immersion fluid manifold (2040) of the present example further includes a recess (2044) under and around central opening (2045). Recess (2044) is surrounded by a rim (2046) at the bottom of body (2042). As best seen in FIG. 31, recess (2044) has an elliptical shape having a major axis (A1) and a minor axis (A2). By way of example only, the major axis (A1) may be approximately 18 mm while the minor axis (A2) may be approximately 14 mm. Alternatively, any other suitable length may be used for the major axis (A1) or the minor axis (A2). During an image capture process, and as will be described in greater detail below, immersion fluid manifold (2040) is oriented such that the major axis (A1) of recess (2044) is oriented along the path of relative motion between flow cell (1030) and objective lens assembly (2000); while the minor axis (A2) of recess (2044) is oriented transversely relative to the path of relative motion between flow cell (1030) and objective lens assembly (2000). In the reference axes provided in FIG. 31 and in FIGS. 38A-38G, this path of relative motion during the

image capture process is along the x-dimension. As will also be described in greater detail below, the elliptical shape and orientation of recess (2044) may promote retention of a volume of immersion fluid (IF) in the space between objective lens element (2002) and upper surface (1032) of flow cell (1030), including while there is relative motion between flow cell (1030) and objective lens assembly (2000) during an image capture process.

[00147] As best seen in FIG. 32, immersion fluid manifold (2040) of the present example further includes a first port (2050), a first channel (2052), a second port (2056), and a second channel (2058). First port (2050) is positioned on a laterally facing region of body (2042) and is configured to couple with immersion fluid source (3000) via fluid conduit (3002), as described above with reference to FIG. 12. Immersion fluid manifold (2040) thus receives immersion fluid via fluid conduit (3002) and first port (2050). In some versions, first port (2050) includes an outwardly extending rigid fitting and/or other structural feature to facilitate coupling with fluid conduit (3002). First channel (2052) extends from first port (2050) to recess (2044), providing a path for communication of immersion fluid from immersion fluid source (3000) to reach recess (2044). Thus, a volume of immersion fluid from immersion fluid source (3000) may be communicated to the space between objective lens element (2002) and upper surface (1032) of flow cell (1030) via fluid conduit (3002), first port (2050), and first channel (2052).

[00148] Second port (2056) is also positioned on a laterally facing region of body (2042) and is configured to couple with suction source (3010) via fluid conduit (3012), as described above with reference to FIG. 12. Immersion fluid is thus drawn from immersion fluid manifold (2040) via fluid conduit (3012) and second port (2056). In some versions, second port (2056) includes an outwardly extending rigid fitting and/or other structural feature to facilitate coupling with fluid conduit (3012). Second channel (2058) extends from second port (2056) to recess (2044), providing a path for communication of immersion fluid from recess (2044). Thus, suction source (3010) may be activated to draw/remove a volume of immersion fluid from the space between objective lens element (2002) and upper surface (1032) of flow cell (1030) via second channel (2058), second port (2056), and fluid conduit (3012). This drawing/removal of immersion fluid may be provided during a de-priming step of operation as described in greater detail below. In addition, or in the alternative,

such drawing of immersion fluid by suction source (3010) may be provided simultaneously with communication of immersion fluid from immersion fluid source (3000), such as during circulation of immersion fluid within the space between objective lens element (2002) and upper surface (1032) of flow cell (1030).

[00149] E. Example of Air Blade Manifold

[00150] FIGS. 33-35 show features of air blade manifold (2060) in greater detail. As shown, air blade manifold (2060) of this example includes a body (2062) having a mount portion (2064) and an arc portion (2066). Mount portion (2064) is configured to couple with beam (1018) via one or more fasteners (e.g., pins, screws, etc.). As noted above with reference to FIG. 11, beam (1018) is secured to frame (1010) via mount (1016). In other versions, air blade manifold (2060) is secured to frame (1010) (or is otherwise secured relative to objective lens assembly (2000)) using other structures or arrangements.

[00151] As best seen in FIG. 35, a primary channel (2068) is formed in body (2062). Primary channel (2068) is configured to fluidically couple with fluid conduit (3034). As described above with reference to FIG. 13, fluid conduit (3034) is configured to receive pressurized air from pressurized air source (3020) via fluid conduit (3022) and valve (3030). In some versions, body (2062) includes an outwardly extending rigid fitting and/or other structural feature to facilitate coupling of primary channel (2068) with fluid conduit (3022). As also best seen in FIG. 35, a plurality of channels (2072) is also in fluid communication with primary channel (2068). Channels (2072) are oriented along respective radii emanating from a single center point.

[00152] Each channel (2072) terminates in a respective opening (2070), such that pressurized air communicated to primary channel (2068) via fluid conduit (3022) will pass through channels (2072) and exit air blade manifold (2060) via openings (2070). Openings (2070) are positioned along arc portion (2066). Openings (2070) are all positioned along the same x-y plane with each other in this example, though openings (2070) may alternatively have any other suitable arrangement. In the present example, the angular extent of the array of openings (2070) is less than 180 degrees. Arc portion (2066) is positioned to face the space between objective lens element (2002) and upper surface (1032) of flow cell (1030). When pressurized air

is communicated from pressurized air source (3020) to air blade manifold (2060) as described above, the pressurized air is expelled via openings (2070) into the space between objective lens element (2002) and upper surface (1032) of flow cell (1030). This pressurized air creates a substantially flat air blade that may tend to clear any residual immersion fluid on objective lens assembly (2000) and/or upper surface (1032) of flow cell (1030).

[00153] As noted above, channels (2072) are oriented along respective radii emanating from a single center point; and openings (2070) are all oriented toward this single center point. In some versions, this single center point is positioned along the optical axis of objective lens assembly (2000), such that channels (2072) and openings (2070) are all oriented toward the central region of the space between objective lens element (2002) and upper surface (1032) of flow cell (1030). Thus, the air expelled via openings (2070) is substantially directed toward the central region of the space between objective lens element (2002) and upper surface (1032) of flow cell (1030). This inwardly oriented airflow pattern may enhance the ability of the air blade formed by air blade manifold (2060) to remove residual immersion fluid from objective lens assembly (2000) and/or upper surface (1032) of flow cell (1030) as described in greater detail below. In some other versions, channels (2072) and openings (2070) are positioned along a straight line rather than an arc, such that airflow pattern of the air blade formed by air blade manifold (2060) is not centrally focused on the central region of the space between objective lens element (2002) and upper surface (1032) of flow cell (1030). In some cases, such a non-centrally focused airflow pattern may be less efficient or effective than a centrally focused airflow pattern; but may nevertheless be acceptable.

[00154] While air blade manifold (2060) is used to remove residual immersion fluid from objective lens assembly (2000) and/or upper surface (1032) of flow cell (1030) in the present example, other structures or techniques may be used to remove residual immersion fluid from objective lens assembly (2000) and/or upper surface (1032) of flow cell (1030). Such other structures or techniques may be used in addition to, or in lieu of, using air blade manifold (2060). Such other structures or techniques may include absorbent features, non-absorbent wiping features, and/or any other suitable structures or techniques. In some other versions, air blade manifold (2060) is omitted, and no other additional features are provided to remove residual

immersion fluid from objective lens assembly (2000) and/or upper surface (1032) of flow cell (1030). In some such versions, the de-priming provided via suction source (3010) and immersion fluid manifold (2040) may sufficiently remove immersion fluid from objective lens assembly (2000) and/or upper surface (1032) of flow cell (1030).

[00155] F. Example of Use of Imaging Assembly with Immersion Microscopy Features

[00156] FIGS. 36A-36B schematically depict an example of an imaging process where imaging assembly (1040) is used to capture images with fluorescent emissions from fluorophore labels of nucleotides in flow cell (1030). As shown, flow cell (1030) includes eight channels (1034). By way of example only, each channel (1034) of flow cell (1030) may be configured and operable like each channel (130) of flow cell (128) described above, like each channel (430) of flow cell (400) described above, like each channel (480) of flow cell (450) described above, or otherwise. At the beginning of an imaging process, imaging assembly (1040) is positioned at a first end of a first channel (1034a), as shown in FIG. 36A. The ellipse (1036) in FIG. 36A represents the footprint of rim (2046) of immersion fluid manifold (2040) over upper surface (1032) of flow cell (1030).

[00157] Through the imaging process, flow cell (1030) moves relative to imaging assembly (1040) along the x-y plane, allowing imaging assembly (1040) to capture numerous images along the length and width of first channel (1034a). As noted above, this relative movement is provided by one or more actuators of sample stage (1020); while imaging assembly (1040) remains stationary along the x-y plane. As also noted above, some other variations may provide driven movement of imaging assembly (1040) along the x-y plane; while flow cell (1030) remains stationary along the x-y plane. In either scenario, after imaging assembly (1040) has captured sufficient images along first channel (1034a), the relative position between imaging assembly (1040) and flow cell (1030) continues to change to allow imaging assembly (1040) to capture images along second channel (1034b), along third channel (1034c), along fourth channel (1034d), along fifth channel (1034e), along sixth channel (1034f), along seventh channel (1034g), and along eighth channel (1034h). At the end of this imaging process, imaging assembly (1040) is positioned

at a second end of a eighth channel (1034h), as shown in FIG. 36B.

[00158] FIGS. 37 and 38A-38G provide an example of how air curtain manifold (2020), immersion fluid manifold (2040), and air blade manifold (2060) may be utilized during an imaging process such as the process described above with reference to FIGS. 36A-36B. As shown in FIG. 38A, this process may begin with activation (block 3050 of FIG. 37) of an air curtain via air curtain manifold (2020). As noted above, this may be accomplished via activation of pressurized air source (3020), with valve (3030) in an operational state where valve (3030) directs the pressurized air to fluid conduit (3032). In some versions, the air curtain remains activated continuously throughout the process until the air blade is activated as described in greater detail below with reference to FIG. 38F.

[00159] After the air blade has been initially activated, and as shown in FIG. 38B, the process may continue with positioning (block 3052 of FIG. 37) of flow cell (1030) under objective lens assembly (2000). In some versions, flow cell (1030) is driven into this position via sample stage (1020) while imaging assembly (1040) remains stationary. In some other versions, imaging assembly (1040) is driven to position (e.g., via mount (1042)) while flow cell (1030) remains stationary. In either scenario, once flow cell (1030) is suitably positioned under objective lens assembly (2000), the frustoconical air curtain expelled from air curtain manifold (2020) is oriented downwardly and outwardly relative to the central longitudinal axis of objective lens assembly (2000) as described above, such that the air curtain blows away any fluid, debris, etc. on upper surface (1032) of flow cell (1030) before such fluid, debris, etc. would otherwise reach the field of view under objective lens assembly (2000). As the air curtain reaches upper surface (1032), the pressurized air is deflected outwardly by upper surface (1032). The stage of operation shown in FIG. 38B may correspond with an initial part of the stage of operation shown in FIG. 36A.

[00160] With flow cell (1030) suitably positioned under objective lens assembly (2000), and as shown in FIG. 38C, immersion fluid manifold (2040) is primed (block 3054 of FIG. 37). While immersion fluid source (3000) is not shown in FIG. 38C, this priming step includes communication of immersion fluid from immersion fluid source (3000) to recess (2044) via conduit (3002) first port (2050) and first channel

(2052). This priming step results in a volume of immersion fluid (IF) in the space between objective lens element (2002) and upper surface (1032) of flow cell (1030). By way of example only, the volume of immersion fluid (IF) may be approximately 125 μL. Alternatively, the volume of immersion fluid (IF) may have any other suitable size.

