US 20190168213A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2019/0168213 A1

PULITZER et al. (43) Pub. Date: Jun. 6, 2019

(54) **SYSTEM AND METHOD FOR DETERMINING EFFICACY AND DOSAGE USING PARALLEL/SERIAL DUAL MICROFLUIDIC CHIP**

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(21) Appl. No.: **16/186,513**

(22) Filed: **Nov. 10, 2018**

Related U.S. Application Data

(60) Provisional application No. 62/584,653, filed on Nov. 10, 2017.

Publication Classification

(51) **Int. Cl.**

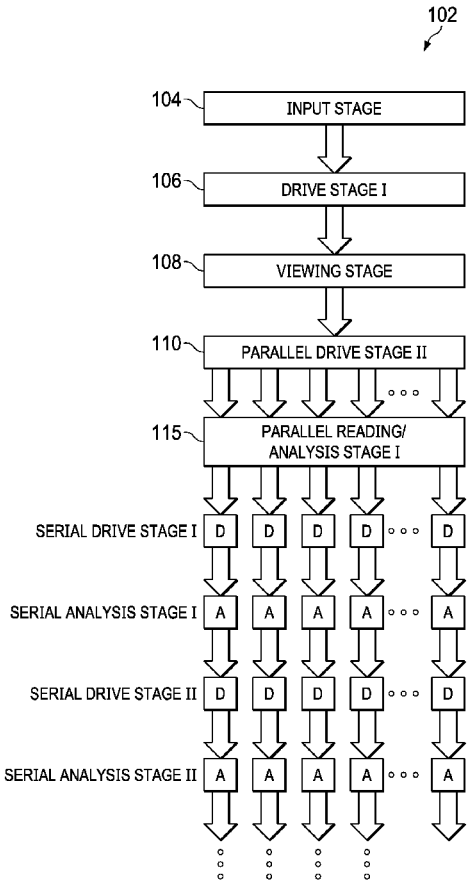
B01L 3/00	(2006.01)
F04B 43/04	(2006.01)
G01N 33/483	(2006.01)
F16K 99/00	(2006.01)

(52) **U.S. Cl.**

CPC ***B01L 3/50273*** (2013.01); ***F04B 43/046*** (2013.01); ***G01N 33/4833*** (2013.01); ***F16K 99/0015*** (2013.01); *F16K 2099/0084* (2013.01); ***B01L 3/502738*** (2013.01); *B01L 2200/0605* (2013.01); *B01L 2400/0638* (2013.01); ***B01L 3/502715*** (2013.01)

(57) **ABSTRACT**

A method for determining a treatment agent and dosage level for a biologic material includes the biologic sample is pumped into each of a first plurality of parallel pathways from the first reservoir using a micro-pump. A separate treatment agent of the plurality of treatment agents is applied within each of the first plurality of parallel pathways. The treatment agent providing a best treatment efficacy for the predetermined biologic material within the biologic sample is determined. A second portion of the biologic sample is pumped into a selected second parallel pathway associated with the determined treatment agent of a second plurality of parallel pathways from the first reservoir using a second micro-pump. The determined treatment agent at a plurality of different dosage levels is applied within the selected second parallel pathway. A dosage level of the plurality of different dosage levels of the determined treatment agent is determined.



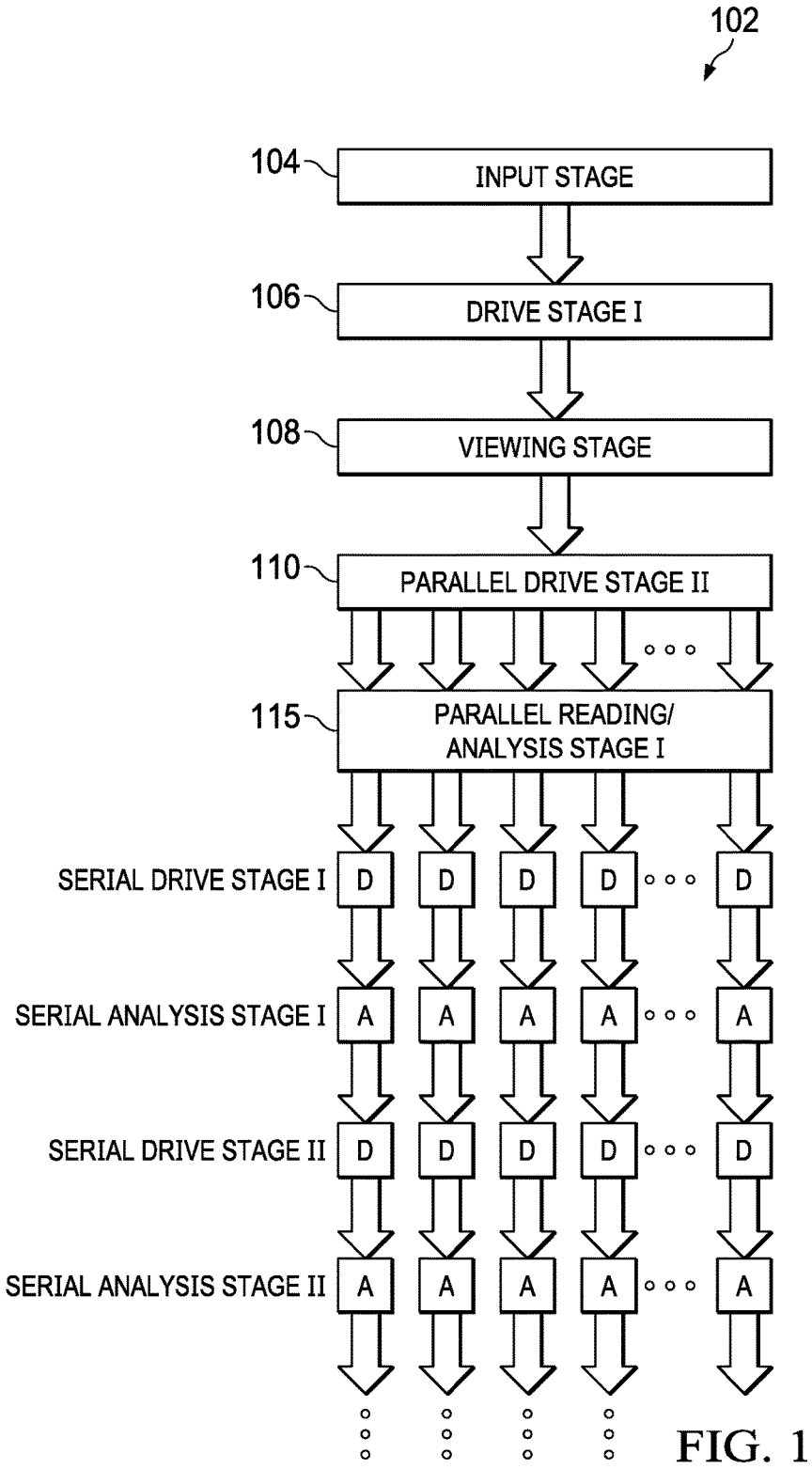

102
104
INPUT STAGE
106
DRIVE STAGE I
108
VIEWING STAGE
110
PARALLEL DRIVE STAGE II
115
PARALLEL READING/
ANALYSIS STAGE I
SERIAL DRIVE STAGE I
SERIAL ANALYSIS STAGE I
SERIAL DRIVE STAGE II
SERIAL ANALYSIS STAGE II
D
A

FIG. 1

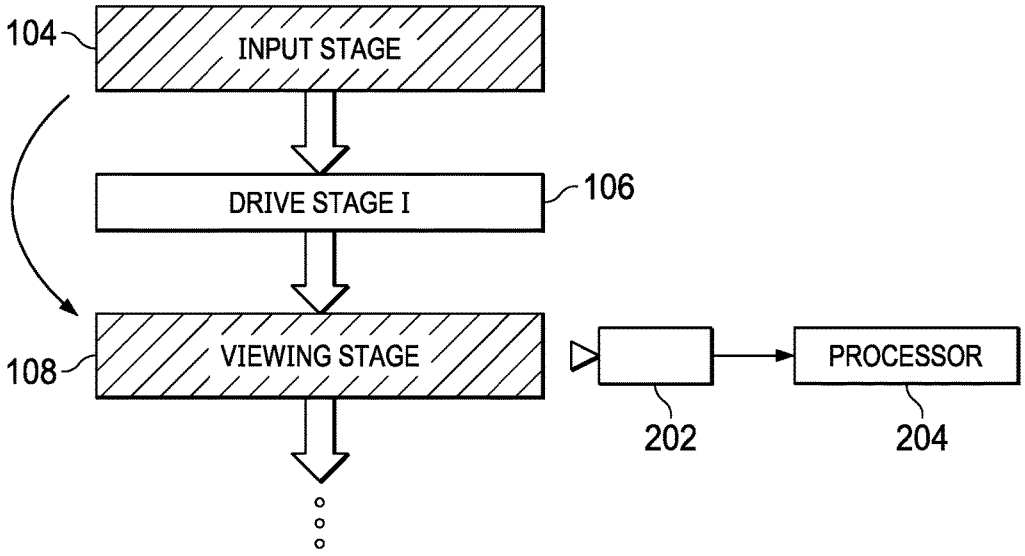

104
INPUT STAGE
DRIVE STAGE I
106
108
VIEWING STAGE
PROCESSOR
202
204

FIG. 2A

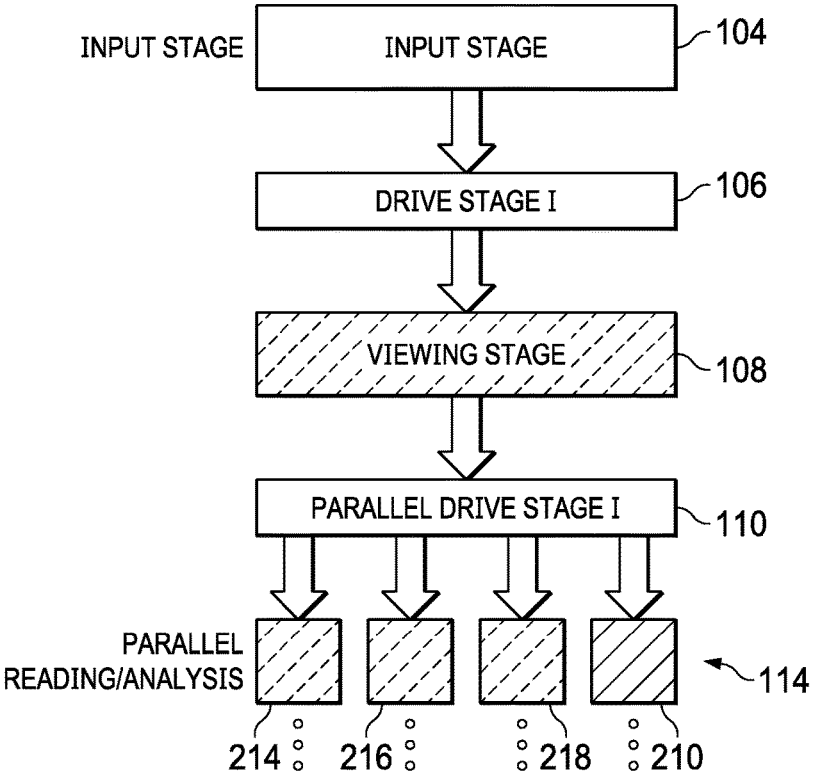

INPUT STAGE
INPUT STAGE
104
DRIVE STAGE I
106
VIEWING STAGE
108
PARALLEL DRIVE STAGE I
110
PARALLEL
READING/ANALYSIS
114
214
216
218
210

FIG. 2B

FIG. 2C

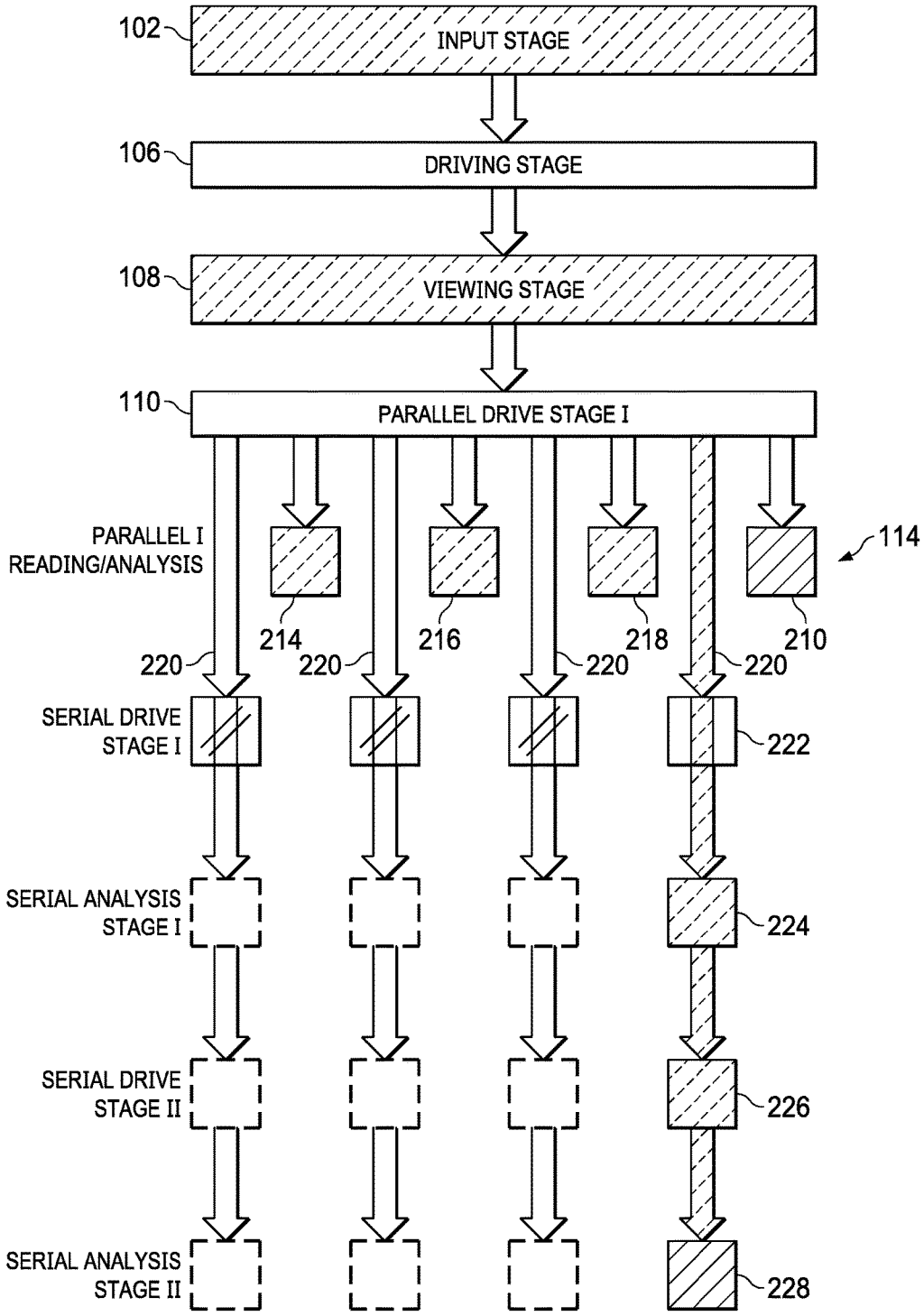
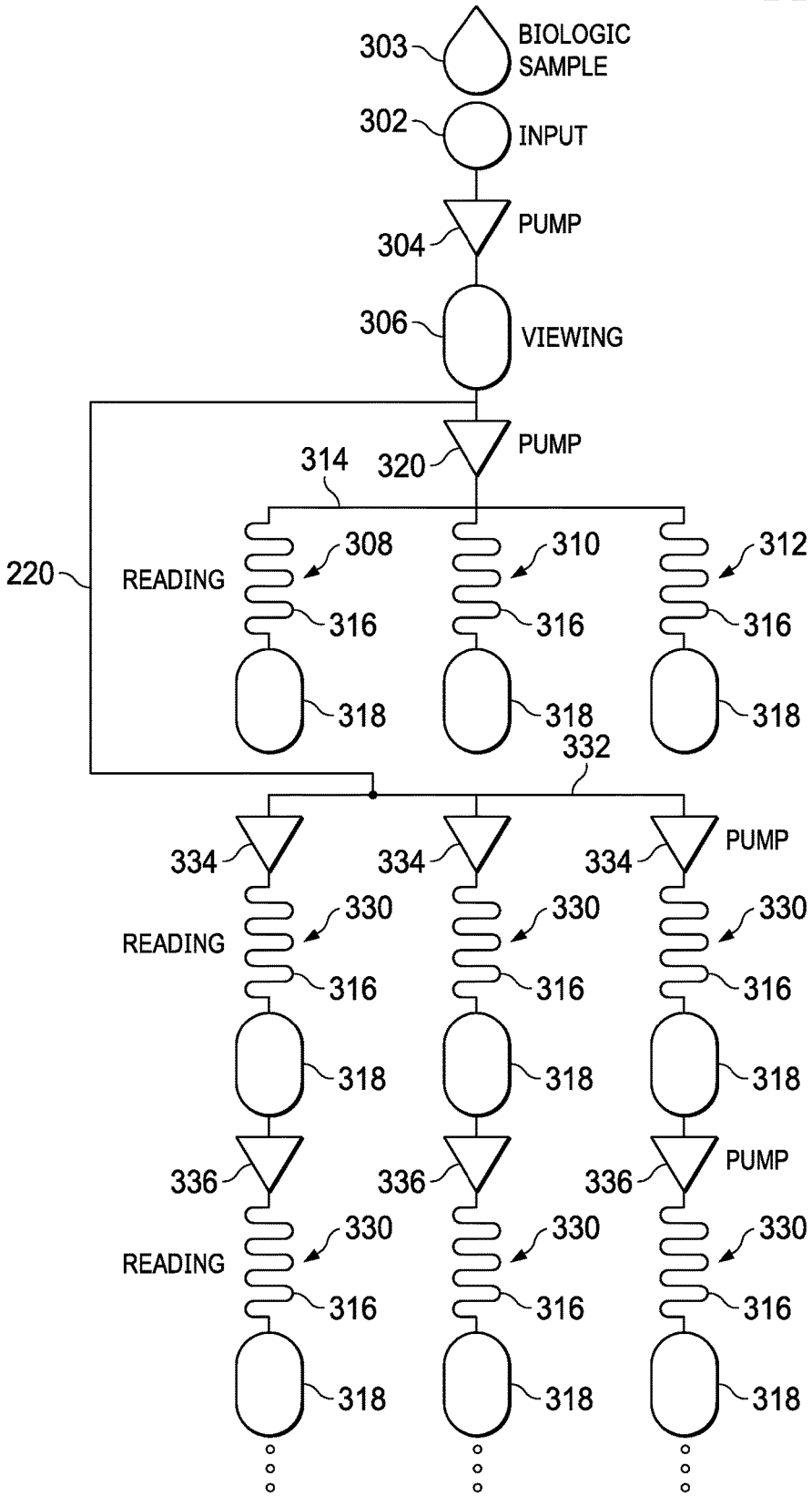

102
INPUT STAGE
106
DRIVING STAGE
108
VIEWING STAGE
110
PARALLEL DRIVE STAGE I
PARALLEL I READING/ANALYSIS
114
214
216
218
210
220
220
220
220
SERIAL DRIVE STAGE I
222
SERIAL ANALYSIS STAGE I
224
SERIAL DRIVE STAGE II
226
SERIAL ANALYSIS STAGE II
228

FIG. 3A


303
BIOLOGIC SAMPLE
302
INPUT
304
PUMP
306
VIEWING
314
320
PUMP
220
READING
308
310
312
316
318
332
334
PUMP
330
336

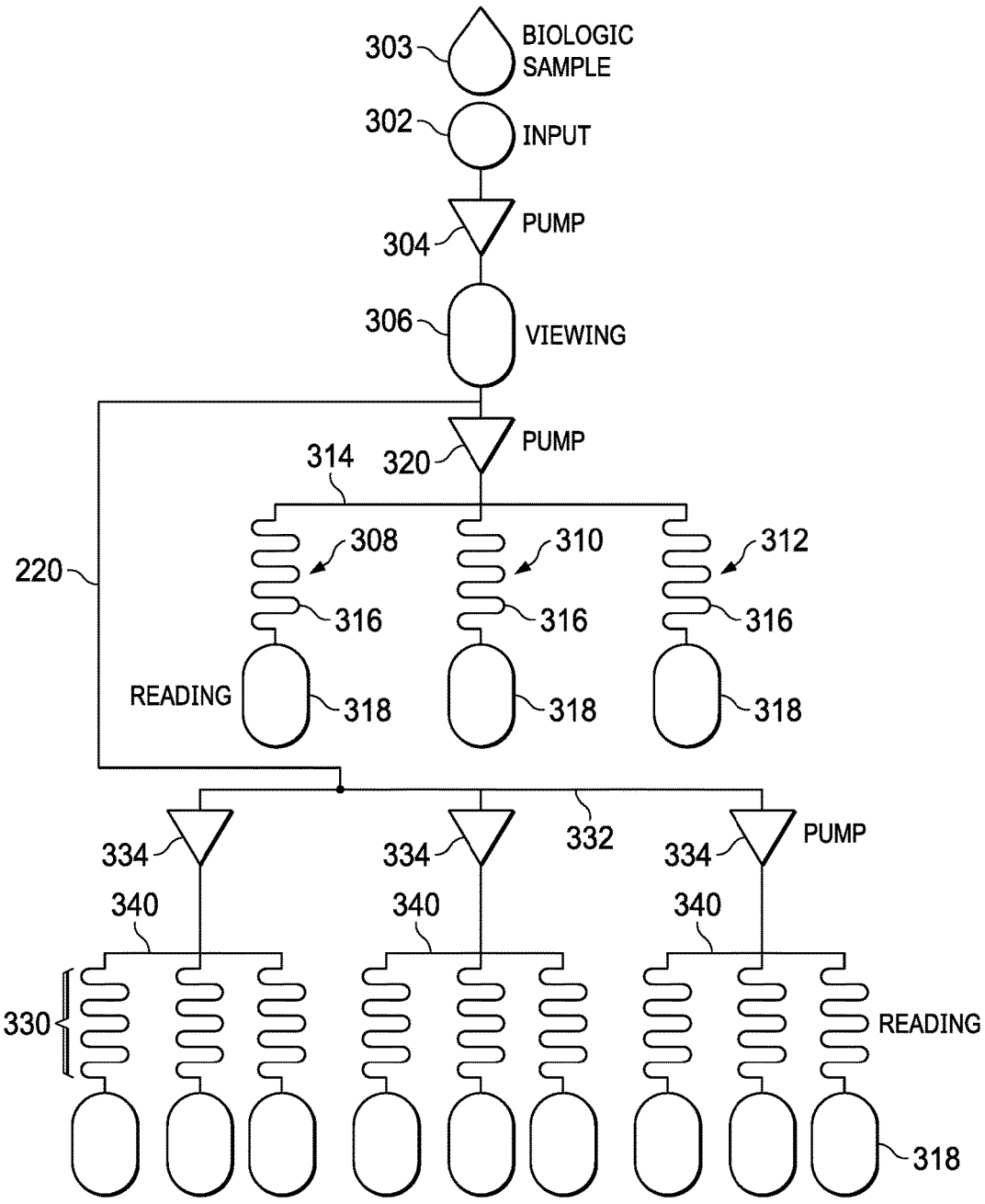

303
BIOLOGIC SAMPLE
302
INPUT
304
PUMP
306
VIEWING
314
320
PUMP
220
308
310
312
316
316
316
READING
318
318
318
334
334
332
334
PUMP
340
340
340
330
READING
318

FIG. 3B

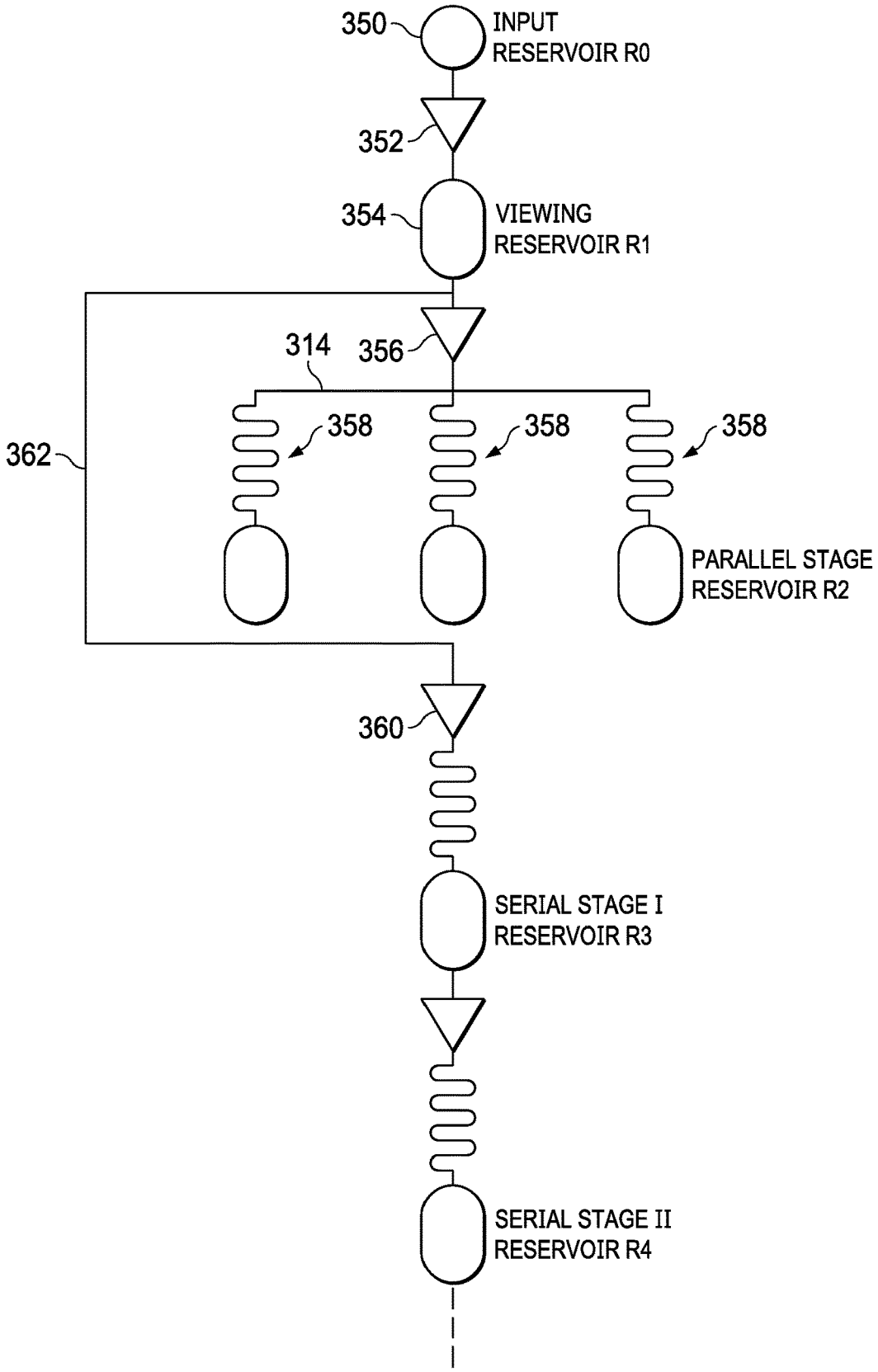

350
INPUT
RESERVOIR R0
352
354
VIEWING
RESERVOIR R1
314
356
358
358
358
362
PARALLEL STAGE
RESERVOIR R2
360
SERIAL STAGE I
RESERVOIR R3
SERIAL STAGE II
RESERVOIR R4

