US 20230405585A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2023/0405585 A1

YAMANA et al. (43) Pub. Date: Dec. 21, 2023

(54) **APPARATUSES WITH FLUIDIC CHANNEL GEOMETRIES FOR SAMPLE TO ANSWER PCR ANALYSIS AND METHODS OF USING SAME**

(71) Applicant: **FORMULATRIX, INC**, Bedford, MA (US)

(72) Inventors: **Kabir YAMANA**, Bedford, MA (US); **Michael NILSSON**, Bedford, MA (US)

(73) Assignee: **Formulatrix International Holding Ltd.**, Dubai (AE)

(21) Appl. No.: **18/032,463**

(22) PCT Filed: **Oct. 19, 2021**

(86) PCT No.: **PCT/US2021/055649**

§ 371 (c)(1),
(2) Date: **Apr. 18, 2023**

Related U.S. Application Data

(60) Provisional application No. 63/093,640, filed on Oct. 19, 2020.

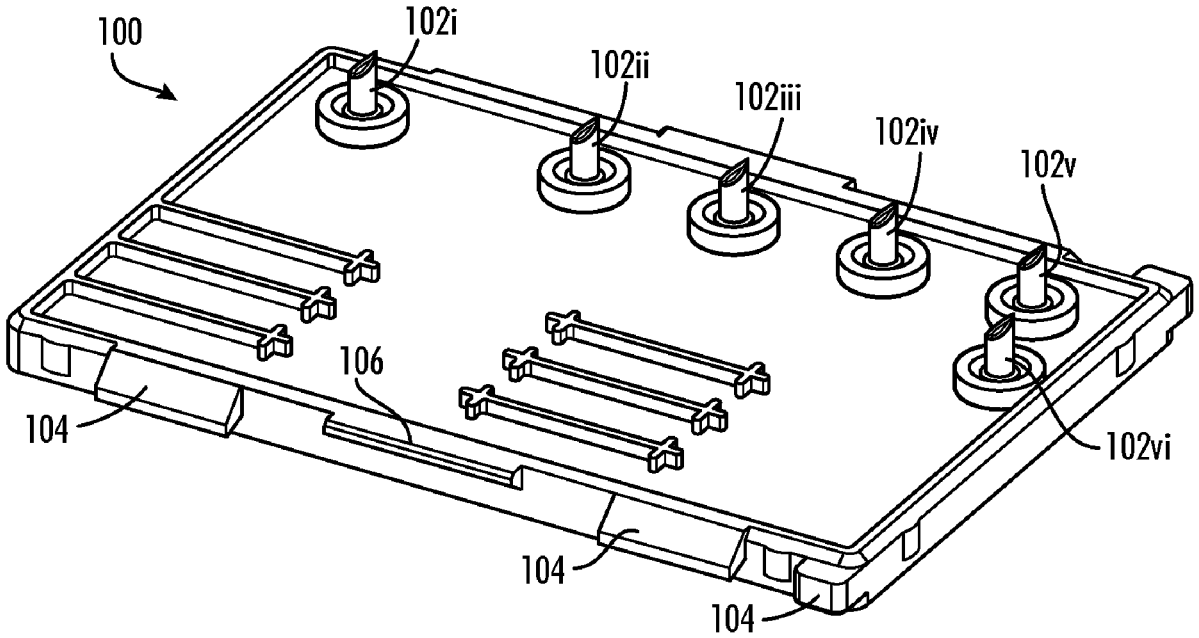
Publication Classification

(51) **Int. Cl.**
B01L 3/00 (2006.01)

(52) **U.S. Cl.**
CPC ... ***B01L 3/502715*** (2013.01); ***B01L 3/502761*** (2013.01); *B01L 2200/027* (2013.01); *B01L 2200/10* (2013.01); *B01L 2300/044* (2013.01); *B01L 2300/0887* (2013.01); *B01L 2400/043* (2013.01); *B01L 2400/0683* (2013.01); *B01L 2300/0672* (2013.01); *B01L 2300/0883* (2013.01)

(57) **ABSTRACT**

Various embodiments for a chip for use in a real-time qPCR system are disclosed. The chip can include at least one port for receiving a sample into the chip; at least one channel in fluidic communication with the at least port; a plurality of magnetically active beads disposed within the at least one channel that capture DNA/RNA from the sample as the sample passes through the at least one channel; and an optical inspection region in fluidic communication with the at least one channel for performing an optical analysis of the sample containing the eluted DNA/RNA previously captured on the magnetic beads.