[00161] In some versions, immersion fluid is continuously circulated to this space through continuous deposition of immersion fluid from immersion fluid source (3000) and continuous drawing-off of immersion fluid by suction source (3010). In some such versions, the immersion fluid is provided at a certain temperature, or within a certain temperature range, to regulate the temperature of objective lens element (2002) and/or flow cell (1030). In addition, or in the alternative, one or more thermal elements (e.g., within objective lens assembly (2000), within immersion fluid manifold (2040), and/or elsewhere) may be used to regulate the temperature of objective lens element (2002), the volume of immersion fluid (IF), and/or flow cell (1030).

[00162] As also shown in FIG. 38C, some versions may include a machine vision sensor (1038) (e.g., camera or other optical sensor, etc.) that is oriented to view the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030). Data from such a machine vision sensor (1038) may thus indicate whether/when a sufficient volume of immersion fluid (IF) has been deposited in the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030). In some such versions, machine vision sensor (1038) and immersion fluid source (3000) may be in communication with the same controller (e.g., controller (114, 308)), such that the controller may issue a command signal to cause immersion fluid source (3000) to continue depositing immersion fluid into the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030) until feedback data from machine vision sensor (1038) indicates that a sufficient volume of immersion fluid (IF) has reached the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030).

[00163] Once the priming has provided a sufficient volume of immersion fluid (IF) in the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030), and as shown in FIG. 38D, the process may proceed to a scan (block 3056

of FIG. 37) of flow cell (1030). During this scanning process, relative movement may be provided between flow cell (1030) and imaging assembly (1040), such that imaging assembly (1040) captures images along the length and width of each channel (1034) of flow cell (1030) as described above with reference to FIGS. 36A-36B. As noted above, this relative movement is provided by one or more actuators of sample stage (1020), such that flow cell (1030) moves along the x-y plane while imaging assembly (1040) remains stationary along the x-y plane. As also noted above, some other variations may provide driven movement of imaging assembly (1040) along the x-y plane; while flow cell (1030) remains stationary along the x-y plane.

[00164] As shown in FIG. 38D, the volume of immersion fluid (IF) may remain in the space between objective lens element (2002) and upper surface (1032) during the relative movement between flow cell (1030) and imaging assembly (1040), though the volume of immersion fluid (IF) may encounter some degree of lateral deformation due to shearing forces. The volume of immersion fluid (IF) may be sufficiently maintained between objective lens element (2002) and upper surface (1032) even when the relative movement between flow cell (1030) and imaging assembly (1040) is substantially fast (e.g., between approximately 20 mm/s and approximately 100 mm/s, or faster than approximately 100 mm/s).

[00165] As noted above, various features may promote retention of the volume of immersion fluid (IF) in the space between objective lens element (2002) and upper surface (1032) during the relative movement between flow cell (1030) and imaging assembly (1040). Such features may include annular recess (2012) in annular distal face (2010) of housing (2006) of objective lens assembly (2000). Such features may also include a hydrophilic coating on distal face (2004) of objective lens element (2002) and/or on distal face (2010) of housing (2006). Such features may also include a hydrophobic coating (e.g., fluoropolymers, diamond-like nanocomposites, diamond-like nanostructures, amorphous carbon, flouronated diamond like nanocomposites, etc.) on upper surface (1032) of flow cell (1030). Such features may also include the elliptical shape of recess (2044) on the underside of immersion fluid manifold (2040), which may tend to minimize the surface tension of the immersion fluid. Such features may also include a recessed spacing of distal face (2004, 2005) of objective lens element (2002, 2003) relative to annular

distal face (2010) of housing (2006). Such features may also include a concave recess formed in distal face (2005) of objective lens element (2003).

[00166] As noted above, some versions may include a machine vision sensor (1038) that is oriented to view the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030). In some such versions, machine vision sensor (1038) may continue to optically monitor the space between objective lens assembly (2000) and upper surface (1032) to confirm that a sufficient volume of immersion fluid (IF) remains in the space between objective lens assembly (2000) and upper surface (1032) during the scanning process. If feedback from machine vision sensor (1038) indicates that there is no longer a sufficient volume of immersion fluid (IF) in the space between objective lens assembly (2000) and upper surface (1032) (or that the volume of immersion fluid (IF) is approaching an insufficient level), a controller (e.g., controller (114, 308)) may issue a command signal to cause immersion fluid source (3000) to deposit additional immersion fluid into the space between objective lens assembly (2000) and upper surface (1032) until a suitable volume of immersion fluid (IF) is again achieved. Such a feedback loop may be maintained throughout the scanning process. It should also be noted that the air curtain remains activated throughout the scanning process to substantially prevent any other fluid, debris, etc. on upper surface (1032) of flow cell (1030) from reaching the field of view under objective lens assembly (2000) during the scanning process.

[00167] After flow cell (1030) has been sufficiently scanned by imaging assembly (1040), with a sufficient number of images captured, the process may proceed to a de-priming step (block 3058 in FIG. 37) as shown in FIG. 38E. In some versions, the relative movement between flow cell (1030) and imaging assembly (1040) has ceased when the de-priming step is initiated. To provide the de-priming, suction source (3010) is activated to draw the volume of immersion fluid (IF) out of the space between objective lens assembly (2000) and upper surface (1032).

[00168] In versions where machine vision sensor (1038) is positioned to optically monitor the space between objective lens assembly (2000) and upper surface (1032), feedback from machine vision sensor (1038) may be used to determine when suction source (3010) has removed as much immersion fluid from the space

between objective lens assembly (2000) and upper surface (1032) that can be removed via immersion fluid manifold (2040). In some other versions (e.g., where machine vision sensor (1038) is omitted), suction source (3010) may be activated for a predetermined duration, without factoring any machine vision feedback; then be deactivated after expiration of the predetermined duration. In some instances, and as shown in FIG. 38E, there may be some residual immersion fluid (RF) on objective lens assembly (2000) and/or on upper surface (1032).

[00169] After the de-priming by suction source (3010) and immersion fluid manifold (2040), the air blade may be activated (block 3060 of FIG. 37), as shown in FIG. 38F. As noted above, this may be accomplished via continued activation of pressurized air source (3020), with valve (3030) being switched to an operational state where valve (3030) directs the pressurized air to fluid conduit (3034). This switching of valve (3030) will cease flow of pressurized air to air curtain manifold (2020), to redirect the pressurized air to air blade manifold (2060). As described above, the air blade provided via air blade manifold (2060) is oriented toward the space between objective lens assembly (2000) and upper surface (1032), such that the air blade effectively blows away the residual immersion fluid (RF) on objective lens assembly (2000) and/or on upper surface (1032). In the example shown in FIG. 38F, suction source (3010) is not activated while the air blade is activated. In some other versions, suction source (3010) remains activated as the air blade is activated.

[00170] In versions where machine vision sensor (1038) is positioned to optically monitor the space between objective lens assembly (2000) and upper surface (1032), feedback from machine vision sensor (1038) may be used to determine when the air blade has removed the residual immersion fluid (RF) from objective lens assembly (2000) and/or upper surface (1032). In some such versions, a controller (e.g., controller (114, 308)) may maintain activation of the air blade until feedback from machine vision sensor (1038) indicates that the air blade has removed the residual immersion fluid (RF) from objective lens assembly (2000) and/or upper surface (1032). In some other versions (e.g., where machine vision sensor (1038) is omitted), the air blade may be activated for a predetermined duration, without factoring any machine vision feedback; then be deactivated after expiration of the predetermined duration.

[00171] As noted above, the use of the air blade to remove the residual immersion fluid (RF) from objective lens assembly (2000) and/or upper surface (1032) may be supplemented or substituted with use of an absorbent feature to remove such residual immersion fluid (RF) and/or a wiping feature to wipe away such residual immersion fluid (RF) and/or any other suitable feature(s).

[00172] After the residual immersion fluid (RF) has been removed from objective lens assembly (2000) and/or upper surface (1032), and as shown in FIG. 38G, flow cell (1030) may be removed (block 3062 of FIG. 37). In some cases, there are no additional flow cells (1030) to scan, such that the process may then end (block 3066 of FIG. 37). In some other cases, there is at least one more flow cell (1030) to scan. In such cases, the process may return to the beginning steps of activating the air curtain (block 3050 of FIG. 37) as shown in FIG. 38A, positioning the next flow cell (block 3052 of FIG. 37) as shown in FIG. 38B, etc., cycling through the above-described steps of FIGS. 37 and 38A-38G until all flow cells (1030) have been scanned.

[00173] While the present example provides a fixed position of air blade manifold (2060) throughout the process of FIGS. 37 and 38A-38G, there may be some variations where air blade manifold (2060) is moved during at least part of the process of FIGS. 37 and 38A-38G. Such movement of air blade manifold (2060) may be provided by mount (1016), which may include one or more motors, servos, or other actuators, etc. as noted above. In some such versions, air blade manifold (2060) may be positioned substantially away from imaging assembly (1040) and flow cell (1030) during the initial positioning (block 3052 of FIG. 37) of flow cell (1030), during the priming step (block 3054 of FIG. 37), during the scanning step (block 3056 of FIG. 37), and during the de-priming step (block 3058 of FIG. 37). Positioning air blade manifold (2060) substantially away from imaging assembly (1040) and flow cell (1030) may allow objective lens assembly (2000) to get closer to upper surface (1032) of flow cell (1030) in some cases, which may in turn facilitate use of a smaller volume of immersion fluid (IF). Air blade manifold (2060) may be moved into the position shown in FIG. 38F to provide the activation of the air blade (block 3060 of FIG. 37) as described above; then be moved back out of the way before or after removal (block 3062 of FIG. 37) of flow cell (1030).

[00174] Some sequencing systems may perform a preliminary scan of a flow cell (1030) to map out the tilt and topography of the flow cell (1030), to thereby gather calibration data for subsequent scans. In such preliminary scans, even if images are captured during the preliminary scans, such images may tend to not be used for gathering nucleotide sequencing data—only for calibration purposes. In some such systems, the air curtain may be continuously activated throughout the preliminary scan to provide an initial sweep of debris, etc. from upper surface (1032) of flow cell; before the process of FIG. 37 and 38A-38G is carried out to obtain images that will be used for gathering nucleotide sequencing data, etc. In some such preliminary scans, immersion fluid may also be used as described above.

[00175] G. Examples of Additional Immersion Microscopy Features, Aspects, and Variations

[00176] As noted above, the temperature of the immersion fluid may be regulated to thereby regulate the temperature of objective lens element (2002) and/or the temperature of flow cell (1030). For instance, in some cases, objective lens assembly (2000) may tend to reach an operating temperature that is substantially higher than the operating temperature that is desired in flow cell (1030). In some such cases, the volume of immersion fluid (IF) between objective lens element (2002) and flow cell (1030) may tend to undesirably conduct this heat from objective lens assembly (2000) to flow cell (1030). In some cases, heat transferred from objective lens assembly (2000) to flow cell (1030) may tend to adversely affect nucleotide sequencing processes occurring in flow cell (1030). In some other cases (e.g., where the desired operating temperature of flow cell (1030) is higher than the desired operating temperature of objective lens assembly (2000)), the combination of objective lens assembly (2000) and the volume of immersion fluid (IF) between objective lens element (2002) and flow cell (1030) may tend to form a heat sink that undesirably draws heat away from flow cell (1030), which may also adversely affect nucleotide sequencing processes occurring in flow cell (1030). In addition, or in the alternative, heat communicated from flow cell (1030) to objective lens element (2002) via the immersion fluid may tend to induce thermal aberration/deformation effects in objective lens element (2002), which may adversely impact the quality of images obtained through objective lens element (2002).