FIG. 3C

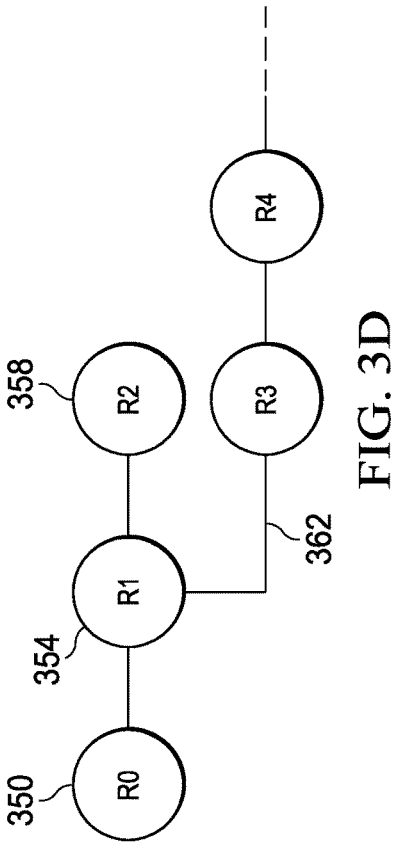

R4
R3
R2
R1
R0
358
362
354
350

FIG. 3D

402
404
406
408
412
410
414
416
418
420
423
422
426
428
430
432

FIG. 4A

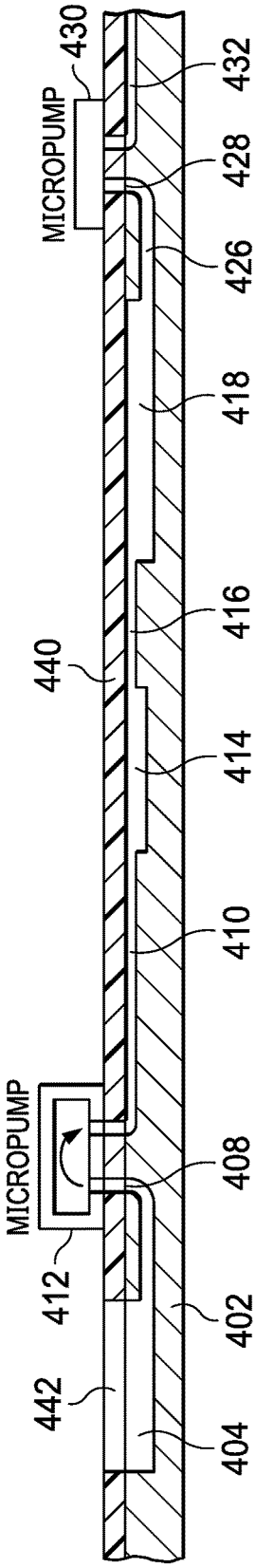

MICROPUMP
430
432
428
426
418
416
440
414
410
MICROPUMP
408
412
402
442
404

FIG. 4B

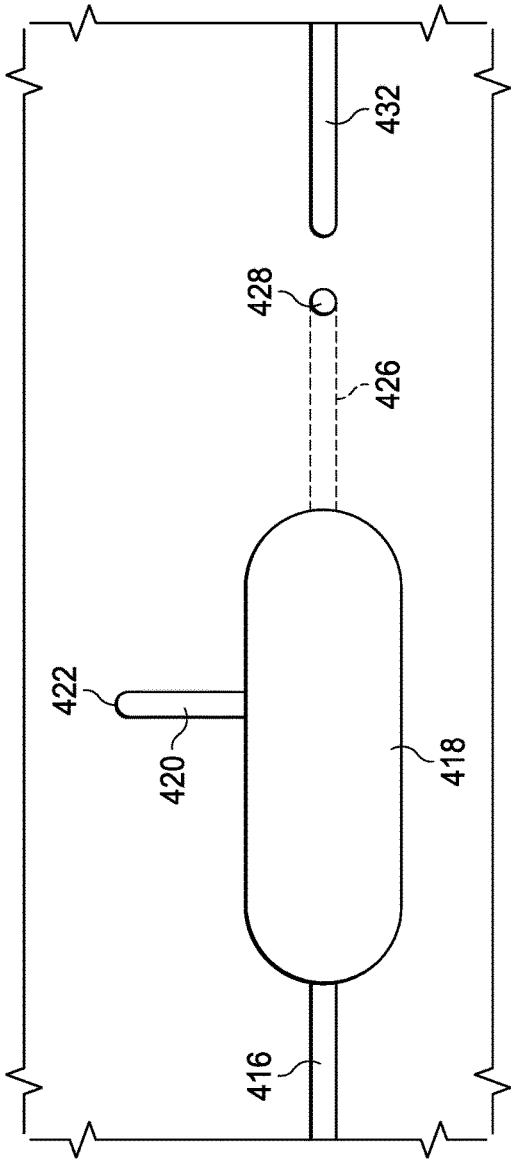

432
428
426
422
420
418
416

FIG. 4C

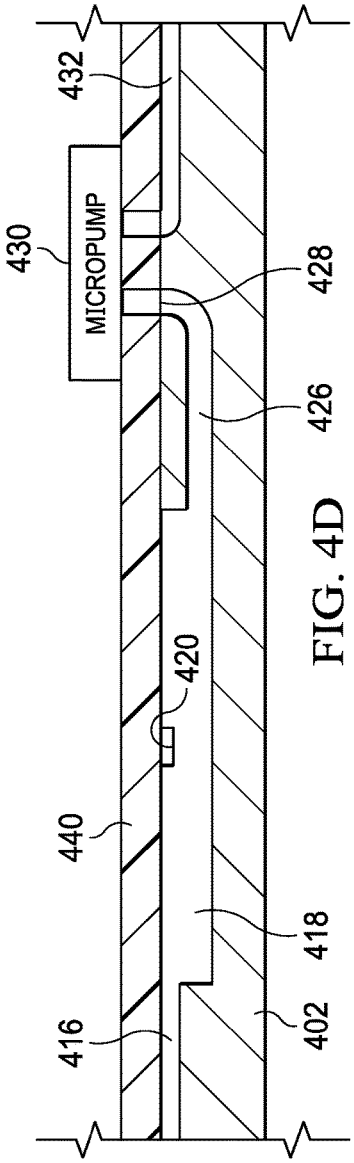

432
430
MICROPUMP
428
426
420
440
418
416
402

FIG. 4D

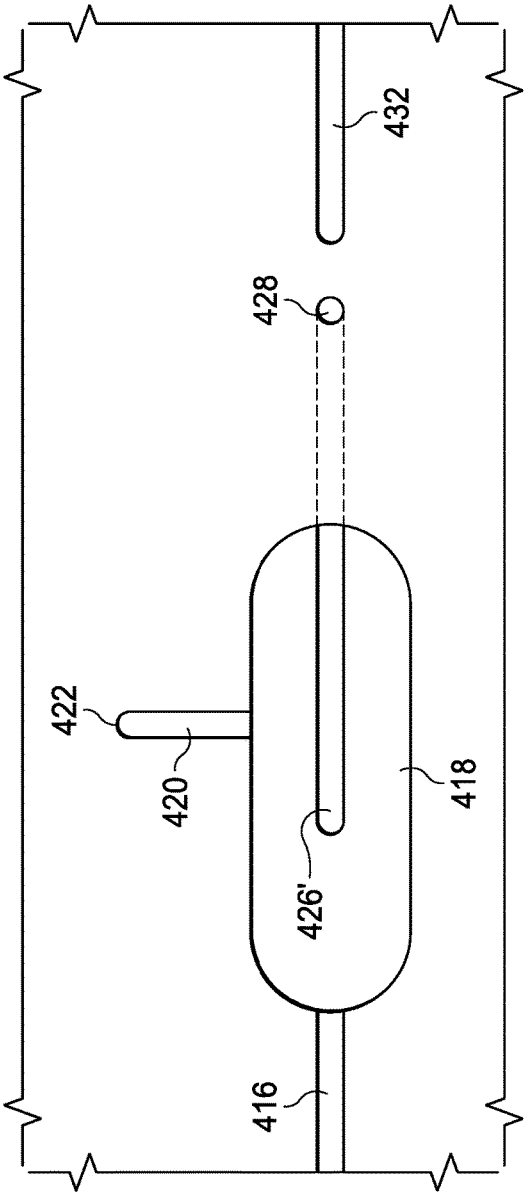

432
428
422
420
418
426'
416

FIG. 4E

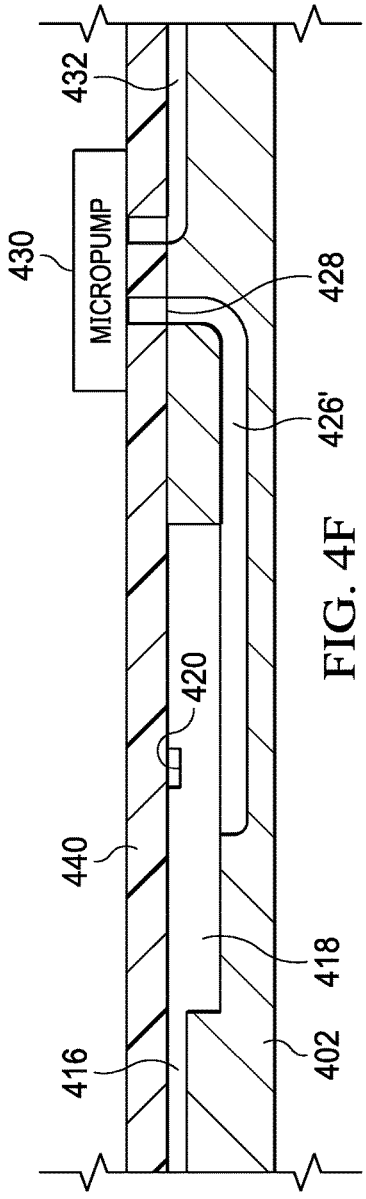

432
MICROPUMP
430
428
426'
420
440
418
416
402

FIG. 4F

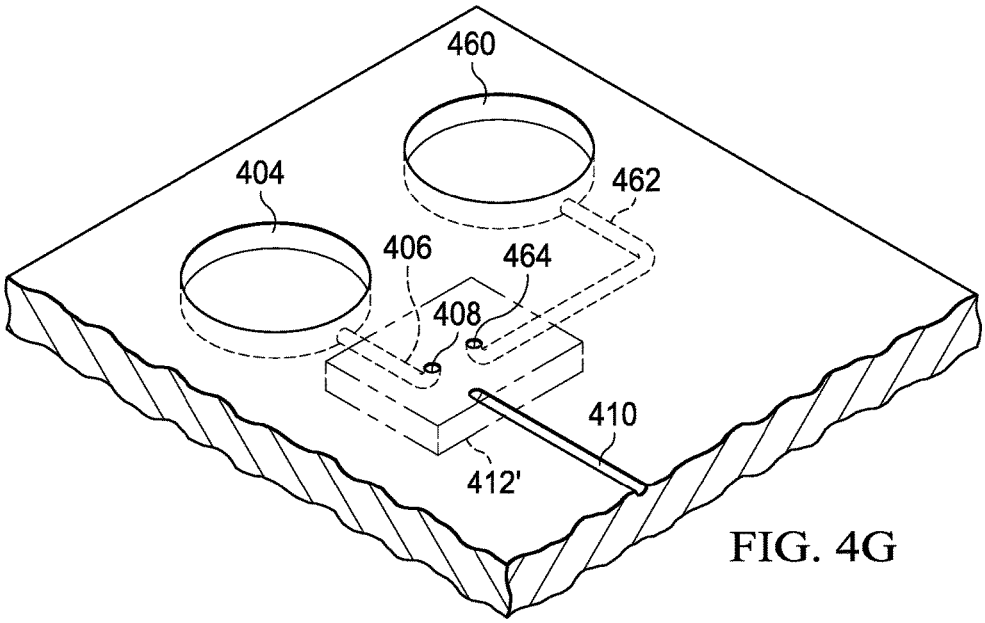


FIG. 4G

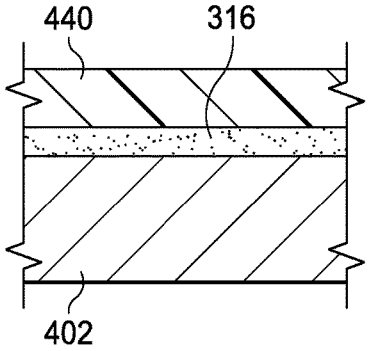


FIG. 5A

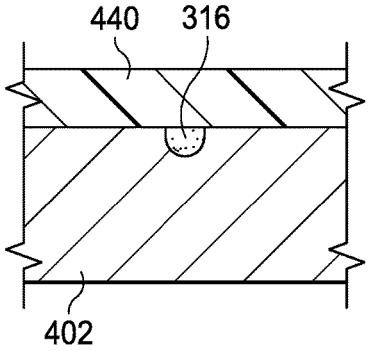


FIG. 5B

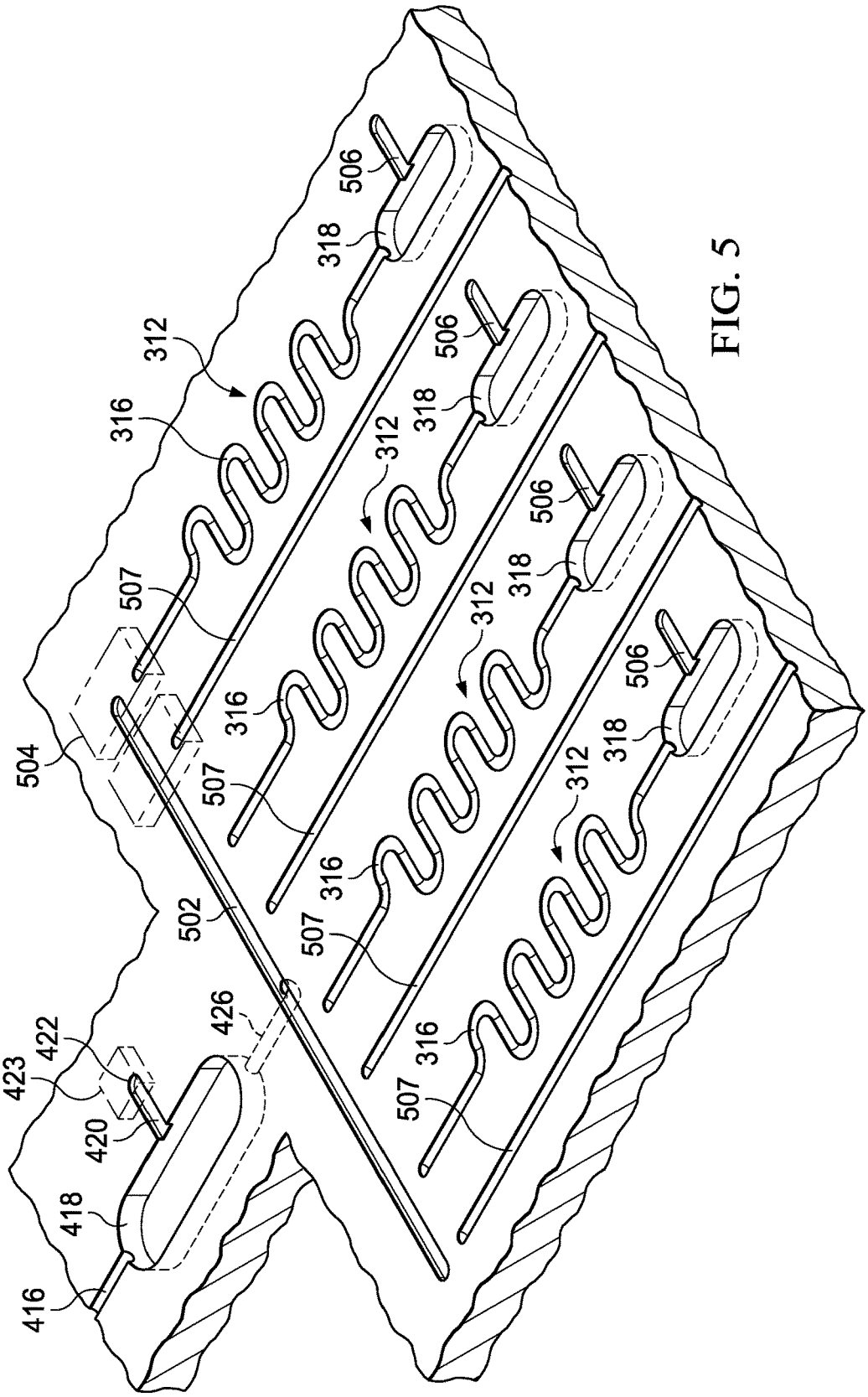


FIG. 5

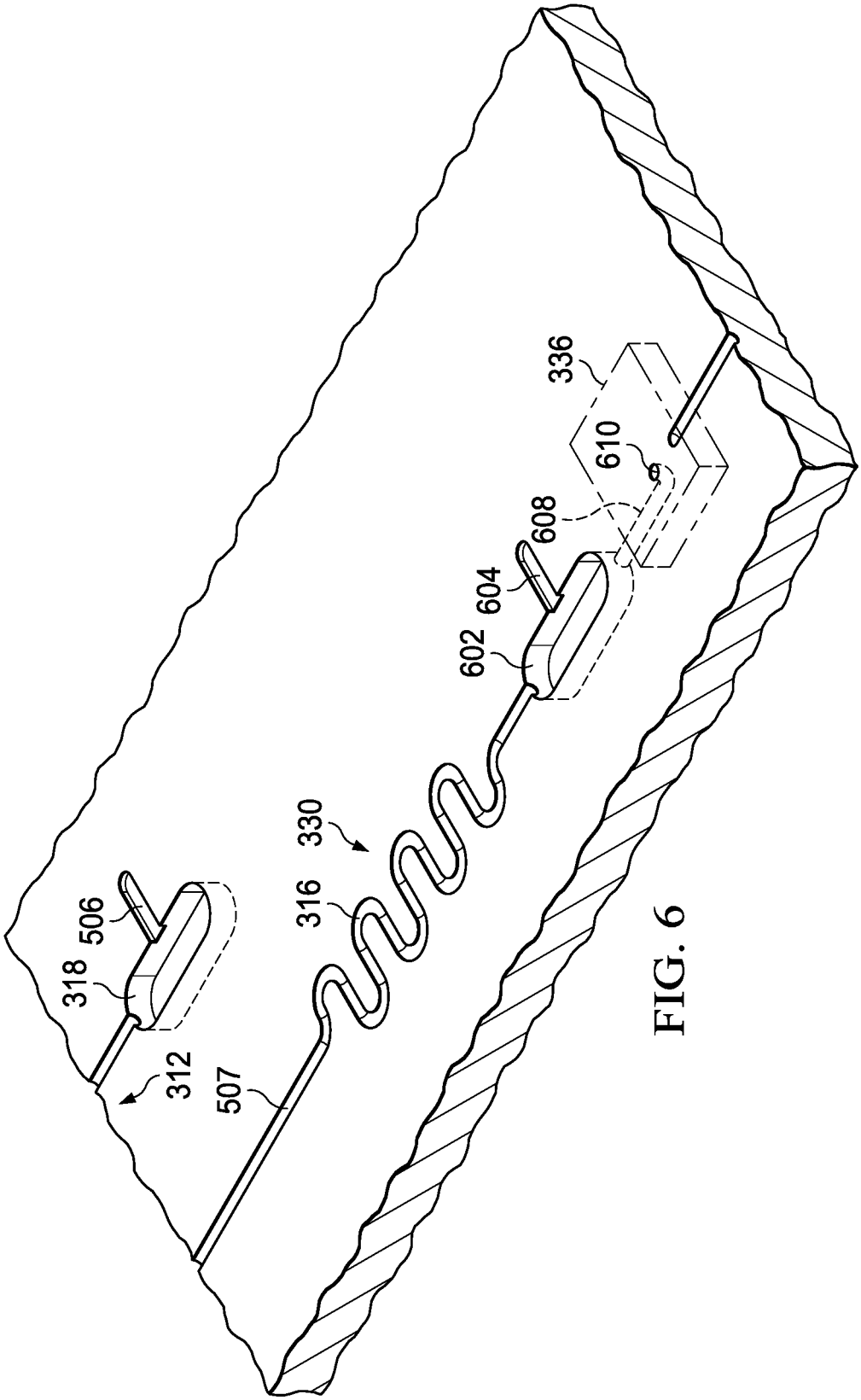


FIG. 6

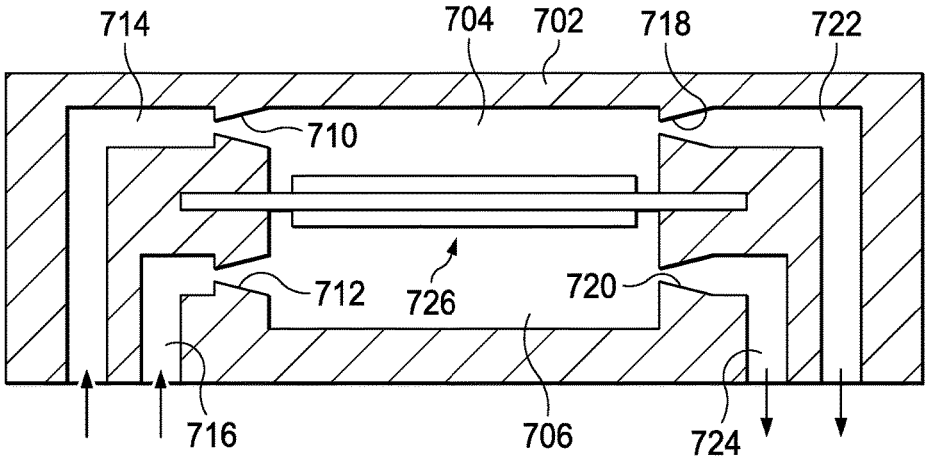


FIG. 7A

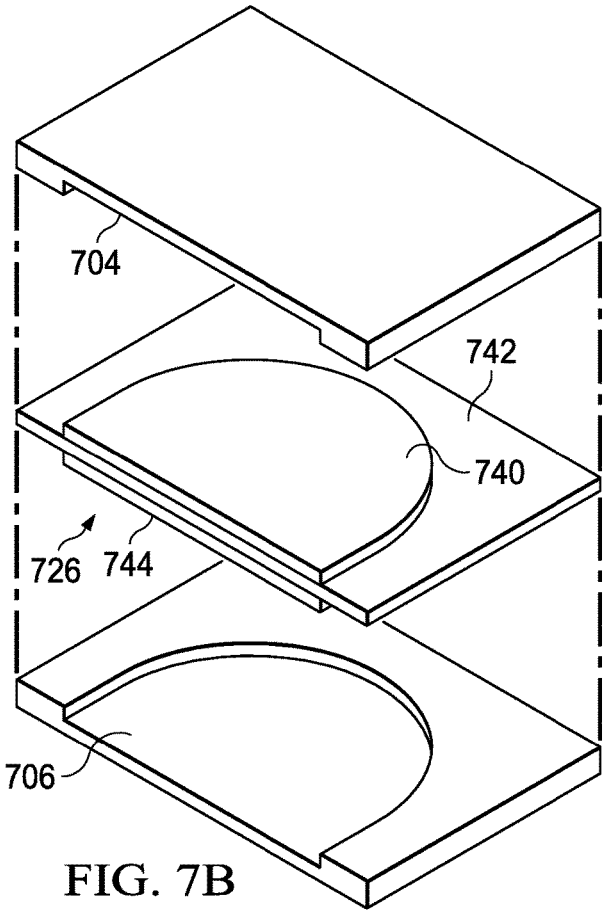


FIG. 7B

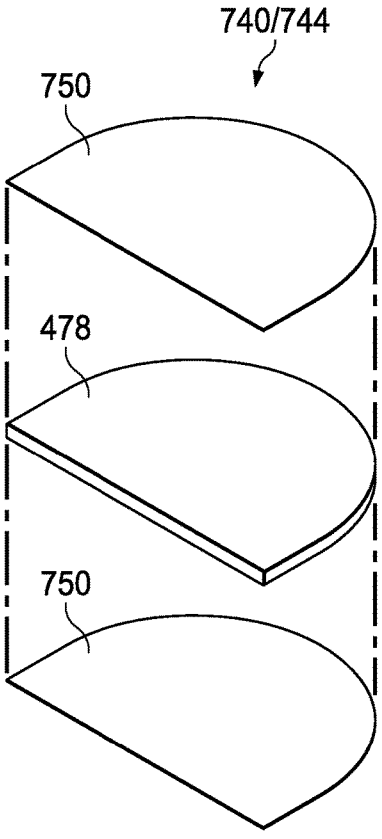


FIG. 7C

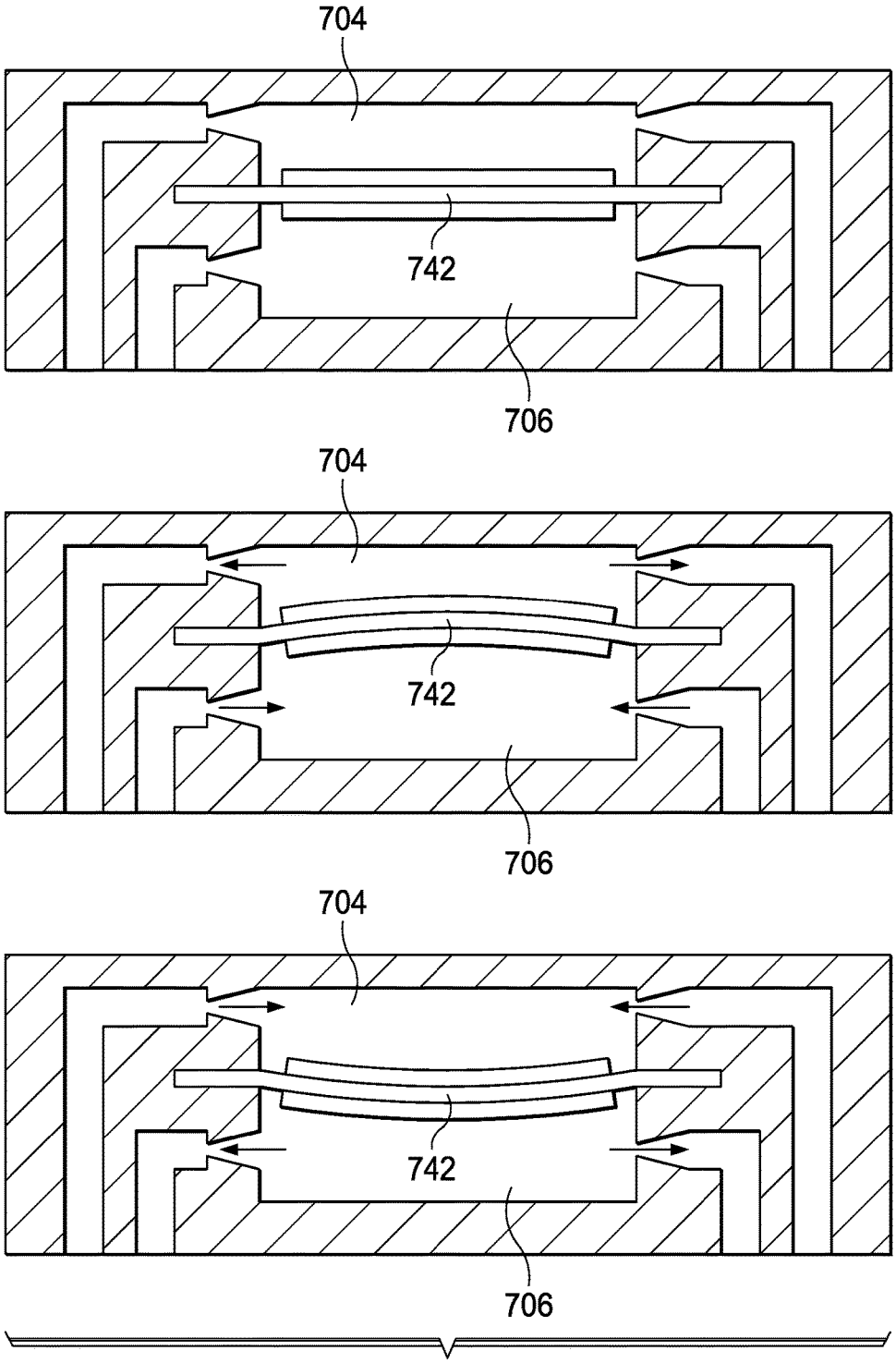


FIG. 7D

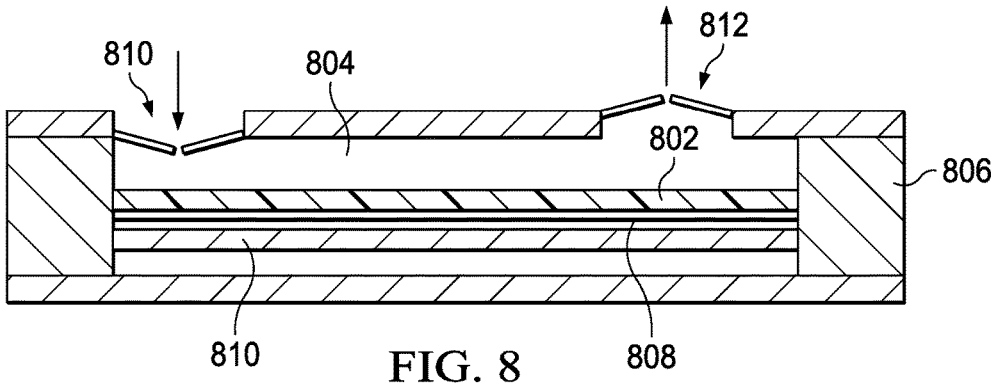

810
804
812
802
806
810
808

FIG. 8

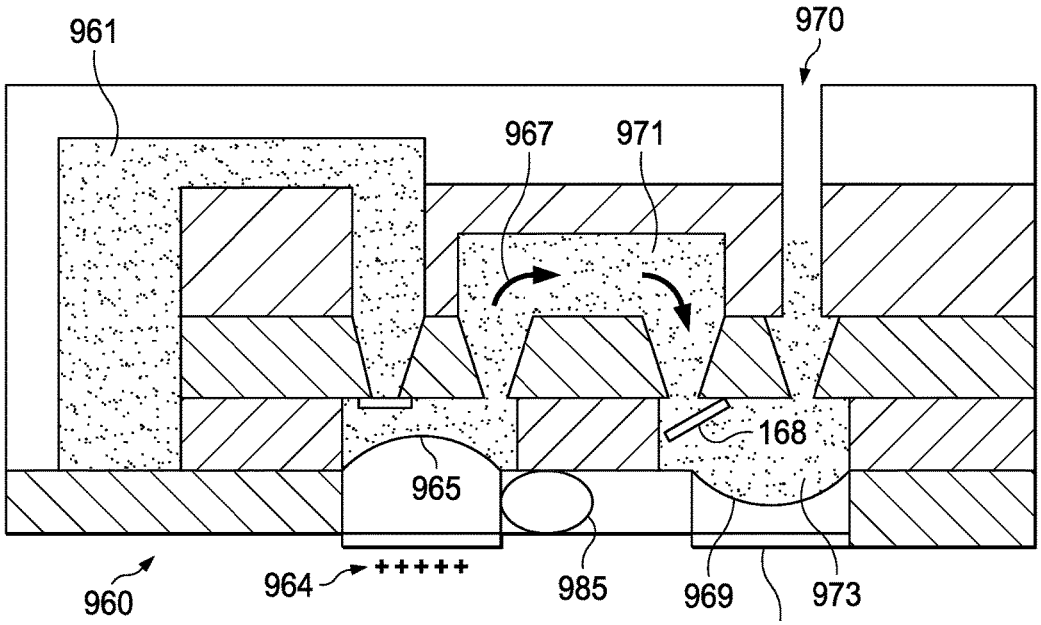

961
970
967
971
168
965
960
964
+++++
985
969
973
968

FIG. 9

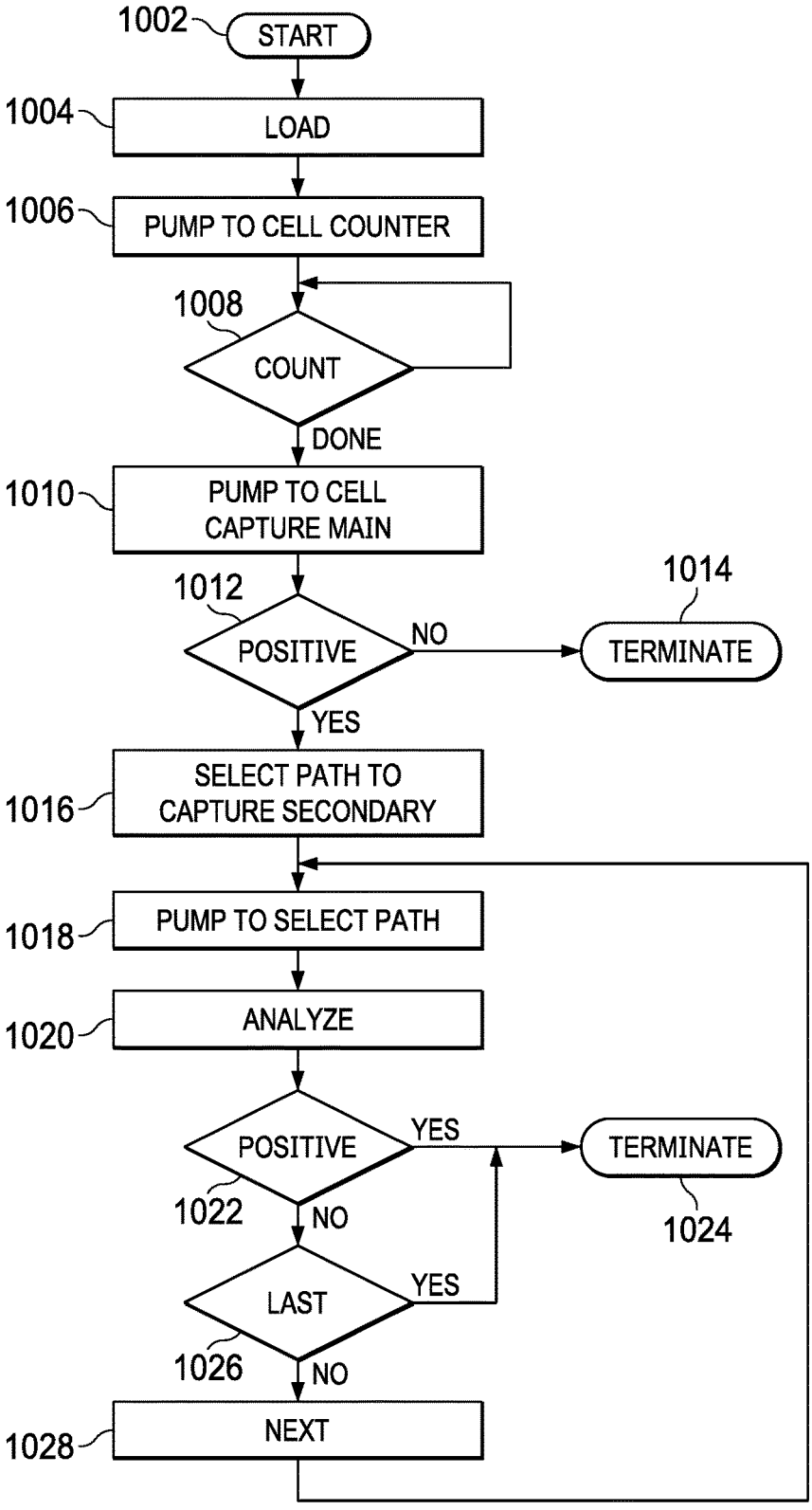

1002
START
1004
LOAD
1006
PUMP TO CELL COUNTER
1008
COUNT
DONE
1010
PUMP TO CELL CAPTURE MAIN
1012
POSITIVE
NO
1014
TERMINATE
YES
1016
SELECT PATH TO CAPTURE SECONDARY
1018
PUMP TO SELECT PATH
1020
ANALYZE
POSITIVE
YES
TERMINATE
1022
NO
1024
LAST
YES
1026