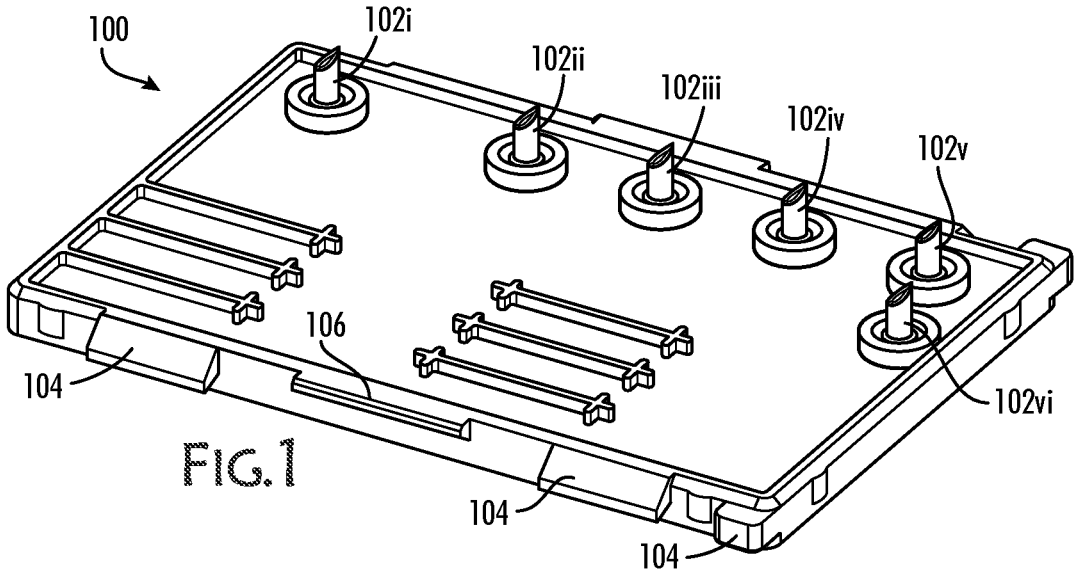

100
102i
102ii
102iii
102iv
102v
102vi
104
106
104
104

FIG.1

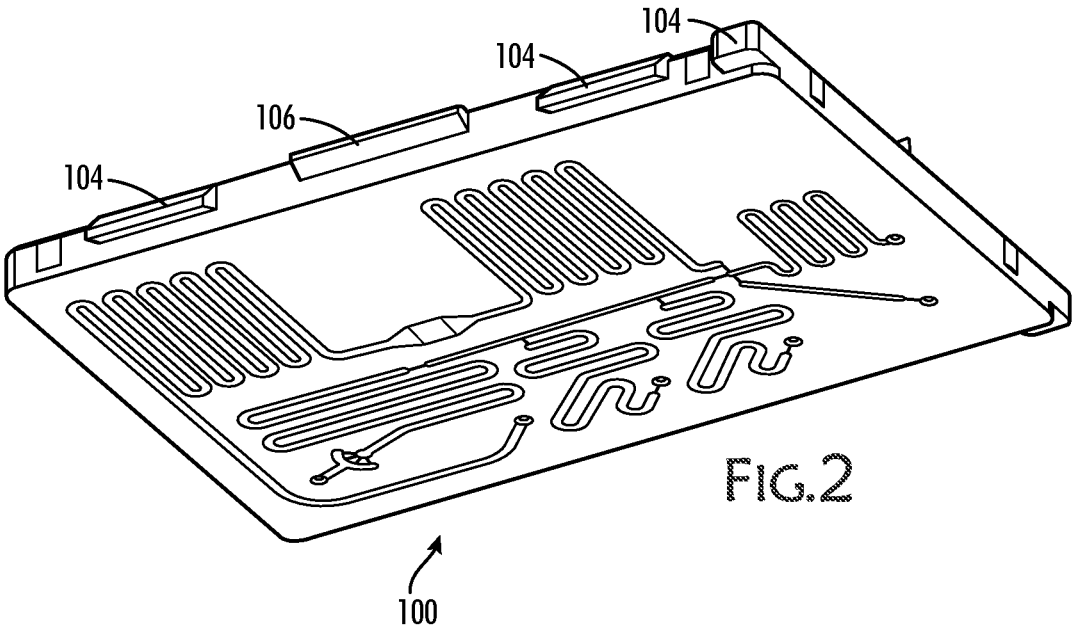

104
104
106
104
100

FIG.2

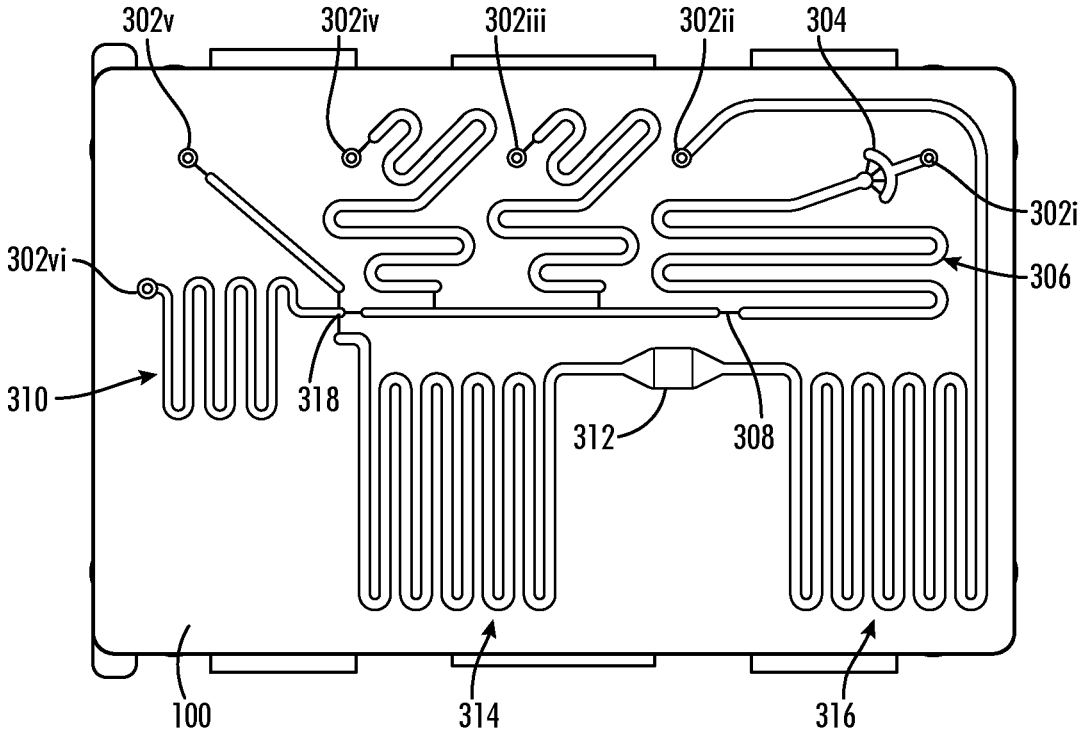

302v
302iv
302iii
302ii
304
302i
302vi
306
310
318
312
308
100
314
316

FIG. 3

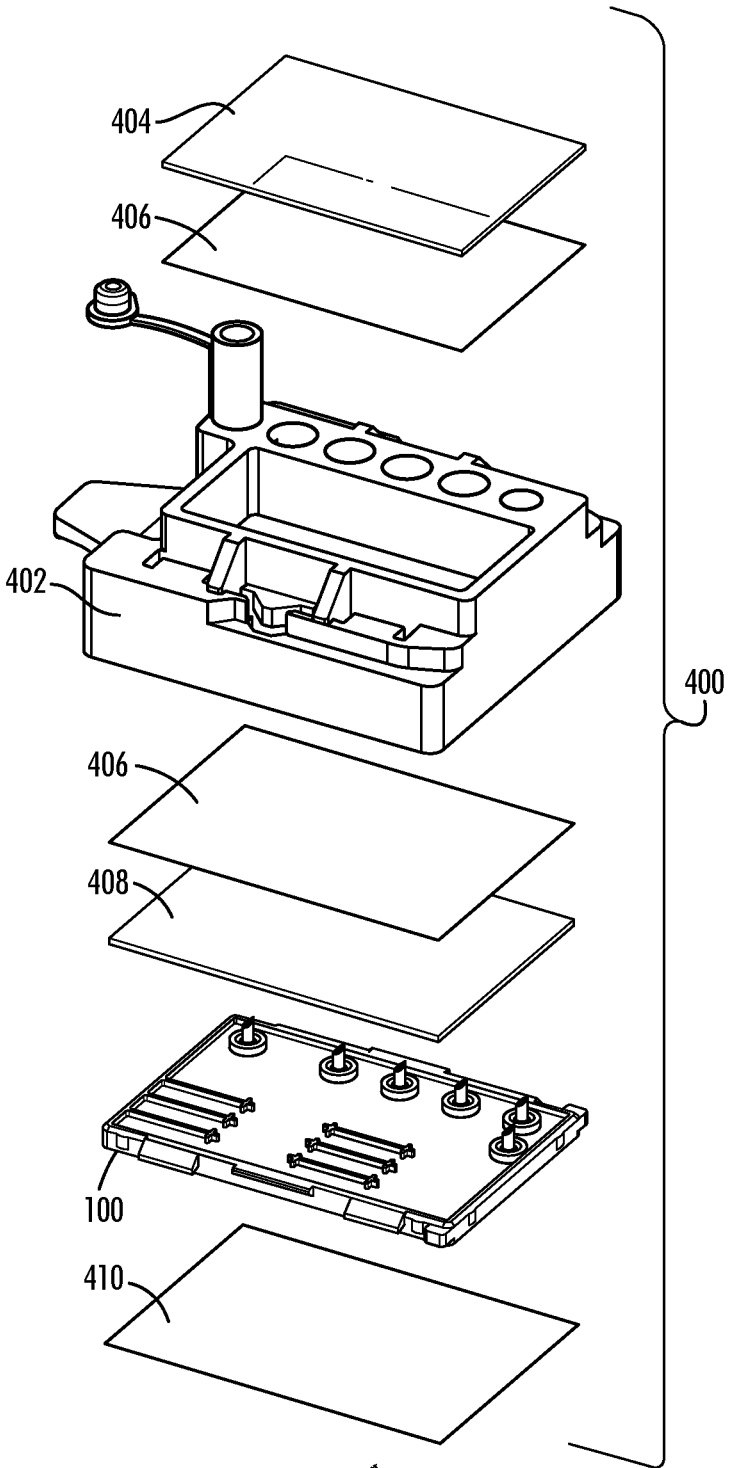


FIG.4

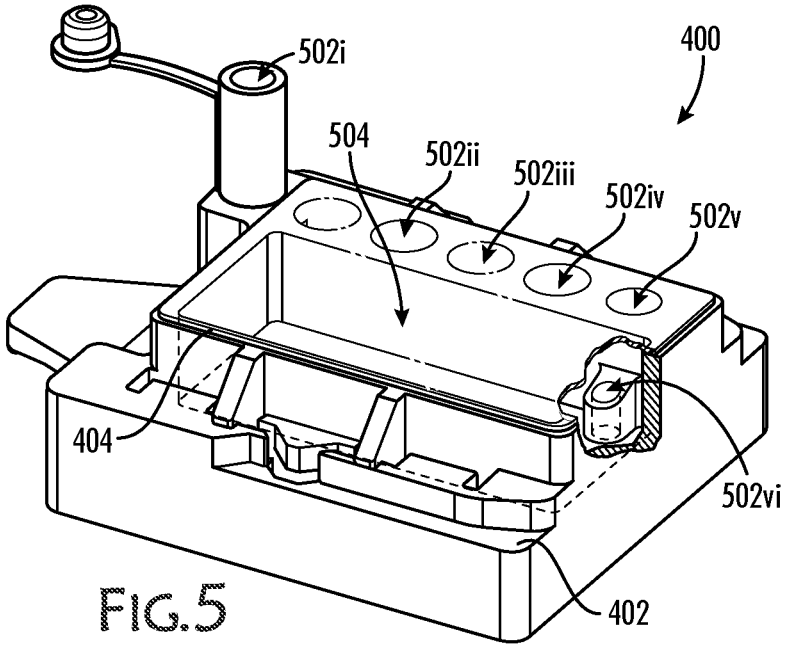

502i
400
504
502ii
502iii
502iv
502v
404
502vi
