

MICROFLUIDIC REACTION STRUCTURES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Patent Application No. 63/489,422 filed March 9, 2023 and U.S. Provisional Patent Application No. 63/489,677 filed March 10, 2023. The disclosure of each application is incorporated herein for all purposes by reference in its entirety.

TECHNICAL FIELD

[0002] The present disclosure relates to devices and methods that allow running reactions in parallel in centrifugal microfluidics.

BACKGROUND

[0003] Currently, 70% of all medical decisions rely on lab based diagnostics but today, the diagnostic process is disjointed from how care is delivered. The primary care system requires patients to travel to external phlebotomy sites to draw blood which is sent to labs via courier, and processed overnight. This means that lab results reach health care professionals long after the patient has left. This friction in care delivery and disease management leads to tremendous waste in the healthcare system:

- a. Patients often delay getting lab tests or fail to adhere to lab testing, or subsequent care recommendations
- b. The gap in the diagnostic process leads to missed tests, missed diagnosis, a lack of intervention, and ultimately poor outcomes
- c. Health care professionals waste time tracing lab orders to patient encounter notes. When intervention is needed, more time is wasted in reaching out to patients and driving subsequent steps in the patient's care pathway.

[0004] These problems are even more acute when caring for rural populations or patients belonging to groups facing adverse social-determinants of health, where there are many challenges in ensuring successful follow ups from an initial patient encounter.

[0005] Several companies have built point-of-care instruments to bridge this divide. However, these instruments are limited to single types of tests and fail to completely meet the workflow needs of primary care providers for a single system that produces simple, comprehensive, and fast test results. A product to meet these needs is currently under development. It achieves this through a highly automated workflow enabled through the use of centrifugal microfluidics discs.

[0006] Centrifugal microfluidics are used in clinical chemistry, immunoassays, hematology, medicine, biomedical research and other fields. Many of these applications involve a large number of reactions. To allow for the large number of reactions, existing microfluidics are often configured with multiple structures arranged sequentially to avoid contamination. Such configurations require having a radial offset between each structure, leading to at least two problems: limitation on the number of structures per cartridge and slightly different operation parameters for each structure.

[0007] Accordingly, there remains a need for improved devices and methods for performing a large number of reactions in centrifugal microfluidics.

SUMMARY

[0008] The present disclosure addresses these and other needs in the art by providing devices and methods that can run reactions in parallel, and in many cases, with controlled reconstitution of dry reagents and intermediate light-based readout.

[0009] In one aspect, the present disclosure provides a device rotatable around a rotational axis. The device includes a reaction chamber having an inlet to receive a fluid and a first well to serve as both reagent and readout wells. A first reagent is disposed in the first well when the device is made. A radial position of a meniscus of the fluid in the reaction chamber depends at least in part on a rotational speed of the device. The first well has a substantially flat wall perpendicular to the rotational axis to allow reproducible light transmission.

[0010] In some embodiments, the reaction chamber further includes a second well and an intermediate chamber between the first and second wells. In some such embodiments, a second reagent is disposed or placed in the second well when the device is made.

[0011] In some embodiments, the first and/or second reagent are lyophilized. In some embodiments, the first and/or second reagent are in bead form. In some embodiments, the second reagent is different than the first reagent.

[0012] In some embodiments, the reaction chamber is unvented to promote homogeneous mixing of the first reagent with the fluid. For instance, in some such embodiments, the reaction chamber includes an outlet connected to an air chamber. In an embodiment, the first well is connected to the air chamber. In some embodiments, the second well is connected to the air chamber.

[0013] In some embodiments, the device further includes an upstream chamber positioned radially inwards of the reaction chamber and connected to the inlet of the reaction chamber. In some embodiments, the inlet of the reaction chamber is located at the first well, and the second well is positioned radially inwards of the first well. In some embodiments, the outlet is located at the second well.

[0014] In some embodiments, the reaction chamber, the upstream chamber, the air chamber, or a combination thereof are configured to allow the initial fill of the first well with the fluid without wetting the second well.

[0015] In another aspect, the present disclosure provides a device rotatable around a rotational axis. The device includes a plurality of reaction structures arranged circumferentially over at least a portion of the device. Each reaction structure in the plurality of reaction structures includes an aliquot chamber and a reaction chamber positioned radially outwards of the aliquot chamber. The aliquot chamber has an outlet and the reaction chamber has an inlet connected to the outlet of the aliquot chamber. In some embodiments, the device also includes one or more connecting siphons that are radially leveled with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures. Each respective connecting siphon in the one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures.

[0016] In some embodiments, for each reaction structure in the plurality of reaction structures, the reaction chamber includes a first well. In some embodiments, for each reaction structure in the plurality of reaction structures, a first reagent is disposed in the first well when the device is made. In some embodiments, for each reaction structure in the plurality of reaction structures, the first well serves as both reagent and readout wells. In some embodiments, the first wells of the plurality of reaction structures are radially leveled with each other.

[0017] In some embodiments, for each reaction structure in at least a subset of the plurality of reaction structures, the reaction chamber includes a second well and an intermediate chamber between the first and second wells. In some embodiments, for each reaction structure in at least the subset of the plurality of reaction structures, a second reagent is disposed in the second well when the device is made. In some embodiments, the second wells of at least the subset of the plurality of reaction structures are radially leveled with each other. In some embodiments, the inlet of the reaction chamber is located at the first well, and the second well is positioned radially inwards of the first well.

[0018] In some embodiments, each respective reaction structure in the plurality of reaction structures includes an air chamber, and the reaction chamber of the respective reaction structure has an outlet connected to the air chamber.

[0019] In some embodiments, the device further includes an overflow chamber, and an overflow channel connecting the aliquot chamber of a last reaction structure in the plurality of reaction structures to the overflow chamber. In some such embodiments, the overflow channel includes a siphon portion and the siphon portion includes an inlet and a crest. In some embodiments, the inlet of the siphon portion of the overflow channel is radially leveled with an inlet and an outlet of each connecting siphon in the one or more siphons. In some embodiments, the crest of the siphon portion of the overflow channel is leveled with or radially outwards of a crest of each connecting siphon in the one or more siphons. In some embodiments, the overflow channel further includes a U-channel having an outlet connected to the overflow chamber. In some embodiments, a portion of the U-channel is outwards of the overflow channel outlet.

[0020] In a further aspect, the present disclosure provides a method including (A) obtaining a device rotatable around a rotational axis. The device includes a plurality of reaction structures and one or more connecting siphons. Reaction structures in the plurality of reaction structures

are arranged circumferentially over at least a portion of the device. Each reaction structure in the plurality of reaction structures includes an aliquot chamber and a reaction chamber positioned radially outwards of the aliquot chamber. The aliquot chamber has an outlet and the reaction chamber has an inlet connected to the outlet of the aliquot chamber. The one or more connecting siphons are radially leveled with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures. Each respective connecting siphon in the one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures. The method also includes (B) rotating the device at a speed to fill a first portion of the reaction chamber of each reaction structure in the plurality of reaction structures with a fluid. The method further includes (C) increasing the speed to empty the fluid from each corresponding connecting siphon in the one or more connecting siphons. In addition, the method includes (D) decreasing the speed to create a gas volume trapped inside of each corresponding connecting siphon in the one or more connecting siphons, thereby interrupting fluidic connection between the plurality of reaction structures.

[0021] In some embodiments, the increasing (C) moves at least a portion of the fluid out of the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned outwards of the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby emptying the fluid from each corresponding connecting siphon in the one or more connecting siphons.

[0022] In some embodiments, the decreasing (D) moves at least a portion of the fluid contained in the reaction chamber of each respective reaction structure in the plurality of reaction structures to the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned inwards of the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby wetting the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons with the gas volume trapped inside of each corresponding connecting siphon.

[0023] In some embodiments where the first portion of the reaction chamber of each reaction structure in the plurality of reaction structures includes a first well with a first reagent, the

method further includes (E) repeating the increasing (C) and decreasing (D) one or more times to promote mixing of the fluid with the first reagent. In some embodiments, the method further includes (F) detecting, for each reaction structure in the plurality of reaction structure, light transmitted through the first well or a wall of the first well of the reaction chamber. In some embodiments, the detecting (F) is performed without the repeating (E) (*e.g.*, without mixing).

[0024] In some embodiments, the method further includes (G) increasing the speed to fill a second portion of the reaction chamber of each reaction structure in at least a subset of the plurality of reaction structures, and (H) performing the decreasing (D). In some embodiments wherein the second portion of the reaction chamber of each reaction structure in at least the subset of the plurality of reaction structures includes a second well with a second reagent, the method further includes (I) repeating the increasing (G) and performing (H) one or more times to promote mixing of the fluid with the second reagent. In some embodiments, the method further includes (J) detecting, for each reaction structure in at least the subset of the plurality of reaction structure, light transmitted through the first well or a wall of the first well of the reaction chamber.