[00177] By appropriately controlling the temperature of the immersion fluid before it reaches the space between objective lens element (2002) and flow cell (1030), the immersion fluid may mitigate the thermal transfer that would otherwise occur between objective lens assembly (2000) and flow cell (1030). Moreover, in versions where the immersion fluid is circulated within the space between objective lens element (2002) and flow cell (1030) during the scanning process, the circulation of the immersion fluid may further facilitate use of the immersion fluid to mitigate the thermal transfer that would otherwise occur between objective lens assembly (2000) to flow cell (1030). Some variations of immersion fluid manifold (2040) may provide one or more temperature sensors in fluid communication with the immersion fluid. Such temperature sensors may be in electrical communication with a controller (e.g., controller (114, 308)), and the controller may drive a heating element and/or a cooling element to maintain the immersion fluid at a desired temperature; or within a desired temperature range.

[00178] In the example provide above with reference to FIGS. 37 and 38A-38G, a de-prime step (block 3058 of FIG. 37) is performed to remove the volume of immersion fluid (ID) from the space between objective lens element (2002) and upper surface (1032) of flow cell (1030) by applying suction through immersion fluid manifold (2040) as shown in FIG. 38E. In some other variations, rather than providing this de-priming step in this fashion, relative movement is provided between flow cell (1030) and imaging assembly (1040) along the x-y plane until recess (2044) of immersion fluid manifold (2040) passes over an edge of flow cell (1030) and is no longer positioned over upper surface (1032) of flow cell (1030). In some cases, a substantial amount of immersion fluid may remain in place in recess (2044). Such an amount of immersion fluid may remain in place due to a combination of surface tension and the hydrophobic properties of immersion fluid manifold (2040) and objective lens element (2002), etc. To the extent that there is any residual immersion fluid on upper surface (1032) of flow cell (1030), such residual immersion fluid may then be removed using the air blade from air blade manifold (2060), using an absorbent member, using a wiping member, and/or using any other suitable structures or techniques. The process may then proceed to removal (block 3062 of FIG. 37) of the flow cell (1030); and if applicable, eventually to positioning (block 3052 of FIG. 37) of the next flow cell (1030). At that stage, the substantial

amount of immersion fluid that remained in place in recess (2044) from the prior scanning process may effectively pre-prime immersion fluid manifold (2040) during the priming step (block 3054 of FIG. 37) for this next flow cell (1030). If needed, additional immersion fluid may be communicated to the space between objective lens element (2002) and upper surface (1032) of flow cell (1030) to complete the priming process, but such an additional amount of immersion fluid may be less than the amount that would have otherwise been required in the absence of the pre-prime provided from the substantial amount of immersion fluid that remained in place in recess (2044) from the prior scanning process. Machine vision sensor (1038) may be used to detect the amount of immersion fluid; and to provide feedback informing any replenishment of immersion fluid that might be needed in the space between objective lens element (2002) and upper surface (1032).

[00179] As yet another example, relative movement may be provided between flow cell (1030) and imaging assembly (1040) along the z-dimension to further separate objective lens assembly (2000) from upper surface (1032) of flow cell (1030). In some cases, a substantial amount of immersion fluid may remain in place in recess (2044), due to a combination of surface tension and the hydrophobic properties of immersion fluid manifold (2040) and objective lens element (2002), etc. To the extent that there is any residual immersion fluid on upper surface (1032) of flow cell (1030), such residual immersion fluid may then be removed using the air blade from air blade manifold (2060), using an absorbent member, using a wiping member, and/or using any other suitable structures or techniques. The process may then proceed to removal (block 3062 of FIG. 37) of the flow cell (1030); and if applicable, eventually to positioning (block 3052 of FIG. 37) of the next flow cell (1030). As noted above, the substantial amount of immersion fluid that remained in place in recess (2044) from the prior scanning process may effectively pre-prime immersion fluid manifold (2040) during the priming step (block 3054 of FIG. 37) for this next flow cell (1030).

[00180] While the examples described above utilize a machine vision sensor (1038) to detect the amount of immersion fluid in the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030), other features may be used, in addition to or in lieu of a machine vision sensor (1038), to detect the amount of immersion fluid in the space between objective lens assembly (2000) and upper surface (1032)

of flow cell (1030). For example, immersion fluid manifold (2040) may include electrodes that are exposed within recess (2044) to contact immersion fluid in recess (2044). Such electrodes may be positioned at or near the opposing ends of the major axis (A1) of recess (2044). When the immersion fluid fills recess (2044) to a point where the immersion fluid simultaneously contacts both electrodes, the immersion fluid may complete an electrical circuit between the electrodes. In versions where the electrodes form a capacitor, the electrodes may be covered in an additional insulator to prevent electrolysis of the immersion fluid, which may otherwise lead to fouling of surfaces in contact with the immersion fluid.

[00181] The foregoing example with two electrodes provides an indication of whether the immersion fluid level has reached a certain threshold (i.e., a point at which the immersion fluid contacts both electrodes. In some other variations, more than two electrodes may be provided at different positions (e.g., along rim (2046) surrounding recess (2044) and/or elsewhere on immersion fluid manifold (2040)). Such multi-electrode versions may provide multi-point immersion fluid level measurements, thereby generating signals indicating different volumes of immersion fluid in the space between objective lens assembly (2000) and upper surface (1032) of flow cell (1030). Regardless of how many electrodes are provided, it may be desirable in some instances to position one electrode between first channel (2052) and rim (2046) and another electrode between second channel (2058) and rim (2046). Such positioning may enable those electrodes to provide immersion fluid level sensing before channels (2052, 2058) become dry, which may tend to undesirably introduce bubbles in the immersion fluid.

[00182] As yet another example, force sensing may be used to measure deformation of the volume of immersion fluid (IF) against upper surface (1032) of flow cell (1030). As deformation occurs upon contact, additional force may be applied in the z-direction to compensate for the deformation. In some versions, the additional force is applied in the z-direction via sample stage (1020). In some other versions, the additional force is applied in the z-direction via mount (1042). In some cases, once the immersion fluid is provided in the priming step (block 3054 of FIG. 37), the introduced immersion fluid may impart a sudden increase in force on imaging assembly (1040) along the z-dimension. This initial force along the z-dimension may provide a baseline against which subsequent force data may be tracked, such

that immersion fluid induced forces falling a certain degree below this baseline may indicate deformation in the volume of immersion fluid (IF) that warrants compensation. In other words, if the forces imparted against imaging assembly (1040) by the immersion fluid along the z-dimension falls below a certain threshold during scanning (block 3056 of FIG. 37), additional force may be applied to imaging assembly (1040) or flow cell (1030) along the z-dimension until the forces imparted against imaging assembly (1040) by the immersion fluid along the z-dimension return to the baseline.

[00183] As yet another example, laser beams may be passed through objective lens element (2002), reflect from the sample in flow cell (1030), and be passed back into imaging assembly (1040) for image capture. The laser beams may intercept surfaces at different positions along the z-dimension. The angle of the incident beam results in lateral shifts of the reflected beam as it returns through the optical path, depending on the height of the surface intercepts. The reflected beam is imaged as a spot on a sensor in imaging assembly (1040), and the different surfaces have spots that are laterally shifted on the sensor. In cases where immersion fluid is disposed as a droplet on objective lens element (2002) before the immersion fluid also contacts upper surface (1032) of flow cell (1030), the reflected spots may tend to form an odd pattern, as the first surface (immersion fluid droplet) is curved, and subsequent surfaces will observe a beam that is focused by the curvature of the immersion fluid droplet. In any case, once the droplet touches upper surface (1032) of flow cell (1030), the spot profile on the sensor will change dramatically (to the expected numbers of spots). This threshold may be used to measure the height of the immersion fluid droplet.

[00184] As yet another example, a collimated beam of light may be output via objective lens element (2002). This collimated beam would then be focused by a droplet of immersion fluid disposed on objective lens element (2002) before the immersion fluid also contacts upper surface (1032) of flow cell (1030). When relative movement is provided between imaging assembly (1004) and flow cell (1030) along the z-dimension, this focused collimated beam would be incident on upper surface (1032) of flow cell (1030), producing a cats-eye reflection (e.g., in a similar manner to an autocollimator), creating a spot on an image sensor in imaging assembly (1004). The droplet of immersion fluid on the objective lens element

(2002) may act as a lens, such that the characteristics of the cats-eye reflection may indicate the radius of curvature of the droplet of immersion fluid on the objective lens element (2002). Thus, as the radius of curvature of the droplet changes, the focal length of the lens effectively formed by the droplet would change. Similarly, the reflection, radius of curvature, and focal length associated with the droplet would change as the droplet engages upper surface (1032) of flow cell (1030). The reflection profile before engagement between the droplet and upper surface (1032) of flow cell (1030) may provide a baseline indicating height of the droplet, such that changes in the reflection profile after engagement between the droplet and upper surface (1032) of flow cell (1030) may indicate changes in the height of the droplet.

[00185] VII. Examples of Combinations

[00186] The following examples relate to various non-exhaustive ways in which the teachings herein may be combined or applied. The following examples are not intended to restrict the coverage of any claims that may be presented at any time in this application or in subsequent filings of this application. No disclaimer is intended. The following examples are being provided for nothing more than merely illustrative purposes. It is contemplated that the various teachings herein may be arranged and applied in numerous other ways. It is also contemplated that some variations may omit certain features referred to in the below examples. Therefore, none of the aspects or features referred to below should be deemed critical unless otherwise explicitly indicated as such at a later date by the inventors or by a successor in interest to the inventors. If any claims are presented in this application or in subsequent filings related to this application that include additional features beyond those referred to below, those additional features shall not be presumed to have been added for any reason relating to patentability.

[00187] Example 1

[00188] An apparatus comprising: an imaging assembly, the imaging assembly including: an objective lens having a bottom surface, and an immersion fluid assembly, the immersion fluid assembly including: a first port to introduce immersion fluid under the bottom surface of the objective lens, a second port to remove immersion fluid from under the bottom surface of the objective lens, and a sidewall defining an

immersion fluid retention region under the objective lens, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension; and an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal dimension.

[00189] Example 2

[00190] The apparatus of Example 1, further comprising a flow cell having an upper surface under the fluid retention region, the immersion fluid assembly to maintain immersion fluid between the bottom surface of the objective lens and the upper surface of the flow cell.

[00191] Example 3

[00192] The apparatus of Example 2, the upper surface including a hydrophobic material.

[00193] Example 4

[00194] The apparatus of any of Examples 2 through 3, the flow cell further comprising a plurality of channels, each channel of the plurality of channels including a plurality of reaction sites.

[00195] Example 5

[00196] The apparatus of Example 4, the plurality of reaction sites including a plurality of nucleotides.

[00197] Example 6

[00198] The apparatus of any of Examples 4 through 5, at least some of the channels of the plurality of channels being oriented along the first horizontal dimension.