NO
1028
NEXT

FIG. 10

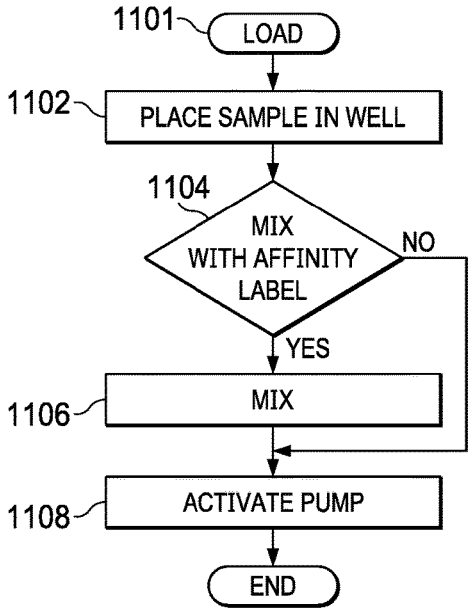

1101
LOAD
1102
PLACE SAMPLE IN WELL
1104
MIX WITH AFFINITY LABEL
NO
YES
1106
MIX
1108
ACTIVATE PUMP
END

FIG. 11

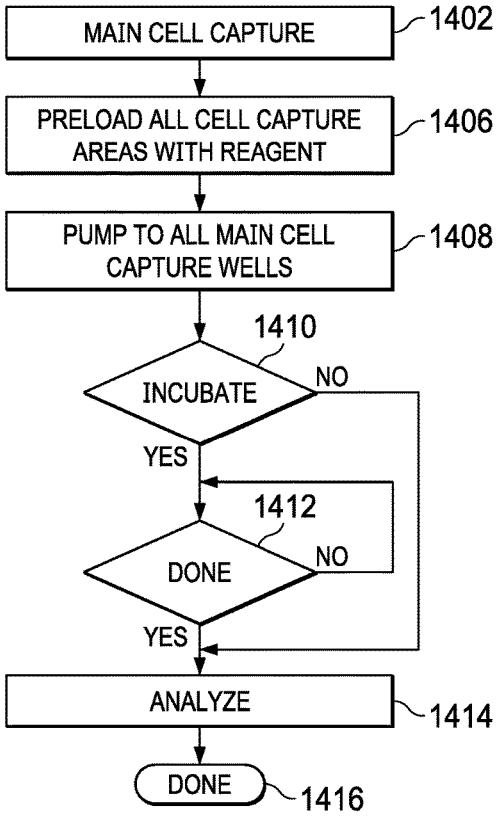

MAIN CELL CAPTURE
1402
PRELOAD ALL CELL CAPTURE AREAS WITH REAGENT
1406
PUMP TO ALL MAIN CELL CAPTURE WELLS
1408
1410
INCUBATE
NO
YES
1412
DONE
NO
YES
ANALYZE
1414
DONE
1416

FIG. 14

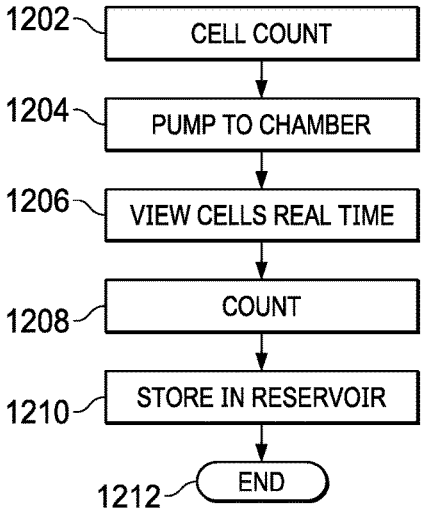


FIG. 12

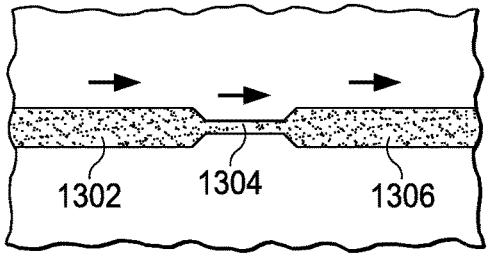


FIG. 13A

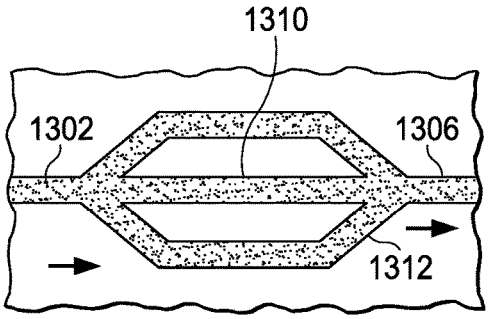


FIG. 13B

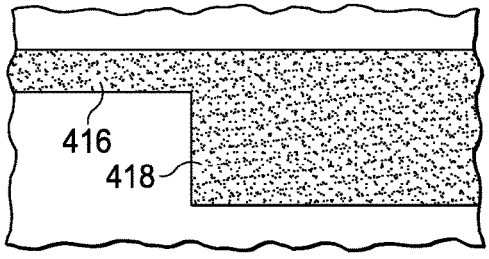


FIG. 13C

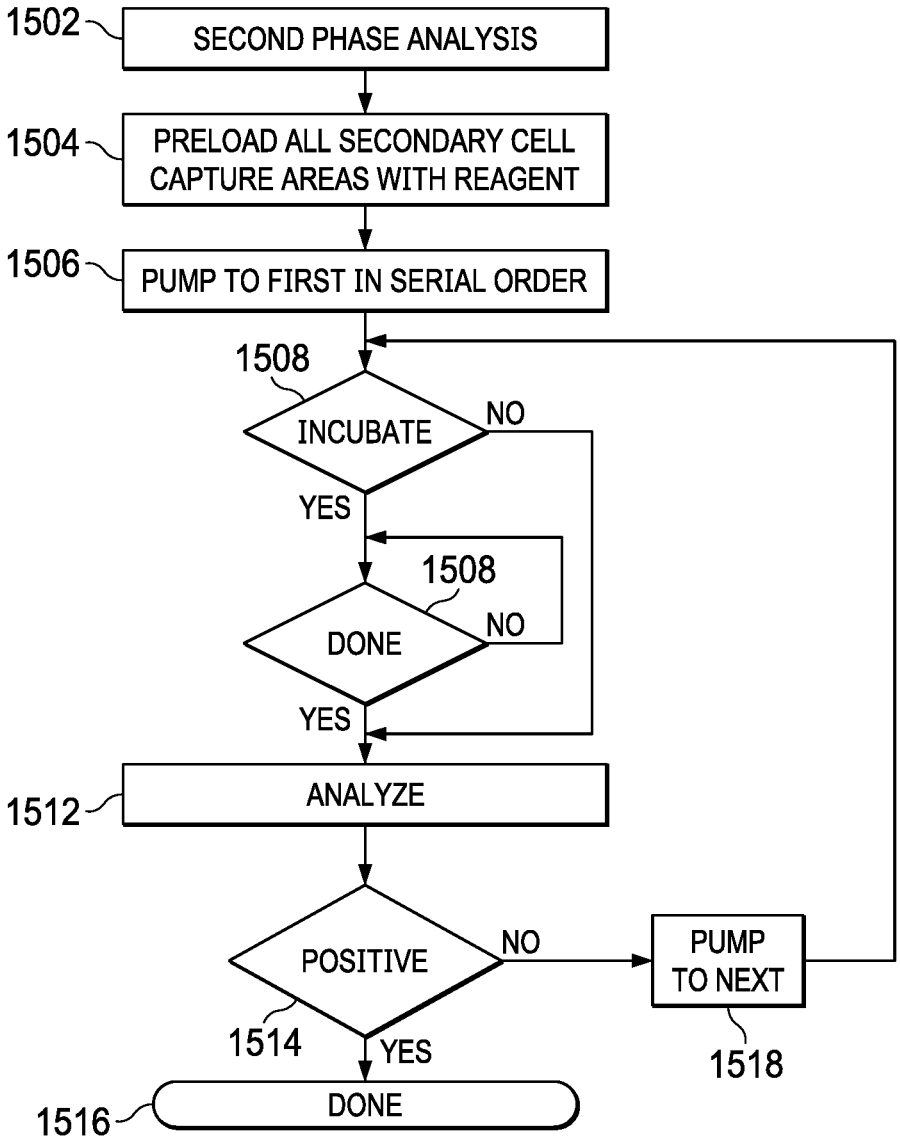
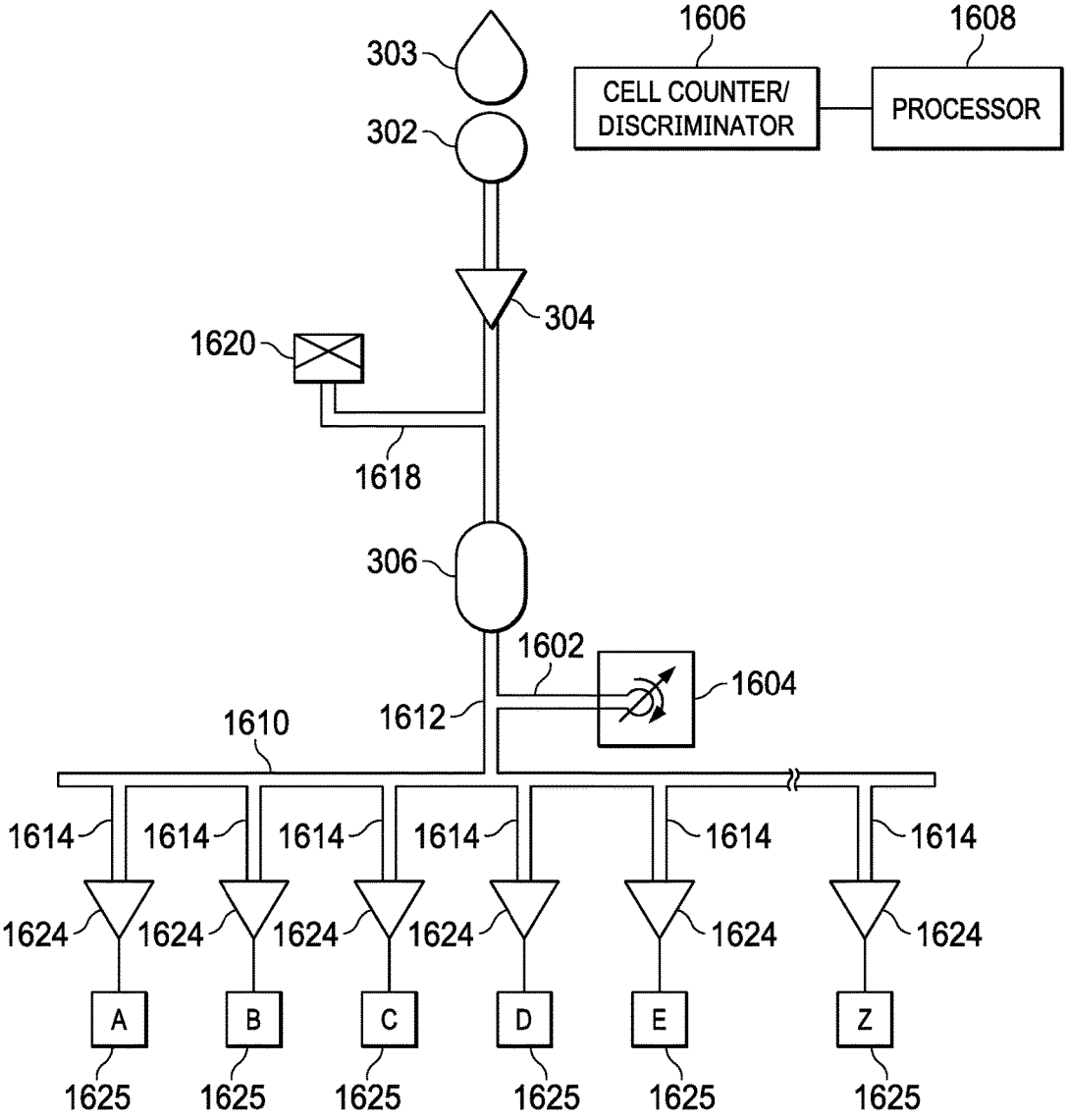

1502
SECOND PHASE ANALYSIS
1504
PRELOAD ALL SECONDARY CELL CAPTURE AREAS WITH REAGENT
1506
PUMP TO FIRST IN SERIAL ORDER
1508
INCUBATE
NO
YES
1508
DONE
NO
YES
1512
ANALYZE
POSITIVE
NO
PUMP TO NEXT
1514
YES
1518
1516
DONE

FIG. 15

FIG. 16


1606
1608
303
CELL COUNTER/
DISCRIMINATOR
PROCESSOR
302
304
1620
1618
306
1602
1604
1612
1610
1614
1624
A
B
C
D
E
Z
1625

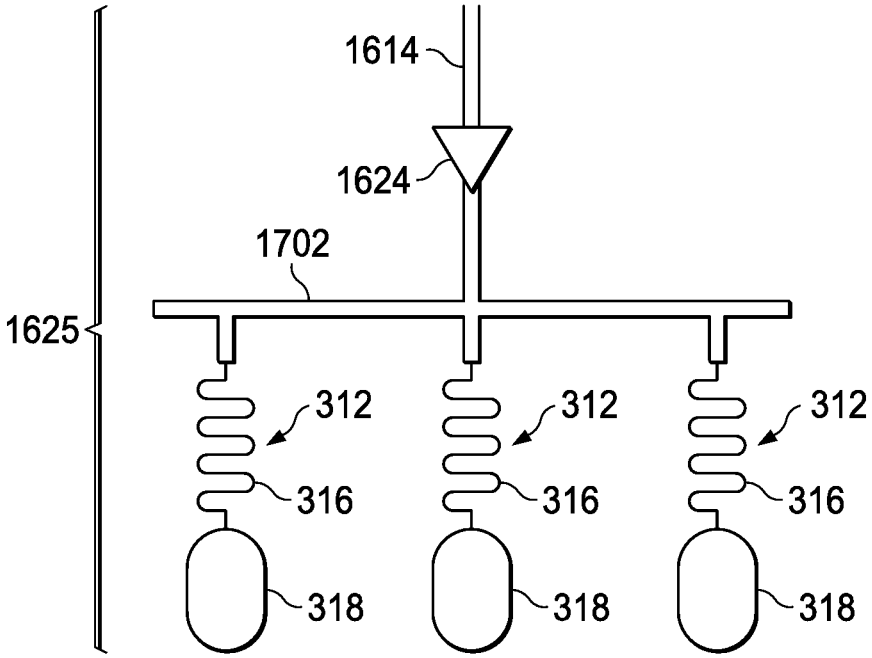


FIG. 17

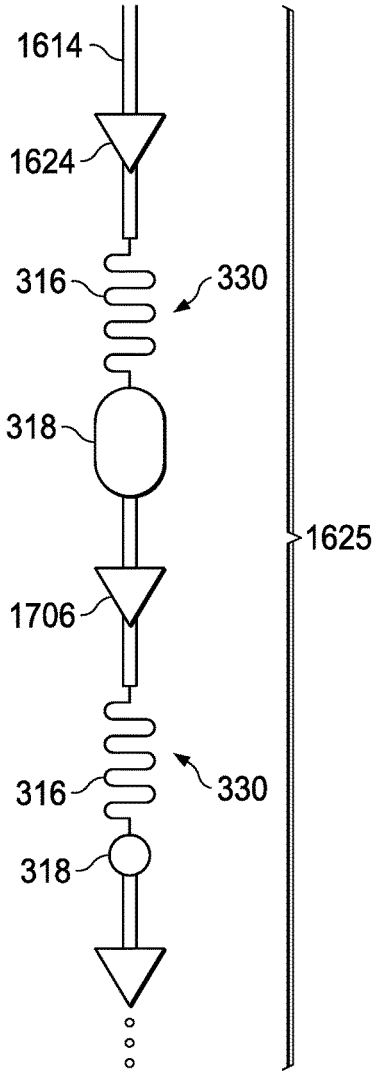

1614
1624
316
330
318
1625
1706
316
330
318

FIG. 18

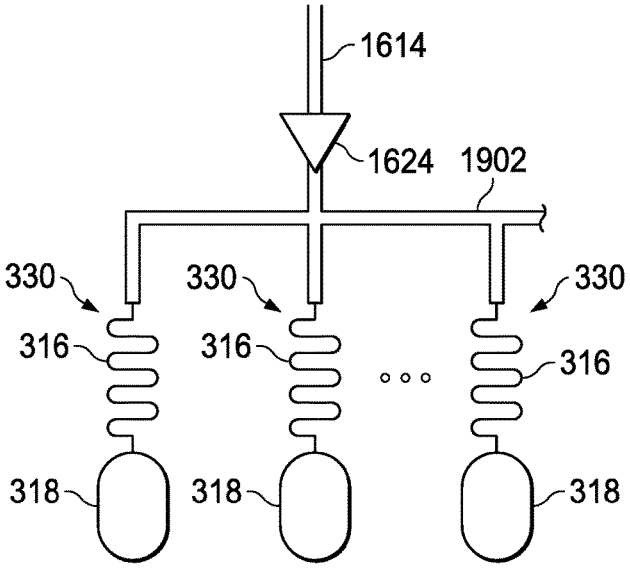

1614
1624
1902
330
330
330
316
316
316
318
318
318

FIG. 19

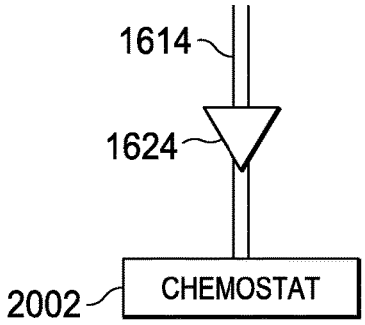


FIG. 20A

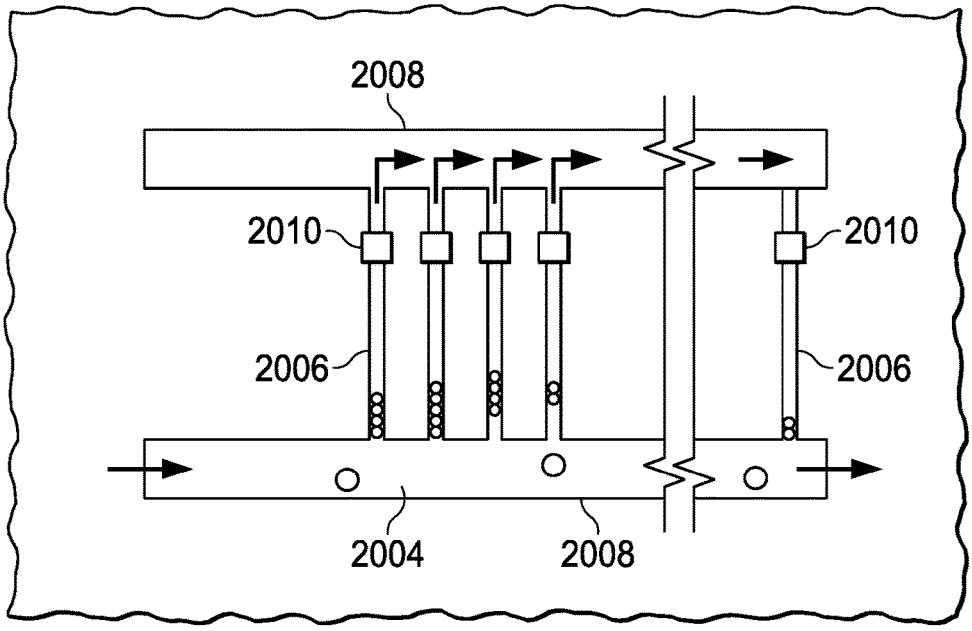


FIG. 20B

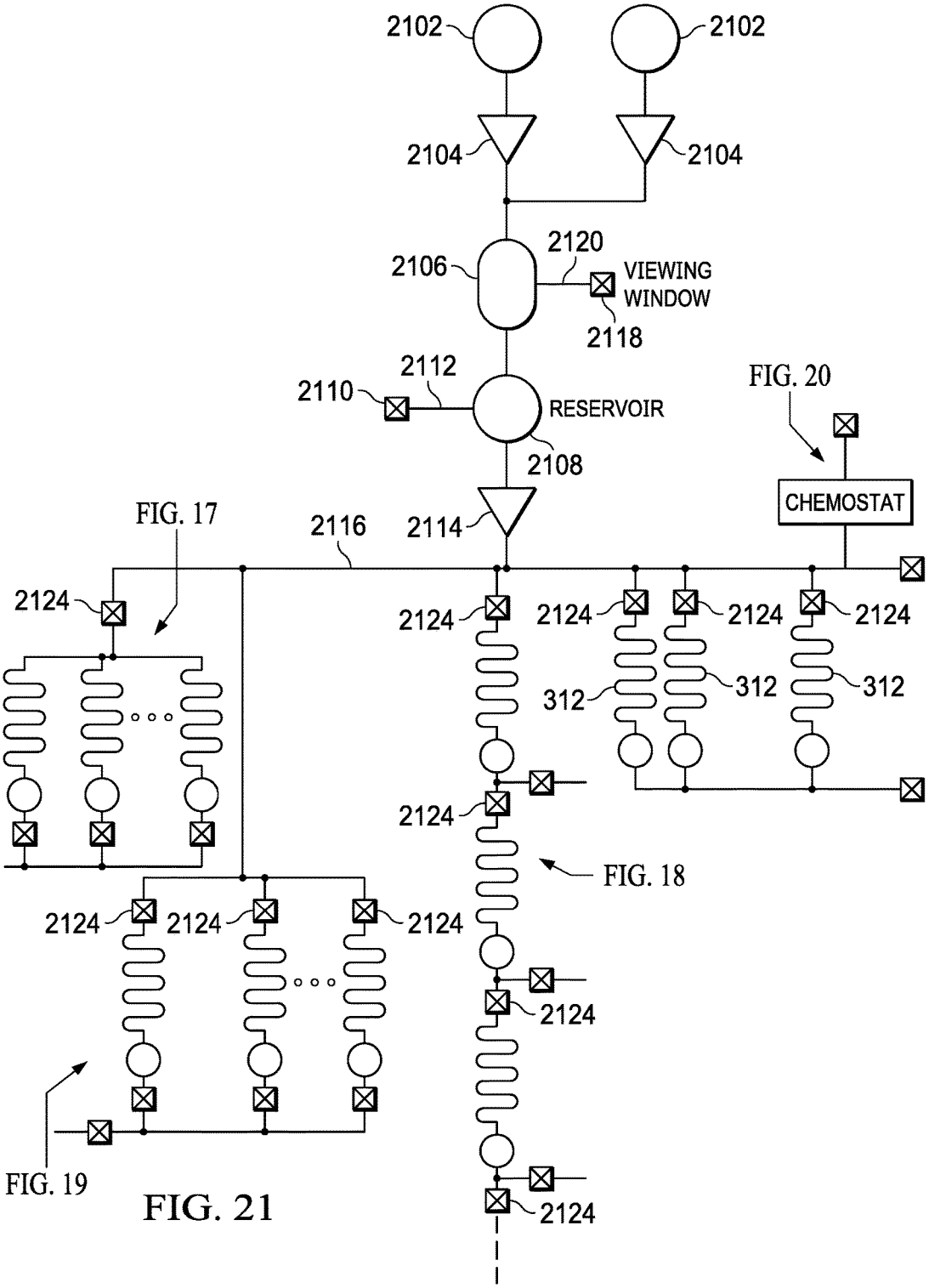

2102
2102
2104
2104
2106
2120
VIEWING WINDOW
2118
2112
2110
RESERVOIR
2108
2116
2114
FIG. 17
FIG. 20
CHEMOSTAT
2124
312
FIG. 18
FIG. 19

