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Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2812269 C 2019/04/09

(11)(21) 2 812 269

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2011/09/21
(87) Date publication PCT/PCT Publication Date: 2012/03/29
(45) Date de délivrance/Issue Date: 2019/04/09
(85) Entrée phase nationale/National Entry: 2013/03/21
(86) N° demande PCT/PCT Application No.: US 2011/052550
(87) N° publication PCT/PCT Publication No.: 2012/040327
(30) Priorité/Priority: 2010/09/22 (US61/385,431)

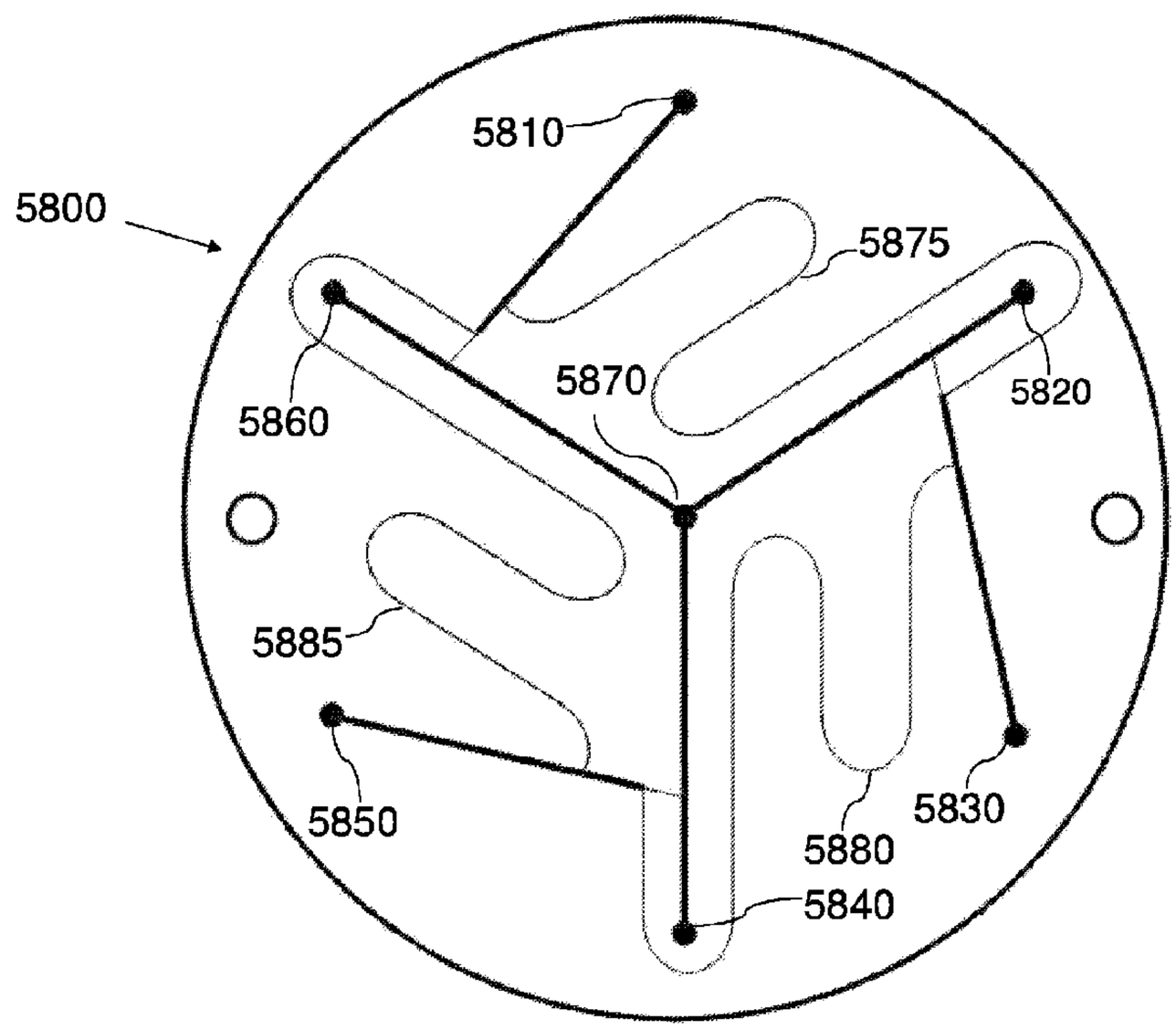
(51) Cl.Int./Int.Cl. *B01D 15/14* (2006.01)

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(54) Titre : PROCEDES ET DISPOSITIFS DE RETROBALAYAGE DESTINES A LA CHROMATOGRAPHIE
(54) Title: BACKFLUSH METHODS AND DEVICES FOR CHROMATOGRAPHY



(57) Abrégé/Abstract:
Chromatography systems include a micro fluidic device and that implement one or more methods to direct sample to a desired fluid flow path. A method of modulating flow of a fluid in a chromatography system including a micro fluidic device fluidic ally coupled to an injector is described. In certain examples, the method comprises controlling fluid pressure of the system to permit a sample to flow from the injector and into the microfluidic device and to a first fluid flow path and a second fluid flow path each fluidically coupled to the micro fluidic device at a separate outlet port of the micro fluidic device, and altering the pressure of the system to reverse the flow of the sample in at least one of the first fluid flow path and the second fluid flow path to backflush sample in the fluid flow path where the pressure is reversed.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 March 2012 (29.03.2012)

PCT

(10) International Publication Number
WO 2012/040327 A1

(51) International Patent Classification:
B01D 53/02 (2006.01)

(21) International Application Number:
PCT/US2011/052550

(22) International Filing Date:
21 September 2011 (21.09.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/385,431 22 September 2010 (22.09.2010) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) **Title**: BACKFLUSH METHODS AND DEVICES FOR CHROMATOGRAPHY

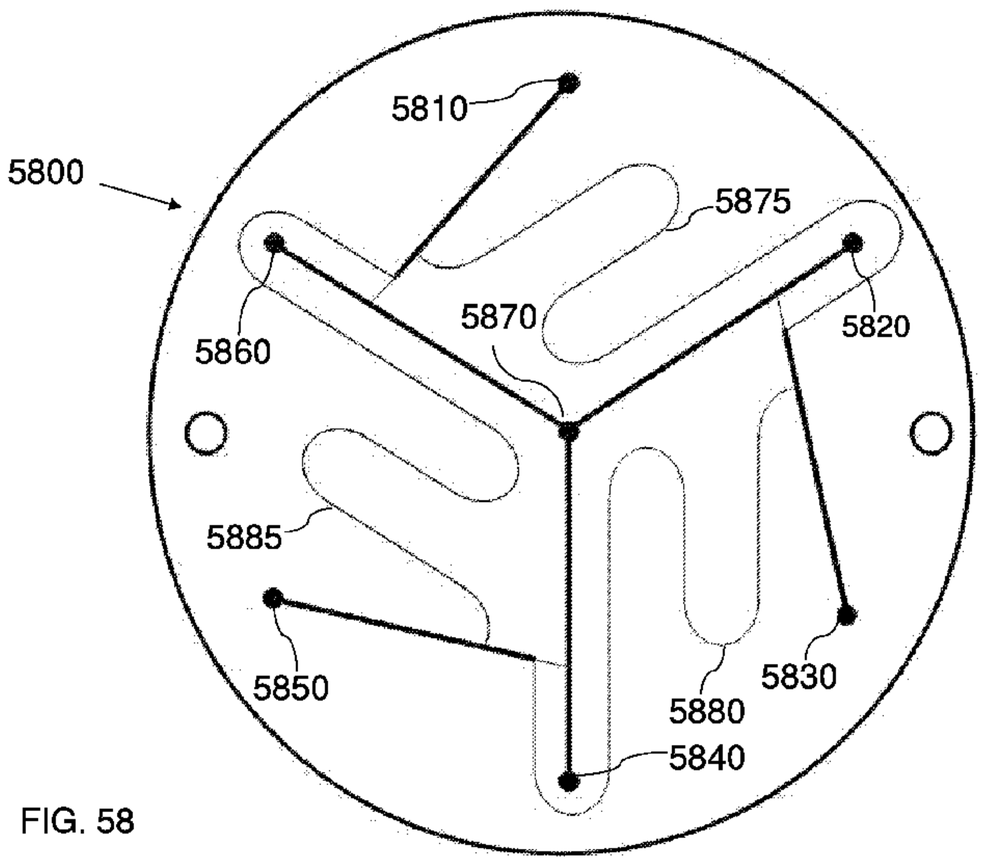


FIG. 58

(57) **Abstract**: Chromatography systems include a micro fluidic device and that implement one or more methods to direct sample to a desired fluid flow path. A method of modulating flow of a fluid in a chromatography system including a micro fluidic device fluidic ally coupled to an injector is described. In certain examples, the method comprises controlling fluid pressure of the system to permit a sample to flow from the injector and into the microfluidic device and to a first fluid flow path and a second fluid flow path each fluidically coupled to the micro fluidic device at a separate outlet port of the micro fluidic device, and altering the pressure of the system to reverse the flow of the sample in at least one of the first fluid flow path and the second fluid flow path to backflush sample in the fluid flow path where the pressure is reversed.

WO 2012/040327 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

BACKFLUSH METHODS AND DEVICES FOR CHROMATOGRAPHY

PRIORITY APPLICATION

[0001] This application claims priority to, and the benefit of, U.S. Provisional Application No. 61/385,431 filed on September 22, 2010.

RELATED APPLICATION

[0002] This application is related to commonly assigned application number US 12/472,948 and U.S. Provisional Application No. 61/354,526 filed on June 14, 2010.

TECHNOLOGICAL FIELD

[0003] Certain features, aspect and embodiments are directed to chromatography systems. In particular, certain embodiments are directed to backflush techniques that can be used in chromatography systems that include a microfluidic device to control fluid flow to one or more other components in the system.

BACKGROUND

[0004] Separations of complex samples can be difficult with existing chromatography systems. In particular, samples having peaks that elute closely can be difficult to separate. In addition, there may also be a need for backflushing, heartcutting, column switching and detector switching.