[00199] Example 7

[00200] The apparatus of Example 6, each channel of the plurality of channels being spaced apart from the other channel of the plurality of channels along the second horizontal dimension.

[00201] Example 8

[00202] The apparatus of any of Examples 4 through 7, the actuation assembly to drive relative movement between the flow cell and the imaging assembly to successively position the imaging assembly over each channel of the plurality of channels.

[00203] Example 9

[00204] The apparatus of any of Examples 1 through 8, the bottom surface of the objective lens being flat.

[00205] Example 10

[00206] The apparatus of any of Examples 1 through 9, the bottom surface of the objective lens including a concave surface.

[00207] Example 11

[00208] The apparatus of any of Examples 1 through 10, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

[00209] Example 12

[00210] The apparatus of Example 11, the air curtain manifold being positioned to encircle the objective lens.

[00211] Example 13

[00212] The apparatus of Example 12, the objective lens being centered along a longitudinal axis, the air curtain manifold being centered along the longitudinal axis.

[00213] Example 14

[00214] The apparatus of any of Examples 11 through 13, the air curtain manifold defining a central opening.

[00215] Example 15

[00216] The apparatus of Example 14, a portion of the imaging assembly being positioned within the central opening of the air curtain manifold.

[00217] Example 16

[00218] The apparatus of Example 15, the central opening being sized to define a gap

between the air curtain manifold and the portion of the imaging assembly positioned within the central opening.

[00219] Example 17

[00220] The apparatus of any of Examples 11 through 16, the air curtain manifold having an annular shape with a circumferentially extending surface, with openings positioned along the circumferentially extending surface, the air curtain manifold to expel pressurized air outwardly relative to the objective lens via the openings positioned along the circumferentially extending surface.

[00221] Example 18

[00222] The apparatus of Example 17, the circumferentially extending surface being angled along a vertical plane.

[00223] Example 19

[00224] The apparatus of any of Examples 17 through 18, the air curtain manifold further including an annular channel in fluid communication with the openings positioned along the circumferentially extending surface.

[00225] Example 20

[00226] The apparatus of any of Examples 11 through 19, further comprising a frame, the imaging assembly being secured to the frame, the air curtain manifold being secured to the frame independently of the imaging assembly.

[00227] Example 21

[00228] The apparatus of any of Example 11 through 20, the air curtain manifold to expel pressurized air outwardly relative to the objective lens in a form of a frustoconical air curtain.

[00229] Example 22

[00230] The apparatus of Example 21, the frustoconical air curtain being centered along a longitudinal axis, the objective lens being centered along the longitudinal axis.

[00231] Example 23

[00232] The apparatus of any of Examples 11 through 22, the air curtain manifold being positioned to expel pressurized air downwardly toward the flow cell.

[00233] Example 24

[00234] The apparatus of any of Examples 1 through 23, the imaging assembly further comprising an objective lens housing supporting the objective lens, the objective lens housing having a distal face surrounding an outer perimeter of the objective lens.

[00235] Example 25

[00236] The apparatus of Example 24, the distal face including a hydrophilic material.

[00237] Example 26

[00238] The apparatus of any of Examples 24 through 25, the bottom surface of the objective lens including a hydrophilic material.

[00239] Example 27

[00240] The apparatus of any of Examples 24 through 26, the distal face further including at least one annular recess spaced outwardly from the outer perimeter of the objective lens.

[00241] Example 28

[00242] The apparatus of any of Examples 1 through 27, further comprising an air blade manifold, the air blade manifold to expel pressurized air toward a space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00243] Example 29

[00244] The apparatus of Example 28, the air blade manifold including a plurality of openings to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00245] Example 30

[00246] The apparatus of Example 29, the plurality of openings being positioned along an arc.

[00247] Example 31

[00248] The apparatus of any of Examples 29 through 30, the air blade manifold further comprising a plurality of channels in fluid communication with the plurality of openings.

[00249] Example 32

[00250] The apparatus of Example 31, each channel in fluid communication with the plurality of openings being oriented along a respective radius extending toward a central point such that the radii along which the plurality of openings are oriented all extend toward the central point.

[00251] Example 33

[00252] The apparatus of Example 32, the central point being positioned to correspond with a central region of the space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00253] Example 34

[00254] The apparatus of any of Examples 28 through 33, the air blade manifold to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell in a form of a substantially flat air blade.

[00255] Example 35

[00256] The apparatus of any of Examples 28 through 34, further comprising a frame, the imaging assembly being secured to the frame, the air blade manifold being secured to the frame independently of the imaging assembly.

[00257] Example 36

[00258] The apparatus of any of Examples 28 through 35, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

[00259] Example 37

[00260] The apparatus of Example 36, further comprising a pressurized air source, the pressurized air source to provide pressurized air to the air blade manifold, the

pressurized air source further to provide pressurized air to the air curtain manifold.

[00261] Example 38

[00262] The apparatus of Example 37, further comprising a valve, the valve to direct pressurized air to a selected one of either the air blade manifold or the air curtain manifold.

[00263] Example 39

[00264] The apparatus of any of Examples 1 through 38, further comprising a sensor to monitor a presence of immersion fluid under the bottom surface of the objective lens.

[00265] Example 40

[00266] The apparatus of Example 39, the sensor comprising an optical sensor.

[00267] Example 41

[00268] The apparatus of Example 40, the optical sensor being positioned and oriented to view the immersion fluid under the bottom surface of the objective lens along a horizontal dimension.

[00269] Example 42

[00270] The apparatus of Example 40, the optical sensor being positioned and oriented to view light transmitted through the immersion fluid and reflected back through the objective lens.

[00271] Example 43

[00272] The apparatus of any of Examples 39 through 42, the sensor comprising a set of electrodes.

[00273] Example 44

[00274] The apparatus of any of Examples 39 through 43, the sensor comprising a force sensor.

[00275] Example 45

[00276] An apparatus comprising: an imaging assembly, the imaging assembly including:

an objective lens assembly, the objective lens assembly including: an objective lens element having a bottom surface, and a housing having a bottom surface adjacent to the bottom surface of the objective lens element, the bottom surface of the housing including a fluid retention feature, and an immersion fluid assembly, the immersion fluid assembly including: a first port to introduce immersion fluid under the objective lens assembly, a second port to remove immersion fluid from under the objective lens assembly, and a sidewall defining an immersion fluid retention region under the objective lens assembly; the fluid retention feature of the housing of the objective lens assembly being laterally interposed between the objective lens element of the objective lens assembly and the sidewall of the immersion fluid assembly.

[00277] Example 46

[00278] The apparatus of Example 45, the fluid retention feature including an annular recess surrounding the objective lens element.

[00279] Example 47

[00280] The apparatus of Example 46, the fluid retention feature being positioned on the bottom surface of the housing.

[00281] Example 48

[00282] The apparatus of any of Examples 45 through 47, the bottom surface of the housing including a hydrophilic material.

[00283] Example 49

[00284] The apparatus of any of Examples 45 through 48, the bottom surface of the objective lens element including a hydrophilic material.

[00285] Example 50

[00286] The apparatus of any of Examples 45 through 49, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension.

[00287] Example 51

[00288] The apparatus of Example 50, further comprising an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a path.

[00289] Example 52

[00290] The apparatus of Example 51, the path including a horizontal path in a direction parallel to the first horizontal dimension.

[00291] Example 53

[00292] The apparatus of any of Examples 45 through 52, further comprising a flow cell having an upper surface under the fluid retention region, the immersion fluid assembly to maintain immersion fluid between the bottom surface of the objective lens and the upper surface of the flow cell.

[00293] Example 54

[00294] The apparatus of Example 53, the upper surface including a hydrophobic material.

[00295] Example 55

[00296] The apparatus of any of Examples 53 through 54, the flow cell further comprising a plurality of channels, each channel of the plurality of channels including a plurality of reaction sites.

[00297] Example 56

[00298] The apparatus of Example 55, the plurality of reaction sites including a plurality of nucleotides.

[00299] Example 57

[00300] The apparatus of any of Examples 55 through 56, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension, at least some of the channels of the plurality of channels being oriented along the first horizontal dimension.

[00301] Example 58

[00302] The apparatus of Example 57, each channel of the plurality of channels being

spaced apart from the other channel of the plurality of channels along the second horizontal dimension.

[00303] Example 59

[00304] The apparatus of any of Examples 55 through 58, further comprising an actuation assembly to drive relative movement between the flow cell and the imaging assembly to successively position the imaging assembly over each channel of the plurality of channels.

[00305] Example 60

[00306] The apparatus of any of Examples 45 through 59, the bottom surface of the objective lens element being flat.

[00307] Example 61

[00308] The apparatus of any of Examples 45 through 60, the bottom surface of the objective lens element including a concave surface.

[00309] Example 62

[00310] The apparatus of any of Examples 45 through 61, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens element.

[00311] Example 63

[00312] The apparatus of Example 62, the air curtain manifold being positioned to encircle the objective lens assembly.

[00313] Example 64

[00314] The apparatus of Example 63, the objective lens element being centered along a longitudinal axis, the air curtain manifold being centered along the longitudinal axis.

[00315] Example 65

[00316] The apparatus of any of Examples 62 through 64, the air curtain manifold defining a central opening.

[00317] Example 66

[00318] The apparatus of Example 65, a portion of the imaging assembly being positioned within the central opening of the air curtain manifold.

[00319] Example 67

[00320] The apparatus of Example 66, the central opening being sized to define a gap between the air curtain manifold and the portion of the imaging assembly positioned within the central opening.

[00321] Example 68

[00322] The apparatus of any of Examples 62 through 67, the air curtain manifold having an annular shape with a circumferentially extending surface, with openings positioned along the circumferentially extending surface, the air curtain manifold to expel pressurized air outwardly relative to the objective lens via the openings positioned along the circumferentially extending surface.

[00323] Example 69

[00324] The apparatus of Example 68, the circumferentially extending surface being angled along a vertical plane.

[00325] Example 70

[00326] The apparatus of any of Examples 68 through 69, the air curtain manifold further including an annular channel in fluid communication with the openings positioned along the circumferentially extending surface.

[00327] Example 71

[00328] The apparatus of any of Examples 62 through 70, further comprising a frame, the imaging assembly being secured to the frame, the air curtain manifold being secured to the frame independently of the imaging assembly.

[00329] Example 72

[00330] The apparatus of any of Example 62 through 71, the air curtain manifold to expel pressurized air outwardly relative to the objective lens element in a form of a frustoconical air curtain.

[00331] Example 73

[00332] The apparatus of Example 72, the frustoconical air curtain being centered along a longitudinal axis, the objective lens element being centered along the longitudinal axis.

[00333] Example 74

[00334] The apparatus of any of Examples 62 through 73, further comprising an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a path, the air curtain manifold being positioned to expel pressurized air downwardly toward the flow cell.

[00335] Example 75

[00336] The apparatus of any of Examples 45 through 74, further comprising an air blade manifold, the air blade manifold to expel pressurized air toward a space between the bottom surface of the objective lens element and an upper surface of the flow cell.

[00337] Example 76

[00338] The apparatus of Example 75, the air blade manifold including a plurality of openings to expel pressurized air toward the space between the bottom surface of the objective lens element and an upper surface of the flow cell.