FIG. 21

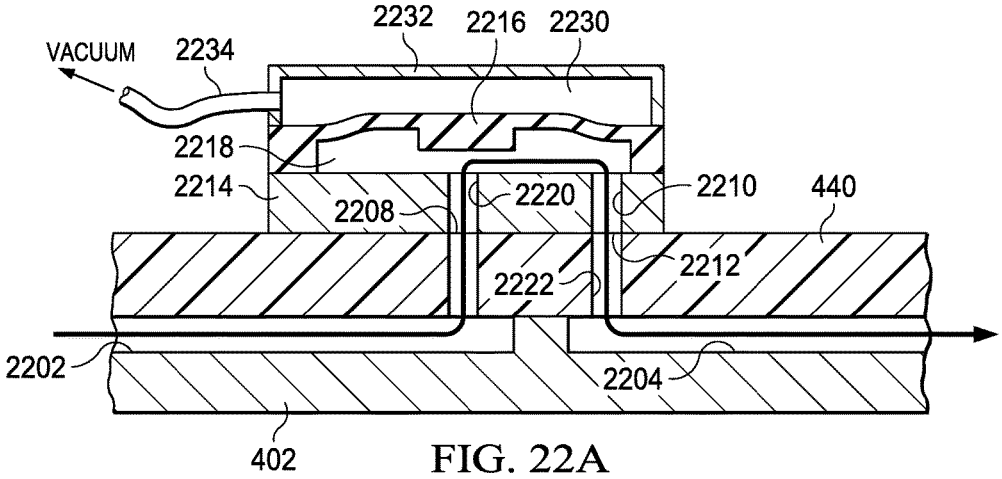


FIG. 22A

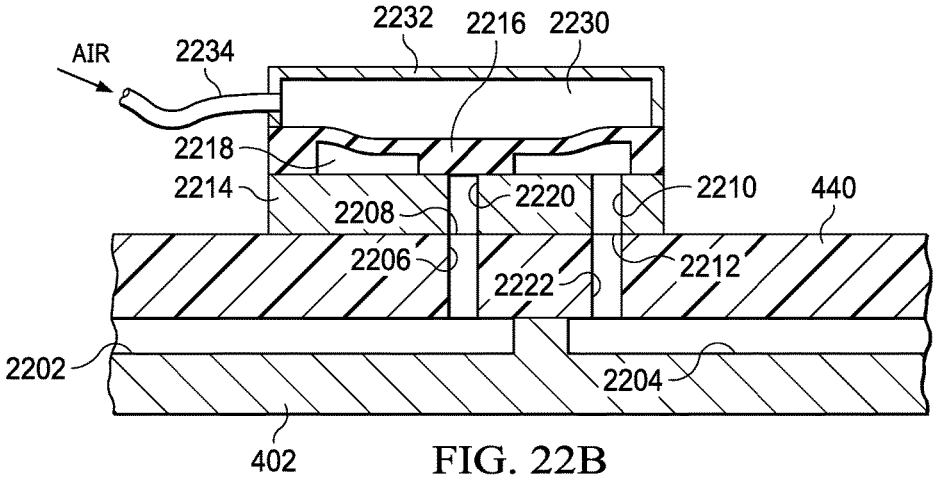


FIG. 22B

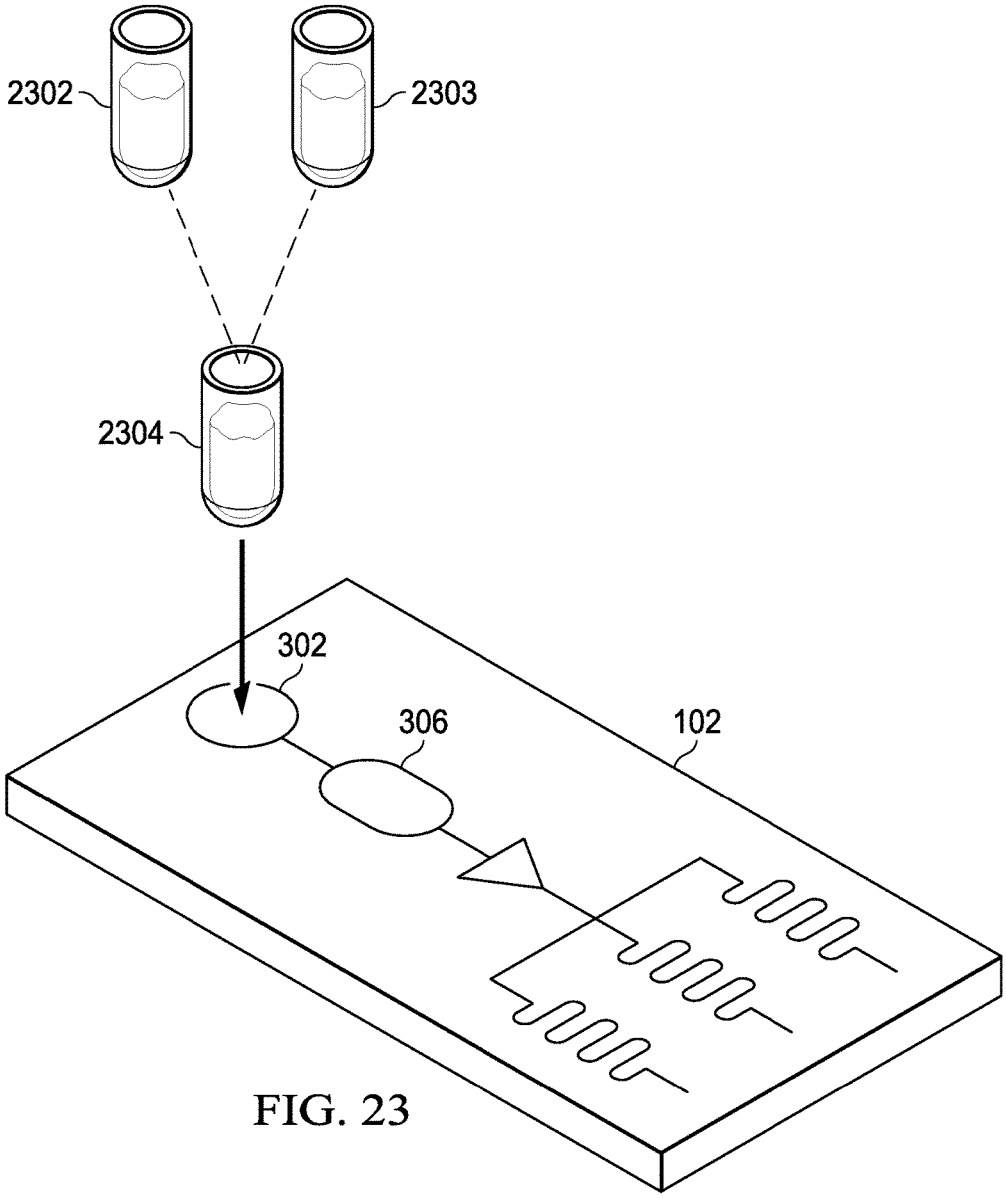


FIG. 23

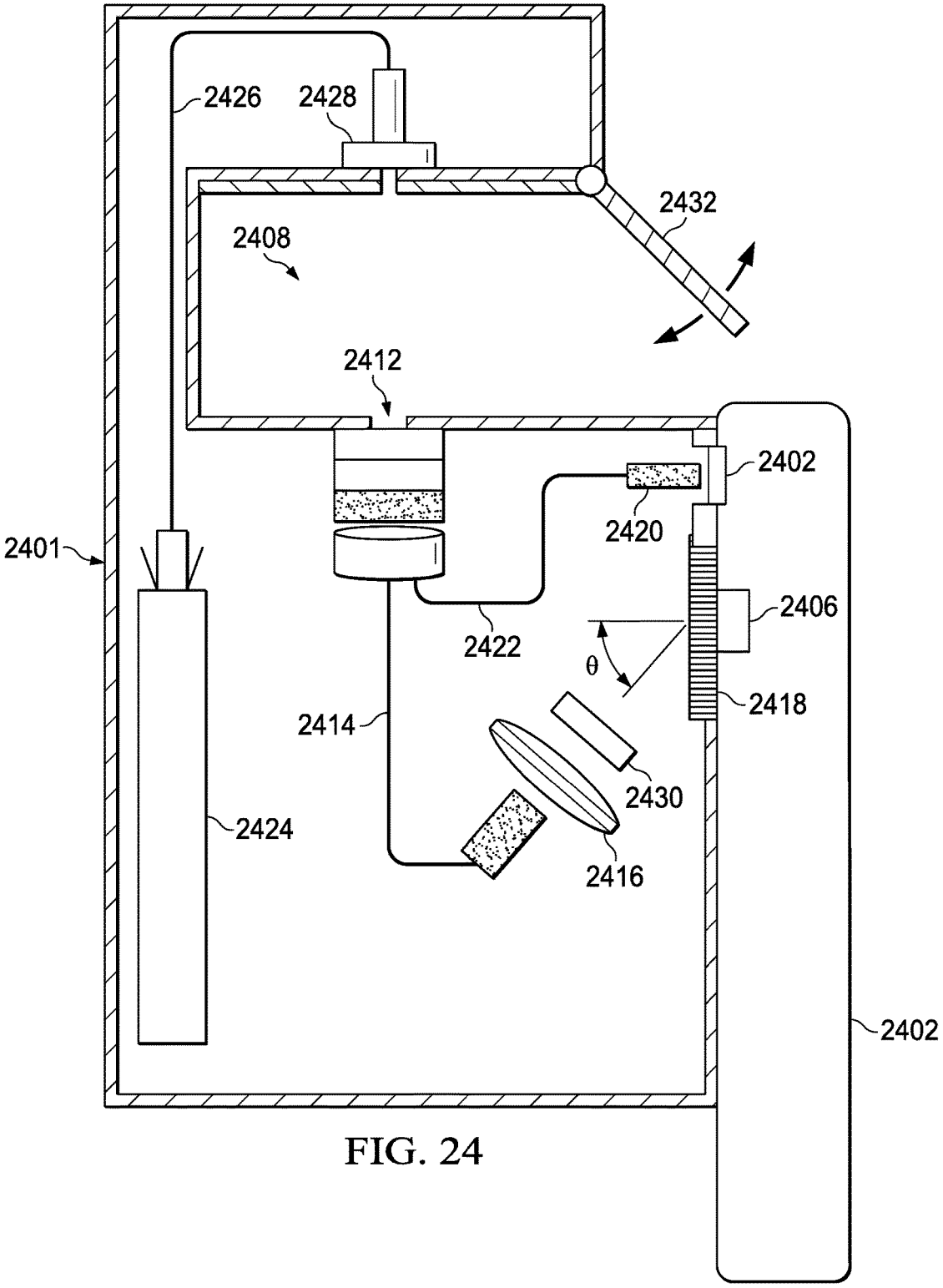

2426
2428
2432
2408
2412
2402
2420
2401
2406
2422
θ
2418
2414
2430
2424
2416
2402