402

FIG.5

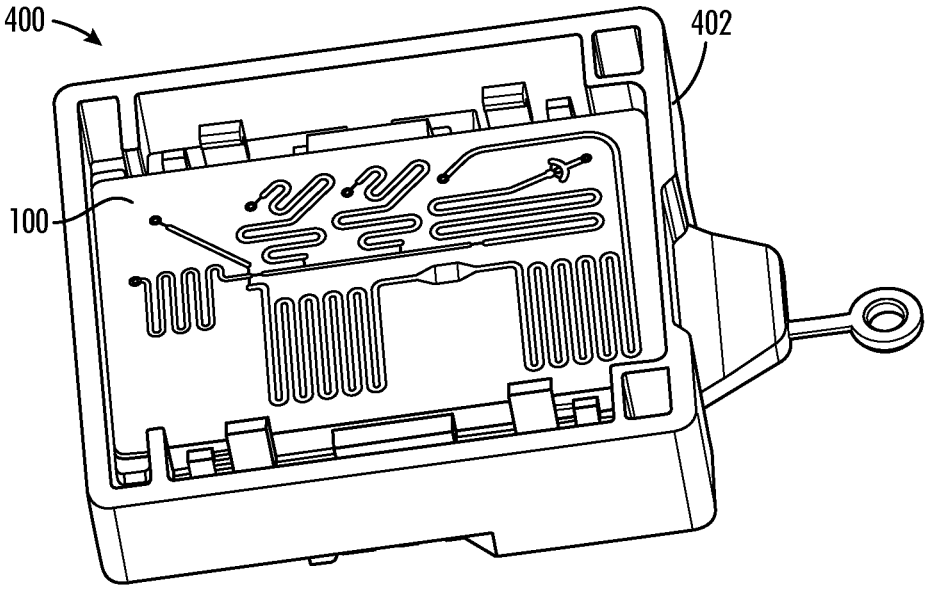

400
402
100

FIG.6

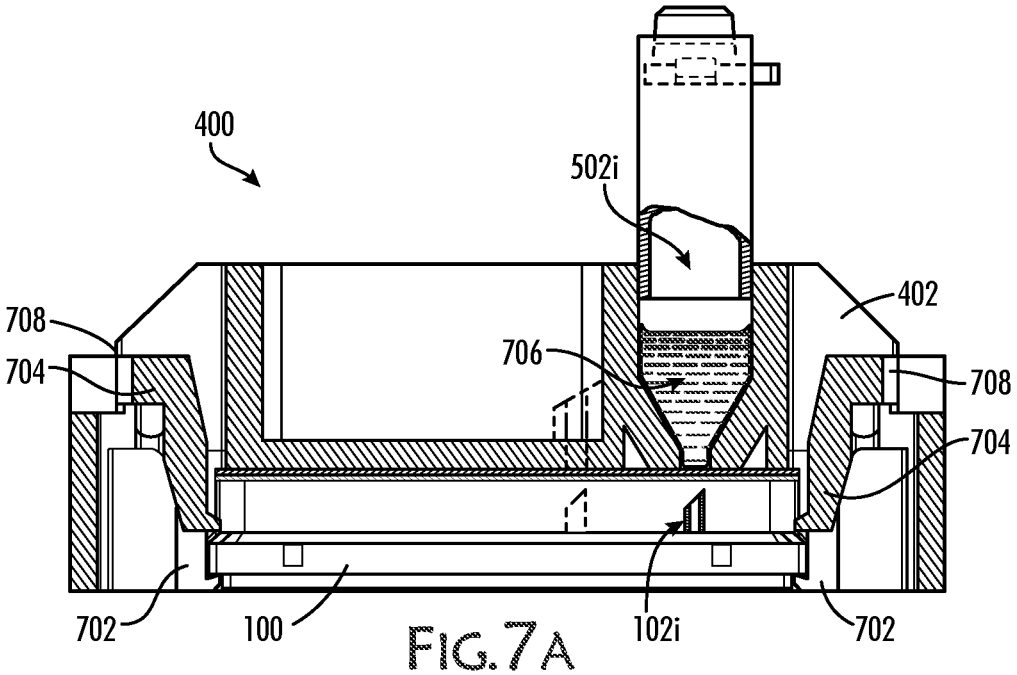

400
502i
402
708
706
704
708
704
702
100
102i
702

FIG. 7A

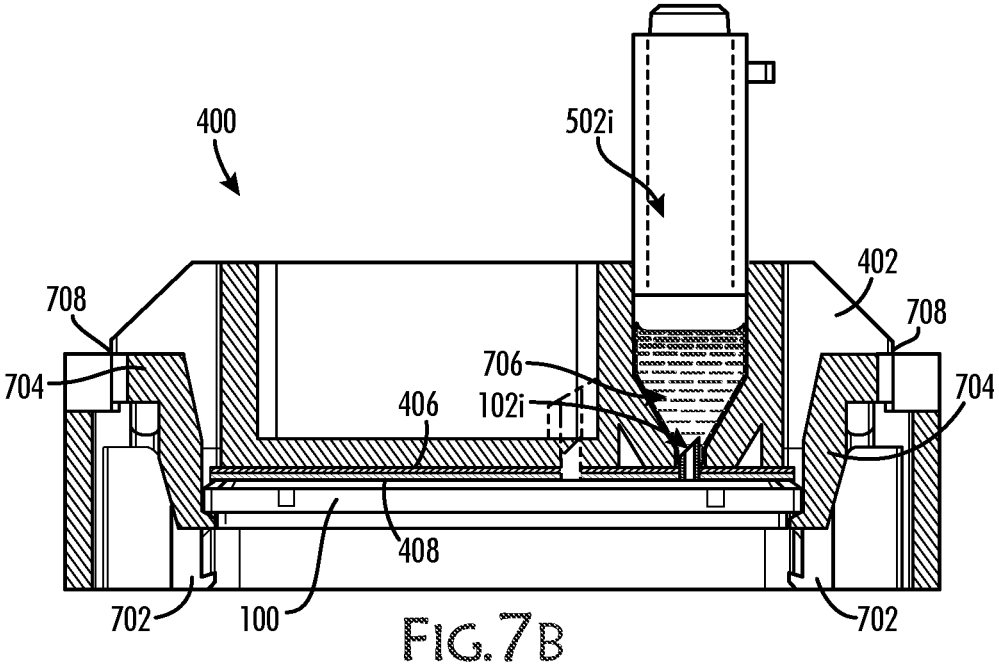

400
502i
402
708
708
706
704
704
406
102i
408
702
100
702

FIG. 7B

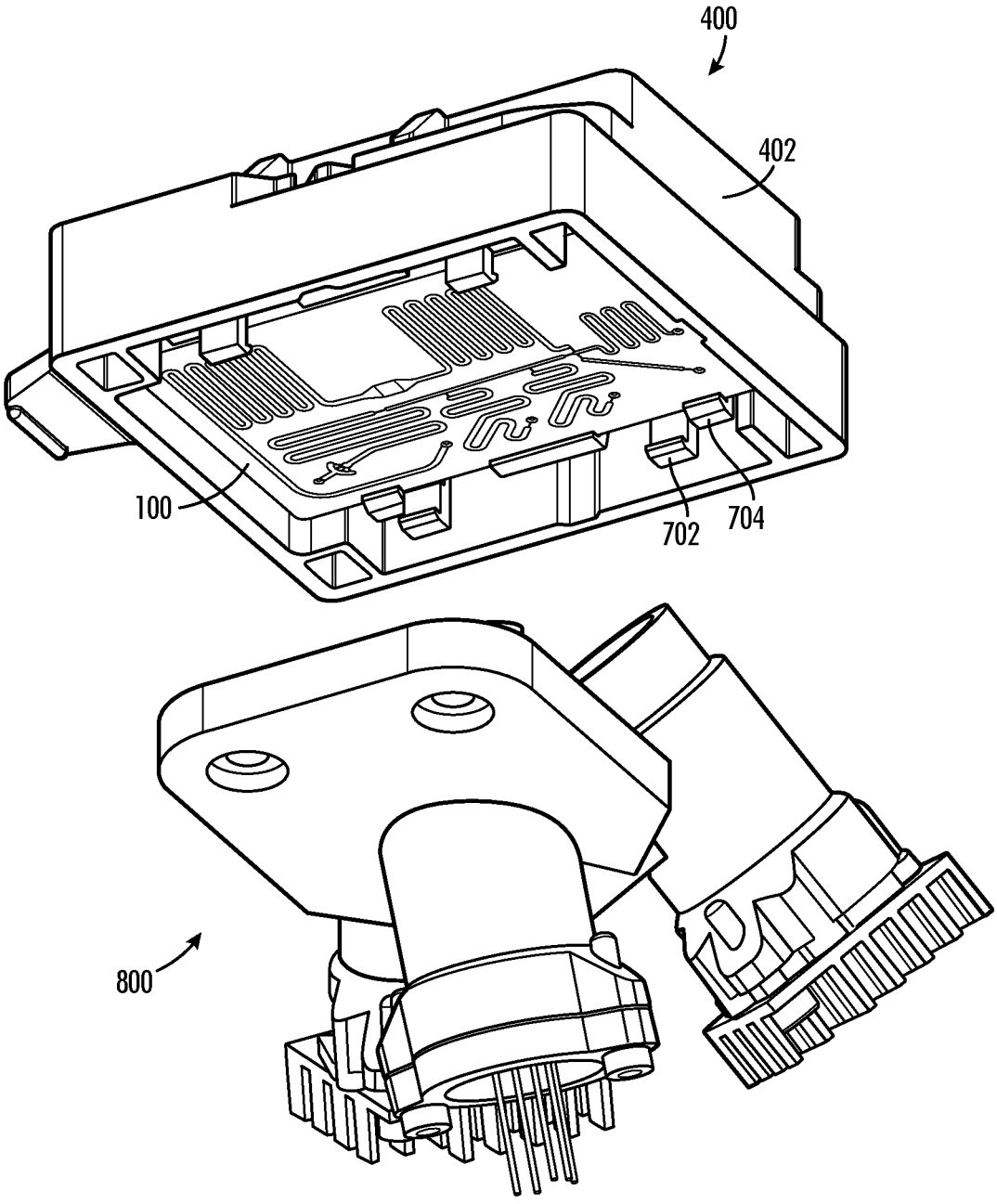


FIG.8

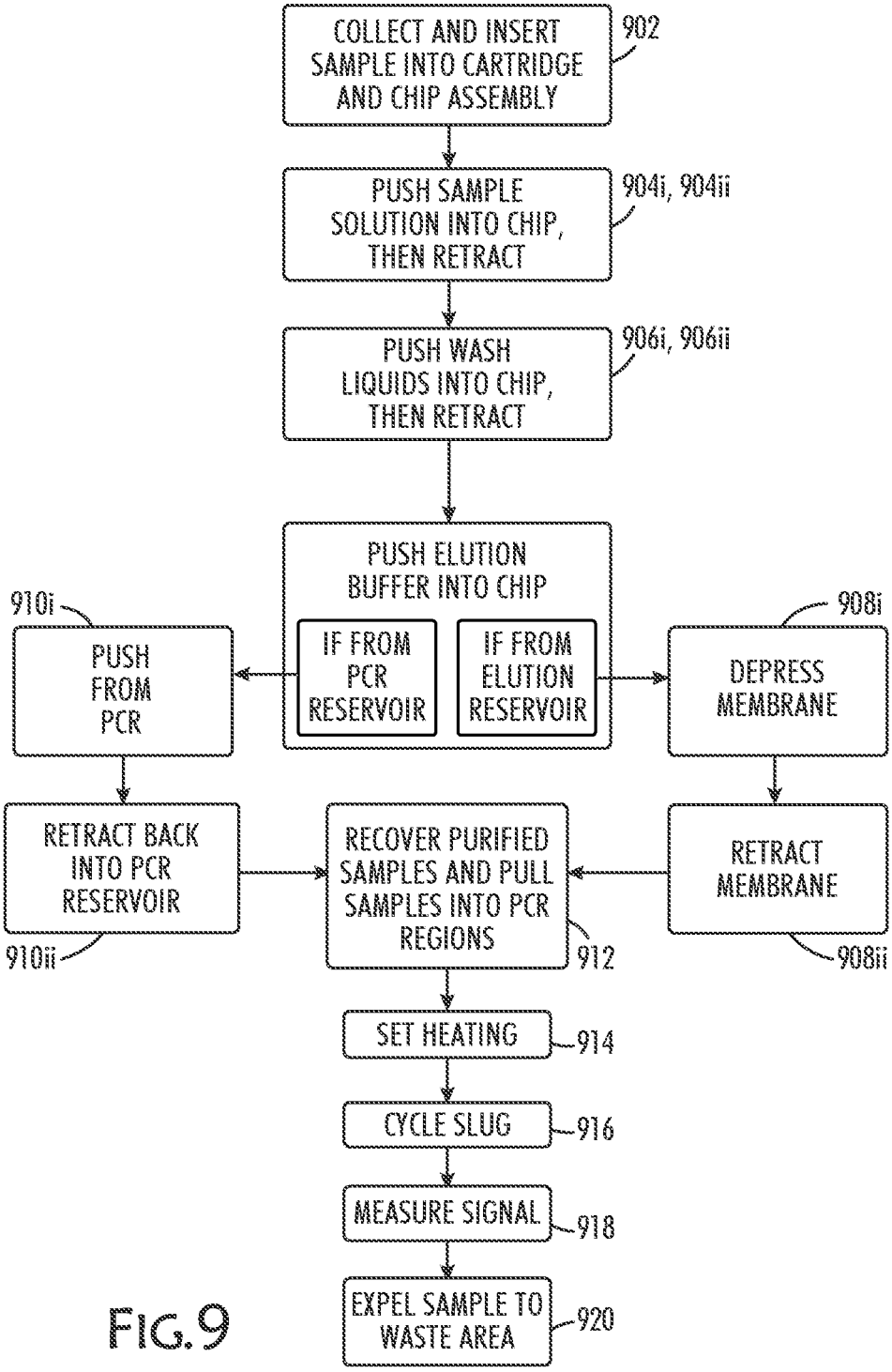

COLLECT AND INSERT SAMPLE INTO CARTRIDGE AND CHIP ASSEMBLY
902
PUSH SAMPLE SOLUTION INTO CHIP, THEN RETRACT
904i, 904ii
PUSH WASH LIQUIDS INTO CHIP, THEN RETRACT
906i, 906ii
PUSH ELUTION BUFFER INTO CHIP
IF FROM PCR RESERVOIR
IF FROM ELUTION RESERVOIR
910i
PUSH FROM PCR
908i
DEPRESS MEMBRANE
RETRACT BACK INTO PCR RESERVOIR
910ii
RECOVER PURIFIED SAMPLES AND PULL SAMPLES INTO PCR REGIONS
912
RETRACT MEMBRANE
908ii
SET HEATING
914
CYCLE SLUG
916
MEASURE SIGNAL
918
EXPEL SAMPLE TO WASTE AREA
920