SUMMARY

[0005] In an aspect, a method of modulating flow of a fluid in a chromatography system including a microfluidic device fluidically coupled to an injector is described. In certain examples, the method comprises controlling fluid pressure of the system to permit a sample to flow from the injector and into the microfluidic device and to a first fluid flow path and a second fluid flow path each fluidically coupled to the microfluidic device at a separate outlet port of the microfluidic device, and altering the pressure of the system to reverse the flow of the sample in at least one of the first

fluid flow path and the second fluid flow path to backflush sample in the fluid flow path where the pressure is reversed.

[0006] In certain embodiments, the method can include configuring each of the first fluid flow path and the second fluid with a chromatography column. In other embodiments, the method can include altering the pressure of the system to reverse flow of the sample after a portion of the sample has eluted from the chromatography column in the first fluid flow path to reverse the sample flow. In additional embodiments, the method can include controlling the pressure of the system to direct the reversed sample flow to the chromatography column in the second fluid flow path. In further embodiments, the method can include altering the pressure of the system to reverse flow of the sample after a portion of the sample has eluted from the chromatography column in the first fluid flow path and been detected by a detector fluidically coupled to the chromatography column in the first fluid flow path to reverse the sample flow. In some examples, the method can include controlling the pressure of the system to direct the reversed sample flow to the chromatography column in the second fluid flow path. In additional examples, the method can include configuring the microfluidic device as a splitter, a two-way switching device or a three-way switching device. In further examples, the method can include configuring the microfluidic device with an inlet port and two outlet ports each fluidically coupled to the inlet port. In some examples, the microfluidic device can be a splitting device, a two-way switched device, a three-way switched device or other suitable microfluidic device.

[0007] In another aspect, a system comprising a microfluidic device comprising an input port and at least two outlet ports each fluidically coupled to the inlet port, a first fluid flow path fluidically coupled to one outlet port of the microfluidic device, a second fluid flow path fluidically coupled to the other outlet port of the microfluidic device, a first restrictor in the first fluid flow path, the first restrictor configured to be fluidically coupled to a first pressure source, and a second restrictor in the second fluid flow path, the second restrictor configured to be fluidically coupled to a second pressure source is disclosed.

[0008] In certain embodiments, the system can include a microfluidic device configured to be fluidically coupled to a pressure source through an additional port of the microfluidic device, in which the additional port is fluidically coupled to the inlet port and the at least two outlet ports of the microfluidic device. In some embodiments,

the system can include pressure sources configured to control flow of sample in the system. In other embodiments, the second pressure source can be configured with a higher pressure to direct sample from the microfluidic device to the first fluid flow path. In certain examples, the first pressure source can be configured with a higher pressure to direct sample from the microfluidic device to the second fluid flow path. In some examples, the first and second pressure sources can be operative in a first condition to direct sample from the microfluidic device to both the first and second fluid flow paths and can be operative in a second condition to direct sample to only one of the first and second fluid flow paths. In some examples, the system can be configured to permit sample flow during the first condition followed by sample flow during the second condition after a single injection of sample into the system. In certain examples, the microfluidic device can be a splitting device, a two-way switched device, a three-way switched device or other suitable microfluidic device.

[0009] In an additional aspect, a method of backflushing column effluent in a chromatography system including two different fluid flow paths each fluidically coupled to a microfluidic device is provided. In certain examples, the method comprises altering the pressure in one of the fluid flow paths to backflush column effluent in that fluid flow path to the other fluid flow path.

[0010] In certain embodiments, the method can include performing the backflushing step before all column effluent is detected. In other embodiments, the method can include performing the backflushing step after at least some column effluent is detected. In some examples, the microfluidic device can be a splitting device, a two-way switched device, a three-way switched device or other suitable microfluidic device.

[0011] In another aspect, a method of backflushing in a chromatography system including two different fluid flow paths each fluidically coupled to a microfluidic device, the method comprising altering the pressure in one of the fluid flow paths to reverse sample flow in that fluid flow path and direct the reversed sample flow to the other fluid flow path is provided.

[0012] In certain examples, the method can include performing the backflushing step after a portion of the sample in one of the fluid flow paths is detected. In other examples, the method can include performing the backflushing step by increasing the pressure in a first fluid flow path to reverse the sample flow and direct the sample to the second fluid flow path. In some examples, the microfluidic device can be a

splitting device, a two-way switched device, a three-way switched device or other suitable microfluidic device.

[0013] In an additional aspect, a method of backflushing in a chromatography system including two different fluid flow paths each fluidically coupled to a microfluidic device is provided. In certain examples, the method comprises selecting pressures in the system to permit sample flow into a first fluid flow path fluidically coupled to the microfluidic device, the first fluid flow path comprising a first chromatography column fluidically coupled to the microfluidic device and a first restrictor fluidically coupled to the first chromatography column, and adjusting pressures in the system to reverse the sample flow in the first fluid flow path and direct the reversed sample flow into a second fluid flow path fluidically coupled to the microfluidic device, the second fluid flow path comprising a second chromatography column fluidically coupled to the microfluidic device and a second restrictor fluidically coupled to the second chromatography column is provided.

[0014] In certain embodiments, the method can include adjusting the pressures in the system to reverse the sample flow after a portion of the sample has eluted from the first chromatography column and been detected with a detector fluidically coupled to the first restrictor. In other embodiments, the method can include adjusting the pressure in the system to reverse the reversed sample flow in the second fluid flow path to direct the twice-reversed flow into the first fluid flow path. In some examples, the microfluidic device can be a splitting device, a two-way switched device, a three-way switched device or other suitable microfluidic device.

[0014a] In an additional aspect, there is provided a method of modulating flow of a fluid in a chromatography system including a microfluidic device comprising a laminated wafer body fluidically coupled to an injector, the method comprising: controlling fluid pressure of the system to permit a sample to flow from the injector and into the laminated wafer body of the microfluidic device and to a first fluid flow path and a second fluid flow path each fluidically coupled to the laminated wafer body of the microfluidic device at a separate outlet port of the laminated wafer body of the microfluidic device; and altering the pressure of the system to

reverse the flow of the sample in at least one of the first fluid flow path and the second fluid flow path to backflush sample in the fluid flow path where the pressure is reversed.

[0014b] In an additional aspect, there is provided a system comprising: a microfluidic device comprising a laminated wafer body comprising an input port and at least two outlet ports each fluidically coupled to the inlet port; a first fluid flow path fluidically coupled to one outlet port of the laminated wafer body of the microfluidic device; a second fluid flow path fluidically coupled to the other outlet port of laminated wafer body of the microfluidic device; a first restrictor in the first fluid flow path, the first restrictor configured to be fluidically coupled to a first pressure source; and a second restrictor in the second fluid flow path, the second restrictor configured to be fluidically coupled to a second pressure source.

[0014c] In an additional aspect, there is provided a method of backflushing column effluent in a chromatography system including two different fluid flow paths each fluidically coupled to a laminated wafer body microfluidic device, the method comprising altering pressure in the laminated wafer body microfluidic device to alter pressure in one of the fluid flow paths to backflush column effluent in that fluid flow path to the other fluid flow path.

[0014d] In an additional aspect, there is provided a method of backflushing in a chromatography system including two different fluid flow paths each fluidically coupled to a laminated wafer body microfluidic device, the method comprising: selecting pressures in the system to permit sample flow into a first fluid flow path fluidically coupled to the laminated wafer body microfluidic device, the first fluid flow path comprising a first chromatography column fluidically coupled to the laminated wafer microfluidic device and a first restrictor fluidically coupled to the first chromatography column; and adjusting pressures in the system to reverse the sample flow in the first fluid flow path and direct the reversed sample flow into a second fluid flow path fluidically coupled to the laminated wafer body microfluidic device, the second fluid flow path comprising a second chromatography column fluidically coupled to the microfluidic device and a second restrictor fluidically coupled to the second chromatography column.

[0015] Additional features, aspects, examples and embodiments are described in more detail below.

BRIEF DESCRIPTION OF THE FIGURES

[0016] Certain illustrative embodiments are described in detail below with reference to the accompanying figures in which:

[0017] FIGS. 1A and 1B are schematics used to describe the general principles of operation of a microfluidic device, in accordance with certain examples;

[0018] FIG. 2 is an illustration of a chromatography system having a midpoint union, in accordance with certain examples;

[0019] FIGS. 3A-3C show a user interface that can be used in a chromatography system to implement flow control, in accordance with certain examples;

[0020] FIGS. 4A-4E are diagrams showing fluid flow in the system under various conditions, in accordance with certain examples;

[0021] FIG. 5 is a chromatography system that includes a pneumatic controller, in accordance with certain examples;

[0022] FIG. 6 is a chromatography system that includes a switching valve and two detectors, in accordance with certain examples;

[0023] FIG. 7 is a chromatography system that includes two detectors and a splitter, in accordance with certain examples;

[0024] FIG. 8A is graph showing the change in flow rate as a function of the error in column internal diameter, in accordance with certain examples;

[0025] FIG. 8B is a graph showing the change in flow rate as a function of the error in column length, in accordance with certain examples;

[0026] FIGS. 9A and 9B are illustrations of chromatography systems including a microfluidic device and a switching valve, in accordance with certain examples;

[0027] FIG. 10 is an illustration of a chromatography system including three detectors, in accordance with certain examples;

[0028] FIG. 11 is a cross-section of a microfluidic device showing the internal microchannel, in accordance with certain examples;

[0029] FIG. 12 is a cross-section of a microfluidic device including four outlet ports, in accordance with certain examples;

[0030] FIG. 13 is a cross-section of a microfluidic device including an elongated portion in the microchannel, in accordance with certain examples;

[0031] FIGS. 14A and 14B are cross-section of a microfluidic device including two outlet ports and three outlet ports, respectively, each arranged in series with the other outlet ports, in accordance with certain examples;

[0032] FIGS. 15A and 15B show other illustration of microfluidic devices, in accordance with certain examples;

[0033] FIG. 16 is a schematic of a chromatography system that includes a single detector fluidically coupled to a microfluidic device, in accordance with certain examples;

[0034] FIG. 17 is a schematic of a chromatography system that includes two detectors each fluidically coupled to a microfluidic device, in accordance with certain examples;