FIG. 24

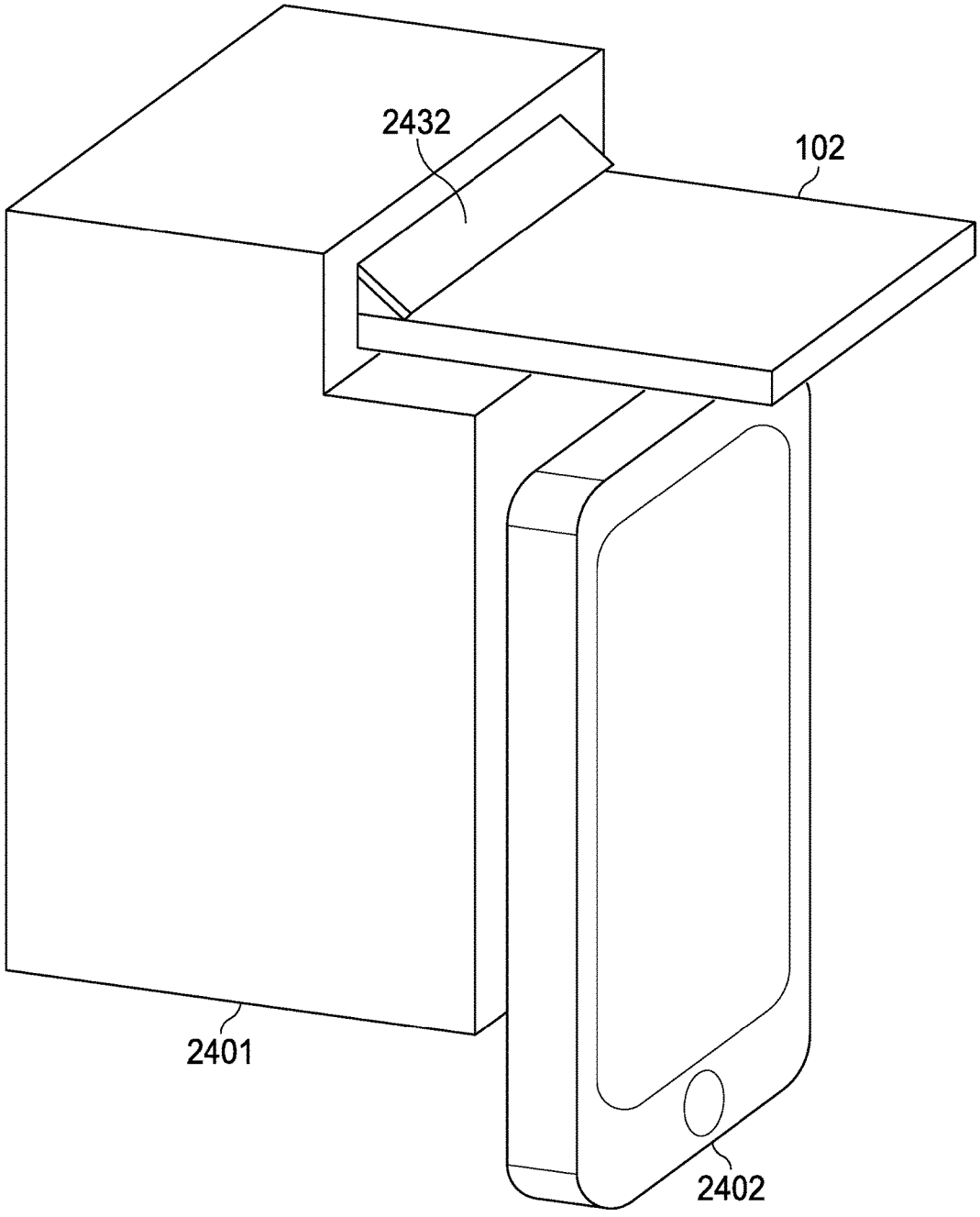


FIG. 25

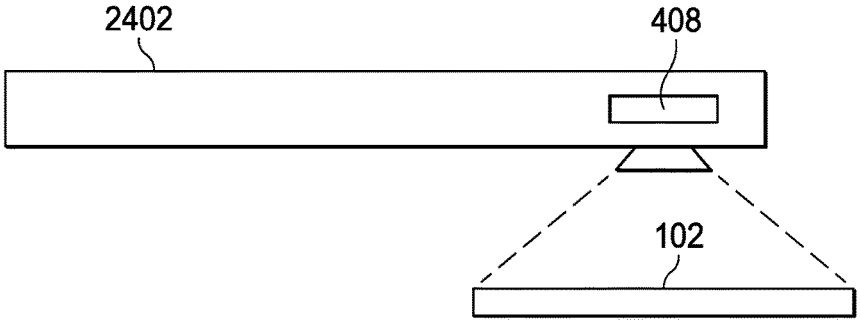


FIG. 26

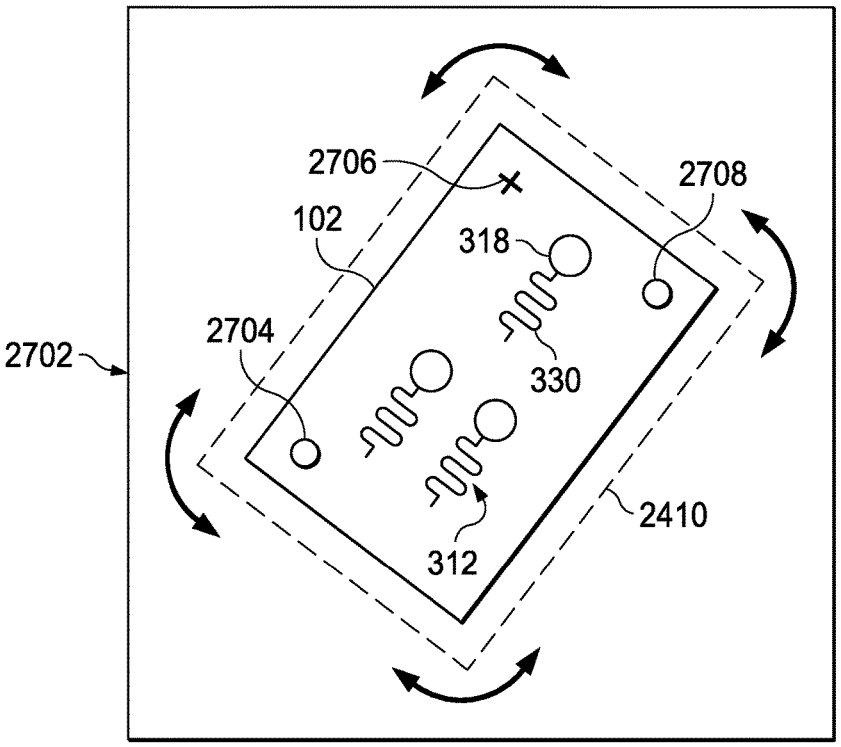


FIG. 27

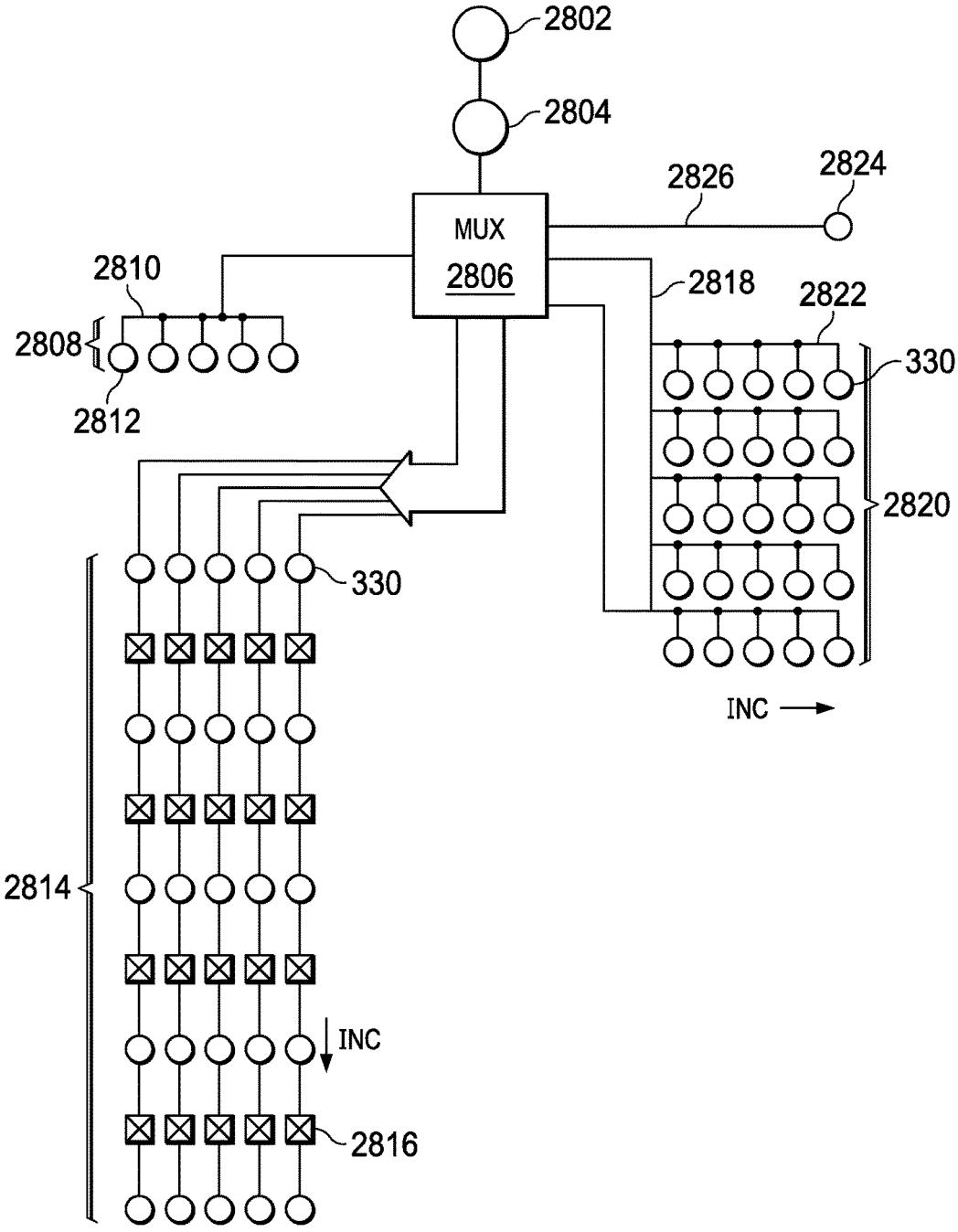

2802
2804
2826
2824
MUX
2806
2810
2818
2822
2808
330
2812
2820
330
INC
2814
INC
2816

FIG. 28A

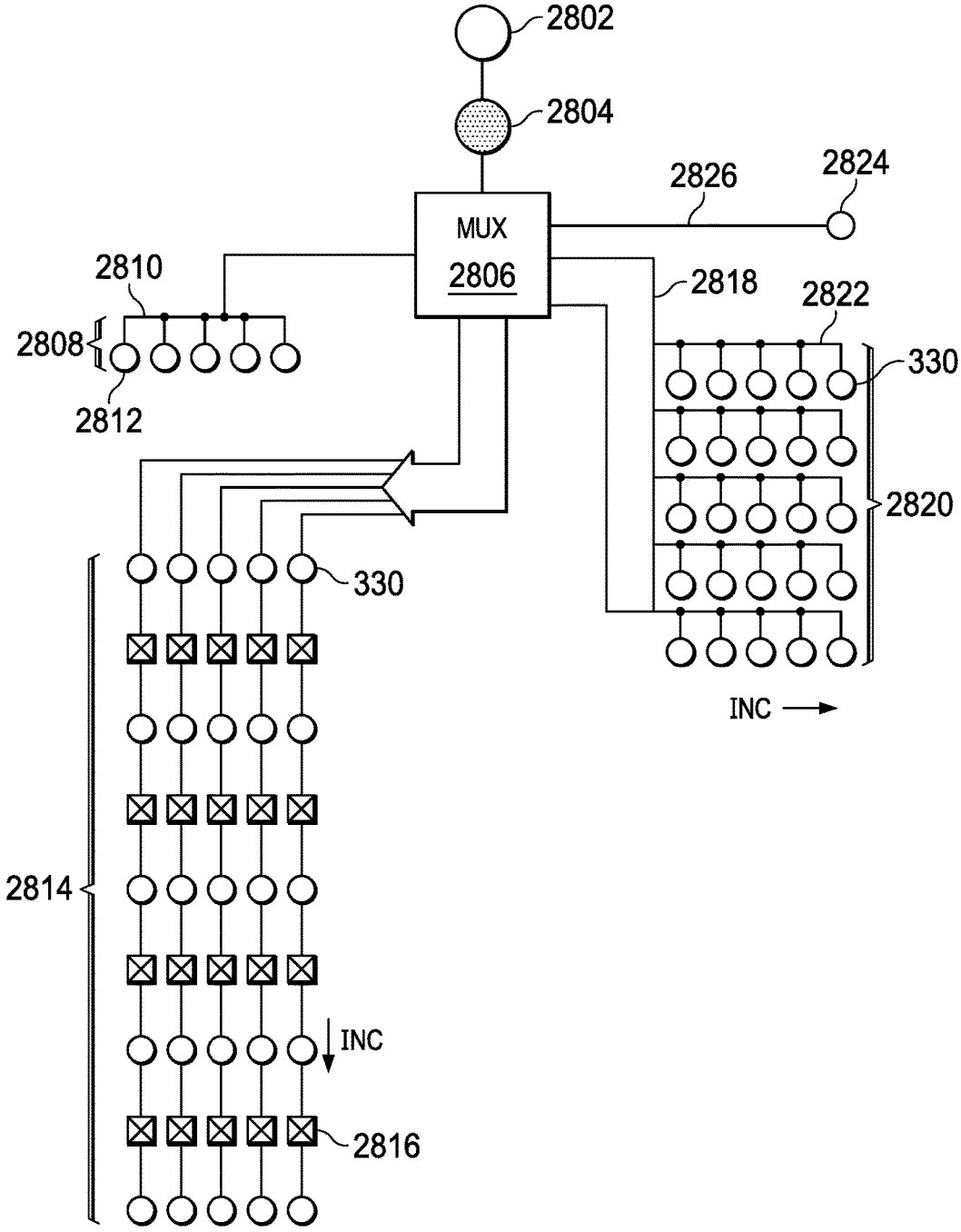

2802
2804
2826
2824
MUX
2806
2810
2818
2822
2808
330
2812
2820
330
INC
2814
INC
2816

FIG. 28B

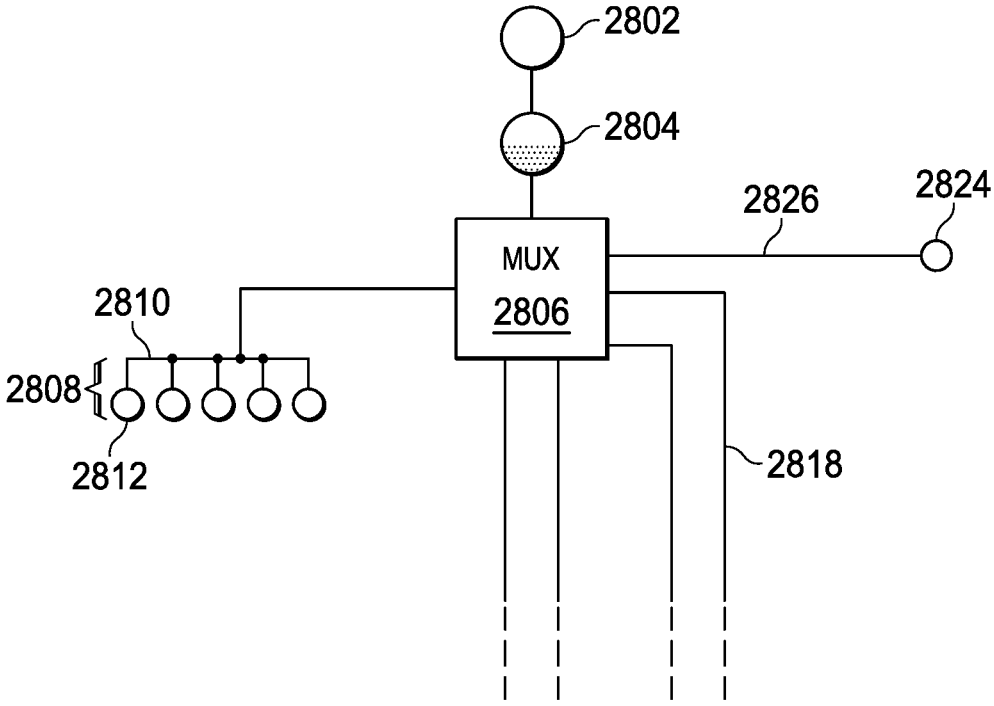


FIG. 28C

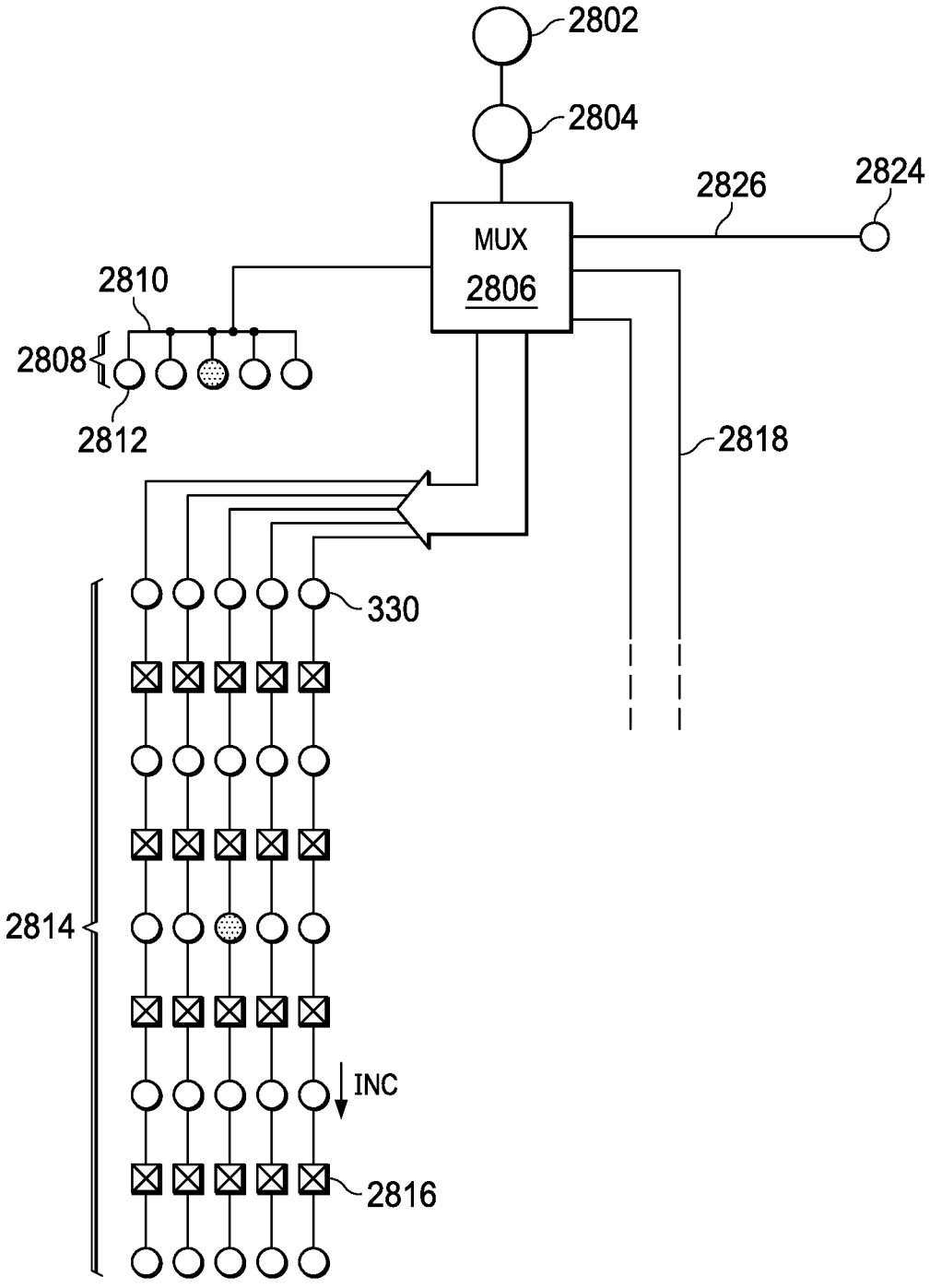

2802
2804
2826
2824
MUX
2806
2810
2808
2812
2818
330
2814
INC
2816

FIG. 28D

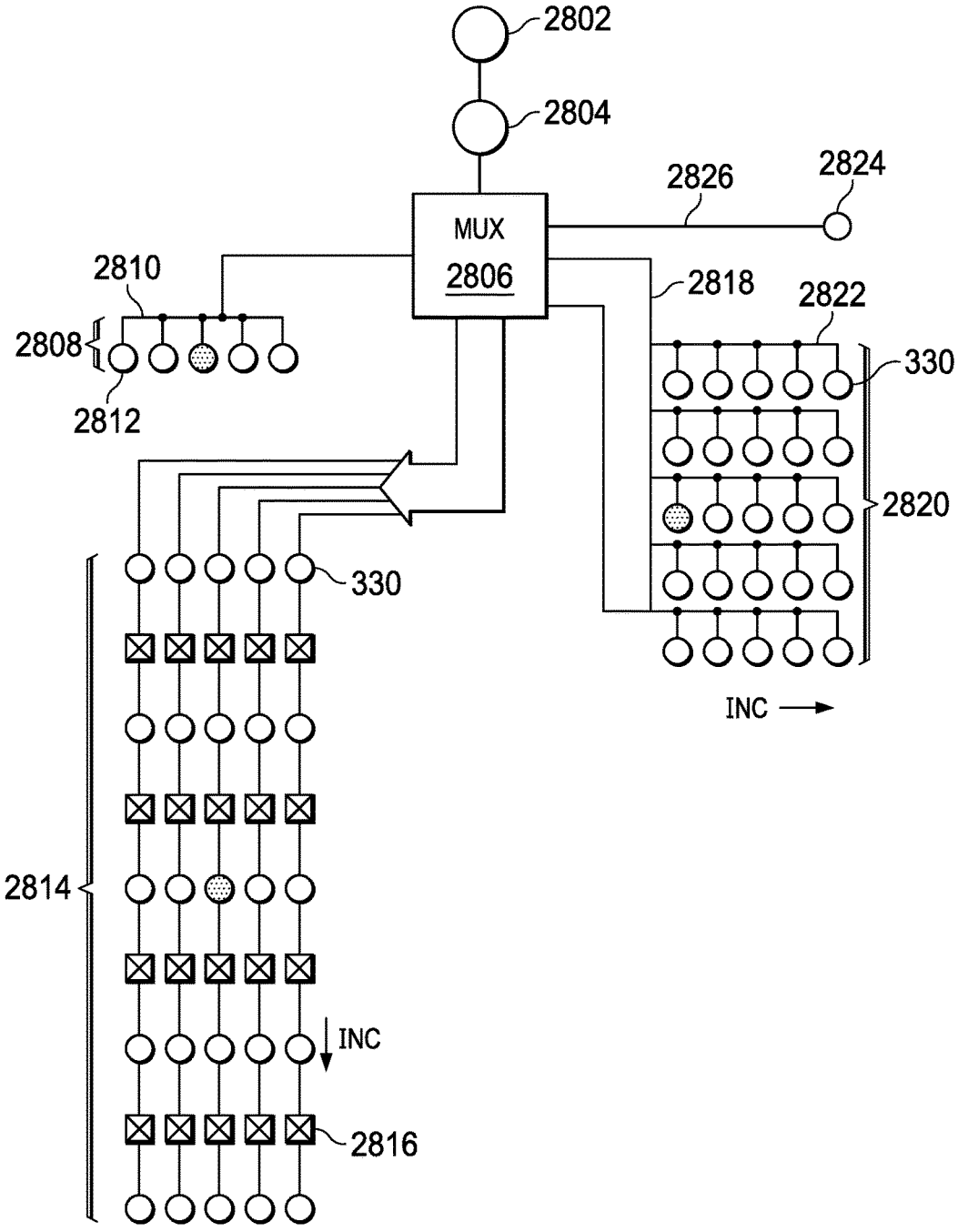

2802
2804
2826
2824
MUX
2806
2810
2818
2822
2808
330
2812
2820
330
INC
2814
INC
2816

FIG. 28E

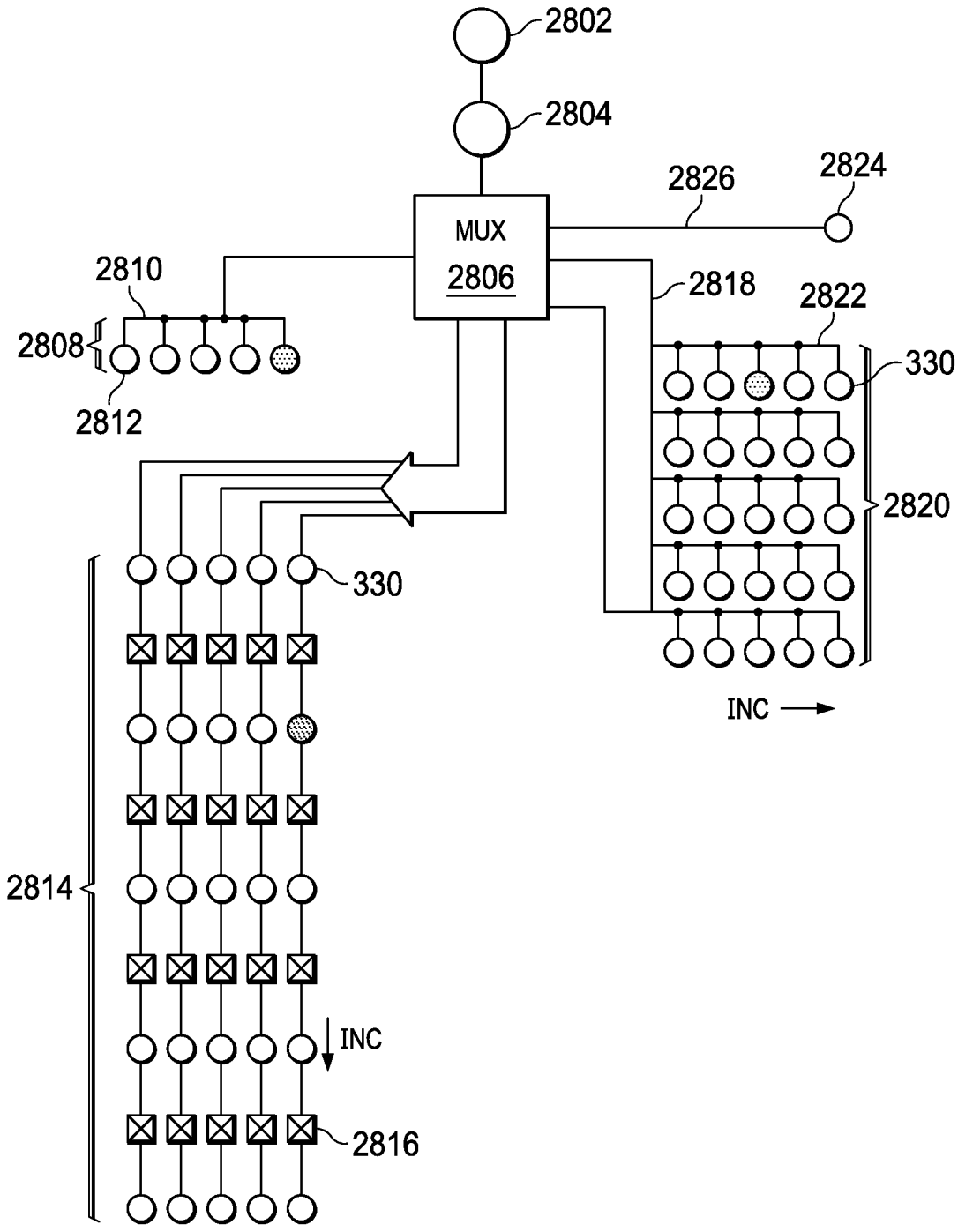

2802
2804
2826
2824
MUX
2806
2810
2818
2822
2808
330
2812
2820
330
INC
2814
INC
2816

FIG. 28F

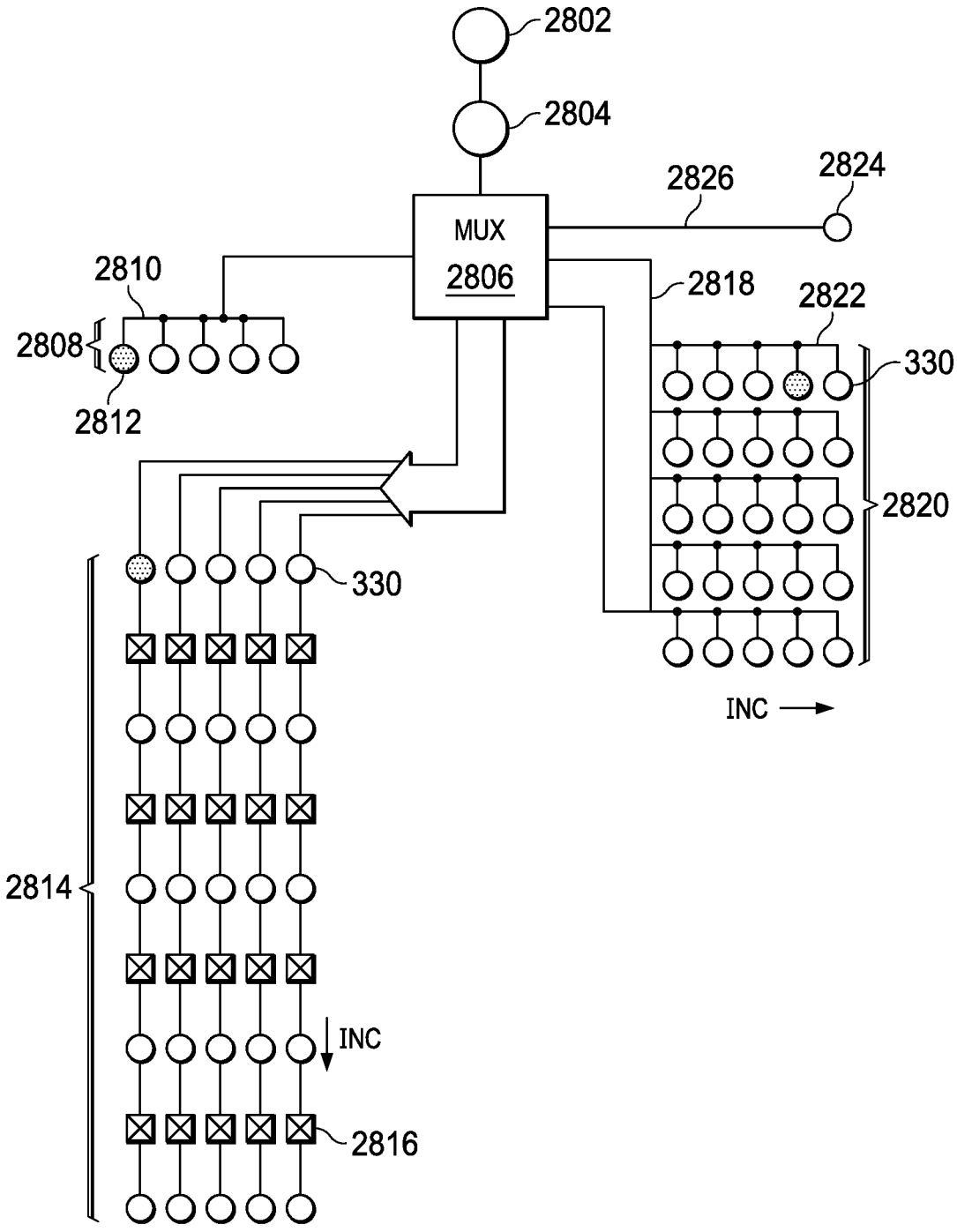

2802
2804
2826
2824
MUX
2806
2810
2818
2822
2808
330
2812
2820
330
INC
2814
INC
2816

FIG. 28G

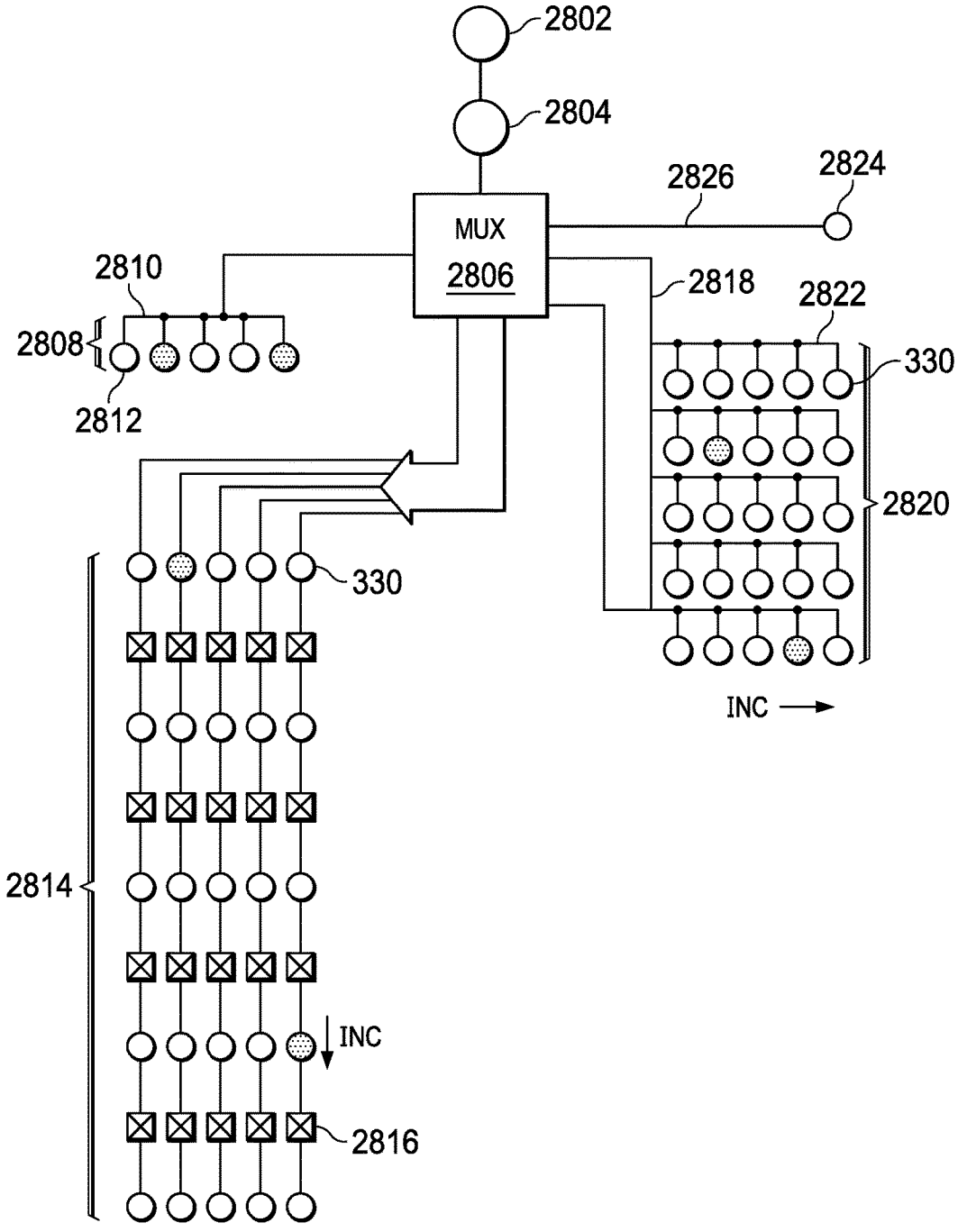

2802
2804
2826
2824
MUX
2806
2810
2818
2822
2808
330
2812
2820
330
INC
2814
INC
2816

FIG. 28H

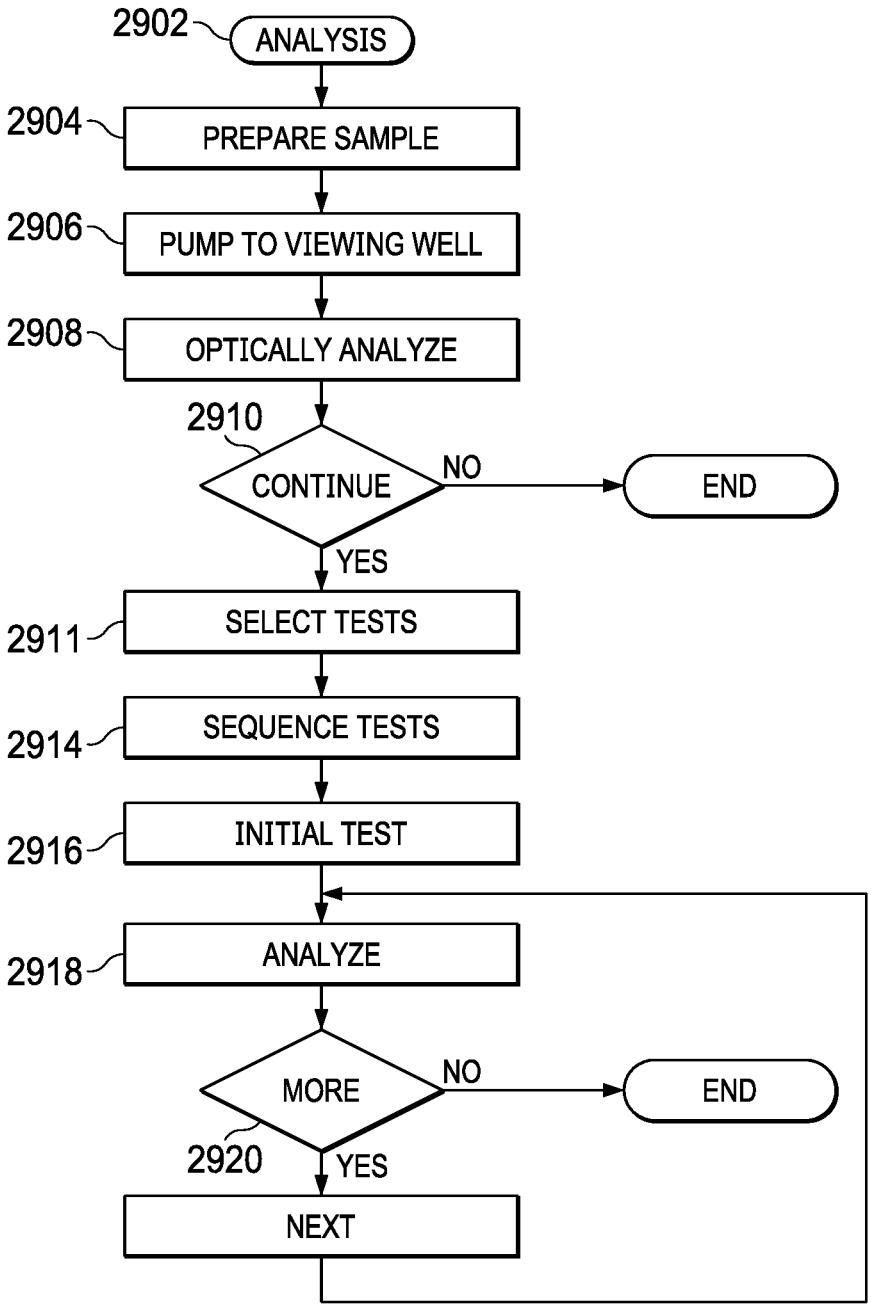

2902
ANALYSIS
2904
PREPARE SAMPLE
2906
PUMP TO VIEWING WELL
2908
OPTICALLY ANALYZE
2910
CONTINUE
NO
END
YES
2911
SELECT TESTS
2914
SEQUENCE TESTS
2916
INITIAL TEST
2918
ANALYZE
MORE
NO
END
2920
YES
NEXT

FIG. 29

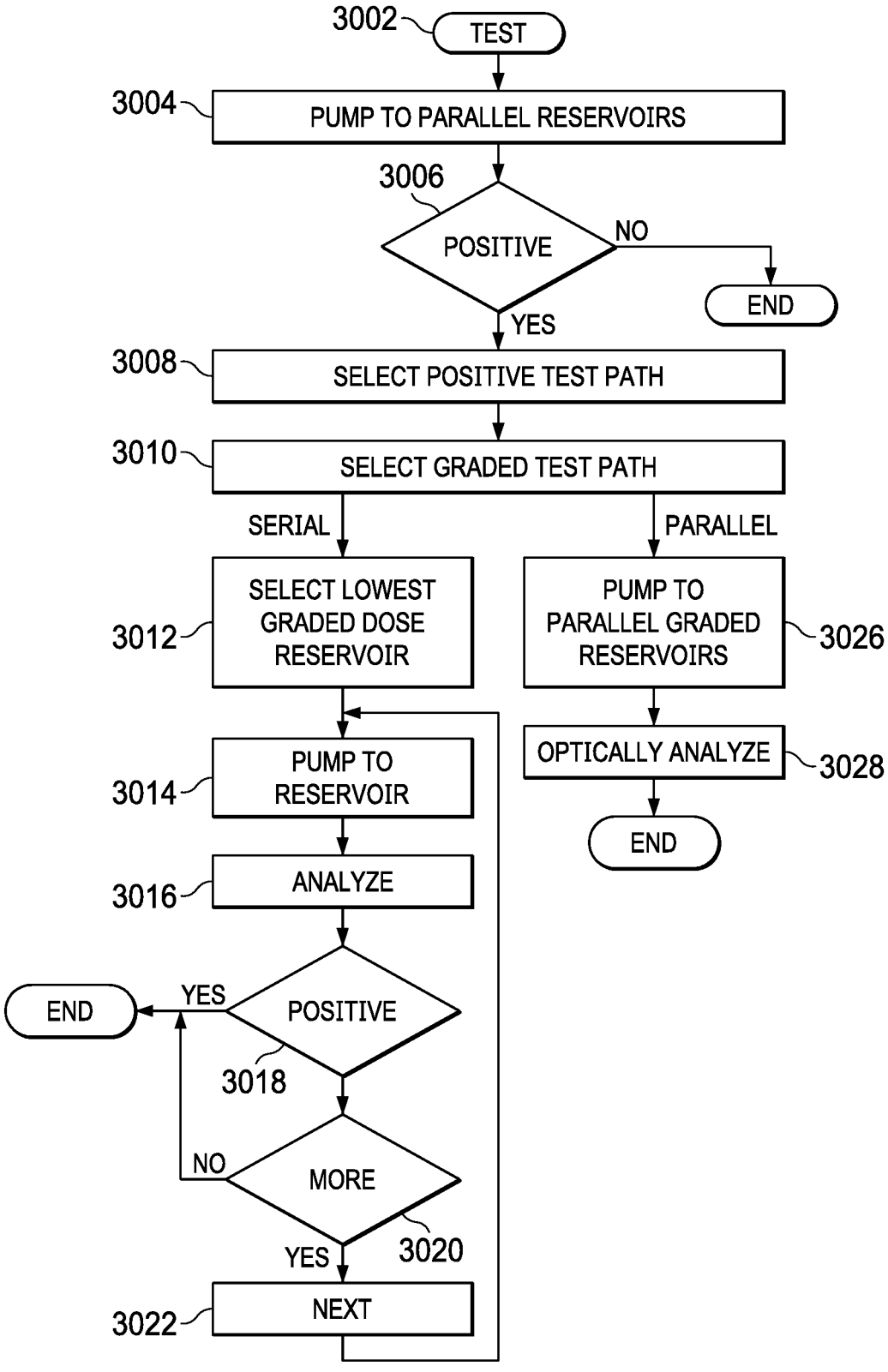

3002
TEST
3004
PUMP TO PARALLEL RESERVOIRS
3006
POSITIVE
NO
END
YES
3008
SELECT POSITIVE TEST PATH
3010
SELECT GRADED TEST PATH
SERIAL
PARALLEL
SELECT LOWEST GRADED DOSE RESERVOIR
3012
PUMP TO PARALLEL GRADED RESERVOIRS
3026
PUMP TO RESERVOIR
3014
OPTICALLY ANALYZE
3028
END
ANALYZE
3016
END
YES
POSITIVE
3018
NO
MORE
3020
YES
NEXT
3022

FIG. 30

SYSTEM AND METHOD FOR DETERMINING EFFICACY AND DOSAGE USING PARALLEL/SERIAL DUAL MICROFLUIDIC CHIP

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to and the benefit of U.S. Provisional Application No. 62/584,653, filed Nov. 10, 2017, and entitled SYSTEM AND METHOD FOR DETERMINING EFFICACY AND DOSAGE USING PARALLEL/SERIAL DUAL MICROFLUIDIC CHIP, the contents of which are incorporated by reference herein in their entirety.