FIG. 9

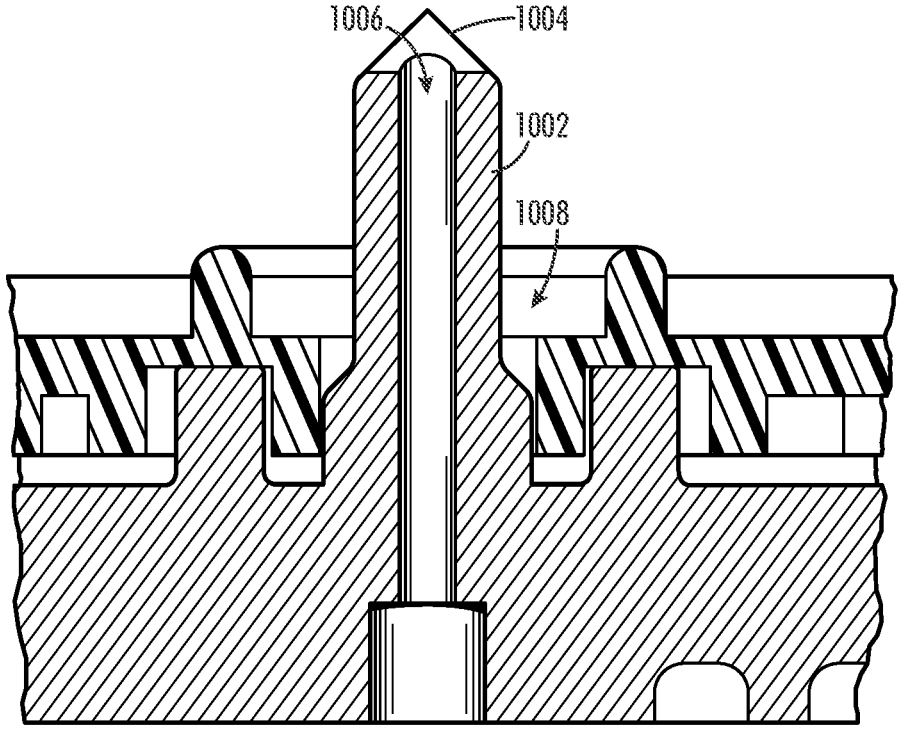


FIG.10

APPARATUSES WITH FLUIDIC CHANNEL GEOMETRIES FOR SAMPLE TO ANSWER PCR ANALYSIS AND METHODS OF USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority under Article 8 PCT of U.S. Provisional Patent Application No. 63/093,640 filed Oct. 19, 2020 and entitled "Point of Collection qPCR System." This application is also related to PCT applications entitled "Fluidic Detection and Control Algorithm for PCR Analysis," "Disposable Cartridge for Reagent Storage and Methods Using Same," and "Method and Apparatus for Controlling Fluid Volumes to Achieve Separation and PCR Amplification," and U.S. Design application Ser. No. 29/812,034 entitled "Fluidic Channel Geometries of a Chip," all filed concurrently on Oct. 19, 2021 and listing the same Applicant, Formulatrix, Inc. The contents of the above applications are all incorporated by reference as if fully set forth herein in their entireties.

FIELD

[0002] The present invention, in some embodiments thereof, relates to real-time, quantitative polymerase chain reaction (qPCR) and, more particularly, but not exclusively, to apparatuses and methods for improving the efficiency of qPCR processing and analysis.

BACKGROUND

[0003] There are a variety of different approaches to reducing the entire sample extraction, purification, and RT-qPCR processes onto a small and disposable format. One implementation can be found on the Roche Cobas Liat platform. This platform utilizes a small disposable transfer pipette to pipette a sample solution from a storage buffer into reagent storage consumable. The reagents required to run the assay are sealed in a tube with separate sections. During the course of the assay, specific sections are ruptured to introduce the appropriate reagents at the correct times in the correct sequence. This requires complicated and manual sample handling that occurs before the system can be used. Additionally, all the fluidics takes place in concealed reservoirs with no fluidic channels.

[0004] Another approach is using electrowetting approaches with two-phase fluidics, such as oil and water/aqueous. This approach was commercialized by NuGen (Mondrian), Advanced Liquid Logic, Illumina (NeoPrep) to keep reagents specifically for NGS library prep separate and introduce them with prescribed electrowetting sequences. This works for some sequences but was largely a commercial failure. Baebies is now attempting to use this technology for PCR testing with their FINDER platform.

[0005] The QIAstat-Dx is a system that employs multiple physical partitions and physical moving barriers or other actuation features to physically move or direct liquids. The instrument of this system either directly or indirectly actuates fluidic motion in a consumable to move liquids from one area to another through channels present in the consumable.

[0006] Another approach is a centrifugal device, so-called "cd-microfluidics", using different rotational speeds, interfacial features to accomplish liquid motion. See ufluidix.com/circle/whats-a-discman-and-how-is-it-a-medical-diagnostic-device-cd-microfluidics/. This is useful for some workflows, but qPCR relies on imaging the on-going PCR reaction at every thermal cycle. In addition, the DNA-Nudge system uses a rotation valve to control the sequence of reagent additions, but then ultimately performs PCR on discrete volumes in fixed locations.

SUMMARY

[0007] According to an aspect of some embodiments of the present invention there is provided a chip for use in a real-time qPCR system, comprising: at least one port for receiving a sample into the chip; at least one channel in fluidic communication with the at least port; a plurality of magnetically active beads disposed within the at least one channel that capture DNA/RNA from the sample as the sample passes through the at least one channel; and an optical inspection region in fluidic communication with the at least one channel for performing an optical analysis of the sample containing the eluted DNA/RNA previously captured on the magnetic beads.

[0008] In an embodiment of the invention, the chip further comprises at least one additional port for receiving at least one of wash fluid and elution fluid into the chip.

[0009] In an embodiment of the invention, the chip further comprises at least one inlet corresponding to and in fluidic communication with the at least one port and located on a top surface of the chip.

[0010] In an embodiment of the invention, the chip further comprises at least one magnetically active region configured to be magnetically active with the magnetically active beads.

[0011] In an embodiment of the invention, one magnetically active region is positioned upstream of the optical inspection region.

[0012] In an embodiment of the invention, the chip further comprises at least one heated region.

[0013] In an embodiment of the invention, one heated region is positioned on each side of the optical inspection region.

[0014] In an embodiment of the invention, the chip further comprises at least one filter disposed within the at least one channel.

[0015] In an embodiment of the invention, the at least one channel is 0.5 mm deep and 0.5 mm wide.

[0016] In an embodiment of the invention, the chip further comprises at least one burst valve.

[0017] In an embodiment of the invention, the at least one burst valve is 0.1 mm deep and 0.1 mm wide.

[0018] In an embodiment of the invention, the chip further comprises at least one chip stop disposed on and protruding from an exterior surface of the chip.

[0019] In an embodiment of the invention, the chip further comprises an exit valve for discharging the sample from the chip.

[0020] According to a further aspect of some embodiments of the present invention there is provided a cartridge and chip assembly, comprising: a cartridge including at least one fluid reservoir; a chip disposed beneath the cartridge with an inlet and a port corresponding to the at least one fluid reservoir; and an elastic membrane disposed on top of the cartridge.

[0021] In an embodiment of the invention, the assembly has a first configuration wherein the chip is held between at least one lower clip and at least one upper clip of the

cartridge such that the inlet is not in fluid communication with the at least one fluid reservoir.

[0022] In an embodiment of the invention, the assembly has a second configuration wherein the chip is held between at least one upper clip of the cartridge and the cartridge such that the inlet is in fluid communication with the at least one fluid reservoir.

[0023] In an embodiment of the invention, the assembly further comprises at least one release located on the cartridge for transitioning the cartridge and chip assembly from a first configuration to a second configuration.

[0024] In an embodiment of the invention, the assembly further comprises an exit valve and outlet of the chip in fluid communication with a waste area of the cartridge.

[0025] In an embodiment of the invention, the assembly further comprises at least one of at least one foil seal, a compressible layer, and an optically transparent seal.

[0026] According to a further aspect of some embodiments of the present invention there is provided a method of using a cartridge and chip assembly, comprising: collecting and inserting a sample into a sample reservoir of a cartridge of the cartridge and chip assembly; pushing the sample from the sample reservoir into a chip of the cartridge and chip assembly by way of an inlet and a port of the chip; mixing the sample with magnetically active beads and then trapping the beads in the chip; retracting the sample from the chip back into the reservoir; pushing at least one wash fluid from at least one wash fluid reservoir in the cartridge; retracting the at least one wash fluid from the chip back into the at least one wash fluid reservoir; pushing an elution buffer into the chip, from an elution reservoir of the cartridge by depressing an elastic membrane, or from a PCR reservoir of the cartridge; retracting the elution buffer by, retracting the elastic member, or retracting the elution buffer into the PCR reservoir, thereby creating a purified sample; recovering the purified sample and pulling the purified sample into at least one heated region of the chip; setting a temperature for the at least one heated region; cycling the purified sample past an optical inspection region of the chip; and measuring a signal taken from the purified sample at the optical inspection region.