[00339] Example 77

[00340] The apparatus of Example 76, the plurality of openings being positioned along an arc.

[00341] Example 78

[00342] The apparatus of any of Examples 76 through 77, the air blade manifold further comprising a plurality of channels in fluid communication with the plurality of openings.

[00343] Example 79

[00344] The apparatus of Example 78, each channel in fluid communication with the plurality of openings being oriented along a respective radius extending toward a

central point such that the radii along which the plurality of openings are oriented all extend toward the central point.

[00345] Example 80

[00346] The apparatus of Example 79, the central point being positioned to correspond with a central region of the space between the bottom surface of the objective lens element and an upper surface of the flow cell.

[00347] Example 81

[00348] The apparatus of any of Examples 75 through 80, the air blade manifold to expel pressurized air toward the space between the bottom surface of the objective lens element and an upper surface of the flow cell in a form of a substantially flat air blade.

[00349] Example 82

[00350] The apparatus of any of Examples 75 through 81, further comprising a frame, the imaging assembly being secured to the frame, the air blade manifold being secured to the frame independently of the imaging assembly.

[00351] Example 83

[00352] The apparatus of any of Examples 75 through 82, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens element.

[00353] Example 84

[00354] The apparatus of Example 83, further comprising a pressurized air source, the pressurized air source to provide pressurized air to the air blade manifold, the pressurized air source further to provide pressurized air to the air curtain manifold.

[00355] Example 85

[00356] The apparatus of Example 84, further comprising a valve, the valve to direct pressurized air to a selected one of either the air blade manifold or the air curtain manifold.

[00357] Example 86

[00358] The apparatus of any of Examples 45 through 85, further comprising a sensor to monitor a presence of immersion fluid under the bottom surface of the objective lens element.

[00359] Example 87

[00360] The apparatus of Example 86, the sensor comprising an optical sensor.

[00361] Example 88

[00362] The apparatus of Example 87, the optical sensor being positioned and oriented to view the immersion fluid under the bottom surface of the objective lens element along a horizontal dimension.

[00363] Example 89

[00364] The apparatus of Example 88, the optical sensor being positioned and oriented to view light transmitted through the immersion fluid and reflected back through the objective lens element.

[00365] Example 90

[00366] The apparatus of any of Examples 86 through 89, the sensor comprising a set of electrodes.

[00367] Example 91

[00368] The apparatus of any of Examples 86 through 90, the sensor comprising a force sensor.

[00369] Example 92

[00370] An apparatus comprising: an imaging assembly, the imaging assembly including: an objective lens having a bottom surface; an immersion fluid assembly, the immersion fluid assembly including: a first port to introduce immersion fluid under the bottom surface of the objective lens, a second port to remove immersion fluid from under the bottom surface of the objective lens, and a sidewall defining an immersion fluid retention region under the objective lens; and an air delivery assembly, the air delivery assembly being coupled with the objective lens assembly, the air delivery assembly to deliver pressurized air to one or both of the objective

lens or an upper surface of a flow cell to thereby clear one or both of debris or immersion fluid relative to one or both of the objective lens or an upper surface of a flow cell.

[00371] Example 93

[00372] The apparatus of Example 92, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension.

[00373] Example 94

[00374] The apparatus of Example 93, further comprising an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal dimension.

[00375] Example 95

[00376] The apparatus of any of Examples 92 through 94, further comprising a flow cell having an upper surface under the fluid retention region, the immersion fluid assembly to maintain immersion fluid between the bottom surface of the objective lens and the upper surface of the flow cell.

[00377] Example 96

[00378] The apparatus of Example95, the upper surface including a hydrophobic material.

[00379] Example 97

[00380] The apparatus of any of Examples 95 through 96, the flow cell further comprising a plurality of channels, each channel of the plurality of channels including a plurality of reaction sites.

[00381] Example 98

[00382] The apparatus of Example 97, the plurality of reaction sites including a plurality of nucleotides.

[00383] Example 99

[00384] The apparatus of any of Examples 97 through 98, at least some of the channels of the plurality of channels being oriented along the first horizontal dimension.

[00385] Example 100

[00386] The apparatus of Example 99, each channel of the plurality of channels being spaced apart from the other channel of the plurality of channels along the second horizontal dimension.

[00387] Example 101

[00388] The apparatus of any of Examples 97 through 100, the actuation assembly to drive relative movement between the flow cell and the imaging assembly to successively position the imaging assembly over each channel of the plurality of channels.

[00389] Example 102

[00390] The apparatus of any of Examples 92 through 101, the bottom surface of the objective lens being flat.

[00391] Example 103

[00392] The apparatus of any of Examples 92 through 102, the bottom surface of the objective lens including a concave surface.

[00393] Example 104

[00394] The apparatus of any of Examples 92 through 103, the air delivery assembly comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

[00395] Example 105

[00396] The apparatus of Example 104, the air curtain manifold being positioned to encircle the objective lens.

[00397] Example 106

[00398] The apparatus of Example 105, the objective lens being centered along a longitudinal axis, the air curtain manifold being centered along the longitudinal axis.

[00399] Example 107

[00400] The apparatus of any of Examples 104 through 20+, the air curtain manifold defining a central opening.

[00401] Example 108

[00402] The apparatus of Example 107, a portion of the imaging assembly being positioned within the central opening of the air curtain manifold.

[00403] Example 109

[00404] The apparatus of Example 108, the central opening being sized to define a gap between the air curtain manifold and the portion of the imaging assembly positioned within the central opening.

[00405] Example 110

[00406] The apparatus of any of Examples 104 through 109, the air curtain manifold having an annular shape with a circumferentially extending surface, with openings positioned along the circumferentially extending surface, the air curtain manifold to expel pressurized air outwardly relative to the objective lens via the openings positioned along the circumferentially extending surface.

[00407] Example 111

[00408] The apparatus of Example 110, the circumferentially extending surface being angled along a vertical plane.

[00409] Example 112

[00410] The apparatus of any of Examples 110 through 111, the air curtain manifold further including an annular channel in fluid communication with the openings positioned along the circumferentially extending surface.

[00411] Example 113

[00412] The apparatus of any of Examples 104 through 112, further comprising a frame, the imaging assembly being secured to the frame, the air curtain manifold being secured to the frame independently of the imaging assembly.

[00413] Example 114

[00414] The apparatus of any of Example 104 through 113, the air curtain manifold to expel pressurized air outwardly relative to the objective lens in a form of a frustoconical air curtain.

[00415] Example 115

[00416] The apparatus of Example 114, the frustoconical air curtain being centered along a longitudinal axis, the objective lens being centered along the longitudinal axis.

[00417] Example 116

[00418] The apparatus of any of Examples 104 through 115, the air curtain manifold being positioned to expel pressurized air downwardly toward the flow cell.

[00419] Example 117

[00420] The apparatus of any of Examples 92 through 116, the imaging assembly further comprising an objective lens housing supporting the objective lens, the objective lens housing having a distal face surrounding an outer perimeter of the objective lens.

[00421] Example 118

[00422] The apparatus of Example 117, the distal face including a hydrophilic material.

[00423] Example 119

[00424] The apparatus of any of Examples 117 through 118, the bottom surface of the objective lens including a hydrophilic material.

[00425] Example 120

[00426] The apparatus of any of Examples 117 through 119, the distal face further including at least one annular recess spaced outwardly from the outer perimeter of the objective lens.

[00427] Example 121

[00428] The apparatus of any of Examples 92 through 120, the air delivery assembly comprising an air blade manifold, the air blade manifold to expel pressurized air

toward a space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00429] Example 122

[00430] The apparatus of Example 121, the air blade manifold including a plurality of openings to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00431] Example 123

[00432] The apparatus of Example 122, the plurality of openings being positioned along an arc.

[00433] Example 124

[00434] The apparatus of any of Examples 122 through 123, the air blade manifold further comprising a plurality of channels in fluid communication with the plurality of openings.

[00435] Example 125

[00436] The apparatus of Example 124, each channel in fluid communication with the plurality of openings being oriented along a respective radius extending toward a central point such that the radii along which the plurality of openings are oriented all extend toward the central point.

[00437] Example 126

[00438] The apparatus of Example 125, the central point being positioned to correspond with a central region of the space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00439] Example 127

[00440] The apparatus of any of Examples 121 through 126, the air blade manifold to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell in a form of a substantially flat air blade.

[00441] Example 128

[00442] The apparatus of any of Examples 121 through 127, further comprising a frame, the imaging assembly being secured to the frame, the air blade manifold being secured to the frame independently of the imaging assembly.

[00443] Example 129

[00444] The apparatus of any of Examples 121 through 128, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

[00445] Example 130

[00446] The apparatus of Example 129, further comprising a pressurized air source, the pressurized air source to provide pressurized air to the air blade manifold, the pressurized air source further to provide pressurized air to the air curtain manifold.

[00447] Example 131

[00448] The apparatus of Example 130, further comprising a valve, the valve to direct pressurized air to a selected one of either the air blade manifold or the air curtain manifold.

[00449] Example 132

[00450] The apparatus of any of Examples 92 through 131, further comprising a sensor to monitor a presence of immersion fluid under the bottom surface of the objective lens.

[00451] Example 133

[00452] The apparatus of Example 132, the sensor comprising an optical sensor.

[00453] Example 133

[00454] The apparatus of Example 133, the optical sensor being positioned and oriented to view the immersion fluid under the bottom surface of the objective lens along a horizontal dimension.

[00455] Example 134

[00456] The apparatus of Example 133, the optical sensor being positioned and oriented to

view light transmitted through the immersion fluid and reflected back through the objective lens.

[00457] Example 135

[00458] The apparatus of any of Examples 132 through 134, the sensor comprising a set of electrodes.

[00459] Example 136

[00460] The apparatus of any of Examples 132 through 135, the sensor comprising a force sensor.

[00461] Example 137

[00462] A method comprising: positioning an imaging assembly in relation to a flow cell, the imaging assembly comprising: an objective lens having a bottom surface, and an immersion fluid assembly, the immersion fluid assembly including: a first port, and a sidewall defining an immersion fluid retention region under the objective lens, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension; depositing immersion fluid via the first port to a space between the bottom surface of the objective lens and an upper surface of the flow cell; and providing relative movement between the flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal dimension, the immersion fluid remaining in the space between the bottom surface of the objective lens and the upper surface of the flow cell during the relative movement between the flow cell and the imaging assembly.

[00463] Example 138

[00464] The method of Example 137, further comprising activating an air curtain, the air curtain comprising pressurized air oriented toward the upper surface of the flow cell and away from the objective lens.

[00465] Example 139

[00466] The method of Example 138, the act of activating the air curtain being performed

before the act of depositing immersion fluid.

[00467] Example 140

[00468] The method of any of Examples 138 through 139, the act of activating the air curtain being performed while providing relative movement between the flow cell and the imaging assembly.

[00469] Example 141

[00470] The method of any of Examples 138 through 140, the air curtain having a frustoconical shape.

[00471] Example 142

[00472] The method of any of Examples 138 through 141, the air curtain being provided via an air curtain manifold positioned about the imaging assembly.

[00473] Example 143

[00474] The method of any of Examples 137 through 142, further comprising activating an air blade, the air blade comprising pressurized air oriented toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00475] Example 144

[00476] The method of Example 143, the act of activating the air blade being performed after providing relative movement between the flow cell and the imaging assembly.