TECHNICAL FIELD

[0002] The present invention pertains in general to a microfluidics lab-on-chip system and, more particularly, to the use of a microfluidics chip and testing at the point of care.

BACKGROUND

[0003] The emergence and spread of antibiotic-resistant bacteria are aggravated by incorrect prescription and use of antibiotics. Courts have this problem is the fact that there is no sufficiently fast diagnostic test to guide correct antibiotic prescription at the point of care. Currently, some fluid sample is retrieved from a patient and forwarded to a lab for testing to determine a specific treatment regimen. As a safeguard, the patient is sometimes initially given large doses of a general antibiotic until a more specific antibiotic can be determined to target the specific bacteria. This can take upwards of two or three days, as the process requires growing the bacteria in some culture medium and observing its response to various antibiotics.

SUMMARY

[0004] The present invention disclosed and claimed herein, in one aspect, comprises a method for determining a treatment agent and dosage level for a predetermined biologic material receives a biologic sample containing the predetermined biologic material for treatment by one of a plurality of treatment agents. The biologic sample containing the predetermined biologic material is held within a first reservoir. A portion of the biologic sample is pumped into each of a first plurality of parallel pathways from the first reservoir using a micro-pump. A separate treatment agent of the plurality of treatment agents is applied within each of the first plurality of parallel pathways to the portion of the biologic sample within the parallel pathway. The treatment agent of the plurality of treatment agents providing a best treatment efficacy for the predetermined biologic material within the biologic sample is determined responsive to the plurality of treatment agents applied to the portion of the biologic sample within each of the first plurality of parallel pathways. A second portion of the biologic sample is pumped into a selected second parallel pathway associated with the determined treatment agent of a second plurality of parallel pathways from the first reservoir using a second micro-pump. The determined treatment agent at a plurality of different dosage levels is applied within the selected second parallel pathway to the second portion of the biologic sample within the second parallel pathway. A dosage level of the plurality of different dosage levels of the determined treatment agent is determined with respect to the predetermined biologic material providing the best treatment efficacy. An output indicating the treatment agent and the dosage level of the treatment agent providing the best treatment efficacy is then provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] For a more complete understanding, reference is now made to the following description taken in conjunction with the accompanying Drawings in which:

[0006] FIG. **1** illustrates a high-level view of a microfluidics chip of the present disclosure;

[0007] FIGS. **2**A-**2**C illustrate detailed views of the multiple stages of analysis provided by the microfluidics chip of FIG. **1**;

[0008] FIGS. **3**A-**3**D illustrate diagrammatic views of the various cell capture regions and the interspersed pumps for the microfluidics chip of FIG. **1**;

[0009] FIGS. **4**A-**4**G illustrates detailed views of the first viewing stage;

[0010] FIG. **5** illustrates a detailed view of the first parallel driving stage;

[0011] FIGS. **5**A and **5**B illustrate details of the coating applied to the micro channels in the first driving stage;

[0012] FIG. **6** illustrates a detail of the serial driving stage;

[0013] FIGS. **7**A-**7**D illustrate detailed views of a valveless nozzle/diffuser micropump;

[0014] FIG. **8** illustrates a detailed view of a piezoelectric micropump;

[0015] FIG. **9** illustrates a detailed view of a multi-chamber micropump with check valves;

[0016] FIG. **10** illustrates a flowchart for the high-level operation of the microfluidics chip;

[0017] FIG. **11** illustrates a flowchart for the initial loading operation of the fluid sample;

[0018] FIG. **12** illustrates a flowchart for the viewing or cell counter stage of analysis;

[0019] FIGS. **13**A-**13**C illustrate diagrammatic use for the cell counter;

[0020] FIG. **14** illustrates a flowchart for the main parallel stage of analysis;

[0021] FIG. **15** illustrates the serial stage of analysis;

[0022] FIG. **16** illustrates a simple fight diagrammatic view of the microfluidics chip;

[0023] FIG. **17** illustrates a simplified diagrammatic view of a parallel module;

[0024] FIG. **18** illustrates simplified diagrammatic view of a serial module;

[0025] FIG. **19** illustrates a simplified diagrammatic view of a serial module arranged in parallel;

[0026] FIGS. **20**A and **20**B illustrated a diagrammatic view of an embodiment utilizing a chemostat;

[0027] FIG. **21** illustrates a diagrammatic you have the microfluidics chip utilizing valves;

[0028] FIGS. **22**A-**22**B illustrate cross-sectional views of a micro valve

[0029] FIG. **23** illustrates a diagrammatic view of preparing a biologic sample and disposing it in the well on the microfluidic chip;

[0030] FIG. **24** illustrates a cross-sectional view of an RT-lamp interfaced with a cell phone;

[0031] FIG. **25** illustrates a perspective view of the RT lamp interfaced with a microfluidic chip and a cell phone;

[0032] FIG. **26** illustrates a side view of a cell phone interfacing with the micro fluidic chip;

[0033] FIG. **27** illustrates a window view of the camera and the alignment process;

[0034] FIGS. **28**A-**28**H illustrate multiple views of a diagram of the microfluidic chip in schematic form and various loading and analysis steps associated there with;

[0035] FIG. **29** illustrates a flowchart for the overall analysis process utilizing the microfluidic chip; and

[0036] FIG. **30** illustrates a flowchart to pick in the details of the test path.

DETAILED DESCRIPTION

[0037] Referring now to the drawings, wherein like reference numbers are used herein to designate like elements throughout, the various views and embodiments of a System and Method for Determining Efficacy and Dosage Using Parallel/Serial Dual Microfluidic Chip is illustrated and described, and other possible embodiments are described. The figures are not necessarily drawn to scale, and in some instances the drawings have been exaggerated and/or simplified in places for illustrative purposes only. One of ordinary skill in the art will appreciate the many possible applications and variations based on the following examples of possible embodiments.

[0038] Referring now to FIG. **1**, there is illustrated a diagrammatic view of a microfluidics chip **102** at a high-level view. There is provided in the microfluidics chip **102** an input stage **104** that is operable to receive a biological specimen. As used herein, a "sample" must be capable of flowing through microfluidic channels of the system embodiments described hereinbelow. Thus, any sample consisting of a fluid suspension, or any sample that be put into the form of a fluid suspension, that can be driven through microfluidic channels can be used in the systems and methods described herein. For example, a sample can be obtained from an animal, water source, food, soil, air, etc. If a solid sample is obtained, such as a tissue sample or soil sample, the solid sample can be liquefied or solubilized prior to subsequent introduction into the system. If a gas sample is obtained, it may be liquefied or solubilized as well. The sample may also include a liquid as the particle. For example, the sample may consist of bubbles of oil or other kinds of liquids as the particles suspended in an aqueous solution.

[0039] Any number of samples can be introduced into the system for analysis and testing, and should not be limited to those samples described herein. A sample can generally include any suspensions, liquids, and/or fluids having at least one type of particle, cellular, droplet, or otherwise, disposed therein. In some embodiments, a sample can be derived from an animal such as a mammal. In a preferred embodiment, the mammal can be a human. Exemplary fluid samples derived from an animal can include, but are not limited to, whole blood, sweat, tears, ear flow, sputum, bone marrow suspension, lymph, urine, brain fluid, cerebrospinal fluid, saliva, mucous, vaginal fluid, ascites, milk, secretions of the respiratory, intestinal and genitourinary tracts, and amniotic fluid. In other embodiments, exemplary samples can include fluids that are introduced into a human body and then removed again for analysis, including all forms of lavage such as antiseptic, bronchoalveolar, gastric, peritoneal, cervical, athroscopic, ductal, nasal, and ear lavages. Exemplary particles can include any particles contained within the fluids noted herein and can be both rigid and deformable. In particular, particles can include, but are not limited to, cells, alive or fixed, such as adult red blood cells, fetal red blood cells, trophoblasts, fetal fibroblasts, white blood cells, epithelial cells, tumor cells, cancer cells, hematopoeitic stem cells, bacterial cells, mammalian cells, protists, plant cells, neutrophils, T lymphocytes, CD4+, B lymphocytes, monocytes, eosinophils, natural killers, basophils, dendritic cells, circulating endothelial, antigen specific T-cells, and fungal cells; beads; viruses; organelles; droplets; liposomes; nanoparticles; and/or molecular complexes. In some embodiments, one or more particles such as cells, may stick, group, or clump together within a sample.

[0040] In some embodiments, a fluid sample obtained from an animal is directly applied to the system described herein at the input stage, while in other embodiments, the sample is pretreated or processed prior to being delivered to a system. For example, a fluid drawn from an animal can be treated with one or more reagents prior to delivery to the system or it can be collected into a container that is preloaded with such a reagent. Exemplary reagents can include, but are not limited to, a stabilizing reagent, a preservative, a fixant, a lysing reagent, a diluent, an anti-apoptotic reagent, an anti-coagulation reagent, an anti-thrombotic reagent, magnetic or electric property regulating reagents, a size altering reagent, a buffering reagent, an osmolality regulating reagent, a pH regulating reagent, and/or a cross-linking agent.

[0041] At this point in the process, a finite amount of biofluids is disposed in the reservoir ready for transferring to subsequent stages. This amount of fluid is then transferred to another stage via a driving stage **106** in order to transfer this biofluid to another reservoir, that associated with a viewing stage **108**. At this stage, a technician can examine the biofluid and determine the makeup of the biofluid, discriminate cells, etc. in order to make certain decisions as to going forward with remaining tests. The microfluidic chip then transfers the biofluid at the viewing stage **108** to a parallel analysis stage **115** through a parallel driving stage **110** wherein the biofluid is divided among a plurality of parallel path this for analysis of the reaction of the material in the biofluid with different reagents in a reading. This requires a certain amount of the biofluid to be transferred to this analysis stage. Thereafter, a decision is made as to whether to transfer the remaining biofluid from the viewing stage **108**, in order to perform more testing and/or analysis on the biofluid. At this stage the process, only one of the multiple second stage or serial stage path is selected. One reason for this is that there is only a finite amount of biofluid available and there is no need for testing along paths that are associated with previous decisions indicating that the results will be negative along these paths. Each of these serial passes associated with one of the parallel paths. Thus, if there are five parallel paths, there will be five serial paths. Note that the term "serial path" is a term meaning that it is within the serial decision tree and it need not actually be a plurality of serial paths that are linked together in a serial manner, although they could be and are in some embodiments described hereinbelow. It is necessary to perform the testing/analysis along each of the five parallel paths, but a decision at this point indicates that only one of the serial paths will be required for the testing/analysis purpose. This will be described in more detail hereinbelow.

[0042] Referring now to FIGS. **2**A-**2**C, there are illustrated diagrammatic views of the various stages of the process. With specific reference to FIG. **2**A, there is illustrated a diagrammatic view of first viewing stage, wherein the amount of biofluid stored in the input stage reservoir **104** is driven to the viewing stage **108** reservoir. At this stage, optical device **202**, for example, can be used to view the cells disposed within the medium. This medium could actually be the actual biofluid that was provided in the sample from the human/animal or could be some diluted version thereof. However, this biofluid will contain some cellular material or some particulate of interest. This can be viewed with the out device **202** and then passed to a processor **204**, or a human could analyze the results. With utilization of the processor **204**, the actual form of biofluid, and analog form, is transferred to a digital form. This could be in the form of cell counting for verification of a particular cell. As will be described hereinbelow, affinity labels can be associated with each of the cells or particulates in the biofluid and this could facilitate visual recognition of different characteristics or different types of cells, such as proteins, bacteria, etc. Each of these cellular materials can have a particular affinity label associated there with that allows it to be visually identified via some characteristics such as florescence or even magnetic properties associated with the affinity label. Again, this will be described hereinbelow. Although an optical device **202** was illustrated and described, any other type of device for analyzing the characteristics of a particular affinity labeled cell can be utilized, such as some type of magnetometer, etc.

[0043] Referring now to FIG. **2**B, there is illustrated the next parallel drive stage. At this stage, a micropump is utilized in the parallel drive stage **110** to pump at least a portion of the biofluid stored in the reservoir associated with the viewing stage **108** is transferred to all of the parallel reading/analysis paths. In this step, it can be seen that a portion of the biofluid in the reservoir associated with the viewing stage **108** and is biofluid exists in each of these parallel paths for analysis. There is an indication in one of these parallel paths, associated with the reservoir **210**, that shows a positive indication of a reaction of some type that is viewable. If, for example, this were bacteria, one reagent could be an antibiotic in a large dosage that would destroy the particular target bacteria and this would be recognized by an observer. The other three paths, associated with reservoirs **214**, **216** and **218** (an example of **4** paths), would have no reaction and, as such, would not have affected the bacteria associated therewith. In this example, a high level of concentrated antibiotic is provided that would destroy the bacteria, but at this level of analysis, there is no indication provided as to the actual dosage of that antibiotic that would destroy the bacteria, other than the fact that a large dosage of this particular antibiotic will destroy the target bacteria. It is important to keep in mind that this particular biofluid may have multiple and different bacteria, proteins, etc. contained therein.

[0044] Referring now to FIG. **2**C, there is illustrated a diagrammatic view of the final serial stage of analysis/testing. Since the first stage of testing/analysis transferred some of the biofluid from the viewing stage **108** to the parallel stages **114**, there is still some biofluid remaining in the viewing stage **108**. This is a selectively transferred to one of the serial paths, that associated with the testing reservoir **210**. There are provided a plurality of bypass channels **220** associated with each of the serial paths and only the bypass channel **220** associated with the reservoir **210** in the parallel path **114** will be selected for transferring biofluid to this particular serial path associated with the reservoir **210** for testing. It will first be pumped to be a micropump in a serial drive stage **222** to a first serial reservoir **224** for testing/analysis. If the test is negative, it can then be passed to a subsequent serial driving stage **226** to a subsequent serial reservoir **228** for testing/analysis and so on. As will be described hereinbelow, there can be provided a single bypass path **220** which is connected to a manifold associated with each of the serial paths and each of the manifolds can be associated with each of the different reservoirs for testing, i.e., at this point the testing is parallel to all of the subsequent testing reservoirs. In the mode illustrated in this FIG. **2**C, it is necessary to transfer all of the necessary biofluid, i.e., typically the remaining biofluid in the viewing stage reservoir **108**, to the reservoir **224** and pass all of that biofluid to the next reservoir **228** and so on. Thus, at each stage, all of the biofluid transferred in the subsequent stages is tested at each subsequent stage. In a parallel configuration, the remaining biofluid in the viewing stage **108** would be required to be divided among the different testing reservoirs at each of the subsequent stages. This will be described in more detail hereinbelow.

[0045] Referring now to FIGS. **3**A-**3**D, there are illustrated diagrammatic views of the process and fluid flow. In FIG. **3**A come there is illustrated an overall process flow for the embodiment described hereinabove. This embodiment, there is provided an input well **302** for receiving the biologic sample indicated by numeral **303**. This constitutes a finite volume that must be transferred via a micropump to a viewing reservoir **306**. At this point, substantially all of the biofluid is transferred from the reservoir **302** to the viewing reservoir **306**. This is the first stage of the process. The second stage of the process is illustrated as providing three separate testing reservoirs **308**, **310**, **312**, attached at one to a microchannel manifold **314**. Each of the testing reservoirs **308**, **310**, **312**, as will be described hereinbelow, is comprised of a serpentine microchannel **316** attached at one end to the manifold **314** and at the other end to a viewing reservoir **318**. A micropump **320** is provided for transferring biofluid from the viewing reservoir **306** to the manifold **314**. This will be divided among the three testing reservoirs **308**, **310**, **312** and substantially even amounts. The biofluid will traverse the serpentine microchannel **316**, which is coated with a particular reagent, one example being an antibiotic. In this example, the antibiotic is at a very high concentrated level, each of the testing reservoirs **308**, **310** and **312** having a different antibiotic associated there with. Only a portion of the biofluid in the viewing reservoir **306** will be transferred to these three testing reservoirs **308**, **310** and **312** for testing/analysis and viewing at the associated viewing reservoir **318**. The serpentine shape, when used with a medium containing cells such as in a biologic sample, facilitates and enhances mixing due to the increased interfacial contact area between the cells within the biofluid sample.

[0046] The next step of testing/analysis will be selected only upon a positive test occurring within one of the three testing reservoirs **308**, **310** and **312**. However, each of the testing reservoirs **308**, **310** and **312** has associated there with a subsequent group of testing reservoirs. In this embodiment, each of the subsequent testing reservoirs is comprised of a plurality of sub reservoirs **330**, each of the sub reservoirs

330 being configured identical to the testing reservoirs 308, 310 and 312, with a serpentine microchannel region 316 and a viewing reservoir 318. A single bypass microchannel 220 is provided to connect viewing reservoir 306 to a sub reservoir manifold 332. Each of the particular sub reservoir paths have associated there with a separate micropump 334. Only one of these micropumps 334 is selected for transferring the remaining portion of the biofluid stored in the viewing reservoir 306 to the selected path. In this embodiment, the remaining portion of the biofluid is transferred to the first reservoir 330 bypassing the biofluid through the serpentine microchannel 316 to the associated viewing reservoir 318. This particular microchannel will have coating of antibiotic, in this example above, at a relatively low dose. If the bacteria, for example, do not react accordingly with this level of antibiotic, it can be recognized as such in the viewing reservoir 318. It is noted that the antibiotic associated with the coating on the walls of the microchannel 330 at this dosage will not be picked up by the bacteria and, as such, the bacteria in the viewing reservoir 318 for the first sub reservoir 330 in the selected path will still be intact. It can then be pumped from the reservoir 318 associated with the first testing reservoir 330 in the chain to a subsequent testing reservoir 330 with a subsequent micropump 336. This subsequent sub reservoir will have a concentration of antibiotic in its serpentine microchannel 316 that is at a higher level. As the level increases, a gradient is tested for, such that the dosage can be gradually increased until the bacteria are destroyed. If, for example, the bacteria were associated with an affinity label that made it fluoresce, this would be recognized. It could also be that there are multiple bacterial types contained within the biofluid that are each associated with a different affinity label and this could be recognized. It could, in fact, the case that one type of bacteria perfected at a first dosage level of the antibiotic and a second bacteria were affected at a another dosage level of the antibiotic.

[0047] Referring now to FIG. 3B, there is illustrated a diagrammatic view of an alternate process flow. This will work substantially identical to the embodiment of FIG. 3A, come up until the operation at the manifold 332 associated with the sub reservoirs. In this embodiment, the three micropumps 334 each feed a sub reservoir manifold 340. Each of the sub reservoir manifolds 340 is connected to a plurality of the sub reservoirs 330 associated with each path. In this embodiment, there are only illustrated three sub reservoirs 330 for each of the sub reservoir manifolds 340, although each path could have a different number of sub reservoirs 330 associated therewith. The difference between these two embodiments is that, at this point, the amount of biofluid remaining in the viewing reservoir 306 now must be divided amongst all of the sub reservoirs attached on one end thereof to the associated sub reservoir manifold 340 selected by the activated one of the micropumps 334. This will result in potentially less biofluid being available for the testing/analysis step. This will also mean that each of the viewing reservoirs 318 associated there with will have a smaller volume associated therewith.

[0048] Referring now to FIG. 3C, there is illustrated a diagrammatic view that provides a simplified diagram of the transfer from reservoir to reservoir. In this illustration, the input stage is illustrated as an input reservoir 350 labeled R0. A micropump 352 is operable to transfer the contents of this input reservoir, the biofluid, to a second reservoir, a viewing reservoir 354, labeled R1. A portion of the contents of this reservoir are then transferred via a micropump 356 to a plurality of parallel stage reservoirs 358 labeled R2. This is the first testing/analysis stage. After this stage, the remaining contents of the viewing reservoir 354 are transferred to the subsequent serial stage reservoirs via a pump 360 via a bypass path and microchannel 362. The serial stage reservoirs are labeled R3, R4, etc. This illustration sets forth how the entire contents of the input reservoir R0 are transferred down the chain. This is best illustrated in FIG. 3D. In this illustration, it can be seen that entire contents of reservoir R0 are transferred to reservoir R1. At this point, only a portion of the contents are transferred to reservoir R2. The remaining contents are sequentially transferred to R3, R4, and so on. For this illustration, the entire remaining contents of the reservoir 354, R1, will be transferred down the chain entirely to reservoir R3, then to reservoir R4, and so on. In the alternate embodiment, as described hereinabove, and not illustrated in FIG. 3D, the bypass 362 could be connected to each of the reservoirs R3, R4, etc. in parallel, noting that the remaining contents of the reservoir R1 will then be divided amongst the parallel connected reservoirs R3, R4, etc.

[0049] Referring now to FIGS. 4A-4G, there are illustrated diagrammatic views of the initial processing section associated with the viewing stage 108. There is provided a substrate 402 upon the surface of which are formed a plurality of wells and microchannels. A first well 404 is provided for receiving the biofluid sample in this well has a finite volume associated there with. At the bottom of this well a microchannel 406 extends outward and up to the surface to an opening 408. The purpose of this microchannel 406 extending to the bottom of the well 404 is to ensure that the biofluid can be completely pumped from the well 404. For the formation of this microchannel 406, it might be that the microchannel is formed through the surface of the substrate 402 and then a cover plate (not shown) having a surface that extends down into the open microchannel. An adjacent channel 410 is disposed proximate the opening 408 to provide another opening therefore in order to accommodate a micropump 412 (shown in phantom) interface with the opening 408 and the one end of the microchannel 410 for transferring fluid from the well 404 to the microchannel 410. The microchannel 410 extends along the surface of substrate 402 in order to interface with a viewing well/reservoir 412. As the biofluid passes through the microchannel 410 and the viewing well 412, a desired analysis can be performed on the contents of the biofluid. As described hereinabove, in one example, various cells in the biofluid might consist of different types of bacteria, proteins, etc. and each of these may have associated there with a specific affinity label, which is optically detectable. There are, of course, other means by which affinity labels can be detected. As the cells contained within the biofluid pass through the viewing well/reservoir 414, they can be examined. The viewing well/reservoir 414 on the other side thereof is connected to one side of a microchannel 416, the other side thereof connected to a reservoir 418. Since the micropump 412 must force the biofluid through the microchannels and the viewing well/reservoir 414, there is required the necessity for a holding reservoir 418 to be present. However, initially, this reservoir 418, the microchannel 410 and the viewing well/reservoir 414 will have air disposed therein. This air must be removed. This can be done with a negative pressure of some sort or just a waste gate output to the atmosphere. This is

provided by a waste gate microchannel **420** that is connected to an opening **422** through the cover glass (not shown) or to the side of substrate **402**. A valve **423** could be provided above the opening **422**. As biofluid enters the reservoir **418**, air will be pushed out through the microchannel **420**. It is desirable for this microchannel **422** to have as low a profile as necessary such that only air exits therefrom. Depending upon the size of the cells contained within the biofluid, the microchannel **420** can be significantly smaller and have a lower profile than the microchannels **410** and **416**. Is important to note that, once the micropump **412** transfers the biofluid from the well **404**, the volume transferred will be spread between the two microchannels **410** and **416**, the viewing well **414** and the reservoir **418**. Thus, the reservoir **418** has a significantly larger volume that any of the microchannels **410** and **416** and the viewing well/reservoir **414**. Additionally, it may be that the depth of the wells/reservoirs **404** and **418**, as well as the viewing well reservoir **414** are also as shallow as the microchannels **410** and **416** but significantly wider to accommodate the required volume.

[0050] The outlet of the reservoir **418** is connected from the bottom thereof through a microchannel **426** to an opening **428** on the upper surface of the substrate **402**. This is interfaced with a micropump **430** (in phantom) to an adjacent microchannel **432** for subsequent processing. These micropumps **412** and **430**, although illustrated as being flush with the substrate, will typically be disposed above the cover plate (not shown) with holes disposed through the cover plate. The opening **428** will be a horizontal microchannel associated with the manifold **314** described hereinabove. This will be associated with a plurality of micropumps **430** for each of the parallel paths or the bypass path. A cross-sectional view of the embodiment of FIG. **4**A is illustrated in FIG. **4**B, with a cover plate **440** disposed over the substrate **402** with an opening **442** disposed above the well **404** for receiving the biofluid sample.

[0051] FIGS. **4**C and **4**D illustrate top view and cross-sectional views of the reservoir **418** illustrating how the microchannel **416** feeds biofluid into the top of the reservoir **418**, and the flow path for the biofluid from the reservoir **418** through the microchannel **426** from the bottom of the reservoir **418**. However, it may be that, with capillary action, the depth of the reservoir **418** could be equal to that of the microchannels **416** and **426** such that they are all at the surface of the substrate **402** for ease of manufacturing. When a negative pressure is placed upon the reservoir **418**, air will be pulled into the microchannel **426** through the microchannel **420**. It is possible in this mode that the micropump **412** could be operated to actually create a positive pressure in the microchannel **416** to force the biofluid in the reservoir **418** into the opening **428** through the microchannel **426**. Again, the microchannel **420** would preferably have a dimension that was smaller than the smallest cell size within the biofluid.