[0035] FIG. 18 is a schematic of a chromatography system that includes three detectors each fluidically coupled to a microfluidic device, in accordance with certain examples;

[0036] FIG. 19 is a schematic of a chromatography system that includes four detectors each fluidically coupled to a microfluidic device, in accordance with certain examples;

[0037] FIG. 20 is a schematic of a chromatography system that includes a single detector and a sniffer port each fluidically coupled to a microfluidic device, in accordance with certain examples;

[0038] FIG. 21 is a schematic of a chromatography system that includes two detectors each fluidically coupled to a microfluidic device and where backflushing of a first column can be performed, in accordance with certain examples;

[0039] FIG. 22 is a schematic of a chromatography system that includes two columns and a single detector fluidically coupled to a microfluidic device, in accordance with certain examples;

[0040] FIG. 23 is a schematic of a chromatography system that includes two columns and two detectors each fluidically coupled to a microfluidic device, in accordance with certain examples;

[0041] FIG. 24 is a schematic of a chromatography system that includes three columns and two detectors each fluidically coupled to a microfluidic device, in accordance with certain examples;

[0042] FIG. 25 is a schematic of a chromatography system that includes two detectors each fluidically coupled to a microfluidic device through a column, in accordance with certain examples;

[0043] FIGS. 26A and 26B are illustrations of a microfluidic device that includes a microchannel where all the ports are arranged in series, in accordance with certain examples;

[0044] FIG. 27 is a photograph showing a microfluidic device and two plates to hold the microfluidic device, in accordance with certain examples;

[0045] FIG. 28 is a cross-section of a microfluidic device showing an internal bypass restrictor, in accordance with certain examples;

[0046] FIG. 29 is a schematic of a chromatography system that includes two columns and two detectors each fluidically coupled to a first and a second microfluidic device, in accordance with certain examples;

[0047] FIGS. 30A and 30B are schematics of a chromatography system with a microfluidic device that can provide for crossover flow, in accordance with certain examples;

[0048] FIG. 31 is a schematic of another chromatography system with a microfluidic device that can provide for crossover flow, in accordance with certain examples;

[0049] FIGS. 32A-32D are cross-section sections showing the various layers of the microfluidic device, in accordance with certain examples;

[0050] FIGS. 33A and 33B show a controller that can be used to actuate a switching valve, in accordance with certain examples;

[0051] FIG. 34 is a graph showing the results of a single peak that has been modulated, in accordance with certain examples;

[0052] FIGS. 35A-35C show traces of samples from two different columns (FIGS. 35A and 35B) and modulation of those sample peaks (FIG. 35C), in accordance with certain examples;

[0053] FIG. 36 and 37 are schematics of systems that can be used to perform simultaneous analysis of two chromatograms, in accordance with certain examples;

[0054] FIGS. 38 and 39 are schematics of systems configured for multidimensional separations, multiplexed chromatography or multiplexed detection, in accordance with certain examples;

[0055] FIG. 40 is a cross-section of a microfluidic device that includes a first charging chamber and a second charging chamber, in accordance with certain examples;

[0056] FIG. 41 shows a conventional chromatograph and FIG. 42 shows the prophetic results using modulation and a charging chamber, in accordance with certain examples;

[0057] FIGS 43A and 43B show flow of fluid through a charging chamber using a 2-way switching valve, in accordance with certain examples;

[0058] FIGS. 44A and 44B show flow of fluid through a charging chamber using a 3-way switching valve, in accordance with certain examples;

[0059] FIGS. 45A and 45B show flow of fluid through a first and second charging chamber using a 3-way switching valve, in accordance with certain examples;

[0060] FIG. 46 shows a microfluidic device that includes an enlarged microchannel portion in accordance with certain examples;

[0061] FIG. 47 shows a microfluidic device that includes a microchannel having restrictions therein, in accordance with certain examples;

[0062] FIGS. 48-54 shows illustrations of chromatography systems that can be used, for example, peak splitting, in accordance with certain examples;

[0063] FIG. 55 is a photograph showing the diameter of tubing, in accordance with certain examples;

[0064] FIG. 56 is a graph showing the results of flow rate measurements using the flow control algorithms described herein and using pressure control, in accordance with certain examples;

[0065] FIG. 57 is schematic showing a microfluidic device that includes an internal bypass restrictor, in accordance with certain examples;

[0066] FIG. 58 is an illustration of a three-way switched microfluidic device, in accordance with certain examples;

[0067] FIG. 59 is an illustration of one layer of a laminated three-way switched microfluidic device, in accordance with certain examples;

[0068] FIG. 60 is an illustration of another layer of a laminated three-way switched microfluidic device, in accordance with certain examples;

[0069] FIG. 61 is an illustration of two 3-way switching devices in a three-way switching system, in accordance with certain examples;

[0070] FIG. 62 shows the active switched ports when the switching devices are in different positions, in accordance with certain examples;

[0071] FIG. 63 shows the fluid flow in a system where both switching devices are in a first position (an off position), in accordance with certain examples;

[0072] FIG. 64 shows the fluid flow in a system where the first switching device is in a second position (an on position) and the second switching device is in the first position (an off position), in accordance with certain examples;

[0073] FIG. 65 shows the fluid flow in a system where the first switching device is in the first position (an off position) and the second switching device is in the second position (an on position), in accordance with certain examples;

[0074] FIG. 66 shows the fluid flow in a system where both switching devices are in the second position (an on position), in accordance with certain examples;

[0075] FIG. 67 is a schematic of a system suitable for implementing backflushing techniques as described herein, in accordance with certain examples;

[0076] FIG. 68 is a schematic of a restrictor, in accordance with certain examples;

[0077] FIG. 69 is a schematic of another system suitable for implementing backflushing techniques as described herein, in accordance with certain examples;

[0078] FIG. 70 is a photograph showing two bleed regulators for the PreVent PPC modules attached to a bracket and mounted under the GC top cover, in accordance with certain examples;

[0079] FIG. 71 is a photograph showing the installed system in a GC oven, in accordance with certain examples;

[0080] FIG. 72 is an illustration with instrument parameters where sample is introduced into both columns, in accordance with certain examples;

[0081] FIG. 73A and 73B are chromatograms obtained using the instrument parameters listed in FIG. 72 and an ASTM D-4815 calibration mixture, in accordance with certain examples;

[0082] FIG. 74 is an illustration with instrument parameters where sample is directed to a TCEP column, in accordance with certain examples;

[0083] FIG. 75A and 75B are chromatograms obtained using the instrument parameters listed in FIG. 74 and an ASTM D-4815 calibration mixture, in accordance with certain examples;

[0084] FIG. 76 is an illustration with instrument parameters where sample is directed to a methyl silicone column, in accordance with certain examples;

[0085] FIG. 77A and 77B are chromatograms obtained using the instrument parameters listed in FIG. 76 and an ASTM D-4815 calibration mixture, in accordance with certain examples;

[0086] FIG. 78 is an illustration with instrument parameters where sample is backflushed from a TCEP column into a methyl silicone column, in accordance with certain examples;

[0087] FIGS. 79A and 79B are chromatograms from the TCEP column in FIG.. 78 without and with backflushing, respectively, in accordance with certain examples;

[0088] FIG. 80 is a chromatogram of the methyl silicone column in FIG. 78, in accordance with certain examples;

[0089] FIG. 81 is an illustration with instrument parameters where sample is backflushed from a methyl silicone column to a vent, in accordance with certain examples; and

[0090] FIG. 82 is a chromatogram up to the backflush point used in the system of FIG. 81, in accordance with certain examples.

[0091] It will be understood by the person of ordinary skill in the art, given the benefit of this disclosure, that the exact size and arrangement of the various components shown in the figures can be altered, e.g., enlarged, stretched, reduced, rearranged or otherwise configured differently to provide a desired result or a desired mode of operation. In addition, the particular placement of one component as "upstream" or "downstream" relative to another component may also be altered depending on the desired results or desired methods to be performed using the technology described herein. Unless otherwise noted, fluid flow, e.g., gas flow, is intended to occur generally from left to right in the figures, though other flow directions are possible depending on the exact configuration and pressures used, as described in more detail herein. Where possible arrows may be used in certain instances to show the general direction of fluid flow.

DETAILED DESCRIPTION

[0092] The following description is intended to demonstrate some of the useful, novel and non-obvious subject matter provided by the technology described herein. Such description is not intended to be limiting but rather illustrative of the many configurations, embodiments and uses of the chromatography systems described herein and the components and uses thereof. The exact shape, size and other dimensions of the components shown in the figures can vary depending on the intended use of the device, the desired form factor and other factors that will be selected by the person of ordinary skill in the art, given the benefit of this disclosure.

[0093] In certain embodiments, the devices, methods and systems described herein can be used in fluid chromatography systems. Fluid chromatography systems are intended to include, but not be limited to, gas chromatography systems, liquid chromatography (LC) systems, supercritical fluid (SCF) chromatography systems and combinations of these illustrative fluid chromatography systems. Certain specific examples are described below with particular reference to gas chromatography (GC) systems, but similar principles and configurations may be used with fluid chromatography systems other than GC systems.

[0094] In the systems disclosed herein and illustrated in the figures, the general term "detector" is often used. The detector may be any commonly used GC, LC or SCF detector including, but not limited to, a flame ionization detector (FID), a flame photometric detector (FPD), a thermal conductivity detector (TCD), a thermionic

detector (TID), an electron-capture detector (ECD), an atomic emission detector (AED), a photoionization detector (PI), an electrochemical detector, a fluorescence detector, a UV/Visible detector, an infrared detector, a nuclear magnetic resonance detector or other detectors commonly used with GC, LC or SCF. In addition, the detector may be a mass spectrometer, an external detector such as, for example, a discharge ionization detector (DID) or a sulfur chemiluminescence detector (SCD) or other suitable detectors and devices that can be hyphenated to a gas chromatography device or other fluid chromatography devices, e.g., those using capillary columns. In some examples, two or more detectors can be present.