[0025] The devices, systems and methods of the present disclosure have other features and advantages that will be apparent from, or are set forth in more detail in, the accompanying drawings, which are incorporated herein, and the following Detailed Description, which together serve to explain certain principles of exemplary embodiments of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The accompanying drawings, which are incorporated into and constitute a part of this specification, illustrate one or more exemplary embodiments of the present disclosure and, together with the Detailed Description, serve to explain the principles and implementations of exemplary embodiments of the invention. The accompanying drawings are not necessarily to scale. The specific design features of the present invention as disclosed herein, including, for example, specific dimensions, orientations, locations, and shapes will be determined in part by the particular intended application and use environment. In addition, the components illustrated in the figures are combinable in any useful number and combination.

[0027] In the drawings:

[0028] FIG. 1A is a schematic diagram illustrating a device in accordance with some exemplary embodiments of the present disclosure;

[0029] FIG. 1B is a schematic cross-sectional view taken along the dotted line in FIG. 1A in accordance with an exemplary embodiment of the present disclosure;

[0030] FIG. 1C is a schematic diagram illustrating a reaction structure in accordance with an alternative exemplary embodiment of the present disclosure;

[0031] FIG. 1D is a schematic diagram illustrating a reaction structure in accordance with another alternative exemplary embodiment of the present disclosure;

[0032] FIG. 2A is a schematic diagram illustrating a device in accordance with some exemplary embodiments of the present disclosure;

[0033] FIG. 2B is a partially enlarged view of FIG. 2A;

[0034] FIGS. 3A and 3B are flow charts collectively illustrating a method in accordance with some exemplary embodiments of the present disclosure;

[0035] FIGS. 4A, 4B, 4C, 4D, 4E and 4F are schematic diagrams collectively illustrating a process performed using the device of FIG. 2A in accordance with some exemplary embodiments of the present disclosure;

[0036] FIG. 5A is a schematic diagram illustrating a device (*e.g.*, a disc) in accordance with some exemplary embodiments of the present disclosure;

[0037] FIG. 5B is a schematic diagram illustrating a loading process in accordance with some exemplary embodiments of the present disclosure;

[0038] FIG. 5C is a schematic diagram illustrating a separation and metering process in accordance with some exemplary embodiments of the present disclosure;

[0039] FIG. 5D is a schematic diagram illustrating a metering process in accordance with some exemplary embodiments of the present disclosure;

[0040] FIG. 5E-1 is a schematic diagram illustrating a diluting process in accordance with some exemplary embodiments of the present disclosure;

[0041] FIG. 5E-2 is a partially enlarged view of FIG. 5E-1;

[0042] FIG. 5F-1 is a schematic diagram illustrating a transferring and dissolving process in accordance with some exemplary embodiments of the present disclosure;

[0043] FIG. 5F-2 is a partially enlarged view of FIG. 5F-1;

[0044] FIG. 5G is a schematic diagram illustrating a mixing process in accordance with some exemplary embodiments of the present disclosure;

[0045] FIG. 5H is a schematic diagram illustrating a dissolving process in accordance with some exemplary embodiments of the present disclosure;

[0046] FIG. 5I is a schematic diagram illustrating a mixing and detection process in accordance with some exemplary embodiments of the present disclosure;

[0047] FIG. 6 is a block diagram illustrating a workflow in accordance with some exemplary embodiments of the present disclosure; and

[0048] FIG. 7 is a schematic diagram illustrating a device (*e.g.*, a disc) in accordance with some exemplary embodiments of the present disclosure.

DETAILED DESCRIPTION

[0049] The present disclosure provides systems and methods for running a large number of reactions in parallel. In various embodiments, the systems and methods of the present disclosure allow for controlled reconstitution of dry reagents and for intermediate light-based readout such as at the first step of two reagent reactions. This may be achieved by controlling reaction timings and volumes while minimizing dead volume (*e.g.*, the liquid volume not being read by detection methods). This is critical because a small increase in dead volume can lead to a large amount of space (*e.g.*, cartridge real estate) allocated to non-critical functions and limiting the number of reactions that can be run on a single device (*e.g.*, cartridge).

[0050] Referring now to the drawings, where like reference numerals indicate like elements throughout, there is shown in FIGS. 1A-1C an exemplary device 100 in accordance with some embodiments of the present disclosure. The device 100 is rotatable around a rotational axis 102. The device 100 includes a reaction structure 104 that is configured to enable the use of pneumatic pressure and centrifugal force to control liquid position and to allow for reagent reconstitution in the same chamber where incubation and detection occur. This eliminates the

dead volume and variability associated with a liquid transfer from a reconstitution chamber into a separate detection chamber.

[0051] The reaction structure 104 includes a reaction chamber 110 having an inlet 112 to receive a fluid, *e.g.*, from an upstream chamber or a pipetting port or the like. The reaction chamber 110 is configured such that the radial position 114 of a meniscus of the fluid in the reaction chamber depends at least in part on the rotational speed of the device. The reaction chamber 110 includes one or more wells. As a non-limiting example, FIG. 1A illustrates the reaction chamber 110 having a first well 121 and a second well 122 connected by an intermediate chamber 123 between the first and second wells. In some embodiments, the inlet is located at the first well, and the second well is positioned radially inwards of the first well. As another non-limiting example, FIG. 1C illustrates the reaction chamber 110 having a single well such as the first well 121. It should be noted that the reaction chamber can have more than two, more than three, more than four, or more than five wells.

[0052] At least one of the one or more wells is configured to serve as both reagent and readout wells. For instance, in some embodiments, as illustrated in FIG. 1B, the first well 121 is configured to serve as both reagent and readout wells. In some such embodiments, as illustrated in FIG. 1B, a first reagent 151 is disposed in the first well when the device is made. The first well 121 is made with a substantially flat wall 124 perpendicular to the rotational axis to allow reproducible light transmission. Alternatively, in some embodiments, the second well 122 is configured to serve as both reagent and readout wells, or both of the first and second wells are configured to serve as both reagent and readout wells. In some embodiments, a second reagent 152 is disposed in the second well 122 when the device is made. The second reagent may be the same as or different from the first reagent.

[0053] In some embodiments, the first and/or second reagents are lyophilized. In some embodiments, the first and/or second reagents are in bead form. In some embodiments, the device or at least a portion of the device that includes the reaction chamber is made of an injection molded thermoplastic piece with one or more cavities, where the first and/or second reagents (*e.g.*, lyophilized beads) are placed, followed by a bonding step with a pressure-sensitive-adhesive lined laminate to define the top surface of the chamber as illustrated in FIG. 1B.

[0054] In some embodiments, as illustrated in FIG. 1A, the reaction chamber 110 is unvented to promote homogeneous mixing of the first reagent and/or second reagent with the fluid. For instance, in some embodiments, the reaction structure 104 includes a pressure chamber (e.g., an air chamber) 130 and the reaction chamber includes an outlet 114 connected to the pressure chamber. The outlet may be located at the second well as illustrated in FIG. 1A, at the intermediate chamber as illustrated in FIG. 1C, or any other suitable locations. This allows control of the liquid meniscus position inside the reaction chamber using the spinning speed, and to ensure a homogeneous mixing of the first or second reagent with the fluid in the reaction chamber. For instance, changing the spinning speed moves the fluid inwards or outwards and thus promotes homogeneous mixing of the reagents. This has an added benefit of allowing for precise control of the liquid position across all the reaction chambers of multiple reaction structures at the same time as will be explained in more detail. The only drivers for the liquid position are the spinning speed, chamber geometry and reaction volume.

[0055] In some embodiments, the reaction structure 104 includes an upstream chamber 140 (e.g., an aliquot chamber) positioned radially inwards of the reaction chamber 110 and has an outlet (e.g., the outlet 145, the outlet 146 or both) connected to the inlet of the reaction chamber to provide a fluid to the reaction chamber. In some embodiments, the reaction chamber, the upstream chamber, the pressure chamber (e.g., air chamber), or a combination thereof are configured to allow the initial fill of the first well 121 with the fluid without wetting the second well 122 if it is present. For instance, in some embodiments, the upstream chamber 140 is configured with a first deeper portion 141, a second deeper portion 142 and a shallow portion 143 between the first and second deeper portion. In some embodiments, the intermediate chamber 123 of the reaction chamber is configured to have a relatively small volume but with a relatively longer radial length. This may ensure a minimal variability in the reaction volume during metering even with a large variation in volume on the upstream chamber. This may ensure a minimal variability in the reaction volume during metering even with a large variation in volume of the upstream chamber by ensuring that a variation in radial position of liquid meniscus 116 inside the reaction chamber 110 does not match a large variation in volume inside the reaction chamber 110.

[0056] The reaction structure 104 can be configured to include additional, optional or alternative components. For instance, as a non-limiting example, FIG. 1D illustrates the reaction structure

104 that includes a vent port 160, the first well 121 is connected to the vent port and the second well 122 is connected to the first well 121, the air chamber 130 and the upstream chamber 140. In this embodiment the first well 122 is filled during initial filling, leaving minimal volume in the upstream chamber 140. By decreasing the rotation speed the liquid meniscus will move inwards and wet the siphon crest, filling the second reagent well 122.