[0027] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

[0028] Implementation of the method and/or system of embodiments of the invention can involve performing or completing selected tasks manually, automatically, or a combination thereof. Moreover, according to actual instrumentation and equipment of embodiments of the method and/or system of the invention, several selected tasks could be implemented by hardware, by software or by firmware or by a combination thereof using an operating system.

[0029] For example, hardware for performing selected tasks according to embodiments of the invention could be implemented as a chip or a circuit. As software, selected tasks according to embodiments of the invention could be implemented as a plurality of software instructions being executed by a computer using any suitable operating system. In an exemplary embodiment of the invention, one or more tasks according to exemplary embodiments of method and/or system as described herein are performed by a data processor, such as a computing platform for executing a plurality of instructions. Optionally, the data processor includes a volatile memory for storing instructions and/or data and/or a non-volatile storage, for example, a magnetic hard-disk and/or removable media, for storing instructions and/or data. Optionally, a network connection is provided as well. A display and/or a user input device such as a keyboard or mouse are optionally provided as well.

BRIEF DESCRIPTION OF DRAWINGS

[0030] Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example, are not necessarily to scale and are for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

[0031] In the drawings:

[0032] FIG. **1** is a top perspective view of a chip, in accordance with an exemplary embodiment of the invention;

[0033] FIG. **2** is a bottom perspective view of a chip, in accordance with an exemplary embodiment of the invention;

[0034] FIG. **3** is a bottom view of a chip, in accordance with an exemplary embodiment of the invention;

[0035] FIG. **4** is an exploded view of a cartridge and chip assembly, in accordance with an exemplary embodiment of the invention;

[0036] FIG. **5** is a top perspective view of a cartridge and chip assembly, in accordance with an exemplary embodiment of the invention;

[0037] FIG. **6** is a bottom perspective view of a cartridge and chip assembly, in accordance with an exemplary embodiment of the invention;

[0038] FIGS. **7**A-**7**B are cross-sectional views of a cartridge and chip assembly in different configurations, in accordance with an exemplary embodiment of the invention;

[0039] FIG. **8** is a perspective view of an optical detection unit in use with a cartridge and chip assembly, in accordance with an exemplary embodiment of the invention;

[0040] FIG. **9** is a flowchart of a method of using a chip, in accordance with an exemplary embodiment of the invention; and,

[0041] FIG. **10** is a cross-sectional view of an inlet, in accordance with an exemplary embodiment of the invention.

DETAILED DESCRIPTION

[0042] The present invention, in some embodiments thereof, relates to real-time, quantitative polymerase chain reaction (qPCR) and, more particularly, but not exclusively, to apparatuses and methods for improving the efficiency of qPCR processing and analysis.

[0043] Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details of construction and the arrangement of the components and/or methods set forth in the following description and/or illus-

trated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways.

[0044] Generally, the inventions described herein fully automate the process of qPCR and PCR in one disposable consumable (e.g. cartridge/chip assembly) using a network of fluidic channels with access to bulk reagent reservoirs and/or a waste area. The inventions described herein: minimize/simplify laboratory instrument requirements and/or costs; accelerate the process of sample extraction and/or purification for preparing samples for PCR amplification; and accelerate the qPCR process while still providing an effective level of sensitivity. One way of providing these benefits, as described in exemplary detail herein, is by using membrane-driven reservoirs (in a cartridge of the system combined with a driving motor) to push the appropriate reagents and samples into a chip in the correct sequence. It is envisioned that with intelligently-planned channel design, small constrictions ("burst valves"), and imaging regions that are distinctly different (wider/deeper) that other channel portions, liquids can be introduced in the appropriate order but also to the appropriate regions of the chip in a controlled and predictable manner. Other components of the qPCR system are described with respect to the cross-referenced patent applications in the Related Applications section. An exemplary qPCR system utilizing some or all of these components will be available as a qPCR system from Formulatrix, Inc. of Bedford, MA.

[0045] FIG. **1** is a top perspective view of a chip **100**, in accordance with an exemplary embodiment of the invention. It should be understood that the chip **100** is a component part of a larger qPCR system, wherein when taken together are capable of performing real time qPCR analysis. In an embodiment of the invention, a plurality of inlets **102***i*, **102***ii*, **102***iii*, **102***iv*, **102***v*, and outlet **102***vi* are provided to the chip **100** wherein fluids are introduced to the chip through the inlets, for example from fluid reservoirs or wells located in a cartridge **402** used with the chip **100** (described in more detail below with respect to FIGS. **4**, **5**, **7**A and **7**B) or exit the chip via outlet **102***vi*. More or less inlets could be provided depending on how many or how much volume of the fluids are desired. In an embodiment of the invention, the inlets are configured with a puncturing/sharp upward end, for example by being formed by an angled cut. In some embodiments of the invention, the chip **100** is configured with chip-stop/alignment features **104**, **106** around the perimeter or exterior of the chip **100**, which are used in conjunction with counter-part features of the cartridge **402** ("clips", described in more detail below with respect to FIGS. **7**A-**7**B). In an embodiment of the invention, the chip **100** is injection molded for low cost, easily reproducible, scalable and/or modifiable construction.

[0046] FIG. **2** is a bottom perspective view of the chip **100**, in accordance with an exemplary embodiment of the invention. An exemplary channel configuration is shown in FIG. **2** and further explained with respect to FIG. **3**.

[0047] FIG. **3** is a bottom view of the chip **100**, in accordance with an exemplary embodiment of the invention. In the interests of brevity, the chip **100** of FIG. **3** is described in conjunction with FIG. **9**, a flowchart **900** of a method of using the chip **100**, in accordance with an exemplary embodiment of the invention. A sample swab is previously collected and inserted (**902**) into a sample reservoir **502***i*, shown and described in more detail in FIG. **5**, which contains a lysis agent to simultaneously lyse cells and protect the exposed DNA and RNA fragments from DNAse and RNAse proteomic activity. This solution of the sample and lysis agent is pushed (**904***i*) from the sample reservoir **502***i* into the inlet **102***i* and through a sample port **302***i* of the chip **100** to introduce the solution to the chip **100** and its inventive fluidic channel geometry. It should be noted that throughout the Figures, inlet, port and reservoir/well reference numerals are consistently used wherein the inlet **102***i* of FIG. **1** corresponds to the port **302***i* of FIG. **3**, both of which correspond to the reservoir **502***i* of FIG. **5**, and so on, such that inlet **102***ii*, port **302***ii* and reservoir **502***ii* also correspond, as an example.

[0048] In an embodiment of the invention, the chip **100** has a filter **304** designed into the channel the sample solution is pushed (**904***i*) through to reduce the potential of clogging the chip **100**. After (i.e. downstream of) the filter **304**, there is a length **306** of a channel including a dried-down solution of silica or carboxylate magnetic beads with a surface that captures DNA/RNA from the sample solution. The sample solution passes through this channel length **306** a few times, through the repeated bends, to re-hydrate the beads into the sample solution and ensure the nucleic acid material has time to bind to the beads. In an embodiment of the invention, on-chip magnetic bead extraction concentrates RNA and allows for low master mix usage. Magnetic bead extraction should exhibit excellent sensitivity, when employed as described herein.

[0049] As the sample solution proceeds down the channel, it passes through a constriction region **308** that is shallower and thinner than the nominal channel. In an embodiment of the invention, the nominal channel on the chip is roughly 0.5 mm deep and 0.5 mm wide. This smaller constriction region is 0.1 mm by 0.1 mm wide, in an embodiment. These constriction regions, or "burst valves", are designed to restrict flow of the fluid being pushed, avoid having the fluid go into undesired regions of the chip **100** and/or guide the fluid into a desired region of the chip **100**. By the design of this chip, flow to the exit valve **302***vi* at the end of the magnetic region **310** is generally desired, and as such is encouraged fluidically by the design and/or location in the chip **100** of the burst valves, such as burst valve **308**.