[0095] In certain embodiments, the terms "microfluidic device" or "switch" are used interchangeably herein. Microfluidic devices are described in many different instances and are typically configured to provide fluid flow from at least one inlet port to one or more outlet ports. In some examples, the microfluidic device can be configured to split an inlet flow into two or more outlet flows without any other substantial operation being performed by the microfluidic device. In other instances, the microfluidic device may be configured to provide fluid flow to two or more devices that can be fluidically coupled to outlet ports of the microfluidic device. The microfluidic device can take many different forms such as, for example, a laminated wafer including a plurality of layers that when assembled provide one or more internal microfluidic channels. In other examples, the microfluidic device may be produced by coupling a desired number and amount of tubing, e.g., capillary tubing or other types of tubing, to provide a microfluidic device effective to perform in a desired manner. "Fluidically coupled" is used herein to refer to the case where fluid can flow between two or more components. Fluid flow can be permitted between the components by, for example, switching or opening a valve between the components, whereas fluid flow can be restricted between the components, for example, by switching or closing the valve. Where two or more components are fluidically coupled, fluid is not necessarily flowing between them at all times. Instead, depending on the other components of the system and their operational state, fluid can flow between the two fluidically coupled components under certain configurations and arrangements. In the case of a switching valve positioned between two components, for example, the two components can remain fluidically coupled when the valve is in the closed position even though no fluid is flowing between the components.

[0096] In certain instances a microfluidic device may be referred to as a "splitter," "splitting device, a "two-way" switched device or a "three-way" switched device. Such terminology is for convenience purposes only and is not intended to limit a splitter as only providing splitting, a two-way switched device to only being able to provide two-way switching or a three-way switched device to only being able to provide three-way switching. Microfluidic devices can be daisy chained to each other to provide for increased ways of switching. In addition, the exact number of switching valves in a particular system may be increased or decreased to provide a desired amount of switching.

[0097] In certain embodiments, the flow control algorithms and methods described herein are applicable to restrictors, columns, transfer lines or other tubing such as, for example, capillary tubing. For example, the diameters and lengths of the restrictors, columns, transfer lines, etc. can be determined using the algorithms, and the description provided herein that is directed to a particular device, e.g., a restrictor, may be applied to a different device, e.g., a column, of the system.

[0098] In certain examples, the components described herein can be connected to each other through tubing, fittings, ferrules or other devices that can provide for substantially fluid tight seals and can provide a fluid flow path between two or more selected components. The lengths, diameters and other parameters for such additional components can be determined based on experimentation or using the length and diameter calculations described herein.

[0099] In certain examples, the devices and system described herein can be used in many different types of chromatography systems. In some embodiments, it may be desirable to configure the devices for use in either heartcut or solvent dump systems. In heartcut systems, selected species or peaks in the sample may be sent to two or more different columns or detectors. Heartcut systems may be particularly advantageous where poor resolution of two peaks is achieved. Those peaks can be sent to a different column having a different separation media or mechanism. For example, a first conventional column of 30 meters length with an internal diameter of 0.25 or 0.32 mm can be used to provide a first separation stage. A selected portion of the column effluent can then be passed to a second column having a different stationary phase, length, internal diameter or other characteristics that can be used to separate components in that portion of the first column effluent. In a solvent dump system, the amount of solvent sent to a detector may be reduced. For example, it may

be desirable to reduce the solvent volume sent to a detector such as a mass spectrometer. A crude separation can first be performed on a first column, e.g., a large internal diameter, low resolution column. Only the components of interest can be sent to a second column, which can be a higher resolution column. To account for differences in pressure, one or more restrictors may be used in the system. For example, there is little pressure differential across a large internal diameter column, and pressures needed to direct the reverse flow across an orifice can cause a large reduction in the flow through the first column. To reduce this effect, a restrictor can be used to increase the overall pressure in the system. Use of restrictors and their effects on pressure are described in more detail herein.

[00100] In certain examples, the devices, systems and methods described herein can include a microfluidic device. The microfluidic device can be configured to split flow from a column, to switch the flow between two or more outlet ports or to provide fluid flow to other ports or in other directions. Certain specific configurations of a microfluidic device are described below. These configurations are merely illustrative and other suitable configurations are possible. In certain embodiments, the microfluidic devices described herein are operative to direct gas flow using differential pressures from external gas supplies or pressure regulators. These differential pressures can be used to change the direction of gas flow eluting from a chromatography column between two or more outlet ports. Such operation can have advantages over traditional mechanical-based valve systems including, for example, input and output flow rates are undisturbed resulting in no or little alteration of retention times, the devices can be fabricated from low thermal mass components to avoid or reduce the likelihood of cold spots, there are no moving parts (or few moving parts where one or more valves are present), the internal volumes of the channels in the switch can be minimal to reduce peak dispersion and adsorption effect, the response time is very fast allowing narrow cuts to be switched between outputs which permits it use with modern capillary columns, and internal surfaces can be generally inert and/or deactivated to enable use with labile analytes. Other advantages are also possible depending on the exact configuration of the system.

[00101] In certain examples, the flow rate control described herein may be used by itself or in combination with one or more microfluidic devices. For example, a microfluidic device can be configured as a heartcutting accessory or module that includes one or more microchannels. In other configurations, the microfluidic device

can be configured to split effluent from a column between multiple detectors. Other configurations of a microfluidic device will be recognized by the person of ordinary skill in the art, and certain illustrative configurations are described herein.

[00102] In certain embodiments, the devices described herein can be used to provide blends of fluids, e.g., gas or liquid blends, that can be used in chromatographic separations or can be used, for example to study gas phase reaction kinetics. For example, two or more different gases can be provided in desired amounts using the flow control algorithms described herein. The gases can be mixed in a microfluidic device (or other device). For example, a first gas can be introduced into a first port of a microfluidic device and a second gas can be introduced into a second port of the microfluidic device. The gases can be mixed, e.g., using an internal buffer, charging chamber or other desired internal channel, and outputted to a reaction chamber, detector or other suitable device. It will be within the ability of the person of ordinary skill in the art, given the benefit of this disclosure, to use the microfluidic devices described herein for these and other uses.

[00103] Pressure balanced systems were pioneered by Dr. David Deans of ICI Chemicals in the late 1950s and remain a popular choice in several important GC applications. For example, the PreVent, Protect, MS Vent and Ozone Precursor systems commercially available from PerkinElmer (Waltham, MA) all utilize this technique. These techniques are sufficiently powerful that, in many instances, there is no other way of performing a particular analysis or their use makes significant improvements with respect to throughput or quality of the results.

[00104] With existing pressure balance systems, there are some drawbacks. It is not possible to directly or explicitly control the flow rate of carrier gas through the column. Many users prefer to specify the flow rate rather than the inlet pressure for carrier gas control through the column. In most instances, this gives more consistent chromatographic performance. It is also not feasible to control the flow rate of carrier gas into the detector. The response of most GC detectors is highly sensitive to gas flow rate and so users would prefer to use flow control to minimize baseline drift and provide consistent analyte response. The applied carrier gas pressures can also be very difficult to set up and requires a substantial amount of understanding on the part of the user. Certain embodiments described herein can permit control of the flow rate through a column by controlling or specifying the carrier gas flow rate through a column.

[00105] To facilitate a better understanding of the microfluidic devices described herein, a generalized operation principle of a microfluidic device is described in reference to FIGS. 1A and 1B. Referring to FIG. 1A, effluent from a column enters a T-shaped piece at a site or point 52 in the direction of an arrow 50. A switching valve 65, e.g., a solenoid valve, MEMS device or other suitable devices, can be switched to a first position or modulated open to at least some degree to permit carrier gas from the gas source 67 to flow into the T-shaped piece at point 54. The gas pressure provided by the source 67 is at a slightly higher pressure than the gas pressure at point 52. The pressure at point 54 is slightly higher than at points 52 and 56 such that carrier gas will flow from point 54 towards points 52 and 56 and push or direct effluent from the column toward point 56. The effluent will exit the T-shaped piece at port 58 in a direction as shown by an arrow 57 in FIG. 1A. In addition, carrier gas with substantially no effluent from the column will exit the T-shaped piece at port 60. A needle valve 62 at the center of the device is operative to maintain a trickle flow of carrier gas through the unswept gas line so that sample does not diffuse into those areas from point 56.

[00106] In certain examples, the switching valve 65 can be switched to a second position such that gas flow in the T-shaped piece is altered or reversed. Referring to FIG. 1B, the switching valve 65 is actuated such that gas flow from the pressure source 67 to the point 56 such that pressure at the point 56 is higher than at points 52 and 54. The effluent from the column will exit the T-shaped piece at port 60 in a direction as shown by an arrow 59 in FIG. 1B. In addition, carrier gas with substantially no effluent from the column will exit the T-shaped piece at port 58. The system shown in FIGS. 1A and 1B is designed to operate when the pressure at points 58 and 60 are substantially the same, e.g., when the pressures are balanced at these points. As described in detail below, the microfluidic devices disclosed herein can be used in such pressure balanced systems to direct the flow of species eluting from a column to a desired port fluidically coupled to a detector, vent, column or other component.

[00107] In certain examples where a microfluidic device includes a switching valve, the switching valve may be operative to connect (or disconnect) two or more fluid flow paths such that fluid can flow between the flow paths when connected and fluid flow is restricted when the flow paths are disconnected. Illustrative switching valves include, but are not limited to, a valve such as, for example, a flow control valve, a

solenoid valve or a photovac valve, MEMS devices, metal laminated constructs with a laminated membrane operative to open and close a channel underneath it, electromechanical valves, pneumatically operated membrane valves, motor operated needle valves and other suitable devices that can restrict flow in one state and permit flow in another state. In certain examples, the switching valve can be integrated into the microfluidic devices disclosed herein, whereas in other examples, the switching valve may be separate from the microfluidic device. For example, where the microfluidic device is placed in an oven, the switching valve can be placed external to the oven and coupled to the microfluidic device through suitable supply lines and/or tubing. Such external placement can be particularly desirable where the high oven temperatures can adversely affect performance of the switching valve. In some examples, the switching valve can be surface mounted to an external surface of the oven so that the length of any tubing between the switching valve and the microfluidic device can be reduced.