[00477] Example 145

[00478] The method of any of Examples 143 through 144, further comprising drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00479] Example 146

[00480] The method of Example 145, the immersion fluid being drawn from the space between the bottom surface of the objective lens and the upper surface of the flow cell via a second port of the immersion fluid assembly.

[00481] Example 147

[00482] The method of any of Examples 145 through 146, the act of activating the air blade being performed after drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00483] Example 148

[00484] The method of any of Examples 145 through 147, the the air blade being provided via an air blade manifold positioned adjacent to the imaging assembly.

[00485] Example 149

[00486] The method of Example 148, the air blade manifold having a plurality of openings positioned along an arc, the air blade comprising pressurized air expelled via the plurality of openings such that air flow of the air blade is focused toward a central region, the central region being positioned in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00487] Example 150

[00488] The method of any of Examples 137 through 149, further comprising monitoring, via a sensor, the presence of immersion fluid in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00489] Example 151

[00490] The method of Example 150, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data along a horizontal path.

[00491] Example 152

[00492] The method of any of Examples 150 through 151, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data from light communicated through the objective lens and toward the optical sensor.

[00493] Example 153

[00494] The method of any of Examples 150 through 152, the sensor comprising electrodes, the act of monitoring comprising monitoring whether a circuit is completed between the electrodes.

[00495] Example 154

[00496] The method of any of Examples 150 through 153, the sensor comprising a force sensor, the act of monitoring comprising monitoring a force associated with the immersion fluid.

[00497] Example 155

[00498] The method of any of Examples 137 through 154, further comprising: removing the flow cell from a space beneath the imaging assembly; and positioning another flow cell in the space beneath the imaging assembly.

[00499] Example 156

[00500] The method of any of Examples 137 through 155, further comprising performing nucleotide sequencing in the flow cell.

[00501] Example 157

[00502] A method comprising: positioning an imaging assembly in relation to a flow cell, the imaging assembly comprising: an objective lens having a bottom surface, and an immersion fluid assembly, the immersion fluid assembly including: a first port, and a sidewall defining an immersion fluid retention region under the objective lens; depositing immersion fluid via the first port to a space between the bottom surface of the objective lens and an upper surface of the flow cell; providing relative movement between the flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal dimension, the immersion fluid remaining in the space between the bottom surface of the objective lens and the upper surface of the flow cell during the relative movement between the flow cell and the imaging assembly; and delivering pressurized air to one or both of the objective lens or the upper surface of the flow cell.

[00503] Example 158

[00504] The method of Example 157, the the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension.

[00505] Example 159

[00506] The method of Example 158, the horizontal path being in a direction parallel to the first horizontal dimension.

[00507] Example 160

[00508] The method of any of Examples 157 through 159, the act of delivering pressurized air to one or both of the objective lens or the upper surface of the flow cell comprising activating an air curtain, the air curtain comprising pressurized air oriented toward the upper surface of the flow cell and away from the objective lens.

[00509] Example 161

[00510] The method of Example 160, the act of activating the air curtain being performed before the act of depositing immersion fluid.

[00511] Example 162

[00512] The method of any of Examples 160 through 161, the act of activating the air curtain being performed while providing relative movement between the flow cell and the imaging assembly.

[00513] Example 163

[00514] The method of any of Examples 160 through 162, the air curtain having a frustoconical shape.

[00515] Example 164

[00516] The method of any of Examples 160 through 163, the air curtain being provided via an air curtain manifold positioned about the imaging assembly.

[00517] Example 165

[00518] The method of any of Examples 157 through 164, the act of delivering pressurized air to one or both of the objective lens or the upper surface of the flow cell comprising activating an air blade, the air blade comprising pressurized air oriented toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

[00519] Example 166

[00520] The method of Example 165, the act of activating the air blade being performed after providing relative movement between the flow cell and the imaging assembly.

[00521] Example 167

[00522] The method of any of Examples 165 through 166, further comprising drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00523] Example 168

[00524] The method of Example 167, the immersion fluid being drawn from the space between the bottom surface of the objective lens and the upper surface of the flow cell via a second port of the immersion fluid assembly.

[00525] Example 169

[00526] The method of any of Examples 167 through 168, the act of activating the air blade being performed after drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00527] Example 170

[00528] The method of any of Examples 167 through 169, the the air blade being provided via an air blade manifold positioned adjacent to the imaging assembly.

[00529] Example 171

[00530] The method of Example 170, the air blade manifold having a plurality of openings positioned along an arc, the air blade comprising pressurized air expelled via the plurality of openings such that air flow of the air blade is focused toward a central region, the central region being positioned in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00531] Example 172

[00532] The method of any of Examples 157 through 171, further comprising monitoring, via a sensor, the presence of immersion fluid in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

[00533] Example 173

[00534] The method of Example 172, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data along a horizontal path.

[00535] Example 174

[00536] The method of any of Examples 172 through 173, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data from light communicated through the objective lens and toward the optical sensor.

[00537] Example 175

[00538] The method of any of Examples 172 through 174, the sensor comprising electrodes, the act of monitoring comprising monitoring whether a circuit is completed between the electrodes.

[00539] Example 176

[00540] The method of any of Examples 172 through 175, the sensor comprising a force sensor, the act of monitoring comprising monitoring a force associated with the immersion fluid.

[00541] Example 177

[00542] The method of any of Examples 157 through 176, further comprising: removing the flow cell from a space beneath the imaging assembly; and positioning another flow cell in the space beneath the imaging assembly.

[00543] Example 178

[00544] The method of any of Examples 157 through 177, further comprising performing nucleotide sequencing in the flow cell.

[00545] VIII. Miscellaneous

[00546] While the foregoing examples are provided in the context of a system (100) that may be used in nucleotide sequencing processes, the teachings herein may also be readily applied in other contexts, including in systems that perform other processes (i.e., other than nucleotide sequencing procedures). The teachings herein are thus not necessarily limited to systems that are used to perform nucleotide sequencing processes.

[00547] It is to be understood that the subject matter described herein is not limited in its application to the details of construction and the arrangement of components set forth in the description herein or illustrated in the drawings hereof. The subject matter described herein is capable of other implementations and of being practiced or of being carried out in various ways. Also, it is to be understood that the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. As used herein, an element or step recited in the singular and proceeded with the word "a" or "an" should be understood as not excluding plural of said elements or steps, unless such exclusion is explicitly stated. Furthermore, references to "one example" are not intended to be interpreted as excluding the existence of additional examples that also incorporate the recited features. The use of "including," "comprising," or "having" and variations thereof herein is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

[00548] When used in the claims, the term "set" should be understood as one or more things which are grouped together. Similarly, when used in the claims "based on" should be understood as indicating that one thing is determined at least in part by what it is specified as being "based on." Where one thing is required to be exclusively determined by another thing, then that thing will be referred to as being "exclusively based on" that which it is determined by.

[00549] Unless specified or limited otherwise, the terms "mounted," "connected," "supported," and "coupled" and variations thereof are used broadly and encompass both direct and indirect mountings, connections, supports, and couplings. Further, "connected" and "coupled" are not restricted to physical or mechanical connections or couplings. Also, it is to be understood that phraseology and terminology used herein with reference to device or element orientation (such as, for example, terms like "above," "below," "front," "rear," "distal," "proximal," and the like) are only used to simplify description of one or more examples described herein, and do not alone indicate or imply that the device or element referred to must have a particular orientation. In addition, terms such as "outer" and "inner" are used herein for purposes of description and are not intended to indicate or imply relative importance or significance.

[00550] It is to be understood that the above description is intended to be illustrative, and not restrictive. For example, the above-described examples (and/or aspects thereof) may be used in combination with each other. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the presently described subject matter without departing from its scope. While the dimensions, types of materials and coatings described herein are intended to define the parameters of the disclosed subject matter, they are by no means limiting and instead illustrations. Many further examples will be apparent to those of skill in the art upon reviewing the above description. The scope of the disclosed subject matter should, therefore, be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. In the appended claims, the terms "including" and "in which" are used as the plain-English equivalents of the respective terms "comprising" and "wherein." Moreover, in the following claims, the terms "first," "second," and "third," etc. are used merely as labels, and are not intended to impose numerical requirements on their objects. Further, the limitations of the following claims are not written in means—plus-function format and are not intended to be interpreted based on 35 U.S.C. §112(f) paragraph, unless and until such claim limitations expressly use the phrase "means for" followed by a statement of function void of further structure.

[00551] The following claims recite aspects of certain examples of the disclosed subject matter and are considered to be part of the above disclosure. These aspects may be combined with one another.

What is claimed is:

1. An apparatus comprising:
an imaging assembly, the imaging assembly including:
an objective lens having a bottom surface, and
an immersion fluid assembly, the immersion fluid assembly including:
a first port to introduce immersion fluid under the bottom surface of the objective lens,
a second port to remove immersion fluid from under the bottom surface of the objective lens, and
a sidewall defining an immersion fluid retention region under the objective lens, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension; and
an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal dimension.

2. The apparatus of claim 1, further comprising a flow cell having an upper surface under the fluid retention region, the immersion fluid assembly to maintain immersion fluid between the bottom surface of the objective lens and the upper surface of the flow cell.

3. The apparatus of claim 2, the upper surface including a hydrophobic material.

4. The apparatus of any of claims 2 through 3, the flow cell further comprising a plurality of channels, each channel of the plurality of channels including a plurality of reaction sites.

5. The apparatus of claim 4, the plurality of reaction sites including a plurality of nucleotides.

6. The apparatus of any of claims 4 through 5, at least some of the channels of the plurality of channels being oriented along the first horizontal dimension.

7. The apparatus of claim 6, each channel of the plurality of channels being spaced apart from the other channel of the plurality of channels along the second horizontal dimension.

8. The apparatus of any of claims 4 through 7, the actuation assembly to drive relative movement between the flow cell and the imaging assembly to successively position the imaging assembly over each channel of the plurality of channels.

9. The apparatus of any of claims 1 through 8, the bottom surface of the objective lens being flat.

10. The apparatus of any of claims 1 through 9, the bottom surface of the objective lens including a concave surface.

11. The apparatus of any of claims 1 through 10, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

12. The apparatus of claim 11, the air curtain manifold being positioned to encircle the objective lens.

13. The apparatus of claim 12, the objective lens being centered along a longitudinal axis, the air curtain manifold being centered along the longitudinal axis.

14. The apparatus of any of claims 11 through 13, the air curtain manifold defining a central opening.

15. The apparatus of claim 14, a portion of the imaging assembly being positioned within the central opening of the air curtain manifold.

16. The apparatus of claim 15, the central opening being sized to define a gap between the air curtain manifold and the portion of the imaging assembly positioned within the central opening.

17. The apparatus of any of claims 11 through 16, the air curtain manifold having an annular shape with a circumferentially extending surface, with openings positioned along the circumferentially extending surface, the air curtain manifold to expel pressurized air outwardly relative to the objective lens via the openings positioned along the circumferentially extending surface.

18. The apparatus of claim 17, the circumferentially extending surface being angled along a vertical plane.

19. The apparatus of any of claims 17 through 18, the air curtain manifold further including an annular channel in fluid communication with the openings positioned along the circumferentially extending surface.

20. The apparatus of any of claims 11 through 19, further comprising a frame, the imaging assembly being secured to the frame, the air curtain manifold being secured to the frame independently of the imaging assembly.