[0052] Referring now to FIGS. **4**E and **4**F, there are illustrated top view and cross-sectional views of the reservoir **418** with an alternate embodiment illustrating microchannel **426'** as being beneath the bottom of the reservoir **418** to allow more complete emptying of the reservoir **418**.

[0053] Referring now to FIG. **4**G, there is illustrated an alternate embodiment of inlet wells for receiving the biofluid sample. There is provided the well **404** for receiving the biofluid sample and a second well **464** receiving an additional fluid sample. This fluid sample in well **460** could be some type of dilutant or it could be a medium containing various affinity labels. As noted hereinabove, the fluid sample could have associated there with affinity labels prior to the biofluid sample being disposed in the well **404**. However, it is possible that the microfluidic chip have disposed in the well **460** a medium containing affinity labels, for example. The well **460** would be interfaced through a microchannel **462** to an opening **464** adjacent the opening **408**. A two input, one output, micropump **412'** that interfaces with the microchannel **410**.

[0054] Referring now to FIG. **5**, there is illustrated a diagrammatic view of the microchannel structure associated with the parallel stage of operation. The microchannel **426** is interfaced with a microchannel manifold **502** which corresponds to the opening **428**. This microchannel manifold **502** is interfaced with a plurality of micropumps **504**, corresponding to the micropump **430**. These micropumps **504** are disposed in pairs, each pair associated with one testing reagent. As noted hereinabove, there are provided a plurality of parallel paths, each associated with a reservoir **312** having a serpentine microchannel **316** and a viewing reservoir **318**. The first micropump **504** in the pair of micropumps **504** is connected to one end of the associated serpentine microchannel **316**. When this micropump **504** is activated, biofluid from the reservoir **418** is passed through the manifold microchannel **502** and through the serpentine microchannel **316** to the viewing reservoir **318**. As was the case above, there is provided a waste microchannel **506** for each of the reservoirs **318** to allow air to escape therefrom as biofluid is forced through the microchannel **316**. The micropump **504** associated with this serpentine microchannel **316** will be operated for a sufficient amount of time to transfer sufficient biofluid from the reservoir **418** through the serpentine a channel **316** and finally into the reservoir **318** to fill the reservoir **318**. The microchannel **506** can have some type of valve associated with the opening thereof to prevent the escape of any biofluid therefrom or, alternatively, the dimensions of that microchannel **506** could be small enough to prevent any appreciable amount of cells escaping therefrom. Although not illustrated, the one of the pair of micropumps **504** associated with the parallel stage of operation and associated reservoirs **312** will also be operated to fill the associated serpentine microchannel **316** and reservoir **318**.

[0055] Referring now to FIGS. **5**A and **5**B, there are illustrated cross-sectional views of the serpentine microchannel **316**. As described hereinabove, the sides of these channels **316** are coated with some type of reagent. For example, if a Urinary Tract Infection (UTI) were suspected and were being tested for in the microfluidic chip, the sensitivity for common antimicrobial agents for UTI treatment might include ampicillin (AMP), ciprofloxacin (CIP), and trimethoprim/sulfamethoxazole (SXT), these being three agents that could be tested for and three different paths. The bacteria that might exist within the urine samples from an individual could be any of uropathogenic *E. coli* strains (EC132, EC136, EC137, and EC462). Some prior research has shown that, through antimicrobial resistance profiles of these pathogens that EC132 is resistant to AMP and CIP but not SXT. EC136 is resistant to AMP only. EC137 is sensitive to all the antibiotics tested. EC462 is resistant to AMP and SXT but not CIP. In order to coat sides of the serpentine microchannels **316**, one technique would to have a certain amount of the antibiotic dissolved in sterile water to the serpentine microchannels **316** at different levels. Subse-

quently, the diluted antibiotic is dried by incubation at a desired temperature and desired time. The original diluted antibiotic has a starting concentration of a predetermined μg/ml concentration. The surface area is sufficiently covered such that, when the biofluid passes thereover, it will interact with reagent.

[0056] Referring now to FIG. **6**, there is illustrated a microchannel diagram of the reservoir **330** on the surface of the chip **402**. This is connected by the microchannel **507** from the associated one of the micropumps **504**. After the results in the viewing reservoir **318** have been determined to yield a positive result, for that particular path in the parallel analysis/testing operation, the other of the pair of micropumps **504** is activated and the remaining amount of microfluid from the reservoir **418** is transferred to the reservoir **330**. This will be passed through the serpentine microchannel **316** and stored in the reservoir **318**, labeled **602** in FIG. **6**. This is substantially larger than the reservoir **318** associated with the reservoir **312**. Thus, for this embodiment, the remaining portion of the biofluid from the reservoir **418** will be substantially stored in the reservoir **602**. This will have associated there with a waste microchannel **604** and an outlet microchannel **608** that extends outward from the bottom of the reservoir **602** and up to an opening **610** in the surface of the substrate for interface with the micropump **336**. The micropump **336** is operable, at the next stage of the testing/analysis, to move the contents of the reservoir **602** over to the next reservoir **330** for testing at that next concentration level associated with the next reservoir **330** in the sequence.

[0057] Referring now to FIGS. **7**A-**7**D, there is illustrated an example of a valveless MEMS micropump. The micropump includes a body **702** with two pumping chambers **704** and **706**. At the inlet side of each of the chamber **704** and **706** is disposed a conical inlet **710** and **712**, respectively. The conical inlets **710** and **712** are wider at the pump chamber side and narrower at the inlet side thereof. The inlet sides of conical inlet **710** and **712** are connected to respective inlet channel **714** and **716**. The outlet side of the chambers **704** and **706** are interfaced with conical outlets **718** and **720**, respectively, the conical outlets **718** and **720** having a narrower portion at the outlet of the respective pump chamber **704** and **706** and a wider portion at the respective outlet thereof interfacing with respective outlet channels **722** and **724**. The conical inlets **710** and **712** and outlets **718** and **720** are frustro conical in shape. A piezoelectric membrane and actuator **726** is dispose between the two pumping chambers **704** and **706** and is operable to be extended up into one of the chambers **704** and **706** at one time to increase the pressure therein and at the same time extend away from the other of the chambers **704** and **706** to decrease the pressure therein. The operation is then reversed.

[0058] The piezoelectric membrane and actuator **726** is comprised of a piezoelectric disc **740** on one side of a membrane **742** and a piezoelectric disc **744** on the other side thereof. Each of the piezoelectric discs **740** and **744** are formed by stratifying a layer of use electric material **748** between two layers of conducting material **750**. Piezoelectric material **748** can be made with Piezo Material Lead Zirconate Titanate (PZT-SA), although other piezoelectric materials can be used. The conducting material **60** may be composed of an epoxy such as commercially available EPO-TEK H31 epoxy. The epoxy serves as a glue and a conductor to transmit power to the piezoelectric discs **750**. The piezoelectric discs **750** are secured to the surface of the intermediate layer **748**, so that when a voltage is applied to the membrane **742**, a moment is formed to cause the membrane **742** to deform.

[0059] The operation of the micropump will be described with reference to FIG. **7**D. At rest, the upper chamber **704** and the lower chamber **706** are separated by a diaphragm pump membrane **742**. The diffuser elements **710**, **712**, **718** and **720** are in fluid communication with each respective chamber. Diffuser elements are oriented so that the larger cross-sectional area end of one diffuser element is opposite the smaller cross-sectional area end of the diffuser element on the other side of the chamber. This permits a net pumping action across the chamber when the membrane is deformed.

[0060] The piezoelectric discs are attached to both the bottom and the top of the membrane. Piezoelectric deformation of the plates is varied by varying the applied voltage so as to excite the membrane with different frequency modes. Piezoelectric deformation of the cooperating plates puts the membrane into motion. Adjustments are made to the applied voltage and, if necessary, the choice of piezoelectric material, so as to optimize the rate of membrane actuation as well as the flow rate. Application of an electrical voltage induces a mechanical stress within the piezoelectric material in the pump membrane **742** in a known manner. The deformation of the pump membrane **742** changes the internal volume of upper chamber **704** and lower chamber **706**. As the volume of the upper chamber **704** decreases, pressure increases in the upper chamber **706** relative to the rest state. During this contraction mode, the overpressure in the chamber causes fluid to flow out the upper chamber **704** through diffuser elements on both sides of the chamber. However, owing to the geometry of the tapered diffuser elements, specifically the smaller cross-sectional area in the chamber end of the left diffuser element relative to the larger cross-sectional area of the right diffuser element, fluid flow out of the left diffuser element is greater than the fluid flow out the right diffuser element. This disparity results in a net pumping of fluid flowing out of the chamber to the left.

[0061] At the same time, the volume of the lower chamber **706** increases with the deformation of the pump member **742**, resulting in an under pressure in the lower chamber **706** relative to the rest state. During this expansion mode, fluid enters the lower chamber **706** from both the left and the right diffuser elements. Again owing to the relative cross-sectional geometry of the tapered diffuser elements, fluid flow into the lower chamber **706** through the right diffuser element is greater than the fluid drawn into the lower chamber **706** through the left diffuser element. This results in a net fluid flow through the right diffuser element into the chamber, priming the chamber for the pump cycle.

[0062] Deflection of the membrane **742** in the opposite direction produces the opposite response for each chamber. The volume of the upper chamber **704** is increased. Now in expansion mode, fluid flows into the chamber from both the left and right sides, but the fluid flow from the right diffuser element is greater than the fluid flow from the left diffuser element. This results in a net intake of fluid from the right diffuser element, priming the upper chamber **704** for the pump cycle. Conversely, the lower chamber **706** is now in contraction mode, expelling a greater fluid flow from the lower chamber **706** through the left diffuser element than the right diffuser element. The result is a net fluid flow out of the lower chamber **706** to the left.

[0063] Referring now to FIG. **8**, there is illustrated a cross-sectional view of a piezoelectric micropump with check valves. Membrane **802** is disposed within a pump chamber **804** and secured to a body **806**. A piezoelectric disc **808** is disposed beneath the membrane **802** and electrode **810** is disposed below the piezoelectric disc **808**. Deformation of the membrane **802** with the piezoelectric disc at the appropriate frequency will cause a volume of the pumping chamber **804** to change. An inlet valve **810** allows fluid to flow into the chamber **804** and an outlet valve **812** allows fluid to flow out of the chamber **804**.

[0064] Referring now to FIG. **9**, there is illustrated a micropump **960** in which a nanofabricated or microfabricated fluid flow pathway is formed between structures. A first reservoir **961** terminates with a first gate valve **966** which permits or restricts fluid flow between the first reservoir **961** and a second reservoir **973**. An electrolytic pump **985** drives a first diaphragm **965** which is communication with the second reservoir **973**, to close the first gate valve **966**, and pulls a second diaphragm **969**, which opens a second gate valve **968** to drive fluid from the second reservoir **973** to a third reservoir **973**. The electrolytic pump **985** is driven by electrowetting of a first membrane **962** on the first gate valve **916** side of the pump. By switching to electrowetting of a second membrane **963**, as depicted in FIG. **16**B, fluid within the third reservoir **973** is emitted from an exit opening **170** by actuation of the second diaphragm **969**.

[0065] Referring now to FIG. **10**, there is illustrated a flowchart depicting the overall operation of the system. The process is initiated at a Start block **1002** and then proceeds to a block **1004**, wherein the biofluid sample is loaded. The process enclosed a block **1006**, wherein the biofluid is transferred to the viewing window or the cell counter. The process then flows to a decision block **1008** to determine when the counting operation is done, i.e., when the cells have been discriminated. As noted hereinabove, each of these cells could be associated with, depending on upon the type, a particular affinity label to allow them to be discriminated between within the viewing window. The process then flows to a block **1010** in order to pump the biofluid material to the next phase, that associated with the parallel testing/analysis step. A decision is then made at a block **1012** as to whether this is a positive state, i.e., has any of the biofluid material interacted with a particular reagent to give a positive result. If not, the process is terminated at a block **1014** and, if so, the process flows to a block **1016** in order to capture the biofluid material in a secondary reservoir. Once the path is selected, the appropriate micropump is activated and the biofluid material is pumped to the next reservoir along the secondary path, as indicated by a block **1018**. The process then flows to a block **1022** in order to analyze the results at each secondary reservoir and, if there is a positive result, as indicated by block **1022**, the process is terminated at a block **1024**. If the result is not positive, the process then flows to a block **1026** to determine if that is the last testing reservoir and, if so, the process flows to the terminate block **1024**. If there are more testing/analysis blocks through which to process the biofluid material, the process then flows back to the input of a block **1018** to pump the biofluid serial to the next testing reservoir.

[0066] Referring now to FIG. **11**, there is illustrated a flowchart for the loading operation, which is initiated at a block **1101** and then flows to a block **1102** wherein the sample is placed in the well and then to a decision block **1104** to determine if this is a process wherein the biofluid sample is to be mixed with some other diluted product or an affinity label. If it is to be mixed, the process flows to a block **1106** to mix the biofluid sample and, if not, the process bypasses this step. The process then flows to a block **1108** in order to activate the pump and transferred the biofluid material after mixing to the next reservoir in the process.

[0067] Referring now to FIG. **12**, there is illustrated a flowchart for the process of the cell counting operation, i.e., the operation at the viewing reservoir. This is initiated at a block **1202** proceeds to a block **1204** in order to transfer the biofluid material to the viewing chamber. The process enclosed a block **1206** in order to view the cells in real time as they pass through the various microchannels and viewing window. The process then flows to a block **1208** in order to count the cells. At this stage, the cells can have various affinity labels associated there with such that the target cells can be viewed and discriminated between based upon the affinity labels associated therewith. If, for example, there were multiple types of bacteria contained within the biofluid sample and each of these types of bacteria had associated therewith different affinity label that clips arrest at a different color, they killed be discriminated between. Additionally, proteins would have a different affinity label than a bacteria and this would also allow discrimination between the two types of cells. The process then flows to a block **1210** to store the transferred biofluid in the reservoir and into a block **1212** to terminate.

[0068] Referring now to FIGS. **13**A-**13**C from their illustrated various configurations for the cell counting operation. In the first embodiment of FIG. **13**A, there are provided a three-part microchannel **1302**, a middle section microchannel **1304** and an outlet microchannel section **1306** the middle section **1304** has a diameter that is slightly larger than the largest cell that could be contained within the biofluid. This allows the cells to be transferred in a more orderly manner. The cell viewing would be performed at this middle section microchannel **1304**. In the embodiment of FIG. **13**B, there are provided three varying diameter middle microchannel sections **1308**, **1310** and **1312**, each with different diameters to allow different size cells to flow therethrough. This type of embodiment may facilitate some selection in the cells for viewing. In the embodiment of FIG. **13**C, there is illustrated the above disclose embodiment wherein the microchannel **416** empties into the reservoir **418** and the viewing is basically performed upon the cells within the reservoir **418**.

[0069] Referring now to FIG. **14** come there is illustrated a flowchart for the parallel cell capture in the first testing/analysis stage. This is initiated at a block **1402** and a process and proceeds to a block **1406** in order to preload all of the cell capture areas having reagent associated there with, such that the portion of the biofluid stored in the reservoir **418** is transferred to the reservoirs associated with the parallel cell capture areas. The process enclosed a block **1408** wherein the pump is activated to fill all of the cell capture wells associated with this stage of testing/analysis. The process then flows to a block **1410** to possibly allow the cells to slowly go through the microchannels in order to interact with the reagent. If so, this requires a certain amount of time and this would result in the micropumps operating at a lower rate to allow sufficient time for the cells to flow through the serpentine microchannels **316** to interface with the particular coating on the surfaces thereof. This basically is the amount

of time required for the micropumps to fill up the reservoir **318** associated there with. The length of the serpentine microchannel **316** would determine the amount of time required to fill up the reservoir **318**. Once the reservoir has been filled, as indicated by a block **1412**, then the viewing window in the reservoir **318** is analyzed, as indicated by a block **1414**. The path from the block **1410** to the input of the block **1414** indicates a path by which the micropumps can be run at a higher rate. The process then is terminated at a block **1416**.

[0070] Referring now to FIG. **15**, there is illustrated flowchart for the second phase of the analysis, provided that the first phase indicated a positive result for one of the cell capture areas and the associated reagent. This is initiated a block **1502** and then proceeds to a block **1504** to preload all of the secondary cell capture areas with reagent and into a function block **1506** to pump all of the remaining biofluid material from the reservoir **418** into the first reservoir in the secondary reservoirs **330**. This also goes through and incubate step to allow the micropumps to pump at a slower rate to allow the biofluid material to go through the serpentine microchannel **316** at a slower rate before it enters the associated reservoir **318**. When the reservoir **318** is filled, as indicate a by block **1510**, the contents of the reservoir **318** are analyzed at a block **1512**. If the pump can be run at a faster rate, this is provided by a path around the block **1510**. If the result is positive, as indicated by a block **1514**, then the process is terminated at a block **1516**. If not, the process flows from the block **1514** to a block **1518** in order to the next reservoir **330** in the back to the input of the serpentine microchannel **316** and then float the input of the block **1508**.

[0071] Referring now to FIG. **16**, there is illustrated a simplified diagrammatic view of the microfluidics chip for processing a plurality of modules. The sample **303** is input to the well **302** and then pumped into the viewing window **306**. A waste microchannel **1602** is provided an interface to the viewing window **306** that is interfaced with a micro valve **1604** to allow air to escape, or any bubbles that may be present, from the viewing window **306**. Additionally, the waste microchannel **1602** could interface with an external vacuum source aid in fluid flow. A cell counter/discriminator **1606** is provided for optically viewing the contents of the viewing window **306**, the output thereof processed via a processor **1608**. The outlet of the viewing window **306** is interfaced with a manifold microchannel **1610** through a connecting channel **1612**. At this point, the micro valve **1604** is closed such that the biofluid contained within the viewing window **306** and the interfaced with microchannel manifold **1610** to allow fluid to be pump therefrom to a plurality of distribution paths along distribution microchannels **1614**. It may be that pump **304** would need to be activated in order to reduce the pressure at the upper end of the viewing channel **306** or, alternately, a microchannel **1618** interfaced with a micro valve **1620** could be provided to, when open, relieve the pressure in the upper end of the viewing window **306** to allow biofluid to be pumped therefrom to the microchannel manifold **1610**.

[0072] Each of the distribution microchannels **1614** is interfaced with a separate module via an associated distribution pump **1624** to interface with and associated one of modules **1625**, labeled A-Z, for example. There can be any number of modules provided. However, each module **1625** has associated there with a finite capacity and, therefore, the number of modules **1625** that can be interfaced to the viewing window **306** is a function of the volume of biofluid contained therein and the capacity of the reservoirs of each of the individual modules **1625**, each of the individual modules **1625** potentially having a different capacity, depending upon the configuration thereof. However, selecting among the various distribution pump **1624** can allow desired tests to be done with the available biofluid contained within the viewing window **306**.

[0073] Referring now to FIG. **17** there is illustrated a diagrammatic view of one of the modules **1625** associated with the parallel testing configuration, wherein biofluid is loaded into a plurality of testing reservoirs. The distribution pump **1624** associated there with transfers fluid from the distribution microchannels **1614** to an intermediate microchannel manifold **1702** which is then interface with a plurality of testing reservoirs **312**, as described hereinabove. Each of these testing reservoirs has a serpentine microchannel **312** and a viewing window **318** associated there with. As described hereinabove, each of these testing reservoirs can have a different volume and a different configuration mechanically and can be associated with a different test. They can each have a particular coating of reagent, such as an antibiotic, to interact with the biofluid for testing purposes to determine if there is any reaction of the biofluid in the cells contained therein to the material coated on the sides of the serpentine microchannels **316**. In the operation of this particular module **1625**, all of these testing reservoirs **312** are associated with different reagents and will be loaded in parallel. For this embodiment, will be desirable for each of the reservoir **312** to have the same volume. If, however, they had different volumetric capacities, it would be necessary to have some type of waste gate with a micro valve to allow all of the viewing windows **318** to achieve full capacity.

[0074] Referring now to FIG. **18**, there is illustrated a diagrammatic view of the serially configured wherein a plurality of testing reservoirs **330** our arranged in a series configuration. In this configuration, the associated distribution pump **1625** will transfer biofluid from the microchannel manifold **1610** through the distribution microchannels **1614** to the first of the testing reservoirs **330**. The biofluid will be contained within the viewing chamber **318** and, as noted hereinabove, there will be possible he some type of waste microchannel associated micro valve to allow air/bubbles to escape during filling of the viewing window **318**. Thereafter, a second serial pump **1706** is activated to transfer the contents of the viewing window **318** to a second testing reservoir **330** in the associated serpentine microchannel **316** and viewing window **318**. In this transfer, there may be required a relief microchannel (not shown) at the inlet end thereof to reduce the pressure therein during the pumping operation. This will continue until all of the tests have been done. Each of the serpentine microchannels **316** associated with each of the testing reservoirs **330** will have a graduated increase in the particular reagent to determine the dosage, in this example. It may be that, upon being exposed to the dosage of the reagent in the first testing reservoir **330** that cellular material in the biofluid is somewhat affected by the reagent, i.e., the antibiotic, for example. By moving to a higher concentration of the reagent in the next sequential testing reservoir **330**, this could be accounted for in the overall analysis. It may be that the actual concentration in the next sequential testing chamber **330** is not an exact incremental increase in the reagent. For example, if it was desired to expose the biofluid to reagent increments of 10%,

20%, 30%, etc. in 10% increments, it may be that the first testing chamber **330** has a concentration of 10% and then the second testing chamber has a concentration of possibly 16%, accounting for the fact that the accumulated effect of passing through the 10% testing chamber **330** and the 16% testing chamber **330** effectively provides a 20% accumulated exposure in the second testing chamber **330** and so on.

[0075] Referring now to FIG. **19**, there is illustrated a diagrammatic view of a configuration for providing parallel loading of the serial configuration for the incremental testing. This is similar to the embodiment of FIG. **17**, except that the testing chambers **330** are all interfaced with the associated distribution pump **1625** through a microchannel manifold **1902** in a parallel configuration, such that they are all loaded at the same time, with each having a different concentration of reagent associated there with. In this configuration, however, since all of the testing chambers **330** will be loaded in parallel, there are required to be a sufficient volume of biofluid contained within the viewing window **306** initially to facilitate complete filling of each of the associated viewing windows **318**.

[0076] Referring now to FIGS. **20**A-**20**B come there is illustrated a diagrammatic view of chemostat, wherein the associated distribution pump **1625** transfers biofluid from the distribution microchannel **1614** to a chemostat **2002**. The details of the chemostat **2002** are illustrated in FIG. **20**B. A main microchannel **2004** is interfaced on one and thereof with the output of the distribution pump **1625** associated there with, with the other end of the microchannel **2004** interfaced with a waste gate via a micro valve (not shown). There are a plurality of cell storage microchannels **2006** connected between one surface of the main microchannel **2004** and a waste microchannel **2008**. Each of these cell storage microchannels **2006** associated there with a filter **2010** disposed at the end thereof proximate to the waste microchannel **2008**. Each of the cell storage microchannels **2006** has a size that will receive a particular target cell having a particular dimension, such that the target cell will flow into the cell storage microchannel and cells of smaller size will pass through the associated filter **2010**, which filter **2010** is a microchannel with a diameter that is smaller than that of the target cell. This waste material will flow out through the waste gate or micro valve (not shown) associated with the waste microchannel **2008**. By maintaining a pressure differential between the main microchannel **2004** and the waste microchannel **2008**, the target cells will be stored within the cell storage channels **2006**. Larger cells than the target cells in the main microchannel **2004** will bypass the cell storage microchannels **2006** and pass out of the waste gate associated with the main microchannel **2004**, keeping in mind that there is required to be a lower pressure within the waste microchannel **2008** as compared to the main microchannel **2004**.

[0077] Referring now to FIG. **21**, there is illustrated an embodiment of the microfluidic chip utilizing micro valves as opposed to intermediate micropumps. In this embodiment, there are illustrated a plurality of input wells **2102** for interfacing with an initial micropump **2104** to pump fluid through a viewing window **2106** to a first reservoir **2108**. Having multiple wells **2102** allows multiple samples to be input through the viewing window **2106** or to actually mix two different materials together for flowing through the viewing window **2106** to the reservoir **2108**. The waste gate **2110** can be provided at the reservoir connected thereto via a waste microchannel **2112** to allow air/bubbles to escape. A micropump **2114** is operable to pump fluid from the reservoir **2108** to a main microchannel manifold **2116**. During this pumping operation, some type of pressure relief is required which can either be provided via one of the pumps **2104** being activated or a relief micro valve **2118** Interface with the input end of the viewing window **2106** through a relief microchannel **2120**.

[0078] Interfaced with the main microchannel manifold **2116** is a plurality of distribution micro valves **2124**. These distribution micro valves **2124** can be interfaced with various modules, as described above herein with respect to FIGS. **17**-**20**A/B. The only difference is that the associated distribution pump **1624** has been replaced by a distribution valve **2124**. Additionally, each of the parallel loaded testing reservoirs **312** can be individually associated with one of the distribution valves **2124** to selectively certain ones thereof for testing. Since each one of these testing reservoirs **312**, after selection, is required to be completely filled, by allowing individual selection of the testing reservoirs **312**, certain ones thereof can be eliminated. It may be that, in pre-analyzing the biofluid sample, it can be predetermined that certain ones of the associated reagents in the reservoir **312** are not required the testing/analysis step and can therefore be eliminated from the step of filling. This is opposed to the embodiment of FIG.**17**, wherein all of the testing reservoirs **312** are complete the filled.

[0079] Referring now to FIGS. **22**A-**22**B, there is illustrated cross-sectional views of a micro valve in an open and a closed position. The substrate **402** has cover plate **440** disposed on top thereof. There are provided to microchannels **2202** and **2204** that are to be connected together with the micro valve. The microchannel **2202** is interfaced with a hole **2006** to the surface of the cover plate **440** to an opening **2208**. The microchannel **2204** is interfaced to a hole **2210** to an opening **2212** in the cover plate **440**. The micro valve has a fixed body **2214** with a membrane **2216** disposed on the surface there above to define a pumping chamber **2218**. The pumping chamber **2218** has a hole **2220** interfacing the pumping chamber **2218** with the opening **2208** on the cover plate **440**. Similarly, the hole **2212** is interfaced to the pumping chamber **2218** through a hole **2222**. The membrane **2216** is operable to reciprocate away from the surface of the fixed body **2214** exposing the top of the hole **2220** in the pumping chamber **2218** to allow fluid to flow through the pumping chamber **2218** and down through the opening **2222** through the cover plate **440** and through to the microchannel **2204**. In the closed position, the membrane **2216** is forced down against the upper end of the hole **2220**. A pneumatic cavity **2230** is disposed above the membrane **2216** in a housing **2232** and interfaces with a pneumatic source through a hose **2234**. Thus, by drawing a vacuum in the pneumatic cavity **2230**, the membrane **2216** will be pulled away from the hole **2220** to allow fluid to flow and, when pressurized air is forced into the pneumatic cavity **2230**, and the membrane **2216** is forced down to the surface of the fixed body **2214** to seal the opening **2224** in a closed position.