[00108] In certain embodiments, the microfluidic devices described herein may include, or be configured as, a wafer, a laminate or other suitably configured device that can provide one or more fluid flow paths from an inlet to two or more potential outlets. The device can be configured to provide flow control of species within a column, detector or other portions fluidically coupled to the device. For example, the microfluidic device may be configured with one or more microchannels to provide for switching or selective flow of gas within a system. Illustrative such systems and devices are described in more detail below. Such microfluidic devices can also permit the control of carrier gas flow through a separation column to simplify the overall setup and use of an instrument by an end-user. These and other features and configurations are described by way of illustration using gas chromatography systems and reference to certain specific embodiments. The laminate may include two, three, four, five, six or more distinct layers with each layer being recognizable as a result of a boundary being present between the layers. The laminate can be subjected to post-lamination processes such as heating, annealing, sintering or the like depending on the exact materials used to produce the laminate.

[00109] In a typical capillary column setup, gas flows from the injector in other ways than just out into the column itself. These pathways include, but are not limited to, splitters, septum purge and an occasional minor leak. Because of these other pathways, regulating the rate of carrier gas flow into the injector does not normally

control the actual rate of flow through the column itself. To circumvent this difficulty, most GCs actually control the carrier gas pressure and not explicitly the flow rate. The pressure is applied to deliver the set flow rate according to the Hagen-Poiseuille relationship shown in Equation 1

$$F_o = \frac{\pi \times d_c^4 \times (p_i^2 - p_0^2)}{256 \times L \times \eta \times p_o} \quad (1)$$

where F_o is the flow rate at the outlet, d_c is the internal diameter of the column, L is the column length, η is the viscosity of the carrier gas at the set temperature, p_i is the gas pressure at the inlet and p_o is the gas pressure at the outlet. Using the above equation, a user can enter into the user interface the details of the column geometry (d and L), the carrier gas type (to allow the viscosity to be calculated correctly) and the column outlet pressure (p_o — normally set to ambient pressure or vacuum, for MS systems). The GC system will have knowledge of the column temperature (to enable the viscosity to be calculated) and so it can calculate the inlet pressure (p_i) needed to deliver a required flow rate.

[00110] Once the system is set up and running, the only potential variable is the gas viscosity which changes if the column temperature is increased during an oven temperature program. Using Equation 1, the system can adjust the inlet pressure, p_i, to maintain the set carrier gas flow rate. While this approach has been widely adopted for carrier gas flow rate control in many successful GC designs, it is not entirely accurate for use in pressure balanced systems and so an alternative approach to carrier gas control is desirable.

[00111] In certain examples, a typical pressure balanced system is shown in FIG. 2 such as, for example, a system configured as a single column backflush configuration or a heartcut configuration. The system 100 includes an injector 110 that can provide a split flow in direction 115. A column 120 is fluidically coupled to the injector 110 to receive a carrier gas, from a gas source 105, and any sample that may have been introduced into the system through the injector 110. A pressure balanced system has at least two active components through which a gas is flowing, the column 120 and a restrictor 130.

[00112] The flow rate through the column 120 is generally a function of its inlet pressure at the injector (p_1) and its outlet pressure at the midpoint pressure (p_2) at a midpoint union 125, whereas the flow rate through the restrictor 130 into the detector

135 is controlled by its inlet pressure at the midpoint p_2 and its outlet pressure at the detector (p_o). These two flow rates are not necessarily the same (in fact in most applications, they are desirably different) and may be independently controlled by varying combinations of the pressures p_1 and p_2 using independent gas sources 105 and 122.

[00113] The flow rates of carrier gas through the column 120 and the restrictor 130 may each still be calculated using Equation 1 — they just have differing inlet and outlet pressures. To provide carrier gas flow control within just the column 120, the pressure at the midpoint as the exit pressure can be used.

[00114] In certain examples and referring to FIG. 3A, a graphical user interface screen 310 of a Clarus GC is shown. Currently, the column exit pressure can only be set to ambient (implied if vacuum not selected) or vacuum (for MS, for example). To enable flow control of the column when fitted to a pressure balance system, it would be desirable for the user to have the ability to enter the outlet pressure, for example, as shown in screen 320 in FIG. 3B or in screen 330 in FIG. 3C. Such modification permits a user to explicitly control the flow rate through a GC column in a pressure balanced system to provide a constant gas flow through a column during temperature programmed chromatography.

[00115] In certain examples, flow control through both a column and a restrictor may be performed using the devices and methods described herein. To control the flow through the restrictor, its dimensions and its outlet pressure (ambient or vacuum) should be known or measurable. The remainder of the information will be the same as that for the column. The flow rate can be controlled by setting the restrictor inlet pressure (e.g., the midpoint pressure) according to Equation 1. In some examples, a PPC pressure module such as, for example, those used in a PerkinElmer PreVent system can be used as a carrier supply (with flow rate control algorithms) rather than just a passive pressure regulator. Once the midpoint PPC module is configured, the injector pressure, e.g., the column inlet pressure, would be set to deliver the set flow rate using the PPC midpoint pressure setting for the outlet pressure in Equation 1. The whole process can automatically track an oven temperature program if the column outlet pressure is to be dynamically linked to the midpoint pressure as shown in FIG. 3C. This approach can provide independent control of the gas flows through both the column and the restrictor to provide constant gas flow through the column during temperature programmed chromatography and constant gas flow into the

detector. Independent and explicit control of the two gas flows also provides for a more user friendly setup and operation of a pressure balanced system.

[00116] In accordance with certain examples, to consider some of the improvements flow control can provide, a configuration for pressure balancing is shown in FIG. 4A. Adjustment of the midpoint pressure affects the respective flow rates in the column and restrictor in opposite ways. An increase in the midpoint pressure reduces the flow rate through the column but increases the flow rate through the restrictor. Reducing this pressure has an opposite effect on both flows. Referring to FIG. 4A, a pressure balancing system 400 is shown which includes an injector 410 that has a split flow at point 415, a column 420 fluidically coupled to the injector 410, a midpoint union 425 in a flow path to a restrictor 430, which itself is fluidically coupled to a detector 435. With the midpoint pressure set very low such that p_2 is less than p_1, the flow rate F_c through the column will be higher than the flow rate F_t through the restrictor and so flow of gas will not occur from the midpoint union 425 as shown in FIG. 4A. Instead, gas flowing from the column 420 will flow up the midpoint supply line 422 at a flow rate of F_m causing loss of sample and potential contamination of the pneumatics system.

[00117] In certain examples and referring to FIG. 4B, where the flow rates through the column F_c and the restrictor F_t are the same, then there is substantially no flow to or from the midpoint union 425, e.g., F_m is about zero. Under the situation shown schematically in FIG. 4B, the mid-point pressure is referred to as the natural mid-point pressure and the system can be considered as pressure balanced. This pressure balancing state can serve as a baseline for the pressure settings.

[00118] In certain embodiments and referring to FIG. 4C, where the flow rate F_c through the column is less than the flow rate F_t through the restrictor, for example, by increasing the flow rate F_m at the midpoint union 425, gas will flow through the midpoint regulator 421 into the midpoint union 425 and mix with the column effluent going into the restrictor 430 and the detector 435. As the midpoint pressure is progressively increased, the flow rate of gas from the column will steadily decrease until a point is reached where the midpoint pressure is the same as the injector pressure (see FIG. 4D). The flow rate F_c through the column will become zero and any chromatography would stop. Under these conditions, gas flow into the detector 435 is still being maintained solely by the midpoint regulator 421.

[00119] In certain examples and referring to FIG. 4E, if the midpoint pressure is raised above that of the injector such that p_2 is greater than p_1, then the flow of gas F_c through the column is reversed and may exit the system at the split point 415. This situation is not particularly advantageous for chromatographic separations, but it may be used for backflushing. For example, heavy samples or samples that are difficult to elute from the column can be driven from a column with backflushing after the species of interest have eluted from the column.

[00120] In accordance with certain examples, the natural midpoint of the system can be advantageously used in the methods and configurations disclosed herein. As discussed herein, the natural midpoint represents the threshold between losing sample and diluting the midpoint and so its determination can increase the overall accuracy of the methods and devices described herein. To determine the natural midpoint, a system such as that shown in FIG. 5 can be used. The system 500 includes an injector 510 fluidically coupled to a column 520 and a carrier gas source 505. A midpoint union 525 is between the column 520 and a restrictor 530. The system 500 also includes a pressure transducer 540, a switching valve 550, and a proportional valve 555 each electrically coupled to a controller 545. During normal operation, the pressure transducer 540 can monitor the gas pressure of the gas as it flows in the system. The internal controller 545 uses this information to adjust the proportional valve 555 positioned upstream of the pressure transducer 540. In this way, closed-loop control can maintain the pressure at a set value.

[00121] To establish the natural midpoint pressure, the switching valve 550 is actuated so there is no flow into or out of the midpoint union 525 (assuming that there are no leaks). The flow rates through the column 520 and the restrictor 530 will now be substantially the same. As gas flows through the column 520 and out through the restrictor 530, the pressure at the midpoint will eventually reach a stable value - the natural midpoint pressure. The flow through the column 520 can be calculated using Equation 1 or estimated from tables. If the flow needs to be adjusted, the inlet pressure p_1 can be changed, the midpoint pressure is given time to stabilize and the calculation repeated until the desired flow rate is obtained.

[00122] Once the correct flow rate has been established and the corresponding natural midpoint pressure is known, then the switching valve 550 can be actuated to permit flow of gas and the midpoint pressure can be set to 1 or 2 psi above the natural midpoint pressure. This slight increase in the set pressure over the natural pressure

provides a positive flow of gas from the midpoint regulator to prevent sample from diffusing into the supply line 552. It also serves to maintain the pressure balance as the oven temperature changes. Setting up a pressure balanced system in the traditional way using differential pressure control is a long tedious process which tends to put many potential users off or cause difficulties in the setup and subsequent performance.