[0050] The sample solution is pushed through the chip **100**, and through the magnetic region **310**. During this fluidic move, the magnetic beads are attracted to the wall of the channel against a magnet that is present in the qPCR system. The remaining solution would continue through the channel and out the exit valve **302***vi*, and exit port **502***vi* that exits to a large waste area **504** of the cartridge **402** from which fluid cannot return to the chip **100**. After this push (**904***i*), the membrane **404** on the sample port retracts, due to operation of a specially-design camshaft driven by a membrane-driving motor of the qPCR system, described in at least one of the applications indicated in the Related Applications section, which correspondingly retracts (**904***ii*) the sample solution back into its reservoir **502***i*, optionally due to pneumatic and/or hydraulic pressure (in an embodiment where a second, immiscible fluid is being used to create pressure in addition to or in the alternative to air).

[0051] In operation, it is not unexpected that some sample solution permeates the unintended burst valves, for example at junction **318**, and progresses slightly into other regions of the chip **100**. In this regard, the burst valves are leaky valves. However, this phenomenon is handled by subsequent wash

liquids (ethanol in this embodiment) that enter from the wash reservoirs **502***iii*, **502***iv* through inlets **102***iii*, **102***iv* which also "leak" into those regions. The wash liquids are pushed (**906***i*) into the chip **100** and flow over the magnetically bound beads, removing impurities and flowing into the waste area **504**. In this embodiment, there are two reservoirs **502***iii*, **502***iv* that contain wash liquids that are pushed (**906***i*) into the chip **100** and then are retracted (**906***ii*) back (again by the membrane-driving motor), however, there could be more or less in other embodiments. Optionally, the junction **318** is magnetized to assist with leakage prevention by substantially holding the magnetic beads in place.

[0052] After the magnetically bound beads are washed, an elution buffer is then pushed into the chip **100** and over the magnetic beads. This elution buffer could come from either an elution reservoir **502***v*, through inlet **102***v*, or could alternatively come from the PCR-mix reservoir **502***ii*, through inlet **102***ii*, if elution into a PCR mix is desired. In an embodiment where an elution buffer comes from the elution reservoir **502***v*, the PCR reservoir **502***ii* would be empty and the membrane **404** would be depressed (**908***i*) and held first. In this way, the fluid in the elution reservoir **502***v* would be pushed into the magnet region **310**, and the membrane **404** corresponding to the PCR reservoir **502***ii* would then retract (**908***ii*), pulling the eluted purified sample into the PCR region of the chip **314**.

[0053] In the embodiment where the elution buffer would come from the PCR reservoir **502***ii*, then after the washing steps, the elution buffer would be pushed (**910***i*) from the PCR reservoir **502***ii* all the way through the PCR regions **314**, **316** and into the magnetic region **310** to elute the sample, and then would retract (**910***ii*) back into the PCR reservoir **502***ii* after elution.

[0054] In an embodiment of the invention, the last functional region of this exemplary chip **100** is an optical detection region **312**, also called "the voxel". An optical detection unit **800** of a qPCR system for analyzing the optical detection region **312** is shown as a representative example in FIG. **8**. The optical detection region **312** can be the same dimensions of the channels surrounding it, or it could have varying depths and widths to enhance optical detection of the fluorescent signal. In this embodiment, it is shown to be both wider and deeper than the fluidic channels surrounding it. This is the area where, in some embodiments, specific PCR components (primers, probes, and mastermix) can be optionally dried down for later rehydration as the elution solutions pass over it.

[0055] After the purified samples are recovered from the magnetic region and pulled (**912**) into the PCR regions **314**, **316**, the fluidic control and motion are simple. Heating elements in the qPCR system are set (**914**) to desired temperatures to heat the separate PCR regions **314**, **316** to the desired temperatures for desired protocols. In an exemplary embodiment of the invention, a hot region is optionally set to 95-98° C. and a cool region is optionally set to 55-60° C., and are heated to the specific temperatures by the heating elements. For most RNA workflows, this includes a time and temperature to enable the reverse transcription of the RNA into complementary DNA (cDNA). Then, depending on the PCR reagents being used, there can be the need for a "hot start" at an elevated temperature that is required to remove inhibitors to the PCR enzymes. In some embodiments of the invention, temperatures are defined for optimal amplification of DNA. In other examples one heating element with variability of heating zones may be used, or in additional examples more than two heating elements may be used.

[0056] These steps, which involve positioning the eluted sample liquid volume, or "slug" of liquid, over either heated region for a desired amount of time while the heater is set to the desired temperature. After these steps, the heating elements are set (**914**) to the desired PCR annealing/extension and denaturing temperatures, and the slug is then cycled (**916**) between the two heated regions **314**, **316**, resting for a programmable amount of time in each section to ensure completion of the desired enzymatic activity. Each cycling (**916**) of the slug results in it passing through the optical detection region of the chip **312**. During this transit, the fluorescent signal can be measured (**918**) by the optical detection unit **800** to characterize the qPCR amplification of the signal. Once measuring (**918**) is completed, the slug/sample is expelled (**920**) from the chip via the exit valve **302***vi* and into the waste area **504**.

[0057] In another embodiment of the invention, multiple PCR channels (a plurality of channels) run in parallel through the common PCR heating areas. These could then all pass through a detection region located between the heated regions.

[0058] In some embodiments of the invention, capacitive liquid sensing arrays are positioned in and/or around PCR regions **314**, **316** which, in combination with the magnetic beads, allows for tracking of fluid within the fluidic channels of the chip **100**. In some embodiments, the capacitive sensing arrays are positioned on either side of the optical detection region **312**. In doing so, the relative position of cycling between the two heating regions (heated PCR regions **314**, **316**) may be determined. Further, the capacitive liquid sensing arrays may be positioned on the entry and exit sides of the heated PCR regions **314**, **316**, thus allowing full tracking of the sample as it moves through the amplification process. The capacitive arrays may work independently of the optical detection unit **312**, or in combination with, in detecting and transmitting signals or information to a processing unit (not shown) that controls the membrane-driving motor as well as instrumentation such as screens or diagnostics for a technician.

[0059] FIG. **4** is an exploded view of a cartridge and chip assembly **400**, in accordance with an exemplary embodiment of the invention. FIG. **4** shows the elastic membrane **404**, a foil seal **406**, the cartridge **402** including reservoirs, a second foil seal **406**, a compressible layer **408**, the chip **100**, and a bottom seal **410** (which is optically transparent, in some embodiments of the invention, to enable scanning of the optical detection region **312** by the optical detection unit **800**). In an exemplary embodiment of the invention and as described elsewhere herein, the membrane **404** acts in combination with the reservoirs of the cartridge to form a fluidic seal, effectuating pneumatic and/or hydraulic pressure for moving fluids throughout the chip **100** as described.

[0060] Additionally, the compressible layer **408** can serve to fluidically seal the cartridge **402** to the chip **100** once the cartridge and chip assembly **400** is fully constructed. The fluidic reservoirs on the cartridge **402** are open on both ends, with the top of each reservoir being wide enough for the membrane **404** to deform into it to cause the pneumatic and/or hydraulic pressure used for fluidic motion. The bottom of each reservoir contains an orifice slightly larger than the puncturing feature of the inlets on the chip **100**. The orifice is sealed in its pre-use state.

[0061] FIG. **5** is a top perspective view of a cartridge and chip assembly **400**, including the cartridge **402** and the chip **100** (not shown), in accordance with an exemplary embodiment of the invention. In an embodiment of the invention, the various reservoirs **502***i* (the sample reservoir), **502***ii* (the PCR reservoir), **502***iii* (wash 1 reservoir), **502***iv* (wash 2 reservoir), **502***v* (the elution reservoir), and the exit port **502***vi* into the waste area **504** are shown.

[0062] FIG. **6** is a bottom perspective view of the cartridge and chip assembly **400** wherein the chip **100** can be seen mounted within the cartridge **402**, in accordance with an exemplary embodiment of the invention.