[0057] Referring to FIGS. 2A and 2B, there is shown an exemplary device 200 in accordance with some embodiments of the present disclosure. The device 200 is rotatable around a rotational axis 202. The device 200 includes a plurality of reaction structures, such as the reaction structure 104 disclosed herein. Reaction structures in the plurality of reaction structures are arranged circumferentially over at least a portion of the device. The device 200 can include any suitable number of reaction structures. For instance, the device may include 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 reaction structures. As a non-limiting example, FIGS. 2A and 2B illustrate the device with four reaction structures, *e.g.*, the reaction structures 104-1, 104-2, 104-3 and 104-4.

[0058] A reaction structure in the plurality of reaction structures can be configured the same as or different from another reaction structure in the plurality of reaction structures. In some embodiments, each reaction structure in the plurality of reaction structures is configured substantially the same as other reaction structures in the plurality of reaction structures. In some embodiments, at least one reaction structure in the plurality of reaction structures is configured differently from one or more other reaction structures in the plurality of reaction structures. By way of example, FIGS. 2A and 2B illustrate that the reaction structures 104-2, 104-3 and 104-4 are configured substantially the same as or identical to each other and the reaction structure 104-1 is configured differently from the reaction structures 104-2, 104-3 and 104-4.

[0059] Each reaction structure in the plurality of reaction structures includes an aliquot chamber, such as the upstream chamber 140 disclosed herein, and a reaction chamber, such as the reaction chamber 110 disclosed herein. The aliquot chamber has an outlet (*e.g.*, the outlet 145, the outlet 146 or both) and the reaction chamber has an inlet (*e.g.*, the inlet 112) connected to the outlet of the aliquot chamber. The reaction chamber is positioned radially outwards of the aliquot chamber. In some embodiments, each reaction structure in the plurality of reaction structures includes an air chamber such as the air chamber 130 disclosed herein. For each

respective reaction structure, the reaction chamber has an outlet (*e.g.*, the outlet 114) is connected to the air chamber.

[0060] In some embodiments, for each reaction structure in the plurality of reaction structures, the reaction chamber includes a first well such as the first well 121 disclosed herein. In some embodiments, for each reaction structure in the plurality of reaction structures, a first reagent, such as the first reagent 151, is disposed in the first well when the device is made. In some embodiments, for each reaction structure in the plurality of reaction structures, the first well serves as both reagent and readout wells. In some embodiments, the first wells of the plurality of reaction structures are radially leveled (*e.g.*, at the same radial position) with each other. In some embodiments, for each and every reaction structure in the plurality of reaction structures, the reaction chamber consists of a single well such as the first well 121 disclosed herein. In some embodiments, for each reaction structure in at least a subset of the plurality of reaction structures, the reaction chamber includes a second well, such as the second well 122 disclosed herein, and an intermediate chamber, such as the intermediate chamber 123 disclosed herein, between the first and second wells. In some embodiments, for each reaction structure in at least the subset of the plurality of reaction structures, a second reagent, such as the second reagent 152, is disposed in the second well when the device is made. In some embodiments, for each and every reaction structure in the plurality of reaction structures, the reaction chamber includes both the first and second wells. By way of example, FIGS. 2A and 2B illustrate that the reaction chamber of the reaction structure 104-1 includes a single well (*e.g.*, the first well 121) and the reaction chamber of each of the reaction structures 104-2, 104-3 and 104-4 includes the first and second wells.

[0061] In some embodiments, the second wells of at least the subset of the plurality of reaction structures are radially leveled with each other. In some embodiments, for each of the plurality of reaction structures, the inlet of the reaction chamber is located at the first well, and the second well (if present) is positioned radially inwards of the first well as illustrated in FIGS. 1A and 2A.

[0062] In some embodiments, reaction structures in the plurality of reaction structures are positioned within a range of radius. For instance, in some embodiments, reaction structures in the plurality of reaction structures may be positioned between a first radius and a second radius with respect to the rotational axis. The first radius may be smaller than about 30 mm, smaller

than about 35 mm, smaller than about 40 mm, smaller than about 45 mm, or smaller than 50 mm. The second radius may be greater than about 35 mm, greater than about 40 mm, greater than about 45 mm, greater than 50 mm, greater than 55 mm, or greater than 60 mm. In some specific implementations, the plurality of reaction structures may be positioned within a range of radius that is between about 25 mm and about 45 mm, between about 30 mm and about 50 mm, between about 35 mm and about 55 mm, or between about 40 mm and about 60 mm. In a specific implementation, the plurality of reaction structures may be positioned within a range of radius that is between about 43 mm and about 50 mm.

[0063] The volumes of the first wells (*e.g.*, reaction volumes for a first reaction 1R in the reaction structures) may be the same as or different from the volumes of the second wells (*e.g.*, reaction volumes for a second reaction 2R in the reaction structures). The “1R” denotes a reaction occurred in the first wells or with the first reagents disposed in the first wells. Similarly, the “2R” denotes a reaction occurred in the second wells or with the second reagents disposed in the second wells. In some embodiments, the reaction volumes for the 1R reaction is smaller than the reaction volumes for the 2R reaction. For instance, in some specific implements, the reaction volumes for the 1R reaction may be less than about 10 μL , less than about 15 μL , less than about 20 μL , less than about 25 μL , or less than about 30 μL , and the reaction volumes for the 1R reaction may be greater than about 20 μL , greater than about 25 μL , greater than about 30 μL , greater than about 35 μL , or greater than about 40 μL . In a specific implementation, the reaction volumes for the 1R reaction may be about 15 μL and the reaction volumes for the 2R reaction may be 29 μL . In another specific implementation, the reaction volumes for the 1R reaction may be about half of the reaction volumes for the 2R reaction.

[0064] In some embodiments, a channel (*e.g.*, the channel connecting the upstream chamber to the reaction chamber) may have a width that is between about 100 μm and 150 μm , between about 150 μm and 200 μm , between about 200 μm and 250 μm , or between about 250 μm and 300 μm , and a depth that is between about 50 μm and 100 μm , 100 μm and 150 μm , between about 150 μm and 200 μm , or between about 150 μm and 250 μm . In a specific implementation, a channel may have a width that is between about 120 μm and 175 μm and a depth that is between about 100 μm and 175 μm .

[0065] A chamber may have a constant or varied depth. For instance, a reaction chamber may have a depth at the intermediate chamber different than at the first and/or second wells. Similarly, the upstream chamber may have a depth at the shallow portion that is different than those at the first and/or second deeper portions. In some embodiments, a chamber may have a depth of between about 0.2 mm and about 5 mm. In some embodiments, a chamber may have a depth of between about 0.4 mm and about 3 mm.

[0066] The device 200 also includes one or more connecting siphons 220 radially leveled (*e.g.*, at the same radial positions) with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures. Each respective connecting siphon in the one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures. For instance, in some embodiments, the device includes two reaction structures and one connecting siphon connecting the two reaction structures. In some embodiments, the device includes three reaction structures and two connecting siphons each connecting two adjacent reaction structures. In the embodiment illustrated in FIG. 2A where there are four reaction structures, the device includes three connecting siphons, *e.g.*, the connecting siphons 220-1, 220-2 and 220-3. The connecting siphon 220-1 connects the reaction structure 104-1 and the reaction structure 104-2. The connecting siphon 220-2 connects the reaction structure 104-2 and the reaction structure 104-3. The connecting siphon 220-3 connects the reaction structure 104-3 and the reaction structure 104-4. The connecting siphons 220-1, 220-2 and 220-3 are radially leveled with each other (*e.g.*, the radial positions of the siphons are the same with respect to the rotational axis 202) and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures.

[0067] In some embodiments, the reaction structures (*e.g.*, the reaction chamber, air chamber, and/or aliquot chamber) and/or connecting siphons are tuned to meet the reaction requirements and to minimize dead volume. For instance, in some embodiments, the reaction structures and/or connecting siphons are tuned to keep the reaction volume in a desired range (*e.g.*, within 1% to 10% or within 5% to 15%) in accordance with the reaction kinetics while minimizing dead volume. In some embodiments, the reaction structures and/or connecting siphons are tuned to ensure that the liquid position inside the reaction chamber of each reaction structure completely fills the readout/reconstitution chamber (*e.g.*, the first well) during the initial filling step without wetting the second reagent chamber (*e.g.*, the second well if present).

[0068] For instance, referring to FIGS. 2A and 2B, in some embodiments, for each reaction structure in at least a subset of the plurality of reaction structures, the upstream/aliquot chamber includes a shallow portion, *e.g.*, the shallow portion 143 as illustrated in FIG. 1B, adjacent to the corresponding connecting siphon. In some embodiments, the shallow portion may have a depth that is less than 1000 μm , less than 900 μm , less than 800 μm , less than 700 μm , less than 600 μm , or less than 500 μm . In some embodiments, the shallow portion may have a depth that is between about 100 μm and about 500 μm . In some embodiments, the shallow portion may be at a position within about 2 mm, about 1.5 mm, about 1 mm, or about 0.5 mm in radii of the connecting siphon radius. In some embodiments, to accommodate all the reaction volume necessary, the aliquot chamber includes deeper portions, such as the first deeper portion 141 and the second deeper portion 142, at radial positions both outwards and inwards from the shallow portion. In some embodiments, the first and/or second deeper portions may have a depth that is at least 1 mm, at least 1.5 mm, at least 2 mm, at least 2.5 mm, at least 3 mm, at least 3.5 mm, or at least 4 mm. In some embodiments, the first and/or second deeper portions may have a depth that is about 2 mm to about 3 mm.