[00123] In certain embodiments, with explicit control of the flow rate in the column and the restrictor, system setup is greatly simplified and overall accuracy and precision can be increased. The system can be configured such that the flow rate through the restrictor is less than the flow rate through the column to ensure correct operation. The system user needs only to enter the respective flow rates in the analytical method and the differential flow control can preserve the correct balance between the column and the restrictor and provide a constant flow rate of gas through the column and into the detector. This approach can be used in the simple situation of single column backflushing as described earlier but also with heartcutting and splitting as shown in FIGS. 6 and 7. Referring to FIG. 6 where a heartcut configuration is shown, the system 600 includes an injector 610 fluidically coupled to a pressure regulator 605 through a supply line 607. The injector 610 is fluidically coupled to a column 620 through a supply line 617. The column 620 is fluidically coupled to a microfluidic device 630 through a supply line 622. The microfluidic device 630 is fluidically coupled to a midpoint pressure regulator 635 through a supply line 637. Detectors 650 and 660 are fluidically coupled to the microfluidic device 630 through restrictors 640 and 645, respectively. The restrictors 640 and 645 are desirably matched or substantially the same such that the flow rate F_{r1} through the restrictor 640 is substantially the same as the flow rate F_{r2} through the restrictor 650. Methods of determining restrictor lengths and diameters to provide a desired flow rate are described herein.

[00124] In certain examples and referring to FIG. 7, a pressure regulated splitter system is shown. The system 700 includes an injector 710 fluidically coupled to a pressure regulator 705 through a supply line 707. The injector 710 is fluidically coupled to a column 720 through a supply line 717. The column 720 is fluidically coupled to a midpoint pressure regulator 735 through supply lines 722 and 737 at a union or split 727. Detectors 750 and 760 are fluidically coupled to the midpoint pressure regulator 735, through the union or split 727, through resistors 740 and 745,

respectively. In operation of the system 700, the flow rate can be set for one of the restrictors 740 and 745, and the other restrictor can be displayed and maintained but not controlled independently of the other restrictor. The actual flow rate in both the column and the restrictor can be determined using Equation 1 for the calculations. The length of the column and its diameter may be inputted by the users, based on the column specifications provided by the column supplier. From Equation 1, the column flow rate has a fourth order dependence on column diameter. An error in diameter of the column can lead to a large error as shown prophetically in the graph of FIG. 8A. As shown in FIG. 8A, an error of just 2% in the internal column diameter, e.g., 5 microns on a 250 micron inner diameter column, is enough to cause an inaccuracy of almost 8% in the applied flow rate. With respect to column length, there is a reciprocal relationship between column length between flow and column length as shown in FIG. 8B. In this case, an error of 2% in column length, e.g., about a 60 centimeter error on a 30 meter column, will produce about a 2% error in the calculated flow rate. This error can lead, however, to additional errors in the flow rate assumptions.

[00125] In certain examples, one solution to any potential inaccuracy is to consider the provision of a geometric factor (GF) that can be applied to a particular column. The GF can be approximated using Equation 2.

$$GF = \frac{d_c^4}{L} \qquad (2)$$

[00126] The GF is constant for any given column and should be simple to establish by simple experiment (by either the supplier or the end user). Because this measurement is empirical, it will apply directly to a given column without making any assumptions about its geometry. Inserting the geometric factor in Equation 1 provides Equation 3.

$$F_o = \frac{\pi \times GF \times (p_i^2 - p_0^2)}{256 \times \eta \times p_o} \qquad (3)$$

To calculate the flow rates, the inlet (p,) and outlet (P_o) pressures and the temperature to calculate the viscosity must be entered or known. In a typical configuration, these parameters are known by the controller or entered by the user.

[00127] In accordance with certain examples, the situation with restrictor flow rate control is similar to that of the column. The restrictor is generally much shorter than the column and so it is much easier to measure its length. The internal diameter is normally much smaller and so small errors in its measurement will have a much greater impact on this flow rate of gas passing through it. The application of a GF for the restrictor is therefore just as desirable as it is for the column. One other aspect of the restrictor that can be considered is that part of it (or possibly most of it in the case of an MS) will reside inside the body of the detector. Thus, different sections of the restrictor will be at different temperatures and so Equations 1 and 3 may not be entirely accurate. This aspect can be addressed by using an approach taken to calculate flow rates through a serially connected transfer line and column for the TurboMatrix thermal desorption systems as given in Equation 4 and as described, for example, in commonly assigned U.S. Patent Nos. 7,219,532 and 7,468,095.

(4)
$$F_o = \frac{\pi \times Tr}{256 \times p_o} \times \frac{(p_i^2 - p_o^2)}{(\frac{T_c \times \eta_c}{GF_c}) + (\frac{T_r \times \eta_r}{GF_r})}$$

In equation (4), F_o is the flow rate at the restrictor outlet (at the temperature and pressure at that location), GF_o is the column geometric factor, GF_r is the restrictor geometric factor, d_o is the column internal diameter and L_o is the column length (for determining GF_o), d_r is the transfer line internal diameter and L, is the length of the transfer line (for determining GF_r), $_{lie}$ is the viscosity of the carrier gas in the column, fi_r is the viscosity of the carrier gas within the restrictor, T_o is the absolute temperature of the column, T_r is the absolute temperature of the transfer line, p_1 is the absolute pressure of the carrier gas at the column inlet and P_o is the absolute pressure of the carrier gas at the restrictor outlet. Equation 4 can be used in place of Equation 3 to provide a more accurate calculation of the flow rate.

[00128] In certain examples, the length of a restrictor of a selected internal diameter can be calculated based on the desired flow rate through a column of specified

geometry. Such dimensions can depend, at least in part, on the temperatures and gas pressures desired in the system. One configuration of a system with a restrictor is shown in FIG. 9A. The system of FIG. 9A includes a split injector 905 fluidically coupled to a first column 910 and a carrier gas source 902. A microfluidic device 920 is fluidically coupled to the first column 910 and is configured to direct species eluting from the first column 910 to a desired component. For example, column effluent can be directed to a first detector 930 through a restrictor 925, or column effluent can be directed to a second column 935 and onto a second detector 940 using the microfluidic device 920 and a switching valve 945.

[00129] In certain examples, the dimensions and geometry of the restrictor 925 can be selected to further balance the pressures in the system. A typical restrictor includes a piece of deactivated fused silica tubing of known internal diameter that can be cut to a length calculated to provide substantially the same flow rate of gas the through the column under a particular applied pressure and temperature. The length of the restrictor can be determined by trial and error, where the length of the restrictor is progressively shortened until the correct flow rate is achieved. However, this process is cumbersome and can take a substantial amount of time to determine the proper restrictor length. Incremental shortening of the restrictor may also not take into account the downstream effects of detector temperature on the flow rates through the column and the restrictor, which can have a significant effect on the actual flow rate to cause a pressure imbalance in the system. Where multiple detectors are present and used at different pressures, e.g., an FID (ambient pressure) and a MS detector (vacuum pressure), the pressure imbalancing may be even greater.

[00130] In certain embodiments, the restrictor geometry and length can be calculated to match or substantially match the gas flows in a selected column based on oven and detector temperature and detector operating pressure. Current calculations assume that the restrictor is of uniform length and temperature. The flow rate can be calculated according to equation (5)

$$F_a = \frac{\pi \times d_r^4 \times T_a \times (p_i^2 - p_{or}^2)}{256 \times L_r \times p_a \times \eta \times T_r} \quad (5)$$

where F_a is the restrictor outlet flow rate at ambient temperature and pressure, d_r is the internal diameter of the restrictor, T_a is the ambient absolute temperature, p_i is the carrier gas absolute pressure at the restrictor inlet, p_{or} is the carrier gas absolute pressure at the restrictor outlet, L_r is the length of the restrictor, p_a is the ambient absolute pressure, η is the viscosity of the carrier gas at the restrictor temperature, and T_r is the restrictor absolute temperature.

[00131] To determine the restrictor length to match a desired gas flow in a column, two simultaneous equations based on equation (5) can be used to solve for L_r, which provides equation (6)

(6)
$$L_r = L_c \times \frac{d_r^4}{d_c^4}$$

where d_c is the internal diameter of the column, and L_c is the length of the column. Equation 6 can be used, for example, where the temperature and the applied inlet and outlet pressures are the same between the column and the restrictor.

[00132] Where two or more detectors or a detector and a vent or any two devices operated at different pressure are present, Equation (5) can be used to obtain Equation (7)

(7)
$$L_r = L_c \times \frac{d_r^4 \times (p_i^2 - p_{or}^2)}{d_c^4 \times (p_i^2 - p_{oc}^2)}$$

where p_{oc} is the carrier gas absolute pressure at the column outlet.

[00133] In certain embodiments, to take into account the effect of detector temperature on the gas flow rates through both the column and the restrictor, the relationship shown in Equation (8a) can be used.

(8a)
$$F_a = \frac{\pi \times T_a}{256 \times p_a} \times \frac{(p_i^2 - p_o^2)}{(\frac{T_t \times \eta_t \times L_t}{d_t^4}) + (\frac{T_c \times \eta_c \times L_c}{d_c^4})}$$

In Equation (8a), F_a is the flow rate at the column outlet, d_c is the column internal diameter, d_t is the transfer line (or restrictor) internal diameter, L_c is the column length, L_t is the transfer line (or restrictor) length, η_c is the viscosity of the carrier gas within the column, η_t is the viscosity of the carrier gas within the transfer line (or restrictor), T_c is the absolute temperature of the column, T_t is the absolute temperature of the transfer line (or restrictor), T_a is the absolute ambient temperature, p_i is the absolute pressure of the carrier gas at the inlet, p_o is the absolute pressure of the carrier gas at the outlet and p_a is the absolute ambient pressure. Equation (8a) can be generalized for any number of serially connected columns or restrictors of differing internal diameter, length or temperature, as shown in Equation 8(b).

(8b) $$F_a = \frac{\pi \times T_a}{256 \times p_a} \times \frac{(p_i^2 - p_o^2)}{(\frac{T_1 \times \eta_1 \times L_1}{d_{c1}^4}) + (\frac{T_2 \times \eta_2 \times L_2}{d_{c2}^4}) + \ldots (\frac{T_n \times \eta_n \times L_n}{d_{cn}^4})}$$

The column and the restrictor of uniform diameter are in a GC oven and are at a different temperature than a detector. Equation (8a) can be modified for the restrictor and the column to provide Equations (9) and (10) for the restrictor and the column, respectively.