21. The apparatus of any of claim 11 through 20, the air curtain manifold to expel pressurized air outwardly relative to the objective lens in a form of a frustoconical air curtain.

22. The apparatus of claim 21, the frustoconical air curtain being centered along a longitudinal axis, the objective lens being centered along the longitudinal axis.

23. The apparatus of any of claims 11 through 22, the air curtain manifold being positioned to expel pressurized air downwardly toward the flow cell.

24. The apparatus of any of claims 1 through 23, the imaging assembly further comprising an objective lens housing supporting the objective lens, the objective lens housing having a distal face surrounding an outer perimeter of the objective lens.

25. The apparatus of claim 24, the distal face including a hydrophilic material.

26. The apparatus of any of claims 24 through 25, the bottom surface of the objective lens including a hydrophilic material.

27. The apparatus of any of claims 24 through 26, the distal face further including at least one annular recess spaced outwardly from the outer perimeter of the objective lens.

28. The apparatus of any of claims 1 through 27, further comprising an air blade manifold, the air blade manifold to expel pressurized air toward a space between the bottom surface of the objective lens and an upper surface of the flow cell.

29. The apparatus of claim 28, the air blade manifold including a plurality of openings to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

30. The apparatus of claim 29, the plurality of openings being positioned along an arc.

31. The apparatus of any of claims 29 through 30, the air blade manifold further comprising a plurality of channels in fluid communication with the plurality of openings.

32. The apparatus of claim 31, each channel in fluid communication with the plurality of openings being oriented along a respective radius extending toward a central point such that the radii along which the plurality of openings are oriented all extend toward the central point.

33. The apparatus of claim 32, the central point being positioned to correspond with a central region of the space between the bottom surface of the objective lens and an upper surface of the flow cell.

34. The apparatus of any of claims 28 through 33, the air blade manifold to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell in a form of a substantially flat air blade.

35. The apparatus of any of claims 28 through 34, further comprising a frame, the imaging assembly being secured to the frame, the air blade manifold being secured to the frame independently of the imaging assembly.

36. The apparatus of any of claims 28 through 35, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

37. The apparatus of claim 36, further comprising a pressurized air source, the pressurized air source to provide pressurized air to the air blade manifold, the pressurized air source further to provide pressurized air to the air curtain manifold.

38. The apparatus of claim 37, further comprising a valve, the valve to direct pressurized air to a selected one of either the air blade manifold or the air curtain manifold.

39. The apparatus of any of claims 1 through 38, further comprising a sensor to monitor a presence of immersion fluid under the bottom surface of the objective lens.

40. The apparatus of claim 39, the sensor comprising an optical sensor.

41. The apparatus of claim 40, the optical sensor being positioned and oriented to view the immersion fluid under the bottom surface of the objective lens along a horizontal dimension.

42. The apparatus of claim 40, the optical sensor being positioned and oriented to view light transmitted through the immersion fluid and reflected back through the objective lens.

43. The apparatus of any of claims 39 through 42, the sensor comprising a set of electrodes.

44. The apparatus of any of claims 39 through 43, the sensor comprising a force sensor.

45. An apparatus comprising:

an imaging assembly, the imaging assembly including:

an objective lens assembly, the objective lens assembly including:

an objective lens element having a bottom surface, and

a housing having a bottom surface adjacent to the bottom surface of the objective lens element, the bottom surface of the housing including a fluid retention feature, and

an immersion fluid assembly, the immersion fluid assembly including:

a first port to introduce immersion fluid under the objective lens assembly,

a second port to remove immersion fluid from under the objective lens assembly, and

a sidewall defining an immersion fluid retention region under the objective lens assembly;

the fluid retention feature of the housing of the objective lens assembly being laterally interposed between the objective lens element of the objective lens assembly and the sidewall of the immersion fluid assembly.

46. The apparatus of claim 45, the fluid retention feature including an annular recess surrounding the objective lens element.

47. The apparatus of claim 46, the fluid retention feature being positioned on the bottom surface of the housing.

48. The apparatus of any of claims 45 through 47, the bottom surface of the housing including a hydrophilic material.

49. The apparatus of any of claims 45 through 48, the bottom surface of the objective lens element including a hydrophilic material.

50. The apparatus of any of claims 45 through 49, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to

the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension.

51. The apparatus of claim 50, further comprising an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a path.

52. The apparatus of claim 51, the path including a horizontal path in a direction parallel to the first horizontal dimension.

53. The apparatus of any of claims 45 through 52, further comprising a flow cell having an upper surface under the fluid retention region, the immersion fluid assembly to maintain immersion fluid between the bottom surface of the objective lens and the upper surface of the flow cell.

54. The apparatus of claim 53, the upper surface including a hydrophobic material.

55. The apparatus of any of claims 53 through 54, the flow cell further comprising a plurality of channels, each channel of the plurality of channels including a plurality of reaction sites.

56. The apparatus of claim 55, the plurality of reaction sites including a plurality of nucleotides.

57. The apparatus of any of claims 55 through 56, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension, at least some of the channels of the plurality of channels being oriented along the first horizontal dimension.

58. The apparatus of claim 57, each channel of the plurality of channels being spaced apart from the other channel of the plurality of channels along the second horizontal dimension.

59. The apparatus of any of claims 55 through 58, further comprising an actuation assembly to drive relative movement between the flow cell and the imaging assembly to successively position the imaging assembly over each channel of the plurality of channels.

60. The apparatus of any of claims 45 through 59, the bottom surface of the objective lens element being flat.

61. The apparatus of any of claims 45 through 60, the bottom surface of the objective lens element including a concave surface.

62. The apparatus of any of claims 45 through 61, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens element.

63. The apparatus of claim 62, the air curtain manifold being positioned to encircle the objective lens assembly.

64. The apparatus of claim 63, the objective lens element being centered along a longitudinal axis, the air curtain manifold being centered along the longitudinal axis.

65. The apparatus of any of claims 62 through 64, the air curtain manifold defining a central opening.

66. The apparatus of claim 65, a portion of the imaging assembly being positioned within the central opening of the air curtain manifold.

67. The apparatus of claim 66, the central opening being sized to define a gap between the air curtain manifold and the portion of the imaging assembly positioned within the central opening.

68. The apparatus of any of claims 62 through 67, the air curtain manifold having an annular shape with a circumferentially extending surface, with openings positioned along the circumferentially extending surface, the air curtain manifold to expel pressurized air

outwardly relative to the objective lens via the openings positioned along the circumferentially extending surface.

69. The apparatus of claim 68, the circumferentially extending surface being angled along a vertical plane.

70. The apparatus of any of claims 68 through 69, the air curtain manifold further including an annular channel in fluid communication with the openings positioned along the circumferentially extending surface.

71. The apparatus of any of claims 62 through 70, further comprising a frame, the imaging assembly being secured to the frame, the air curtain manifold being secured to the frame independently of the imaging assembly.

72. The apparatus of any of claim 62 through 71, the air curtain manifold to expel pressurized air outwardly relative to the objective lens element in a form of a frustoconical air curtain.

73. The apparatus of claim 72, the frustoconical air curtain being centered along a longitudinal axis, the objective lens element being centered along the longitudinal axis.

74. The apparatus of any of claims 62 through 73, further comprising an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a path, the air curtain manifold being positioned to expel pressurized air downwardly toward the flow cell.

75. The apparatus of any of claims 45 through 74, further comprising an air blade manifold, the air blade manifold to expel pressurized air toward a space between the bottom surface of the objective lens element and an upper surface of the flow cell.

76. The apparatus of claim 75, the air blade manifold including a plurality of openings to expel pressurized air toward the space between the bottom surface of the objective lens element and an upper surface of the flow cell.

77. The apparatus of claim 76, the plurality of openings being positioned along an arc.

78. The apparatus of any of claims 76 through 77, the air blade manifold further comprising a plurality of channels in fluid communication with the plurality of openings.

79. The apparatus of claim 78, each channel in fluid communication with the plurality of openings being oriented along a respective radius extending toward a central point such that the radii along which the plurality of openings are oriented all extend toward the central point.

80. The apparatus of claim 79, the central point being positioned to correspond with a central region of the space between the bottom surface of the objective lens element and an upper surface of the flow cell.

81. The apparatus of any of claims 75 through 80, the air blade manifold to expel pressurized air toward the space between the bottom surface of the objective lens element and an upper surface of the flow cell in a form of a substantially flat air blade.

82. The apparatus of any of claims 75 through 81, further comprising a frame, the imaging assembly being secured to the frame, the air blade manifold being secured to the frame independently of the imaging assembly.

83. The apparatus of any of claims 75 through 82, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens element.

84. The apparatus of claim 83, further comprising a pressurized air source, the pressurized air source to provide pressurized air to the air blade manifold, the pressurized air source further to provide pressurized air to the air curtain manifold.

85. The apparatus of claim 84, further comprising a valve, the valve to direct pressurized air to a selected one of either the air blade manifold or the air curtain manifold.

86. The apparatus of any of claims 45 through 85, further comprising a sensor to monitor a presence of immersion fluid under the bottom surface of the objective lens element.

87. The apparatus of claim 86, the sensor comprising an optical sensor.

88. The apparatus of claim 87, the optical sensor being positioned and oriented to view the immersion fluid under the bottom surface of the objective lens element along a horizontal dimension.

89. The apparatus of claim 88, the optical sensor being positioned and oriented to view light transmitted through the immersion fluid and reflected back through the objective lens element.

90. The apparatus of any of claims 86 through 89, the sensor comprising a set of electrodes.

91. The apparatus of any of claims 86 through 90, the sensor comprising a force sensor.

92. An apparatus comprising:

an imaging assembly, the imaging assembly including:

an objective lens having a bottom surface;

an immersion fluid assembly, the immersion fluid assembly including:

a first port to introduce immersion fluid under the bottom surface of the objective lens,

a second port to remove immersion fluid from under the bottom surface of the objective lens, and

a sidewall defining an immersion fluid retention region under the objective lens; and

an air delivery assembly, the air delivery assembly being coupled with the objective lens assembly, the air delivery assembly to deliver pressurized air to one or both of the objective lens or an upper surface of a flow cell to thereby clear one or both of debris or

immersion fluid relative to one or both of the objective lens or an upper surface of a flow cell.

93. The apparatus of claim 92, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension.

94. The apparatus of claim 93, further comprising an actuation assembly to drive relative movement between a flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal dimension.

95. The apparatus of any of claims 92 through 94, further comprising a flow cell having an upper surface under the fluid retention region, the immersion fluid assembly to maintain immersion fluid between the bottom surface of the objective lens and the upper surface of the flow cell.

96. The apparatus of claim95, the upper surface including a hydrophobic material.

97. The apparatus of any of claims 95 through 96, the flow cell further comprising a plurality of channels, each channel of the plurality of channels including a plurality of reaction sites.

98. The apparatus of claim 97, the plurality of reaction sites including a plurality of nucleotides.

99. The apparatus of any of claims 97 through 98, at least some of the channels of the plurality of channels being oriented along the first horizontal dimension.

100. The apparatus of claim 99, each channel of the plurality of channels being spaced apart from the other channel of the plurality of channels along the second horizontal dimension.

101. The apparatus of any of claims 97 through 100, the actuation assembly to drive relative movement between the flow cell and the imaging assembly to successively position the imaging assembly over each channel of the plurality of channels.