[0069] In some embodiments, for each reaction structure in at least a subset of the plurality of reaction structures, the maximum angular length (*e.g.*, the arc length) of the upstream/aliquot chamber is defined by the total reaction volume and the need to minimize the space with the minimum boundary defined by the minimum angular length taken up by the connecting siphon and the downstream features of the reaction structure. In some embodiments, for each reaction structure in at least a subset of the plurality of reaction structures, the angular length of the upstream/aliquot chamber may be between about 1 mm and about 5 mm, between about 2 mm and about 6 mm, between about 3 mm and 7 mm, or between about 4 mm and about 8 mm.

[0070] In some embodiments, the device 200 includes an overflow chamber 230 and an overflow channel 240. The overflow channel 240 connects the aliquot chamber of a reaction structure, *e.g.*, the last reaction structure in the plurality of reaction structure, to the overflow chamber. For instance, in the illustrated embodiment, the overflow channel 240 connects the aliquot chamber of the reaction structure 104-4 to the overflow chamber. The overflow channel allows for metering of the reaction volumes inside the reaction structures by overflowing excess volume. In some embodiments, these reaction structures may be loaded with an excess of volume and only the target volume may be retained, the rest being overflowed.

[0071] In some embodiments, the overflow channel includes a siphon portion 241. The siphon portion 241 has an inlet 242 and a crest 243. The inlet 242 is radially leveled with the inlet 221 and the outlet 222 of each connecting siphon in the one or more siphons. The crest 243 is leveled with or slightly radially outwards of the crest 223 of each connecting siphon in the one or more siphons. In some embodiments, the overflow channel further includes a U-channel 244 having an outlet connected to the overflow chamber. In some embodiments, the U-channel 244 includes an outwards segment and an inwards segment as illustrated in FIGS. 2A and 2B. In some embodiments, the U-channel traps liquid after overflowing. The device 200 allows for precise control of the liquid positions across all the reaction chambers at the same time. In particular, it enables the use of pneumatic pressure and centrifugal force to control the liquid positions during each reaction step in parallel across all the reaction chambers of the plurality of reaction structures.

[0072] For instance, during initial filling, each connecting siphon is filled and forms a connection to the next reaction structure, *e.g.*, it simply allows for the liquid to overflow from the reaction structure (*e.g.*, the aliquot chamber) N to the reaction structure N+1. After this initial filling, there is a known volume of the liquid inside each reaction structure. Increase of the rotation speed introduces more volume of the liquid to the reaction chamber and increases the radial position of the liquid on the upstream chamber. This ensures that the connecting siphons are emptied. Lowering the speed afterwards decreases the radial position of the liquid on the upstream channel, wetting the connecting siphons again. As this happens on all reaction structures at the same time, both ends (*e.g.*, the inlet and outlet channels) of the connecting siphons are filled at the same time and an air gap is created inside the connecting siphon. This air gap prevents liquid movement between the reaction structures during the mixing process. It has an added benefit of improving the efficiency of mixing by allowing for a larger speed range during mixing - the lowest speed can be lower than the initial filling speed and this would not be possible if there was a simple overflow connection between the chambers.

[0073] The overflow channel allows metering of the reaction volume for each reaction structure. When all the reaction structures fill up to a certain inner radius and once that radius is inwards of the crest 243 of the siphon portion of the overflow channel, it may prime the siphon portion of the overflow channel and may empty all the liquid inward of the inlet 242 of the siphon portion of the overflow channel to the overflow chamber. By adding the U-channel 244 on the outlet

portion of the overflow channel while defining the radial position of the crest 243 to be close to the target desired radial position of the meniscus on the upstream chamber during filling, a need for additional dead volume to allow for the initial emptying is minimized. This ensures that there is sufficient volume left inside the overflow channel when the siphon breaks to create an air gap.

[0074] Moreover, the device can have different reaction structures for single and multiple reagent reactions which work at exactly the same operation speeds and which can be used in parallel on the same fluidic system. For instance, in some embodiments such as the one illustrated in FIG. 2A, the device has at least one structure (1R structure) with a single well (*e.g.*, the first well) for a single reagent reaction and at least one structure (2R structure) with two wells (*e.g.*, the first and second wells) for two reagent reactions on the same device. These may minimize the reaction volume to be very close to the minimum volume required to reconstitute a lyophilized bead (*e.g.*, 1R structure uses $\sim\frac{1}{2}$ volume of 2R structure). In addition, this provides extreme flexibility when designing new cartridges. Referring to FIGS. 3A and 3B, there is shown a flowchart illustrating an exemplary method 300 for running a number of reactions in parallel in accordance with some embodiments of the present disclosure. In the flowchart, the preferred parts of the method are shown in solid line boxes, whereas additional, optional, or alternative parts of the method are shown in dashed line boxes. It should be noted that the processes disclosed herein and exemplified in the flowchart can be, but do not have to be, executed in full or in the order as they are presented.

[0075] Referring to block 302, in some embodiments, the method 300 includes (A) obtaining a device such as the device 200 disclosed herein (*e.g.*, as illustrated in FIG. 2A). The device is rotatable around a rotation axis. In some embodiments, the device includes a plurality of reaction structures and one or more connecting siphons. Reaction structures in the plurality of reaction structures are arranged circumferentially over at least a portion of the device. Each reaction structure in the plurality of reaction structures includes an aliquot chamber having an outlet and a reaction chamber having an inlet connected to the outlet of the aliquot chamber. The reaction chamber is positioned radially outwards of the aliquot chamber. The one or more connecting siphons are radially leveled with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures. Each respective connecting siphon in the

one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures

[0076] Referring to block 304, in some embodiments, the method 300 includes (B) rotating the device at a speed to fill a first portion (*e.g.*, the first well) of the reaction chamber of each reaction structure in the plurality of reaction structures with a fluid. In some embodiments, the device is rotated at a relatively low speed (*e.g.*, about 2000 rpm to about 2500 rpm, or any other suitable speed depending on the implementation). On one hand, this speed is slow enough to not wet the R2 wells prematurely and fast enough to ensure to fill the first wells of all the reaction structures within the allowed time on the workflow. On the other hand, this speed is high enough to avoid surface effects and ensure a repeatable meniscus position during overflow.

[0077] This process is illustrated in FIGS. 4A-4C. Initially, the rotation of the device causes the fluid to fill the first well of the first reaction structure (*e.g.*, the reaction structure 104-1) as shown in FIG. 4A.

[0078] As more fluid flows into the upstream chamber of the first reaction structure, it overflows into the next reaction structure (*e.g.*, the reaction structure 104-2 via the connecting siphon 220-1) and so forth (*e.g.*, the reaction structure 104-3 via the connecting siphon 220-2 and then the reaction structure 104-4 via the connecting siphon 220-3) as shown in FIG. 4B. The time for filling the first wells of all the reaction structures depends at least in part on the number of reaction structures, the upstream flow rate, the rotation speed, or any combination thereof. It may be long or short. In some embodiments, it may take a few seconds, tens of seconds, a minute or more than a minute. During this period, the positions of the fluid inside the reaction structures may fluctuate, for instance, the radial position of the fluid meniscus in the upstream chamber may fluctuate between the radial positions 402-1 and 402-2 and the radial position of the fluid meniscus in the reaction chamber may fluctuate between the radial positions 404-1 and 404-2. It should be noted that the radial positions illustrated in FIG. 4B is by way of illustration and non-limiting. The actual radial positions will depend at least in part on the application (*e.g.*, the volume of fluid, the speed applied) and the configuration of the reaction structure (*e.g.*, the size and shape of the first well and/or the intermediate chamber).

[0079] As the last reaction structure (*e.g.*, the reaction structure 104-4) is filled with the fluid, the liquid position in the aliquot chambers (*e.g.*, the upstream chamber 140) is inwards of the

overflow siphon crest 406 in FIG. 4C (*e.g.*, the radial position of the crest 243 of the siphon portion of the overflow channel 240). As such, the fluid overflows and empties all the excess volume from all reaction structures into the overflow chamber (*e.g.*, the overflow chamber 230). This leaves a metered reaction volume inside each reaction structure that is defined by the connecting siphons and the overflow channel.

[0080] Referring to block 306, in some embodiments, the method 300 includes (C) increasing the speed to empty the fluid from each corresponding connecting siphon in the one or more connecting siphons. In some embodiments, the increasing (C) moves at least a portion of the fluid out of the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned outwards of the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby emptying the fluid from each corresponding connecting siphon in the one or more connecting siphons.

[0081] For instance, as a non-limiting example, FIG. 4D illustrates the increase of the rotation speed (*e.g.*, from between 2000 rpm and about 2500 rpm to between 2500 rpm and about 3000 rpm, or any other suitable speed depending on the implementation) that moves radially inward the meniscus position 404 of the fluid in each reaction chamber and moves radially outward the meniscus position 402 in each upstream chamber (*e.g.*, the aliquot chamber). In some embodiments, the meniscus position 402 and/or the meniscus position 404 are moved to avoid wetting the R2 chambers (*e.g.*, the second wells).