(9) $$F_a = \frac{\pi \times T_a \times d_r^4}{256 \times p_a} \times \frac{(p_i^2 - p_{or}^2)}{T_{r1} \times \eta_{r1} \times L_{r1} + T_{r2} \times \eta_{r2} \times L_{r2}}$$

In Equation (9), L_{r1} is the length of the restrictor inside the oven, L_{r2} is the length of the restrictor inside the detector, η_{r1} is the viscosity of carrier gas at the oven temperature, η_{r2} is the viscosity of carrier gas at the detector temperature, T_{r1} is the absolute temperature of the oven and T_{r2} is the absolute temperature of the detector.

(10) $$F_a = \frac{\pi \times T_a \times d_c^4}{256 \times p_a} \times \frac{(p_i^2 - p_{oc}^2)}{T_{c1} \times \eta_{c1} \times L_{c1} + T_{c2} \times \eta_{c2} \times L_{c2}}$$

In Equation (10), L_{c1} is the length of the restrictor inside the oven, L_{c2} is the length of the restrictor inside the detector, η_{c1} is the viscosity of carrier gas at the oven temperature, η_{c2} is the viscosity of carrier gas at the detector temperature, T_{c1} is the absolute temperature of the oven and T_{c2} is the absolute temperature of the detector.

[00134] In certain examples, Equation (10) can be used to calculate the pressure to apply to the column to deliver a required flow rate through the column by rearranging it as Equation (11).

$$p_i = \sqrt{\frac{F_a \times 256 \times p_a}{\pi \times T_a \times d_c^4} \times (T_{c1} \times \eta_{c1} \times L_{c1} + T_{c2} \times \eta_{c2} \times L_{c2}) - p_{oc}^2} \quad (11)$$

Once the inlet pressure is calculated for the column at a specific geometry, temperatures and outlet pressure, the length of the restrictor may be calculated using the rearranged form of Equation (9) as shown in Equation (12).

$$L_r = \frac{[\frac{\pi \times T_a \times d_r^4}{256 \times F_a \times p_a} \times (p_i^2 - p_{or}^2) - T_{r2} \times \eta_{r2} \times L_{r2}]}{T_{r1} \times \eta_{r1}} + L_{r2} \quad (12)$$

Using Equation (12), the length of a restrictor of known internal diameter that can provide a desired flow rate to balance the system can be calculated. In particular, the length of a restrictor of known internal diameter to balance the flow rate in another channel taking into account detector length, temperature and pressure of the other detector can be determined. In use, the algorithms can be implemented in software such that a user can enter a desired flow rate and the restrictor lengths and diameters to provide such desired flow rate, under specified column parameters and temperatures, can be displayed in a user interface to facilitate use of the system.

[00135] Certain embodiments described herein include the use of an additional column in the chromatography system. The additional column is used in place of a restrictor and is typically present when heartcutting is desired. Unlike fused silica restrictors, a user is unlikely to cut off pieces of a column to achieve a pressure balance across the microfluidic device. Even if the columns are selected to have the same length and diameter, temperature and pressures differences between two different detectors can disrupt the pressure balancing. When the columns are of a different geometry or length, the pressure imbalance can be even greater.

[00136] One possible solution when multiple columns are present is to use an inline restrictor with the column having the highest flow rate, as shown schematically in FIG. 9B. The system of FIG. 9B includes a second column 955 fluidically coupled to the

first column 910 through the microfluidic device 920. Between a first detector 965 and the second column 955 is a restrictor 960. The system shown in FIG. 9B also includes a third column 970 fluidically coupled to a detector 975. In this configuration, the column 955 has a higher flow rate than the columns 910 and 970. In the configuration shown in FIG. 9B, there are three restrictive zones to consider: the second column 955 at the oven temperature, the restrictor 960 at the oven temperature and the restrictor 960 at the temperature of the first detector 965. Equation (9) can be modified to include these three zones as shown in Equation (13),

$$F_a = \frac{\pi \times T_a}{256 \times p_a} \times \frac{(p_i^2 - p_{or}^2)}{(\frac{T_{r1} \times \eta_{r1} \times L_{r1} + T_{r2} \times \eta_{r2} \times L_{r2}}{d_r^4}) + (\frac{T_{c3} \times \eta_{c3} \times L_{c3}}{d_{c3}^4})} \quad (13)$$

where L_{c3} is the length of the column 955, η_{c3} is the viscosity of the carrier gas in the column 955 and T_{c3} is the absolute temperature of the column 955. To calculate the length of the restrictor to deliver a desired flow rate, Equation (13) can be rearranged to provide Equation (14)

$$L_r = \frac{[d_r^4 \times [\frac{\pi \times T_a}{256 \times F_a \times p_a} \times (p_i^2 - p_{or}^2) - \frac{T_{c3} \times \eta_{c3} \times L_{c3}}{d_{c3}^4}] - T_{r2} \times \eta_{r2} \times L_{r2}]}{T_{r1} \times \eta_{r1}} + L_{r2} \quad (14)$$

In use, Equation (11) can be used to calculate the flow rate through the column without the restrictor. Equation (14) can then be used to calculate the restrictor length to match that flow rate. Also, while not shown, the column 955 can be placed between the restrictor 960 and the first detector 965 and Equations (13) and (14) can be modified based on this rearrangement.

[00137] In certain examples, the restrictor internal diameter (or the internal diameter of other tubing or columns) can be selected to provide a desired flow rate. For a number of GC techniques that use a microfluidic device as described herein, it is important to accurately know the internal diameter of columns and tubes. In practice, the manufacturer's description is assumed to be accurate and is adopted. This can lead to significant errors as most relationships involve a calculation based on the 4th-

power of the internal diameter. In these instances, knowledge of the true internal diameter would be desirable. Equation (15) can be used to approximate the flow rate

$$F_a = \frac{\pi \cdot d_c^{\,4} \cdot T_a \cdot \left(p_i^{\,2} - p_o^{\,2}\right)}{256 \cdot L \cdot p_a \cdot \eta \cdot T_c} \tag{15}$$

where F_a is the flow rate at the column outlet at ambient temperature and pressure, d_c is the internal diameter of the column, L is the length of the column, p_i is the carrier gas pressure at the column inlet, p_o is the outlet pressure, p_a is the ambient pressure, T_c is the column temperature, T_a is the ambient temperature, and η is the viscosity of the carrier gas at the column temperature. For a column or tube at ambient temperature, Equation (15) may be re-arranged to provide Equation (16).

$$F_a = \left[\frac{\pi \cdot d_c^{\,4}}{256 \cdot L \cdot p_a \cdot \eta}\right] \cdot \left[p_i^{\,2}\right] - \left[\frac{\pi \cdot d_c^{\,4} \cdot p_o^{\,2}}{256 \cdot L \cdot p_a \cdot \eta}\right] \tag{16}$$

For a given column or tube, the terms inside the large brackets are constant and so Equation (16) may be represented as Equation (17).

$$F_a = b \cdot \left[p_i^{\,2}\right] - a \tag{17}$$

where a and b are constants. Thus by applying a range of pressures to one end of the column or tube and measuring the flow rate at the other, the value of the constant b can be determined by a least squares statistical fit. Once the value of b is established, the internal diameter may be calculated from Equation (18).

$$d_c = \sqrt[4]{\frac{256 \cdot L \cdot p_a \cdot \eta}{b \cdot \pi}} \tag{18}$$

As shown specifically in Example 1 below, the diameter of the tubing, e.g., columns, restrictors and the like can accurately be determined using these equations. The methods can be implemented in software to provide a calibration protocol where various diameters of tubing, e.g., columns, internal tubing, restrictors, etc. can be

determined to provide for increased accuracy of the system. The calibration can be performed by the chromatography system or the user can determine the diameter of the tubing and enter the calculated diameters into the system for use in controlling or modulating the flow rates as described herein.

[00138] In certain embodiments, to provide for a more user friendly system, the equations noted above may be implemented in software such that a user can enter the column parameters, e.g., length and internal diameter, the oven temperature and the detector temperature and the system can accurately predict the particular pressures needed to accomplish a desired separation run. The software can calculate the flow rate, restrictor lengths and/or diameters based on the inputted parameters, and the user can then insert a restrictor having the calculated length and diameter at a desired site in the system.

[00139] Referring to FIG. 10, an illustrative system including a splitting device, which can be a microfluidic device as described herein, configured to split column effluent to two or more detectors is shown. The system 1000 includes an injector 1010 fluidically coupled to a pressure regulator 1005 through a supply line 1007. The injector 1010 may have a split flow such that a portion of the sample introduced into the injector 1010 is passed to a column 1020 fluidically coupled to the injector 1010 through a supply line 1017 and the rest of the sample is passed along a direction 1015, which may be sent to waste or to another column, for example. The column 1020 is fluidically coupled to a splitting device 1030 through a supply line 1022, which fluidically couples the column 1020 to the splitting device 1030 through an input port on the splitting device 1030. The splitting device 1030 is also fluidically coupled to a midpoint pressure regulator 1025 through a supply line 1027. As shown schematically in FIG. 10, the splitting device 1030 is configured to split effluent flow from the column 1020 into three different flow paths. The splitting device 1030 is fluidically coupled to each of a detector 1050, 1055 and 1060 through a resistor 1035, 1040 and 1045, respectively. During operation of the system 1000, column effluent will enter the input port of the splitting device 1030 and mix with carrier gas supplied from a midpoint pressure regulator 1025. The effluent can then exit through a plurality of output ports of the splitting device to the detectors 1050, 1055 and 1060. While three detectors are shown in FIG. 10, either fewer, e.g., two detectors, or more, e.g., four or more detectors, could be used. It is not necessary to balance the restrictors 1035, 1040 and 1045. The restrictors may take several forms such as any

of those described herein. By selecting restrictors of appropriate length and internal diameter, the column effluent may be split between the attached detectors over a large range of ratios. The use of a midpoint pressure regulator 1025 in the system 1000, provides some advantageous features. By having a midpoint gas supply, the flow rate into each detector can be increased according to the needs of each detector thus providing for flexibility in detector flow rates. The low carrier gas flow rates through narrow bore columns can cause even lower flow rates to flow out of the microfluidic device and limit the range of split ratios that could be used. The mid-point regulator can provide additional gas flow to overcome these issues and allow very narrow-bore columns to be used if desired. Column backflushing may also be performed. The midpoint regulator also provides for the ability to protect an active MS detector while changing columns.