[0063] FIGS. **7**A-**7**B are cross-sectional views of a cartridge and chip assembly **400** in different configurations, in accordance with an exemplary embodiment of the invention. FIG. **7**A shows a first configuration of the chip assembly **400** prior to insertion of the assembly **400** into a qPCR system, but after manufacture. In an embodiment of the invention, the cartridge **402** contains a variety of one-way clips to retain the chip **100** to the cartridge **402**. Lower flexible clips **702** on the cartridge **402** engage the chip-stop/alignment features **104** of the chip **100** and retain the chip **100** to the cartridge **402** after initial assembly of the cartridge and chip assembly **400** in the factory and until it is inserted in the qPCR system. In this first configuration, the lower retaining clips **702** hold the chip up against at least one upper retaining clip **704** of the cartridge **402**. These upper retaining clip **704** keep the chip **100** far enough away from the cartridge **402** to prevent the puncturing/sharp upward ends of the inlets on the chip **100** from pre-maturely puncturing the second foil seal **406** and compressible layer **408** on the bottom of the cartridge **402** (which would unintentionally establish a fluidic connection between the reservoirs of the cartridge **402** and the channels of the chip **100**).

[0064] In an embodiment of the invention, a user would collect their sample on a swab or by some other method, and put it into the sample reservoir **502***i* of the cartridge **402**. In an embodiment of the invention, the swab breaks off in the cartridge **402** or at least deposits the sample in the cartridge, optionally with a registration feature in the sample reservoir **502***i* that engages with the swab, this enabling the sample reservoir **502***i* to be sealed using the attached lid with the swab/sample still in the cartridge **402**. In this manner, the swab/sample is submerged in the pre-stored wet reagents in the sample reservoir **502***i*.

[0065] FIG. **7**B shows a second configuration of the assembly **400** after the insertion of the assembly **400** into a qPCR system. In an embodiment of the invention, when the assembly **400** is inserted into the qPCR system, system hardware features push inward on chip-stop releases **708**, which disengage/rock the upper clips **704** out and away from retaining the chip **100** in the lower position of the first configuration. The qPCR system then pushes on the bottom of the chip **100** by sustaining pressure on the releases **708**, the upper clips **704** and through to the lower clips **702** to mechanically press the chip **100** upwards into the second configuration shown in FIG. **7**B. During this motion, the puncturing/sharp upward ends of the inlets pierce the compressible layer **408** between the chip **100** and cartridge **402**, and then subsequently puncture the second foil layer **406** while the sealing features seal up against the compressible layer **408**. At this point, with the chip **100** sealed up against the cartridge **402**, it also engages the upper retaining clips **704**. The chip-stop functionality is also effectuated by a chip-stop/alignment structure **106** (providing a structure in relief) on the side of the chip **100** that acts to retain the chip **100** in the second configuration.

[0066] In an embodiment of the invention, once the chip **100** is fluidically engaged with the cartridge **402**, pins push on the top flexible membrane **404** to move liquids contained in the cartridge reservoirs into the channel network on the chip **100**. The upper retaining clips **704** keep the chip **100** engaged and connected to the cartridge **402** throughout the course of the assay runtime, as well as afterward for safe disposal.

[0067] FIG. **8** is a perspective view of an optical detection unit **800** in use with a cartridge and chip assembly **400**, in accordance with an exemplary embodiment of the invention. As a sample moves through the chip **100**, by force resulting from the membrane-driving motor pushing pins into the membrane **404**, the fluid crosses the optical detection region **312** wherein the optical detection unit **800** performs analysis on the sample. This type of detection is often referred to as dynamic detection, as the optical detection unit **800** is performing detection as the fluid cycles, and as amplification is occurring in real-time.

[0068] FIG. **10** is a cross-sectional view of an exemplary embodiment of an inlet **1002**, showing a puncturing/sharp upward end **1004** and internal lumen **1006** which fluidically connects a reservoir of the cartridge to the channel network of the chip. In some embodiments of the invention, a tray **1008** is provided around the inlet **1002** to capture and retain any leakage from the piercing/forming a fluidic connection between the inlet and a reservoir.

[0069] The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

[0070] The term "consisting of" means "including and limited to".

[0071] The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

[0072] The term "plurality" means "two or more".

[0073] As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

[0074] Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0075] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges

between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

[0076] As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

[0077] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

[0078] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

[0079] All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

What is claimed is:

1. A chip for use in a real-time qPCR system, comprising:
- at least one port for receiving a sample into the chip;
- at least one channel in fluidic communication with the at least port;
- a plurality of magnetically active beads disposed within the at least one channel that capture DNA/RNA from the sample as the sample passes through the at least one channel; and
- an optical inspection region in fluidic communication with the at least one channel for performing an optical analysis of the sample containing the eluted DNA/RNA previously captured on the magnetic beads.

2. The chip according to claim **1**, further comprising at least one additional port for receiving at least one of wash fluid and elution fluid into the chip.

3. The chip according to claim **1**, further comprising at least one inlet corresponding to and in fluidic communication with the at least one port and located on a top surface of the chip.

4. The chip according to claim **1**, further comprising at least one magnetically active region configured to be magnetically active with the magnetically active beads.

5. The chip according to claim **4**, wherein one magnetically active region is positioned upstream of the optical inspection region.

6. The chip according to claim **1**, further comprising at least one heated region.

7. The chip according to claim **6**, wherein one heated region is positioned on each side of the optical inspection region.

8. The chip according to claim **1**, further comprising at least one filter disposed within the at least one channel.

9. The chip according to claim **1**, wherein the at least one channel is 0.5 mm deep and 0.5 mm wide.

10. The chip according to claim **1**, further comprising at least one burst valve.

11. The chip according to claim **10**, wherein the at least one burst valve is 0.1 mm deep and 0.1 mm wide.

12. The chip according to claim **1**, further comprising at least one chip stop disposed on and protruding from an exterior surface of the chip.

13. The chip according to claim **1**, further comprising an exit valve for discharging the sample from the chip.

14. A cartridge and chip assembly, comprising:
- a cartridge including at least one fluid reservoir;
- a chip disposed beneath the cartridge with an inlet and a port corresponding to the at least one fluid reservoir; and
- an elastic membrane disposed on top of the cartridge.

15. The cartridge and chip assembly according to claim **14**, having a first configuration wherein the chip is held between at least one lower clip and at least one upper clip of the cartridge such that the inlet is not in fluid communication with the at least one fluid reservoir.

16. The cartridge and chip assembly according to claim **14**, having a second configuration wherein the chip is held between at least one upper clip of the cartridge and the cartridge such that the inlet is in fluid communication with the at least one fluid reservoir.

17. The cartridge and chip assembly according to claim **14**, further comprising at least one release located on the cartridge for transitioning the cartridge and chip assembly from a first configuration to a second configuration.

18. The cartridge and chip assembly according to claim **14**, further comprising an exit valve and outlet of the chip in fluid communication with a waste area of the cartridge.

19. The cartridge and chip assembly according to claim **14**, further comprising at least one of at least one foil seal, a compressible layer, and an optically transparent seal.

20. A method of using a cartridge and chip assembly, comprising:
- collecting and inserting a sample into a sample reservoir of a cartridge of the cartridge and chip assembly;
- pushing the sample from the sample reservoir into a chip of the cartridge and chip assembly by way of an inlet and a port of the chip;
- mixing the sample with magnetically active beads and then trapping the beads in the chip;
- retracting the sample from the chip back into the reservoir;
- pushing at least one wash fluid from at least one wash fluid reservoir in the cartridge;

retracting the at least one wash fluid from the chip back into the at least one wash fluid reservoir;
pushing an elution buffer into the chip,
from an elution reservoir of the cartridge by depressing an elastic membrane, or
from a PCR reservoir of the cartridge;
retracting the elution buffer by,
retracting the elastic member, or
retracting the elution buffer into the PCR reservoir, thereby creating a purified sample;
recovering the purified sample and pulling the purified sample into at least one heated region of the chip;
setting a temperature for the at least one heated region;
cycling the purified sample past an optical inspection region of the chip; and
measuring a signal taken from the purified sample at the optical inspection region.

* * * * *