[0082] Referring to block 308, in some embodiments, the method 300 includes (D) decreasing the speed to create a gas volume trapped inside of each corresponding connecting siphon in the one or more connecting siphons, thereby interrupting fluidic connection between the plurality of reaction structures. In some embodiments, the decreasing (D) moves at least a portion of the fluid contained in the reaction chamber of each respective reaction structure in the plurality of reaction structures to the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned inwards of the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby

wetting the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons with the gas volume trapped inside of each corresponding connecting siphon.

[0083] For instance, as a non-limiting example, FIG. 4E illustrates that due to the decreasing of the rotation speed (*e.g.*, from between 2500 rpm and about 3000 rpm to about 900 rpm, or any other suitable speed depending on the implementation), the fluid moves out of the reaction chambers and into the aliquot chambers. In some embodiments, the entire volume of the fluid in the reaction chambers is moved out of the reaction chambers. In some embodiments, only a portion of the volume of the fluid in the reaction chambers is moved out of the reaction chambers. For instance, in some specific implementations, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the volume of the fluid in the reaction chambers is moved out of the reaction chambers. In some other specific implementations, at most 90%, at most 80% or at most 70% of the volume of the fluid in the reaction chambers is moved out of the reaction chambers. In some embodiments, the change of speed promotes mixing. Typically, the higher portion of volume moves in and out, the more efficient the mixing is

[0084] As the fluid moves back into the aliquot chambers, it wets the inlets of the one or more connecting siphons and the overflow channel, forming air gaps between the reaction volumes and between the last reaction volume and the liquid trapped in the overflow channel. The air gaps separate the reaction structures from each other and from the overflow chamber as illustrated in FIG. 2A and 2B.

[0085] Referring to block 310, in some embodiments, the method 300 includes (E) repeating the increasing (C) and decreasing (D) one or more times. For instance, in some embodiments the first portion of the reaction chamber of each reaction structure in the plurality of reaction structures includes a first well with a first reagent, the method includes repeating the increasing (C) and decreasing (D) one or more times to promote mixing of the fluid with the first reagent. In some embodiments, to promote mixing, the increasing (C) and decreasing (D) are repeated at least once, at least 5 times, at least 10 times, at least 15 times, at least 20 times, or any suitable times to meet the requirements of the applications. In some embodiments, the method repeats the mixing step(s) after detection, which can be performed any number of times.

[0086] Referring to block 312, in some embodiments, the method 300 includes (F) detecting, for each reaction structure in the plurality of reaction structure, light transmitted through the first

well or a wall of the first well of the reaction chamber. For instance, in some embodiments, the device is rotated at a speed after the R1 homogenization as illustrated in FIG. 4F, or at any desirable time or stage during the process. The light absorption at one or more wavelengths is read through the readout wells (*e.g.*, the first wells). This may take less than a minute, about a minute, several minutes or any time period depending on the reactions being used and their kinetics.

[0087] Referring to block 312, in some embodiments, the method 300 includes (G) increasing the speed to fill a second portion (*e.g.*, the second well) of the reaction chamber of each reaction structure in at least a subset of the plurality of reaction structures. For instance, as a non-limiting example, FIG. 4G illustrates the increase of the rotation speed (*e.g.*, to about 7500 ~ 8000 rpm or any other suitable speed depending on the applications) to completely fill the second wells and start the second incubation.

[0088] Referring to block 314 and block 316, in some embodiments, the method 300 subsequent to the increasing (H), performing the decreasing (D), and (I) repeating the increasing (G) and performing (H) one or more times. For instance, in some embodiments where the second portion of the reaction chamber of each reaction structure in at least the subset of the plurality of reaction structures includes a second well with a second reagent, the method includes repeating the increasing (G) and performing (H) one or more times to promote mixing of the fluid with the second reagent.

[0089] In some embodiments, to promote mixing, the increasing (G) and performing (H) are repeated at least once, at least 5 times, at least 10 times, at least 15 times, at least 20 times, or any suitable times to meet the requirements of the applications. It should be noted that in the performing (H), the speed can be decreased to the same speed as or different from the speed for promoting the mixing of the fluid in the first wells.

[0090] Referring to block 318, in some embodiments, the method 300 includes (J) detecting, for each reaction structure in at least the subset of the plurality of reaction structure, light transmitted through the first well or a wall of the first well of the reaction chamber. For instance, in some embodiments, the device is rotated at a speed after the R2 homogenization or at any desirable time or stage during the process. The light absorption at one or more wavelengths is read through the readout wells (*e.g.*, the first or second wells). This may take less than a minute,

about a minute, several minutes or any time period depending on the reactions being used and their kinetics.

[0091] The devices and methods of the present disclosure can have additional, optional or alternative components. For instance, in some embodiments, the device may include more than two reagents per reaction chamber (*e.g.*, include one or more additional wells in addition to the first and second wells). Reaction wells themselves can contain more than one bead each. There is no hard limit. It is mostly a tradeoff between the available radial distance, maximum rotation speed and available space for volume aliquoting. In some embodiments, both the first and second wells may be configured as readout wells to allow readouts on both reagent wells on the 2R reaction structure. In some embodiments, emptying may be controlled with an additional channel after the 2R reconstitution to allow for reading while the device is not rotating. In some embodiments, the device may not include the connecting siphon and the reaction chamber of each reaction structure is loaded individually with a controlled volume. In some embodiments, some components are arranged differently to minimize the reaction volume even further.

[0092] Referring to FIG. 7, there is shown a device 700 (*e.g.*, a disc) in accordance with some exemplary embodiments of the present disclosure. The device 700 (*e.g.*, the disc) includes a plurality of units, such as units 710-1, 710-2, 710-3, arranged circumferentially. In some embodiments, the device 700 includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 units. In some embodiments, a unit 710 includes one or more features/components/devices (*e.g.*, the reaction structure 104) disclosed herein. In some embodiments, each unit 710 includes one or more features/components/devices (*e.g.*, the reaction structure 104) disclosed herein. In some embodiments, each unit is identical to another unit in the plurality of units. In some embodiments, at least one unit is different than the other unit(s) in the plurality of units. The device 700 can be used in various applications, including but not limited to clinical chemistry.

[0093] Exemplary Workflow(s)

[0094] FIGS. 5A-5I collectively illustrate an exemplary workflow in accordance with some exemplary embodiments of the present disclosure. While specific specimens (*e.g.*, whole blood) are used in describing the workflow, it should be noted that the present disclosure is not limited thereto. Other samples, such as those disclosed herein, can be used. In addition, the workflow can be automated.

[0095] Referring to FIG. 5A, is a schematic diagram illustrating a device (*e.g.*, a disc) in accordance with some exemplary embodiments of the present disclosure. For clarity, only a portion of the device is shown. The device 500 is rotatable about a rotational axis, such as the vertical rotational axis 503. In some implementations, the device 500 may be rotated, during one or more processes, at a speed of at least about 1000 rpm, at least about 1200 rpm, at least about 1400 rpm, at least about 1600 rpm, at least about 1800 rpm, at least about 2000 rpm, at least about 2200 rpm, at least about 2400 rpm, at least about 2600 rpm, at least about 2800 rpm, at least about 2900 rpm, at least about 3000 rpm, at least about 3500 rpm, at least about 4000 rpm, at least about 4500 rpm, at least about 5000 rpm, at least about 5500 rpm, at least about 6000 rpm, at least about 6500 rpm, or at least about 7000 rpm. In some implementations, the device 500 may be rotated, during one or more processes, at a speed of at most about 500 rpm, at most about 600 rpm, at most about 700 rpm, at most about 800 rpm, at most about 900 rpm, at most about 1000 rpm, at most about 1200 rpm, at most about 1400 rpm, at most about 1600 rpm, at most about 1800 rpm, at most about 2000 rpm, at most about 2200 rpm, at most about 2400 rpm, at most about 2600 rpm, at most about 2800 rpm, at most about 2900 rpm, at most about 3000 rpm, at most about 3500 rpm, at most about 4000 rpm, at most about 4500 rpm, or at most about 5000 rpm.

[0096] The device 500 includes one or more chambers, one or more channels, and/or other features/components. For instance, in the illustrated embodiments, the device 500 includes a vent channel 501, a sample chamber 502, a buffer chamber 504, a blood separation chamber 506, a sample overflow chamber 508, an additional plasma overflow chamber 509, a sample metering chamber 510, a mixing chamber 512, a diluted sample overflow chamber 514, a pneumatic chamber 515, a pneumatic and dilution buffer overflow chamber 516, one or more 1R/detection chambers 518 (*e.g.*, the first well 121 of the reaction chamber 110 depicted in FIG. 1C), one or more 2R R1/detection chambers 520 (*e.g.*, the first well 121 of the reaction chamber 110 depicted in FIG. 1A), one or more 2R R2 chambers 522 (*e.g.*, the first well 122 of the reaction chamber 110 depicted in FIG. 1A), one or more aliquoting chambers 524, and a mixing/metering chamber 526.