102. The apparatus of any of claims 92 through 101, the bottom surface of the objective lens being flat.

103. The apparatus of any of claims 92 through 102, the bottom surface of the objective lens including a concave surface.

104. The apparatus of any of claims 92 through 103, the air delivery assembly comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

105. The apparatus of claim 104, the air curtain manifold being positioned to encircle the objective lens.

106. The apparatus of claim 105, the objective lens being centered along a longitudinal axis, the air curtain manifold being centered along the longitudinal axis.

107. The apparatus of any of claims 104 through 20+, the air curtain manifold defining a central opening.

108. The apparatus of claim 107, a portion of the imaging assembly being positioned within the central opening of the air curtain manifold.

109. The apparatus of claim 108, the central opening being sized to define a gap between the air curtain manifold and the portion of the imaging assembly positioned within the central opening.

110. The apparatus of any of claims 104 through 109, the air curtain manifold having an annular shape with a circumferentially extending surface, with openings positioned along the circumferentially extending surface, the air curtain manifold to expel pressurized

air outwardly relative to the objective lens via the openings positioned along the circumferentially extending surface.

111. The apparatus of claim 110, the circumferentially extending surface being angled along a vertical plane.

112. The apparatus of any of claims 110 through 111, the air curtain manifold further including an annular channel in fluid communication with the openings positioned along the circumferentially extending surface.

113. The apparatus of any of claims 104 through 112, further comprising a frame, the imaging assembly being secured to the frame, the air curtain manifold being secured to the frame independently of the imaging assembly.

114. The apparatus of any of claim 104 through 113, the air curtain manifold to expel pressurized air outwardly relative to the objective lens in a form of a frustoconical air curtain.

115. The apparatus of claim 114, the frustoconical air curtain being centered along a longitudinal axis, the objective lens being centered along the longitudinal axis.

116. The apparatus of any of claims 104 through 115, the air curtain manifold being positioned to expel pressurized air downwardly toward the flow cell.

117. The apparatus of any of claims 92 through 116, the imaging assembly further comprising an objective lens housing supporting the objective lens, the objective lens housing having a distal face surrounding an outer perimeter of the objective lens.

118. The apparatus of claim 117, the distal face including a hydrophilic material.

119. The apparatus of any of claims 117 through 118, the bottom surface of the objective lens including a hydrophilic material.

120. The apparatus of any of claims 117 through 119, the distal face further including at least one annular recess spaced outwardly from the outer perimeter of the objective lens.

121. The apparatus of any of claims 92 through 120, the air delivery assembly comprising an air blade manifold, the air blade manifold to expel pressurized air toward a space between the bottom surface of the objective lens and an upper surface of the flow cell.

122. The apparatus of claim 121, the air blade manifold including a plurality of openings to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

123. The apparatus of claim 122, the plurality of openings being positioned along an arc.

124. The apparatus of any of claims 122 through 123, the air blade manifold further comprising a plurality of channels in fluid communication with the plurality of openings.

125. The apparatus of claim 124, each channel in fluid communication with the plurality of openings being oriented along a respective radius extending toward a central point such that the radii along which the plurality of openings are oriented all extend toward the central point.

126. The apparatus of claim 125, the central point being positioned to correspond with a central region of the space between the bottom surface of the objective lens and an upper surface of the flow cell.

127. The apparatus of any of claims 121 through 126, the air blade manifold to expel pressurized air toward the space between the bottom surface of the objective lens and an upper surface of the flow cell in a form of a substantially flat air blade.

128. The apparatus of any of claims 121 through 127, further comprising a frame, the imaging assembly being secured to the frame, the air blade manifold being secured to the frame independently of the imaging assembly.

129. The apparatus of any of claims 121 through 128, further comprising an air curtain manifold, the air curtain manifold to expel pressurized air outwardly relative to the objective lens.

130. The apparatus of claim 129, further comprising a pressurized air source, the pressurized air source to provide pressurized air to the air blade manifold, the pressurized air source further to provide pressurized air to the air curtain manifold.

131. The apparatus of claim 130, further comprising a valve, the valve to direct pressurized air to a selected one of either the air blade manifold or the air curtain manifold.

132. The apparatus of any of claims 92 through 131, further comprising a sensor to monitor a presence of immersion fluid under the bottom surface of the objective lens.

133. The apparatus of claim 132, the sensor comprising an optical sensor.

133. The apparatus of claim 133, the optical sensor being positioned and oriented to view the immersion fluid under the bottom surface of the objective lens along a horizontal dimension.

134. The apparatus of claim 133, the optical sensor being positioned and oriented to view light transmitted through the immersion fluid and reflected back through the objective lens.

135. The apparatus of any of claims 132 through 134, the sensor comprising a set of electrodes.

136. The apparatus of any of claims 132 through 135, the sensor comprising a force sensor.

137. A method comprising:

positioning an imaging assembly in relation to a flow cell, the imaging assembly comprising:

an objective lens having a bottom surface, and

an immersion fluid assembly, the immersion fluid assembly including:

a first port, and

a sidewall defining an immersion fluid retention region under the objective lens, the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension;

depositing immersion fluid via the first port to a space between the bottom surface of the objective lens and an upper surface of the flow cell; and

providing relative movement between the flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal dimension, the immersion fluid remaining in the space between the bottom surface of the objective lens and the upper surface of the flow cell during the relative movement between the flow cell and the imaging assembly.

138. The method of claim 137, further comprising activating an air curtain, the air curtain comprising pressurized air oriented toward the upper surface of the flow cell and away from the objective lens.

139. The method of claim 138, the act of activating the air curtain being performed before the act of depositing immersion fluid.

140. The method of any of claims 138 through 139, the act of activating the air curtain being performed while providing relative movement between the flow cell and the imaging assembly.

141. The method of any of claims 138 through 140, the air curtain having a frustoconical shape.

142. The method of any of claims 138 through 141, the air curtain being provided via an air curtain manifold positioned about the imaging assembly.

143. The method of any of claims 137 through 142, further comprising activating an air blade, the air blade comprising pressurized air oriented toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

144. The method of claim 143, the act of activating the air blade being performed after providing relative movement between the flow cell and the imaging assembly.

145. The method of any of claims 143 through 144, further comprising drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

146. The method of claim 145, the immersion fluid being drawn from the space between the bottom surface of the objective lens and the upper surface of the flow cell via a second port of the immersion fluid assembly.

147. The method of any of claims 145 through 146, the act of activating the air blade being performed after drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

148. The method of any of claims 145 through 147, the the air blade being provided via an air blade manifold positioned adjacent to the imaging assembly.

149. The method of claim 148, the air blade manifold having a plurality of openings positioned along an arc, the air blade comprising pressurized air expelled via the plurality of openings such that air flow of the air blade is focused toward a central region, the central region being positioned in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

150. The method of any of claims 137 through 149, further comprising monitoring, via a sensor, the presence of immersion fluid in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

151. The method of claim 150, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data along a horizontal path.

152. The method of any of claims 150 through 151, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data from light communicated through the objective lens and toward the optical sensor.

153. The method of any of claims 150 through 152, the sensor comprising electrodes, the act of monitoring comprising monitoring whether a circuit is completed between the electrodes.

154. The method of any of claims 150 through 153, the sensor comprising a force sensor, the act of monitoring comprising monitoring a force associated with the immersion fluid.

155. The method of any of claims 137 through 154, further comprising:
removing the flow cell from a space beneath the imaging assembly; and
positioning another flow cell in the space beneath the imaging assembly.

156. The method of any of claims 137 through 155, further comprising performing nucleotide sequencing in the flow cell.

157. A method comprising:
positioning an imaging assembly in relation to a flow cell, the imaging assembly comprising:
an objective lens having a bottom surface, and
an immersion fluid assembly, the immersion fluid assembly including:
a first port, and
a sidewall defining an immersion fluid retention region under the objective lens;
depositing immersion fluid via the first port to a space between the bottom surface of the objective lens and an upper surface of the flow cell;
providing relative movement between the flow cell and the imaging assembly along a horizontal path in a direction parallel to the first horizontal

dimension, the immersion fluid remaining in the space between the bottom surface of the objective lens and the upper surface of the flow cell during the relative movement between the flow cell and the imaging assembly; and

delivering pressurized air to one or both of the objective lens or the upper surface of the flow cell.

158. The method of claim 157, the the immersion fluid retention region having a first horizontal dimension and a second horizontal dimension orthogonal to the first horizontal dimension, the first horizontal dimension being larger than the second horizontal dimension.

159. The method of claim 158, the horizontal path being in a direction parallel to the first horizontal dimension.

160. The method of any of claims 157 through 159, the act of delivering pressurized air to one or both of the objective lens or the upper surface of the flow cell comprising activating an air curtain, the air curtain comprising pressurized air oriented toward the upper surface of the flow cell and away from the objective lens.

161. The method of claim 160, the act of activating the air curtain being performed before the act of depositing immersion fluid.

162. The method of any of claims 160 through 161, the act of activating the air curtain being performed while providing relative movement between the flow cell and the imaging assembly.

163. The method of any of claims 160 through 162, the air curtain having a frustoconical shape.

164. The method of any of claims 160 through 163, the air curtain being provided via an air curtain manifold positioned about the imaging assembly.

165. The method of any of claims 157 through 164, the act of delivering pressurized air to one or both of the objective lens or the upper surface of the flow cell comprising activating

an air blade, the air blade comprising pressurized air oriented toward the space between the bottom surface of the objective lens and an upper surface of the flow cell.

166. The method of claim 165, the act of activating the air blade being performed after providing relative movement between the flow cell and the imaging assembly.

167. The method of any of claims 165 through 166, further comprising drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

168. The method of claim 167, the immersion fluid being drawn from the space between the bottom surface of the objective lens and the upper surface of the flow cell via a second port of the immersion fluid assembly.

169. The method of any of claims 167 through 168, the act of activating the air blade being performed after drawing the immersion fluid from the space between the bottom surface of the objective lens and the upper surface of the flow cell.

170. The method of any of claims 167 through 169, the the air blade being provided via an air blade manifold positioned adjacent to the imaging assembly.

171. The method of claim 170, the air blade manifold having a plurality of openings positioned along an arc, the air blade comprising pressurized air expelled via the plurality of openings such that air flow of the air blade is focused toward a central region, the central region being positioned in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

172. The method of any of claims 157 through 171, further comprising monitoring, via a sensor, the presence of immersion fluid in the space between the bottom surface of the objective lens and the upper surface of the flow cell.

173. The method of claim 172, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data along a horizontal path.

174. The method of any of claims 172 through 173, the sensor comprising an optical sensor, the act of monitoring comprising obtaining optical data from light communicated through the objective lens and toward the optical sensor.

175. The method of any of claims 172 through 174, the sensor comprising electrodes, the act of monitoring comprising monitoring whether a circuit is completed between the electrodes.

176. The method of any of claims 172 through 175, the sensor comprising a force sensor, the act of monitoring comprising monitoring a force associated with the immersion fluid.

177. The method of any of claims 157 through 176, further comprising:
removing the flow cell from a space beneath the imaging assembly; and
positioning another flow cell in the space beneath the imaging assembly.

178. The method of any of claims 157 through 177, further comprising performing nucleotide sequencing in the flow cell.