[0080] Referring now to FIG. **23**, there is illustrated a process flow for preparing the biologic sample for the microfluidic chip **102**. The preparation of the biologic sample can take many forms. In this example, the raw biologic sample can be preprocessed, depending upon the type of sample that is being considered. For example, if

blood is being tested, the Complete Blood Count (CBC) can be determined, as well as the White Blood Cell Count (WBC), the liver functions and the kidney functions. For urinalysis, the sample can be prepared for testing for WBC's and nitrates, as well as proteins and Bilirubin. There are many well-known processes for preparing biologic samples prior to testing. Once the biologic sample has been prepared a, affinity labels are attached thereto. Typically, there will be a vial **2302** provided with the biologic sample that is mixed with affinity labels in a vial **2304** resulting in the vial **2304** containing a labeled sample. These labels are sometimes referred to as "affinity labels" or "microspheres." These functional polymeric microspheres typically have a diameter of less than 5 μm and have been developed for use with immunological methods. The reagents were initially used as visual markers to identify specific cell types and analyze the distribution of cell surface antigens by scanning electron microscopy. They have also been used, due to their inherent properties, two separate labeled from unlabeled cells by techniques such as centrifugation, a electrophoresis, magnetic chromatography and fluorescence cell sorting. The cells contained within the biologic sample are basically cells bearing defined antigens or receptors, ligands which bind with a high degree of selectivity an affinity to these cell surface sites. The microspheres interact with the specific ligand, which can allow for separation based upon the characteristic properties of the microspheres. This allows for displaying of these labeled cells with the target receptor or antigen with antibiotics or other ligands directly or indirectly bound to the microspheres. Specific types of microspheres or affinity labels can be the type that will fluoresce at a particular wavelength. Thus, specific cells can be identified the optical techniques to identify target cells or differentiate between various types of, for example, bacterial cells and proteins, etc. This labeled sample is then disposed within the well **302** on the microfluidic chip **102** for later processing.

[0081] Referring now to FIG. **24**, there is illustrated a side cross-sectional view of an RT-lamp. The RT-lamp is a Reverse Transcription Loop-mediated isothermal Amplification device, which is an a technique for the amplification of RNA. This combines the advantages of the reverse transcription without of the LAMP technique. The LAMP technique is a single to technique for the application of DNA. This technique is an isothermal nucleic acid application technique, in which a chain reaction is carried out at a constant temperature and does not require a thermal cycler. The target sequences animal five at a constant temperature using either two or three sets of primers and polymerase with high strand displacement activity in addition to a replication activity. The addition of the reverse transcription phase allows for the detection of RNA and provides a one-step nucleic acid amplification method that is used to diagnose infectious diseases caused by bacteria or viruses.

[0082] FIG. **24** illustrates an example in which a multimode instrument **2401** is coupled to a smartphone **2402**. The smartphone **2402** includes an LED **2404** and a camera **2406**. The camera **2406** includes an image sensor, such as a CCD. The instrument **2400** includes a sample chamber **2408** for receiving an optical assay medium. The optical assay medium comprises the labeled biologic sample disposed within the viewing window **306** on the microfluidic chip **102**. The sample chamber **2408** may include a door **2410** that prevents stray light from entering

[0083] The optical assay medium is positioned over a detection head **2412** in the sample chamber **2408**. The instrument **2400** includes an optical output path for receiving an optical output from the optical assay medium in the sample chamber **2408** via the detection head **2412**. The optical output path may include a multimode fiber **2414** that directs light from the detection head **2412** to a cylindrical lens **2416**. The optical output path may further include a wavelength-dispersive element, such as a diffraction grating **2418**, that is configured to disperse the optical output into spatially-separated wavelength components. The optical output path may also include other optical components, such as collimating lenses, filters, and polarizers.

[0084] The instrument **2401** can include a mount for removably mounting the smartphone **2402** in a working position such that the camera **2406** is optically coupled to the optical path, for example, in a predetermined position relative to the diffraction grating **2418**. In this working position, the camera **2406** can receive at least a portion of the dispersed optical output such that different locations are received at different locations on the image sensor.

[0085] The instrument **2401** may also include an input optical path for directing light from a light source to the optical assay medium in the sample chamber **2408**, for example, through the detection head **2412**. In some instances, the LED **2404** on the smartphone **2402** could be used as the light source. To use the LED **2404** as the light source, the input optical path may include a collimating lens **2420** that receives light from the LED **2404** when the smartphone **2402** is mounted to the instrument **2400** in the working position. The input optical path may further include a multimode fiber **2422** that directs the light from the collimating lens **2420** to the detection head **2412**. The input optical path may also include other optical components, such as collimating lenses, filters, and polarizers.

[0086] The instrument **2400** may also include an additional input optical path that directs light form an internal light source, such as a laser **2424**, to the optical assay medium in the sample chamber **2408**. The additional input optical path may include a multimode optical fiber **2426**, as well as collimating lenses, filters, polarizers, or other optical components.

[0087] Referring now to FIG. **25**, there is illustrated the view of the RT-lamp **2401** with a microfluidics chip **102** disposed within the sample chamber **2408**.

[0088] Referring now to FIG. **26**, there is illustrated a side view of the smart phone **2402** interfaced with the microfluidic chip **102** four imaging the surface thereof, which is illustrated in a window view in FIG. **27**. This window view illustrates the viewing window as a box **2702** in which the image of the microfluidic chip **102** is displayed. The application automatically recognizes various markers **2704**, **2706** and **2708** 1 three corners thereof. This will allow orientation of the window with respect to the application. A box **2710** shown in phantom dashes will be oriented by the application running on the smart phone **2402**. Once the box has been oriented visually about the image of the microfluidic chip **102**, then processing can proceed. The processing is basically focusing upon the chip to gain the best optical image of the target sites. The target sites are storage reservoirs **312** and **330**, for example. Each of these will have a viewing well **318** associated there with and these viewing wells **318** will have, in one example, a process biologic sample having affinity labels associated there with that fluoresce. By rec-

ognizing the florescence, the presence of the cell can be determined. The lack of florescence indicates that the cell, a bacteria for example, has been destroyed. This can be a positive test. By examining at each stage of the testing process the chip, a determination can be made as to results in essentially real time. This will be described in more detail hereinbelow. Once the image is believed to be in focus, the user can actually take the picture or the application can automatically determine that the focus is adequate and take that. This is very similar to character recognition techniques that are utilized in recognizing faces in camera images received by the phone.

[0089] Referring now to FIGS. **28**A-**28**H, there are illustrated various images of the microfluidic chip **102** at different stages, this view being a diagrammatic view for simplicity. In this view, there is provided the sample well **2802** which then feeds into the viewing well **2804**. As described hereinabove, there are multiple pumps that allow fluid to be moved from the sample well **2802** over to the viewing well **2804** and these are not shown for simplicity purposes.

[0090] There is provided a multiplexer **2802** which represents the micropumps/valves described hereinabove. The multiplexer **2008** may be associated with one bank **2808** of reservoirs **2802** for the parallel processing stage. These reservoirs **2012** correspond to the reservoirs **312**. This requires that the multiplexer **2806** distribute fluid to a microchannel manifold **2810** and one testing phase. The multiplexer **2006** also is connected via a plurality of microchannels to a bank of reservoirs associated with the serial processing stage to selectively distribute fluid to one of the strings in a second testing phase. This bank of reservoirs includes the reservoirs **330** described hereinabove. Each of these reservoirs **330** is arranged in series such that each has a valve or pump disposed there between. The multiplexer **2806** also interfaces with a bank **2830** of reservoirs, these, in this example, associated with the serial testing/analysis stage and having reservoirs **330** associated there with. In this example, there are provided five test reservoirs in the bank **2808**, wherein each of these test reservoirs has associated there with one serial string of test reservoirs **330** in the bank **2814** and one parallel loaded string of reservoirs **330** in the bank **2820**. Additionally, there is a separate testing reservoir **2824** which could correspond to the cell storage area utilizing a chemostat described hereinabove, which is interfaced with multiplexer **2806** through a microchannel **2826**.

[0091] Referring now to FIGS. **28**B-**28**H, there are illustrated various stages of the loading and analysis. FIG. **28**B illustrates the first step in the process wherein the biologic sample is loaded into the viewing window **2004**. That the step in the process, the microfluidic chip is disposed within the RT lamp **2401** and analyzed to determine the number of cells and the type of cells. If, for example, a certain bacteria were being tested for on this particular microfluidic chip **102**, the lack of bacteria cells, as indicated by the particular affinity labels that would be attached to these particular bacteria cells, would indicate that further testing is not required. However, if the correct cells are labeled and the number of cells is at an appropriate level for testing, then the next step of the process is taken.

[0092] FIG. **28**C illustrates a next step of the process wherein a portion of the contents of the viewing well **2804** are transferred to all of the reservoirs **2812** in the bank **2808**, there being five reservoirs **2812** disposed therein, it being noted that there could be more reservoirs **2012** provided on the microfluidic chip **102**. There will be a certain amount of time required for the pump associated with the multiplexer **2808** to actually move the desire portion of the biologic sample through the manifold **2810** to the reservoirs **2812**. As noted hereinabove, each of the reservoirs **2812** corresponds to the reservoirs **312**, each having a serpentine microchannel **316** and a viewing reservoir **318** associated there with. The micro pumps associated with the multiplexer **2806** in communication with the very small widths of the microchannels can require this process to take upwards of 10 or 20 minutes. After this period of time, the microfluidic chip **102** can be imaged to determine if the cells have been destroyed by the coating on the surfaces of the serpentine channel **316**. (It should be noted that the viewing well **318** could also be coated). If the cells are destroyed, this indicates that the reagent that coats the walls of the microchannel associated with the reservoirs **312** reacted in a manner indicating self-destruction. However, any visual indication in the viewing wells that can be a vehicle for discrimination between interaction with the particular reagent coating the walls of the serpentine microchannels **316** will provide the ability for a decision to be made as to which reagent is required for further testing.

[0093] FIG. **28**D illustrates the next phase of operation, which is the phase in which the dosage level is determined. In the example above, the middle reservoir in the bank **2808** provided a trigger indication that triggered a decision to then test for dosage in the middle string within the bank **2814**. This will require a multiplexer **2806** to only transfer the remaining portion of the biologic sample from the viewing reservoir **2804** into this particular string. As described hereinabove, this process will involve first passing of fluid to the first reservoir **330**, which will take a certain amount of time to actually pump the biologic sample through the microchannels into the viewing window **318**. This can be a multiphase process, which requires viewing at each stage. In this particular example, the third stage of testing in this middle string in the bank of reservoirs **2814** resulted in a perceivable result, i.e., a lack of florescence, for example. At this point, the image will actually show the perceivable result in both the bank **2808** and in the bank **2814**. Thus, in the three phases of testing, the particular cells have been a defined, an indication has been provided as to which of multiple reagents that could possibly provide the desired therapeutic results would be the best choice for the patient and then the third phase of testing provides the actual dosage of that determine reagent. It may be that for ten individuals that had exactly the same symptoms and processed a similarly processed biologic sample for testing in the same way with the microfluidic chip and the RT-lamp **2401** came up with different results. Each individual's particular physiology can vary and, as such, the results could differ. In a typical medical environment, the particular reagent of choice or drug of choice is determined by an individual based upon various criteria. Since the medical professional does not have the test directly in front of them, they might just prescribe, for example, a broad based antibiotic. They might follow that up with testing of a biologic sample in a lab, which could take a number of days just to determine exactly what bacteria is present and what would be the best antibiotic to use in order to attack this particular bacteria. Of course, the broad-spectrum antibiotic might have worked by the time the test results come back. If not, these results might be useful to the medical professional. However, these tests

seldom if ever actually focus in on the dosage that would be preferable for a particular individual. If even the particular antibiotic could be identified which was specific to that particular bacteria tested for and found be present in the biologic sample, the dosage prescribed is typically a medium or high dosage, depending upon the criteria that the medical professional utilizes. However, the medical professional typically generalizes the physiology of any individual and maybe filters that based upon age, gender, etc. However, the individual physiology is not taken into account.

[0094] With use of the present microfluidic chip **102**, the entire testing process can be performed at the Point of Care (POC) in a relatively short amount of time. The result is not only the identification of the best reagent to use but also the dosage. This is all accomplished with a very small amount of biologic sample.

[0095] FIG. **28**E, there is illustrated a potential further processing that can be provided. In this embodiment, the bank **2820** can have a different modification of the antibiotic that was determined from the test associated with the bank **2808**. This modification could be associated with the pH of the antibiotic, wherein it has been determined with respect to some antibiotics that the pH of the antibiotic can affect the efficacy thereof. In this example, it can be seen that the third reservoir with respect to dosage is the one that is selected in the bank **2814** but in the bank **2820**, is the lowest dosage. Thus, the multiplexer **2806** needs to first test the bank **2814** and then test the bank **2820**. However, it should be understood that both the bank **2814** and the bank **2020** could be identical, either serially loaded or parallel loaded, the commonality being that they have a gradually increasing dose of antibiotics that can be tested for.

[0096] FIGS. **28**F-**28**G, there are illustrated two additional examples of two different patients with substantially the same symptoms and utilizing substantially the same process for preparing the biologic sample. With respect to FIG. **28**F, the fifth reservoir and the antibiotic associated there with exhibited the highest efficacy at the highest dose as to destroying the particular bacteria, in the example of the bacteria. The associated dosage determined from testing the biologic sample in the bank **2814** was considered to be the second level of dosage. In the bank **2020**, the third level of dosage was considered to be the lowest dose. With respect to FIG. **28**G, the first reservoir and the antibiotic associated there with was considered to have the highest efficacy with respect to dealing with the particular bacteria and it was the lowest dose in that case when tested in the bank **2814**, as compared to the fourth level dosage in the bank **2820**. It can be seen thus that different patients will have different "fingerprints" associated with the testing of the same biologic sample repaired and substantially same way.

[0097] FIG. **28**H, there is illustrated an alternate embodiment wherein the test performed at the bank **2808** resulted in a slight ambiguity in that the bacteria were killed in two other reservoirs. In this case, the indication would be that either of these antibiotics would work against this particular strain of bacteria. Thus, the next phase the test would require the multiplexer **2808** to distribute the contents of the reservoir **2804** through the microchannels to actually two different strings. Thus, for this type of test to be carried out, it is important that there be sufficient volume in the viewing window **2804**, i.e., sufficient amount of biofluid introduced to the well **2802**, in order to fill both of these reservoirs and allow the testing to progress down to the highest dosage level in either or both of the banks **2814** and **2820**. The results of this test show that, for the rightmost reservoir in the bank **2808** having been determined to be effective at the highest dose, the next of the last dosage was required in order to achieve the desired results, whereas the next to the left reservoir in the bank **2808** having been determined to be effective at the highest dose required only the smallest dose to achieve the results. Therefore, this test shows that, although two antibiotics would work, one would actually work with the lower dose.

[0098] It should also be understood that, in addition to the test being different for the same strain of bacteria in a biologic prepared men substantially the same way, it should also be understood that this particular set of results could be different for different strains of the same bacteria. It may be that, for one strain, one antibiotic would work at a particular dose and, for another strain of the same bacteria, a different antibiotic work or just a different dose of the same antibiotic. The microfluidic chip described and disclosed in the present disclosure allows this determination to be made utilizing a single sample in a parallel/serial testing method at the POC wherein the first step or phase of selection is made among a plurality of potential antibiotics that could arguably target different bacteria and, once a determination is made at the first phase, then the next and serial decision is made to determine dosage at a second phase.

[0099] Referring now to FIG. **29**, there is illustrated a flowchart depicting the overall analysis process. The process is initiated at a block **2902** and then proceeds to a block **2904** wherein the biologic sample is prepared. As described hereinabove, this preparation involves labeling the cells within the biologic sample so that they can be discriminated between or identified. It may be that there are a number of different types of cells such as bacteria of different strains and types, proteins, etc. Different affinity labels can be applied such that multiple cells of different types can be identified. The process then flows to a block **2906** wherein the biologic sample is placed into the sample well and then passed on to the viewing well. At this point, the microfluidic chip is placed into the RT-lamp and optically analyzed, as indicated by process block **2908**. It is at this point in the testing phase that the identification process will identify the potential target cells. Since each of the microfluidic chips has a finite number of reservoirs associated there with for the purpose of testing, the coating is applied to these particular reservoirs for the specific antibiotics or reagents to be tested may not be useful for testing the particular cellular structures that have been identified at this step in the process. However, it should be understood that the number of different banks of testing reservoirs that can be provided on a particular microfluidic chip can be expandable and the could actually be provided for multiple different types of reagents. For example, one set of testing banks may be associated with UTI and another associated with streptococcal bacteria. Recognizing these at this step in utilizing them with a microfluidic chip that can test for both types of bacteria will allow the particular biologic sample, which is quite small, to be routed to the appropriate reservoirs for testing for that specific identify bacteria.

[0100] The decision to proceed is determined at a decision block **2910** and, if testing can proceed with the current microfluidic chip, the process proceeds to a block **2911** to select the particular test that are to be performed. The process then proceeds to sequence through the tests, as

indicated by a block **2914**. This sequencing sequences through the various phases, with the initial test being selected first, as indicated by block **2916**. In the above examples, this is the first parallel phase to determine which among several reagents is most effective against the particular cellular structure of interest. The process then proceeds to a block **2918** in order to analyze the results of this initial test and then to a decision block **2920** to determine if more tests are required or if this is the only test. If the test is negative at this stage and none of the reagents provides any effectiveness indication, the process is terminated or, if this is the last test, the process is terminated. The process, if continued, then selects the next test in the sequence and proceeds back to the input of the block **2918** to continue sequencing through the tests.

[0101] Referring now to FIG. **30**, there is illustrated a flowchart for the testing process. This is initiated at a block **3002** and then proceeds to a block **3004** two first pump a portion of the biologic sample stored in the viewing window through to the parallel reservoirs and load all of the parallel reservoirs for testing/analysis. This may take upwards of 10 or 20 minutes, due to the fact that the micropumps utilized are relatively slow and the diameter of the microchannels is small, thus restricting high flow rates. The process then flows to decision block **3006** to determine if there is been any positive result, i.e., is there any indication that any of the reagents provide an effectiveness indication, either through some color change or the lack of color indicating the destruction of the cells. If there is no result, then the process is terminated and the process flows to a function block **3008** to select the next test path that is associated with the antibiotic having been tested as being effective in the first phase of operation/testing. A process block **3010** and indicates that a graded dosage test path is selected, either the one for loading parallel or the one or loading serially. It should be understood that the parallel loaded graded dosage test path requires all of the reservoirs to be completely filled from the reservoir associated with the viewing window. The serial path, by comparison, allows all of the contents of the viewing window in the reservoir associated there with to be disposed in each reservoir and then sequentially transferred to the next reservoir down the chain and at the higher dosage. However, it should be understood that the system can be configured such that the first reservoir at the lowest dosage is loaded with only a portion of the contents of the viewing window and the reservoir associated there with, analyzed and then a micro valve gate opened to allow the micropumps for pumping fluid to the serial path to operate to continue pushing more biofluid through the first reservoir, thus filling the second and reservoir and so on. In this process, sufficient biofluid must be contained within the viewing window and the reservoir associated there with in order to allow for filling of all of the reservoirs down to the highest dosage rate associated with that serial string.

[0102] In the process, the serial string will first select the lowest graded dose reservoir and a process block **3012** and then pump biofluid to the first reservoir and a process block **3014**, analyze the results a process block **3016**, understanding that it could take **10** to **20** minutes to fill each reservoir. A determination is made at a decision block **3018** as to whether there is a positive result, i.e., was there and an effectiveness determination made at this point, and, if so proceed to a decision block **3020** to determine if there are any higher concentrations to be tested for. If so, the next reservoir selected by opening gate or activating a micropump, as indicated by a process block **3022**, and the proceed back to the process block **3014** in order to pump to this reservoir.

[0103] In the parallel process, a process block **3026** indicates an operation wherein the micropump pumps sufficient biofluid material to the parallel rated reservoirs to fill all of the reservoirs and into a process block **3028** in order to analyze the results.

[0104] It will be appreciated by those skilled in the art having the benefit of this disclosure of a System and Method for Determining Efficacy and Dosage Using Parallel/Serial Dual Microfluidic Chip. It should be understood that the drawings and detailed description herein are to be regarded in an illustrative rather than a restrictive manner, and are not intended to be limiting to the particular forms and examples disclosed. On the contrary, included are any further modifications, changes, rearrangements, substitutions, alternatives, design choices, and embodiments apparent to those of ordinary skill in the art, without departing from the spirit and scope hereof, as defined by the following claims. Thus, it is intended that the following claims be interpreted to embrace all such further modifications, changes, rearrangements, substitutions, alternatives, design choices, and embodiments.

1. A method for determining a treatment agent and dosage level for a predetermined biologic material, comprising:

receiving a biologic sample, the biologic sample containing the predetermined biologic material for treatment by one of a plurality of treatment agents;

holding the biologic sample containing the predetermined biologic material within a first reservoir;

pumping a portion of the biologic sample into each of a first plurality of parallel pathways from the first reservoir using a micro-pump;

applying a separate treatment agent of the plurality of treatment agents within each of the first plurality of parallel pathways to the portion of the biologic sample within the parallel pathway;

determining the treatment agent of the plurality of treatment agents providing a best treatment efficacy for the predetermined biologic material within the biologic sample responsive to the plurality of treatment agents applied to the portion of the biologic sample within each of the first plurality of parallel pathways;

pumping a second portion of the biologic sample into a selected second parallel pathway associated with the determined treatment agent of a second plurality of parallel pathways from the first reservoir using a second micro-pump;

applying the determined treatment agent at a plurality of different dosage levels within the selected second parallel pathway to the second portion of the biologic sample within the second parallel pathway;

determining a dosage level of the plurality of different dosage levels of the determined treatment agent with respect to the predetermined biologic material providing the best treatment efficacy; and

providing an output indicating the treatment agent and the dosage level of the treatment agent providing the best treatment efficacy.

2. The method of claim **1**, wherein the step of applying further comprises:

coating an interior of a portion of each of a plurality of micro-channels interconnecting the first reservoir with a plurality of second reservoirs with one of the plurality of treatment agents; and

passing the biologic sample through the plurality of micro-channels to apply the plurality of treatment agents to the biologic sample.

3. The method of claim **1**, wherein the step of determining further comprises:

holding the portion of the biologic sample treated with one of a plurality of treatment agents in a plurality of second reservoirs; and

detecting efficacy of the plurality of treatment agents on the biologic material within the biologic sample through a plurality of second viewing windows each associated with one of the plurality of second reservoirs.

4. The method of claim **3**, wherein the step of pumping the portion further comprises pumping the portion of the biologic sample through the plurality of micro-channels into the plurality of second reservoirs.

5. The method of claim **1**, wherein the step of applying the determined treatment agent further comprises:

coating an interior of a portion of each of a second plurality of micro-channels interconnecting the first reservoir with each of the plurality of third reservoirs with a predetermined dosage level one of the plurality of treatment agents; and

passing the biologic sample through the micro-channel associated with treatment agent determined to provide the best treatment efficacy to apply the treatment agent determined to provide the best treatment efficacy at one or more dosage levels.

6. The method of claim **1**, wherein the step of determining the dosage level further comprises:

holding the second portion of the biologic sample treated with the treatment agent determined to provide the best treatment efficacy with the at least one dosage level in a plurality of third reservoirs; and

detecting efficacy of the at least one dosage level of the determined treatment agent on the biologic material within the biologic sample through at least one second viewing windows each associated with one of the plurality of third reservoirs.

7. The method of claim **6**, wherein the step of pumping the second portion further comprises pumping the second portion of the biologic sample through the plurality of micro-channels into the plurality of third reservoirs.

8. The method of claim **1**, wherein the step of applying the determined treatment agent further comprises applying the determined treatment agent in series at the plurality of different dosage levels to test an efficacy of the plurality of dosage levels one at a time.

9. The method of claim **1**, wherein the step of applying the determined treatment agent further comprises applying the determined treatment agent at the plurality of different dosage levels in parallel to test an efficacy of the plurality of dosage levels at a same time.

10. The method of claim **1** further comprising the step of applying an affinity label to cells of the detected biologic material within the biologic sample using a cell counter.

11. A method for testing a treatment agent for a predetermined biologic material, comprising:

receiving a biologic sample, the biologic sample containing a unique combination of the predetermined biologic material that must be treated via one of a plurality of treatment agents and a patient's biologic material;

testing a first portion of the biologic sample containing the unique combination of the predetermined biologic material and the patient's biologic material in a plurality of first parallel testing pathways, each of the plurality of first parallel testing pathways applying a different treatment agent to the first portion of the biologic sample;

determining a treatment agent providing a best treatment efficacy responsive to the tests performed in each of the plurality of testing pathways; testing a second portion of the biologic sample containing the unique combination of the predetermined biologic material and the patient's biologic material in a selected pathway of a second plurality of testing pathways, the selected pathway selected responsive to the determined treatment agent providing the best treatment efficacy, the selected pathway applying at least one dosage level of the determined treatment agent providing the best treatment efficacy to the second portion of the biologic sample;

determine a lowest dosage level of the determined treatment agent providing the best treatment efficacy responsive to the applied at least one dosage level; and

providing an indication of the determined treatment agent and the determined lowest dosage level.

12. The method of claim **11**, wherein the step of testing the second portion further comprises applying the determined treatment agent in series at the plurality of different dosage levels to test an efficacy of the plurality of dosage levels one at a time.

13. The method of claim **11**, wherein the step of testing the second portion further comprises applying the determined treatment agent at the plurality of dosage levels in parallel to test an efficacy of the plurality of dosage levels at a same time.

14. A method for determining a treatment agent and dosage level for a predetermined biologic material, comprising:

receiving a biologic sample, the biologic sample containing the predetermined biologic material for treatment by one of a plurality of treatment agents;

holding the biologic sample containing the predetermined biologic material within a first reservoir;

pumping a portion of the biologic sample into each of a first plurality of parallel pathways from the first reservoir using a micro-pump;

applying a separate treatment agent of the plurality of treatment agents within each of the first plurality of parallel pathways to the portion of the biologic sample within the parallel pathway;

holding the portion of the biologic sample treated with one of a plurality of treatment agents in a plurality of second reservoirs;

detecting efficacy of the plurality of treatment agents on the biologic material within the biologic sample through a plurality of second viewing windows each associated with one of the plurality of second reservoirs.

determining the treatment agent of the plurality of treatment agents providing a best treatment efficacy for the

predetermined biologic material within the biologic sample responsive to the detected efficacy of the plurality of treatment agents applied to the portion of the biologic sample;

pumping a second portion of the biologic sample into a selected second parallel pathway associated with the determined treatment agent of a second plurality of parallel pathways from the first reservoir using a second micro-pump;

applying the determined treatment agent at a plurality of different dosage levels within the selected second parallel pathway to the second portion of the biologic sample within the second parallel pathway;

holding the second portion of the biologic sample treated with the treatment agent determined to provide the best treatment efficacy with the at least one dosage level in a plurality of third reservoirs;

detecting efficacy of the at least one dosage level of the determined treatment agent on the biologic material within the biologic sample through at least one second viewing windows each associated with one of the plurality of third reservoirs;

determining a dosage level of the plurality of different dosage levels of the determined treatment agent with respect to the predetermined biologic material providing the best treatment efficacy; and

providing an output indicating the treatment agent and the dosage level of the treatment agent providing the best treatment efficacy.

15. The method of claim **14**, wherein the step of applying further comprises:

coating an interior of a portion of each of a plurality of micro-channels interconnecting the first reservoir with a plurality of second reservoirs with one of the plurality of treatment agents; and

passing the biologic sample through the plurality of micro-channels to apply the plurality of treatment agents to the biologic sample.

16. The method of claim **15**, wherein the step of pumping the portion further comprises pumping the portion of the biologic sample through the plurality of micro-channels into the plurality of second reservoirs.

17. The method of claim **14**, wherein the step of applying the determined treatment agent further comprises:

coating an interior of a portion of each of a second plurality of micro-channels interconnecting the first reservoir with each of the plurality of third reservoirs with a predetermined dosage level one of the plurality of treatment agents; and

passing the biologic sample through the micro-channel associated with treatment agent determined to provide the best treatment efficacy to apply the treatment agent determined to provide the best treatment efficacy at one or more dosage levels.

18. The method of claim **17**, wherein the step of pumping the second portion further comprises pumping the second portion of the biologic sample through the plurality of micro-channels into the plurality of third reservoirs.

19. The method of claim **14**, wherein the step of applying the determined treatment agent further comprises applying the determined treatment agent in series at the plurality of different dosage levels to test an efficacy of the plurality of dosage levels one at a time.

20. The method of claim **14**, wherein the step of applying the determined treatment agent further comprises applying the determined treatment agent at the plurality of different dosage levels in parallel to test an efficacy of the plurality of dosage levels at a same time.

21. The method of claim **14** further comprising the step of applying an affinity label to cells of the detected biologic material within the biologic sample using a cell counter.

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