[0097] While the device 500 is illustrated with specific components (*e.g.*, specific chambers, channels), it should be noted that this is by way of example and is non-limiting. In some

implementations, the device 500 may not include one or more of these specific components. In some implementations, the device 500 may include additional or alternative components such as those disclosed herein. In addition, the device 500 can be used in various applications, including but not limited to clinical chemistry.

[0098] Referring to FIG. 5B, there is illustrated a loading process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, buffer and sample (e.g., whole blood) are loaded to the device 500. For instance, in some implementations, a buffer is loaded into the buffer chamber 504, and the whole blood is loaded into the sample chamber 502.

[0099] Referring to FIG. 5C, there is illustrated a separation and metering process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, in this process, the whole blood is separated, and buffer is metered. For instance, in some implementations, the device 500 is rotated about the vertical rotation axis 503. The whole blood flows from the sample chamber 502 to the blood separation chamber 506, and separates into plasma (radially inward) and cellular fractions (radially outward). The buffer flows from the buffer chamber 504, through the mixing chamber 512, into mixing/metering chamber 526, where it is metered. If there is excess buffer, the excess buffer flows into the pneumatic and dilution buffer overflow chamber 516. In this process, back pressure is built up by the trapped air in the pneumatic and dilution buffer overflow chamber 516. Back pressure in the pneumatic and dilution buffer overflow chamber 516 is a function of the rotational speed of the device 500.

[00100] Referring to FIG. 5D, there is illustrated a metering process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, in this process, the plasma is metered. For instance, in some implementations, the rotational speed of disc 500 is decreased. As the rotational speed of disc 500 is decreased, the metered buffer flows from the mixing/metering chamber 526 to the mixing chamber 512, due to the decreased pressure in the pneumatic and dilution buffer overflow chamber 516. In addition, as the speed of disc 500 decreases, the plasma in the blood separation chamber 506 flows through a connecting siphon towards the sample metering chamber 510. In some implementations, the plasma completely fills the sample metering chamber 510, with the excess plasma, if any, overflows into the

additional plasma overflow chamber 509. At this point in the process, both the plasma and buffer have been metered.

[00101] Referring to FIGS. 5E-1 and 5E-2, there is illustrated a diluting process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, in this process, the metered plasma is diluted and/or mixed with the metered buffer. For instance, in some implementations, the rotational speed of the device 500 is increased. By increasing the rotational speed of the device 500, the metered plasma flows from the sample metering chamber 510 to the mixing chamber 512. Then, the rotational speed of the device 500 is decreased, causing the trapped air in the pneumatic and dilution buffer overflow chamber 516 to expand, thereby pushing the metered buffer from the mixing/metering chamber 526 into the mixing chamber 512. To mix the metered plasma and metered buffer, the rotational speed of the device 500 is increased and decreased, causing the trapped air in the pneumatic and dilution buffer overflow chamber 516 to expand and contract and forcing the mixture of the metered buffer and metered plasma back and forth between the mixing/metering chamber 526 and the mixing chamber 512. Increasing/decreasing the rotational speed of the device 500 can be repeated as desired, programed, or until the solution is adequately/thoroughly mixed.

[00102] Referring to FIGS. 5F-1 and 5F-2, there is illustrated a transferring and dissolving process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, in this process, the diluted plasma buffer mixture is transferred to the outer most detection chambers, dissolving lyophilized reagents in those chambers. For instance, in some implementations, the rotational speed of the device 500 is initially decreased, priming the connecting channel(s) between the mixing chamber 512 and the aliquoting chamber(s) 524. Then, the rotational speed of the device 500 is increased to transfer the diluted plasma buffer mixture into the aliquoting chamber(s) 524, the 2R R1/detection chamber(s) 520, and 1R/detection chamber(s) 518, with excess (if any) flowing into the diluted sample overflow 514. FIG. 5F-1 this step before it's complete, while FIG. 5F-2 shows complete transfer. The diluted plasma buffer mixture does not reach the 2R R2 chambers 522 in this step, due to the back pressure in the pneumatic chamber 515.

[00103] Referring to FIG. 5G, there is illustrated a mixing process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, in this process,

the dissolved lyophilized reagents and diluted plasma buffer mixture are adequately or thoroughly mixed. For instance, in some implementations, the rotational speed of the device 500 is increased and decreased, causing the trapped air in the pneumatic chamber 515 (and/or other pneumatic chambers connected to the 2R R1/detection chamber(s) 520 and 1R/detection chamber(s) 518) to expand and contract, forcing the mixture back and forth between the 2R R1/detection chamber 520, the 1R/detection chamber 518, and the aliquoting chamber 524. Increasing/decreasing the rotational speed of the device 500 can be repeated as desired, programed, or until adequate/thorough mixing is achieved. Once the mixing is complete (*e.g.*, once adequate mixing is achieved), the reaction mixture can be transferred back to the 2R R1/detection chamber(s) 520 and the 1R/detection chamber(s) 518, where optical measurements can be made if desired.

[00104] Referring to FIG. 5H, there is illustrated a dissolving process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, in this process, lyophilized reagents in the 2R R2 chamber(s) 522 are dissolved. For instance, in some implementations, the rotational speed of the device 500 is increased, causing the trapped air in the pneumatic chamber 515 (and/or other pneumatic chambers connected to the 2R R1/detection chamber(s) 520 and the 1R/detection chamber(s) 518) to contract, forcing the mixture into the 2R R2 chamber(s) 522, dissolving lyophilized reagents in the 2R R2 chamber(s) 522.

[00105] Referring to FIG. 5I, there is illustrated a mixing and detection process in accordance with some exemplary embodiments of the present disclosure. In some embodiments, in this process, the mixture of lyophilized reagents and diluted buffer are mixed, and/or optical measurements are made. For instance, in some implementations, the rotational speed of the device 500 is increased and decreased, causing the trapped air in the pneumatic chamber 515 (and/or other pneumatic chambers connected to the 2R R1/detection chamber(s) 520 and the 1R/detection chamber(s) 518) to expand and contract, forcing the mixture back and forth between the 2R R2 chamber 522, the 2R R1/detection chamber 520, the 1R/detection chamber 518, and the aliquoting chamber 524. Increasing/decreasing the rotational speed of the device 500 can be repeated as desired, programed, or until adequate/thorough mixing is achieved. Once the mixing is complete (*e.g.*, once adequate mixing is achieved), the reaction mixture can be transferred back to the 2R R2 chamber 522, the 2R R1/detection chamber 520, and the 1R/detection chamber 518, where optical measurements can be made, if desired.

[00106] In some embodiments, the workflow is complete at this point. The rotation of the device 600 can be stopped, and the device 100 can be discarded.

[00107] Referring to FIG. 6, there is illustrated an exemplary workflow 600 (*e.g.*, workflow) in accordance with some exemplary embodiments of the present disclosure. The workflow 600 can be performed on any devices disclosed herein (*e.g.*, the device 500). The workflow 600 can also be automated.

[00108] In some embodiments, the workflow 600 includes a process 602 that loads a buffer (*e.g.*, water) to the device and a process 606 that loads a sample (*e.g.*, whole blood) to the device. In some embodiments, the process 602 and the process 606 are the same as or similar to those disclosed herein with respect to FIG. 5B.

[00109] In some embodiments, the workflow 600 includes a process 604 that meters the buffer and a process 608 that separates the sample (*e.g.*, separating the whole blood into plasma and cellular fractions). In some embodiments, the process 604 and the process 608 are the same as or similar to those disclosed herein with respect to FIG. 5C.

[00110] In some embodiments, the workflow 600 includes a process 610 that meters the sample or a component of the sample. For instance, in embodiments where the sample is whole blood that has been separated into plasma and cellular fractions, the process 610 meters the plasma. In some embodiments, the process 610 is the same as or similar to those disclosed herein with respect to FIG. 5D.

[00111] In some embodiments, the workflow 600 includes a process 612 that mixes the metered buffer and metered sample (*e.g.*, metered plasma). In some embodiments, the process 612 is the same as or similar to those disclosed herein with respect to FIGS. 5E-1 and 5E-2.

[00112] In some embodiments, the workflow 600 includes a process 614 that aliquots the mixture of the metered buffer and metered sample (*e.g.*, metered plasma). In some embodiments, the process 614 is the same as or similar to those disclosed herein with respect to FIGS. 5F-1 and 5F-2.

[00113] In some embodiments, the workflow 600 includes a process 616 that resuspends the dry R1 bead(s), *e.g.*, dissolving lyophilized reagents in the 2R R1/detection chamber(s) 520,

and 1R/detection chamber(s) 518. In some embodiments, the process 616 is the same as or similar to those disclosed herein with respect to FIGS. 5F-1 and 5F-2.

[00114] In some embodiments, the workflow includes a process 618 that incubates the mixture of the metered buffer, metered plasma and dissolved lyophilized reagents. In some embodiments, the process 618 may be performed under a controlled environment (*e.g.*, with a controlled temperature and/or over a predetermined time period).

[00115] In some embodiments, the workflow 600 includes a process 620 that resuspends the dry R2 bead(s), *e.g.*, dissolving lyophilized reagents in the 2R R2 chamber(s) 522. In some embodiments, the process 620 is the same as or similar to those disclosed herein with respect to FIG. 5H.

[00116] In some embodiments, the workflow 600 includes a process 622 that incubates the mixture of the metered buffer, metered plasma and dissolved lyophilized reagents. Like the process 618, in some embodiments, the process 622 may be performed under a controlled environment (*e.g.*, with a controlled temperature and/or over a predetermined time period).

[00117] In some embodiments, the workflow 600 may include one or more measurements, which may be formed at any suitable stage when desired. For instance, a measurement may be performed to measure an absorbance before, during, or after the lyophilized reagents is dissolved. A measurement may be performed the same as or similar to those disclosed herein with respect to FIGS. 5G and 5I. In some embodiments, the workflow 600 may include a measurement process 624 after resuspending the dry R1 bead(s), a measurement process 626 after the first incubation but before resuspending the dry R2 bead(s), a measurement process 628 after resuspending the dry R2 bead(s) but before the second incubation, a measurement process 630 after the second incubation, or any combination thereof.

[00118] The devices and methods of the present disclosure have a number of advantages. For instance, the devices of the present disclosure are more compact with no active valves. They are independent of coatings/surface tension and the total number of reactions structures and reactions. They are also insensitive to sample/biological variability and are easily prototypical (not dependent on materials, surface roughness, etc.). The devices and methods of the present disclosure allow for well controlled mixing strategy, readout during mixing cycles, and running of multiple reaction methodologies simultaneously.

[00119] The devices and methods disclosed herein can be used in a variety of applications including but not limited to clinical chemistry, immunoassays and hematology. Examples of clinical chemistry, immunoassays and/or hematology are disclosed in WO2018/119437, WO2018/140719, WO2022/029731, and WO 2022/029732, the content of each application is hereby incorporated by reference in its entirety. The devices and methods disclosed herein may be operated or performed by a system similar to those disclosed in U.S. Patent Application No. 17/371,746, the content of which is hereby incorporated by reference in its entirety.

[00120] Illustration of Subject Technology as Clauses

[00121] Various examples of aspects of the disclosure are described as numbered clauses (1, 2, 3, etc.) for convenience. These are provided as examples, and do not limit the subject technology.

[00122] Clause 1. A device comprising: a rotational axis; and a reaction chamber comprising an inlet to receive a fluid and a first well to serve as both reagent and readout wells, wherein (i) a first reagent is disposed in the first well when the device is made, (ii) a radial position of a meniscus of the fluid in the reaction chamber depends at least in part on a rotational speed of the device, and (iii) the first well has a substantially flat wall perpendicular to the rotational axis to allow reproducible light transmission. In some embodiments, the connection between inlet and first well is designed to ensure that some portion of the liquid inside the first well can move back into the inlet.

[00123] Clause 2. The device of Clause 1, wherein the first reagent is lyophilized.

[00124] Clause 3. The device of any preceding Clause, wherein the first reagent is in bead form.

[00125] Clause 4. The device of any preceding Clause, wherein the reaction chamber is unvented to promote homogeneous mixing of the first reagent with the fluid.

[00126] Clause 5. The device of Clause 4, wherein the reaction chamber comprises an outlet connected to an air chamber.

[00127] Clause 6. The device of any preceding Clause, further comprising: an upstream chamber positioned radially inwards of the reaction chamber and connected to the inlet of the reaction chamber.

[00128] Clause 7. The device of any preceding Clause, wherein the first well is connected to an air chamber.

[00129] Clause 8. The device of any one of Clauses 1-4, wherein the reaction chamber further comprises a second well and an intermediate chamber between the first and second wells, wherein a second reagent is disposed in the second well when the device is made. In some embodiments, the reaction structure or chamber is unvented, such that the meniscus level in the aliquot chamber is variable during operation or the like.

[00130] Clause 9. The device of Clause 8, wherein the second reagent is different than the first reagent.

[00131] Clause 10. The device of any one of Clauses 8-9, wherein the inlet is located at the first well, and the second well is positioned radially inwards of the first well.

[00132] Clause 11. The device of Clause 10, wherein the outlet is located at the second well.

[00133] Clause 12. The device of any one of Clauses 8-11, wherein the reaction chamber, the upstream chamber, the air chamber, or a combination thereof are configured to allow the initial fill of the first well with the fluid without wetting the second well.

[00134] Clause 13. A device comprising: a rotational axis; a plurality of reaction structures arranged circumferentially over at least a portion of the device, wherein each reaction structure in the plurality of reaction structures comprises an aliquot chamber having an outlet and a reaction chamber having an inlet connected to the outlet of the aliquot chamber, wherein the reaction chamber is positioned radially outwards of the aliquot chamber; and one or more connecting siphons radially leveled with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures, wherein each respective connecting siphon in the one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures.

[00135] Clause 14. The device of Clause 13, wherein for each reaction structure in the plurality of reaction structures, the reaction chamber comprises a first well.

[00136] Clause 15. The device of Clause 14, wherein for each reaction structure in the plurality of reaction structures, a first reagent is disposed in the first well when the device is made.

[00137] Clause 16. The device of any one of Clauses 14-15, wherein for each reaction structure in the plurality of reaction structures, the first well serves as both reagent and readout wells.

[00138] Clause 17. The device of any one of Clauses 13-16, wherein the first wells of the plurality of reaction structures are radially leveled with each other.

[00139] Clause 18. The device of any one of Clauses 13-17, wherein for each reaction structure in at least a subset of the plurality of reaction structures, the reaction chamber comprises a second well and an intermediate chamber between the first and second wells.

[00140] Clause 19. The device of Clause 18, wherein for each reaction structure in at least the subset of the plurality of reaction structures, a second reagent is disposed in the second well when the device is made.

[00141] Clause 20. The device of any one of Clauses 18-19, wherein the second wells of at least the subset of the plurality of reaction structures are radially leveled with each other.

[00142] Clause 21. The device of any one of Clauses 18-20, wherein the inlet of the reaction chamber is located at the first well, and the second well is positioned radially inwards of the first well.

[00143] Clause 22. The device of any one of Clauses 13-21, wherein each respective reaction structure in the plurality of reaction structures comprises an air chamber, wherein the reaction chamber of the respective reaction structure has an outlet connected to the air chamber.

[00144] Clause 23. The device of any one of Clauses 13-22, further comprising: an overflow chamber; and an overflow channel connecting the aliquot chamber of a last reaction structure in the plurality of reaction structures to the overflow chamber.

[00145] Clause 24. The device of Clause 23, wherein the overflow channel comprises a siphon portion and the siphon portion comprises (optionally or additionally): an inlet radially leveled with an inlet and an outlet of each connecting siphon in the one or more siphons; and a

crest leveled with or radially outwards of a crest of each connecting siphon in the one or more siphons.

[00146] Clause 25. The device of Clause 24, wherein the overflow channel further comprises a U-channel having an outlet connected to the overflow chamber. In some embodiments, the outlet of U-channel is radially outwards of the siphon inlet. In some embodiments, the U-channel outer crest is outwards of the channel outlet.

[00147] Clause 26. A method for using the device of any preceding Clause to mix one or more fluids, resuspend reagents, detect mixing or reaction, or a combination thereof.

[00148] Clause 27. A method comprising: (A) obtaining a device comprising: a rotational axis; and a plurality of reaction structures arranged circumferentially over at least a portion of the device, wherein each reaction structure in the plurality of reaction structures comprises an aliquot chamber having an outlet and a reaction chamber having an inlet connected to the outlet of the aliquot chamber, wherein the reaction chamber is positioned radially outwards of the aliquot chamber; and one or more connecting siphons radially leveled with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures, wherein each respective connecting siphon in the one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures; (B) rotating the device at a speed to fill a first portion of the reaction chamber of each reaction structure in the plurality of reaction structures with a fluid; (C) increasing the speed to empty the fluid from each corresponding connecting siphon in the one or more connecting siphons; and (D) decreasing the speed to create a gas volume trapped inside of each corresponding connecting siphon in the one or more connecting siphons, thereby interrupting fluidic connection between the plurality of reaction structures. In some embodiments, the reaction chamber is unvented

[00149] Clause 28. The method of Clause 27, wherein the increasing (C) moves at least a portion of the fluid out of the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned outwards of either the inlet or outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby emptying the fluid from each corresponding connecting siphon in the one or more connecting siphons.

[00150] Clause 29. The method of any one of Claims 27-28, wherein the decreasing (D) moves at least a portion of the fluid contained in the reaction chamber of each respective reaction structure in the plurality of reaction structures to the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned inwards of the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby wetting the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons with the gas volume trapped inside of each corresponding connecting siphon.

[00151] Clause 30. The method of any one of Claims 27-29, wherein the first portion of the reaction chamber of each reaction structure in the plurality of reaction structures comprises a first well with a first reagent, the method further comprising: (E) repeating the increasing (C) and decreasing (D) one or more times to promote mixing of the fluid with the first reagent.

[00152] Clause 31. The method of any one of Claims 30, further comprising: (F) detecting, for each reaction structure in the plurality of reaction structure, light transmitted through the first well or a wall of the first well of the reaction chamber.

[00153] Clause 32. The method of any one of Claims 27-31, further comprising: (G) increasing the speed to fill a second portion of the reaction chamber of each reaction structure in at least a subset of the plurality of reaction structures; and (H) performing the decreasing (D).

[00154] Clause 33. The method of Claim 32, wherein the second portion of the reaction chamber of each reaction structure in at least the subset of the plurality of reaction structures comprises a second well with a second reagent, the method further comprising: (I) repeating the increasing (G) and performing (H) one or more times to promote mixing of the fluid with the second reagent.

[00155] Clause 34. The method of any one of Claims 33, further comprising: (J) detecting, for each reaction structure in at least the subset of the plurality of reaction structure, light transmitted through the first well or a wall of the first well of the reaction chamber.

[00156] Clause 35. A system for operating the device or performing the method of any preceding Claim.

TERMINOLOGIES AND REFERENCES CITED

[00157] The terminology used herein is for the purpose of describing particular implementations only and is not intended to be limiting of the claims. As used in the description of the implementations and the appended claims, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be understood that the terms “left” or “right”, “top” or “bottom”, “lower” or “upper”, “interior” or “exterior”, “inward” or “outward” and etc. are used to describe features of the exemplary embodiments with reference to the positions of such features as displayed in the figures. It will be understood that, although the terms “first,” “second,” etc. may be used herein to describe various elements, these elements should not be limited by these terms. These terms are only used to distinguish one element from another. For example, a first element could be termed a second element, and, similarly, a second element could be termed a first element, without changing the meaning of the description, so long as the “first element” and the “second element” are renamed consistently.

[00158] As used herein, the term “and/or” as used herein refers to and encompasses any and all possible combinations of one or more of the associated listed items. It will be further understood that the terms “include”, “includes”, “including”, “comprise”, “comprises” and/or “comprising,” when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

[00159] The term “about” or “approximately” is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number, which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number. It should be appreciated that all numerical values and ranges disclosed herein are approximate values and ranges, whether “about” is used in conjunction therewith. It should also be appreciated that the term “about,” as used herein, in conjunction with

a numeral refers to a value that may be $\pm 0.01\%$ (inclusive), $\pm 0.1\%$ (inclusive), $\pm 0.5\%$ (inclusive), $\pm 1\%$ (inclusive) of that numeral, $\pm 2\%$ (inclusive) of that numeral, $\pm 3\%$ (inclusive) of that numeral, $\pm 5\%$ (inclusive) of that numeral, $\pm 10\%$ (inclusive) of that numeral, or $\pm 15\%$ (inclusive) of that numeral. It should further be appreciated that when a numerical range is disclosed herein, any numerical value falling within the range is also specifically disclosed.

[00160] The term “if” used herein is, optionally, construed to mean “when” or “upon” or “in response to determining” or “in response to detecting” or “in accordance with a determination that,” depending on the context. Similarly, the phrase “if it is determined” or “if [a stated condition or event] is detected” used herein is, optionally, construed to mean “upon determining” or “in response to determining” or “upon detecting [the stated condition or event]” or “in response to detecting [the stated condition or event]” or “in accordance with a determination that [a stated condition or event] is detected,” depending on the context.

[00161] When a reference number is given an “ i^{th} ” denotation, the reference number refers to a generic component, set, or embodiment. For instance, a “unit i ” refers to the i^{th} unit in a plurality of units.

[00162] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

CLAIMS

What is claimed is:

1. A device comprising:
a rotational axis; and
a reaction chamber comprising an inlet to receive a fluid and a first well to serve as both reagent and readout wells, wherein (i) a first reagent is disposed in the first well when the device is made, (ii) a radial position of a meniscus of the fluid in the reaction chamber depends at least in part on a rotational speed of the device, and (iii) the first well has a substantially flat wall perpendicular to the rotational axis to allow reproducible light transmission.
2. The device of claim 1, wherein the reaction chamber is unvented to promote homogeneous mixing of the first reagent with the fluid.
3. The device of claim 2, wherein the reaction chamber comprises an outlet connected to an air chamber.
4. The device of claim 3, wherein the first well is connected to the air chamber.
5. The device of claim 3, wherein the reaction chamber further comprises a second well and an intermediate chamber between the first and second wells, wherein a second reagent is disposed in the second well when the device is made.
6. The device of claim 5, wherein the inlet is located at the first well, and the second well is positioned radially inwards of the first well.
7. The device of claim 6, wherein the outlet is located at the second well.
8. A device comprising:
a rotational axis;
a plurality of reaction structures arranged circumferentially over at least a portion of the device, wherein each reaction structure in the plurality of reaction structures comprises an aliquot

chamber having an outlet and a reaction chamber having an inlet connected to the outlet of the aliquot chamber, wherein the reaction chamber is positioned radially outwards of the aliquot chamber; and

one or more connecting siphons radially leveled with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures, wherein each respective connecting siphon in the one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures.

9. The device of claim 8, wherein for each reaction structure in the plurality of reaction structures, the reaction chamber comprises a first well, and a first reagent is disposed in the first well.

10. The device of claim 9, wherein for a respective reaction structure in the plurality of reaction structures, the corresponding first well serves as both reagent and readout wells.

11. The device of claim 8, wherein the first wells of the plurality of reaction structures are radially leveled with each other.

12. The device of claim 9, wherein for each reaction structure in at least a subset of the plurality of reaction structures, the reaction chamber comprises a second well and an intermediate chamber between the first and second wells, and a second reagent is disposed in the second well.

13. The device of claim 12, wherein the second wells of at least the subset of the plurality of reaction structures are radially leveled with each other.

14. The device of claim 12, wherein the inlet of the reaction chamber is located at the first well, and the second well is positioned radially inwards of the first well.

15. The device of claim 8, wherein each respective reaction structure in the plurality of reaction structures comprises an air chamber, wherein the reaction chamber of the respective reaction structure has an outlet connected to the air chamber.

16. The device of claim 8, further comprising:
an overflow chamber; and
an overflow channel connecting the aliquot chamber of a last reaction structure in the plurality of reaction structures to the overflow chamber.
17. The device of claim 16, wherein the overflow channel comprises a siphon portion and the siphon portion comprises:
an inlet radially leveled with an inlet and an outlet of each connecting siphon in the one or more siphons; and
a crest leveled with or radially outwards of a crest of each connecting siphon in the one or more siphons.
18. The device of claim 17, wherein the overflow channel further comprises a U-channel having an outlet connected to the overflow chamber.
19. A method comprising:
(A) obtaining a device comprising:
a rotational axis; and
a plurality of reaction structures arranged circumferentially over at least a portion of the device, wherein each reaction structure in the plurality of reaction structures comprises an aliquot chamber having an outlet and a reaction chamber having an inlet connected to the outlet of the aliquot chamber, wherein the reaction chamber is positioned radially outwards of the aliquot chamber; and
one or more connecting siphons radially leveled with each other and radially inwards of the outlets of the aliquot chambers of the plurality of reaction structures, wherein each respective connecting siphon in the one or more connecting siphons connects the aliquot chambers of corresponding adjacent reaction structures in the plurality of reaction structures;
(B) rotating the device at a speed to fill a first portion of the reaction chamber of each reaction structure in the plurality of reaction structures with a fluid;
(C) increasing the speed to empty the fluid from each corresponding connecting siphon in the one or more connecting siphons; and

(D) decreasing the speed to create a gas volume trapped inside of each corresponding connecting siphon in the one or more connecting siphons, thereby interrupting fluidic connection between the plurality of reaction structures.

20. The method of claim 19, wherein the increasing (C) moves at least a portion of the fluid out of the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned outwards of either the inlet or outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby emptying the fluid from each corresponding connecting siphon in the one or more connecting siphons.

21. The method of claim 19, wherein the decreasing (D) moves at least a portion of the fluid contained in the reaction chamber of each respective reaction structure in the plurality of reaction structures to the aliquot chamber of each respective reaction structure in the plurality of reaction structures such that the fluid contained in the aliquot chamber of each respective reaction structure in the plurality of reaction structures is positioned inwards of the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons, thereby wetting the inlet and outlet of each corresponding connecting siphon in the one or more connecting siphons with the gas volume trapped inside of each corresponding connecting siphon.

22. The method of claim 19, wherein the first portion of the reaction chamber of each reaction structure in the plurality of reaction structures comprises a first well with a first reagent, the method further comprising:

(E) repeating the increasing (C) and decreasing (D) one or more times to promote mixing of the fluid with the first reagent.

23. The method of claim 22, further comprising:

(F) detecting, for each reaction structure in the plurality of reaction structure, light transmitted through the first well or a wall of the first well of the reaction chamber.

24. The method of claim 19, further comprising:

(G) increasing the speed to fill a second portion of the reaction chamber of each reaction structure in at least a subset of the plurality of reaction structures; and

(H) performing the decreasing (D).

25. The method of claim 24, wherein the second portion of the reaction chamber of each reaction structure in at least the subset of the plurality of reaction structures comprises a second well with a second reagent, the method further comprising:

(I) repeating the increasing (G) and performing (H) one or more times to promote mixing of the fluid with the second reagent.

26. The method of claim 25, further comprising:

(J) detecting, for each reaction structure in at least the subset of the plurality of reaction structure, light transmitted through the first well or a wall of the first well of the reaction chamber.