[00140] In certain examples, the system described herein that includes a microfluidic device can be used in many different configurations. For example, it is possible to simultaneously use selective detectors on the same chromatogram. This feature saves time (only one run needed) and eliminates variations (particularly retention times) between different chromatograms. One example is the TO-14 US-EPA air monitoring method where both an FID and ECD are used to monitor different compounds in the same chromatogram. In other configurations, improved dynamic range can be achieved by splitting different amounts to the same type of detector. Some detectors (e.g. FPD) have a very limited dynamic range and so the ability to see large peaks on one detector and small on the other could be useful. In some examples as described herein, single column backflushing can be performed by controlling the flow rate at various points in the system. This process can save time and eliminate extended temperature programs by efficiently removing heavy sample residue from the column after the analytes have eluted. Dual column backflushing can also be performed. For example, one (or more) of the restrictors shown in the system 1000 could be replaced by a GC column. This configuration would enable the first column to be backflushed while chromatography continues on the second column. A mid-point detector can be configured to monitor the passage of peaks between the two columns to aid setup. Dual column backflushing has a big advantage over single column backflushing in that the backflushing occurs simultaneously with the chromatography run thus achieving a substantial time savings. In the case of air-sensitive detectors, such as an MS or ECD, this system can permit those detectors to remain at a detection

temperature, e.g., hot and active, while a column is being exchanged or an injector is serviced. This feature would save significant time and reduce stress on the system and so the down time would be minimized. In addition, three or more column backflushing could also be performed so that chromatography can proceed on one or more other columns while the first (or more than one column) is being backflushed.

[00141] In certain examples, the systems described herein can be used for polarity tuning. In this technique, the respective residence time of compounds within the two columns can be modified by changing the midpoint pressure. This configuration serves to change the effective polarity of the combined columns and enables tweaking or fine control of the columns selectively to achieve difficult separations.

[00142] In accordance with certain examples, one configuration of a microfluidic device is shown in FIG. 11. In this cross-sectional view, the microfluidic device is configured as a wafer 1100 and includes an internal microchannel 1110 that has a variable diameter at different portions of the microchannel 1110. For example, the diameter of the microchannel at area 1125 can be about 300 to about 700 microns, e.g., about 400 to about 600 microns in diameter, the diameter of the microchannel at area 1130 can be about 75 microns to about 300 microns, e.g., about 100 microns to about 200 microns in diameter, the diameter of the microchannel at area 1135 can be about 300 to about 700 microns, e.g., about 400 to about 600 microns in diameter, the diameter of the microchannel at area 1140 can be about 75 microns to about 300 microns, e.g., about 100 microns to about 200 microns in diameter, and the diameter of the microchannel at area 1145 can be about 300 to about 700 microns, e.g., about 400 to about 600 microns in diameter. In certain examples, the diameter of the restricted portion in the microchannel, e.g., area 1140, can be at least two times smaller than the diameter of the adjacent channel portions, e.g., at least three times smaller, at least four times smaller or at least five times smaller, to provide for restricted fluid flow. The wafer 1100 also includes openings or apertures 1115 and 1120 that can couple the wafer to a wafer holder or other device to hold the wafer 1100 in place during operation of the system.

[00143] In certain examples, the microfluidic device can include various ports, e.g., inlet and outlet ports, that can provide a fluidic coupling between the column and the various other components downstream of the microfluidic device. One configuration of such a wafer 1200 is shown in FIG. 12. In this configuration, the ports are arranged in series in a microchannel 1205. A port 1210 is fluidically coupled to a

column. The flow of gas through the wafer 1200 is in the general direction from the port 1210 to ports 1220, 1230, 1240 and 1250. A midpoint pressure regulator can be fluidically coupled to the wafer 1200 at a port 1260. During operation, effluent from the column that enters through the port 1210 will be mixed with a carrier gas from the midpoint regulator entering through the port 1260 and then flow in succession through ports 1220, 1230, 1240 and finally 1250. The wafer 1200 can be coupled to a holder through apertures 1265 and 1270. Depending on the exact configuration, the various restrictors present in the system may take different operational states. One example of restrictor setup using the wafer 1200 is shown in Table 1.

Table 1

	Port			
Number of Detectors	**1220**	**1230**	**1240**	**1250**
1	Closed	Closed	Closed	Restrictor
2	Closed	Closed	Slowest flow restrictor	Fastest flow restrictor
3	Closed	Slowest flow restrictor	Medium flow restrictor	Fastest flow restrictor
4	Slowest flow restrictor	Slowest Medium flow restrictor	Fastest Medium flow restrictor	Fastest flow restrictor

The restrictors are arranged in order of increasing flow rate with the fastest flow rate being at port 1250. The microchannel can be arranged so that the outlet ports are within a single microchannel flow path. To plug or close any particular port, the port may be capped or otherwise blocked using blanking nuts, fittings, ferrules or other suitable devices that can provide a fluid tight seal. When closed, desirably no or little dead volume in the port is created that could prevent peak losses or cause tailing. By using a single wafer as shown in FIG. 12, anywhere from 1-4 detectors can be used without having to change the wafer for each different detector combination.

[00144] In accordance with certain examples, the particular length of the flow path between various ports can vary depending on the desired effect. One configuration of

a wafer having a different flow path configuration is shown in FIG. 13. The wafer 1300 includes a microchannel 1305, a port 1310 that is fluidically coupled to a column (not shown), a port 1360 that is fluidically coupled to a midpoint pressure regulator (not shown) and ports 1320, 1330, 1340 and 1350, each of which may or may not be fluidically coupled to a restrictor and/or detector. A portion 1315 of the microchannel 1305 is elongated to provide an increased flow path length to permit additional mixing of carrier gas from the midpoint regulator port 1360 and the column effluent port 1310. Such increased length can, for example, provide additional time to provide increased sample residence time and a more homogenous mixture of column effluent and carrier gas, which can be used to avoid or reduce diffusional broadening of the analyte peaks as described in more detail herein. In addition, the particular length of the flow path between any two or more of ports 1320, 1330, 1340 and 1350 can be different than the other lengths. When such a different length is present, it may be desirable to alter the restrictor flow rate to balance the flow rates of gas provided to the different detectors. Openings 1365 and 1370 can be used, for example, to attach the microfluidic device to a holder or other device designed to retain the microfluidic device at a desired site or in a desired orientation.

[00145] It will be recognized by the person of ordinary skill in the art, given the benefit of this disclosure that the exact number of ports in the wafer can vary and may be, for example, fewer ports or more ports than the illustrative configurations shown in FIGS. 12 and 13. Illustrative configurations are shown in FIGS. 14A-15B. Referring to FIG. 14A, a wafer includes a column effluent port 1405 fluidically coupled to a midpoint pressure regulator port 1425 and ports 1410 and 1415 each of which can be fluidically coupled to a restrictor and/or detector, for example. Apertures 1430 and 1435 can be used to attach the wafer to a holder or other structures of the microfluidic device. The length of the flow path between the two ports 1410 and 1415 can vary, and the particular restrictor flow rate can be altered to provide substantially the same flow rate through the different ports, if desired. FIG. 14B shows a configuration where the length of the flow path between ports 1410 and 1415 has been lengthened. Such lengthening may be desirable, for example, to provide more spacing for coupling of fittings to the various ports and facilitate overall setup of the device, to provide for increased residence time or other desired performance.

[00146] While two ports 1410 and 1415 are shown in FIGS. 14A and 14B, port 1415 can be omitted and a single port may be present. In the alternative, one or more additional ports can be present to provide fluidic coupling between such additional port(s) and a restrictor(s) and/or detector(s). Two configurations using additional ports are shown in FIGS. 15A and 15B. Referring to FIG. 15A, the wafer includes a column effluent port 1505 fluidically coupled to a midpoint pressure regulator port 1510 and to ports 1520, 1525, 1530, 1535, 1540 and 1545. Apertures 1550 and 1555 can be used to attach the wafer to a holder or other portions of the microfluidic device. FIG. 15B shows a similar arrangement to that of FIG. 15A, but the position of port 1535 has been moved.

[00147] In certain examples, the exact cross-sectional shape and angles of the microchannels can vary. In certain examples, the cross-sectional shape of the microchannel is circular or substantially circular, whereas in other examples, elliptical shapes or other non-circular shapes can be present. Similarly, the angle of the microchannel between two or more ports can vary and where a non-continuous flow path is present, the angle made by the change in direction of the flow path may be a sharp angle or may be a gradual angle such as, for example, an elbow or a curved surface. For example, in fluid chromatography systems where sharp angles may create turbulent flow, the angles can be configured as elbow or gradual turns to avoid or reduce such turbulence.

[00148] In certain examples, the microfluidic devices described herein can be used in many different configurations. FIGS. 16-25 show several illustrative configurations. Referring to FIG. 16, a single detector configuration that can be used, for example, to backflush and in a MS Vent mode is shown. The system 1600 includes an injector 1610 fluidically coupled to a pressure regulator 1605 through a supply line 1607. The injector 1610 is also fluidically coupled to a column 1620 through a supply line 1612. The column 1620 is fluidically coupled to a microfluidic device 1625 through a supply line 1617. The microfluidic device 1625 includes a column effluent port 1627 fluidically coupled to a midpoint pressure regulator 1630 through a port 1633. Gas is provided from the midpoint pressure regulator 1630 to the port 1633 through a supply line 1632. The microfluidic device 1625 includes ports 1635, 1640, 1645 and 1650. In the embodiment of FIG. 16, ports 1635, 1640 and 1645 are closed or plugged such that no gas flows into them. The port 1650 is fluidically coupled to a detector 1660 through a restrictor 1655. In operation, a

sample is introduced into the injector 1610 and species in the sample can be separated using the column 1620. Species elute from the column 1620 and are provided to the detector 1660 through the microfluidic device 1625. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 1600. If desired, any of ports 1635, 1640 or 1645 can be coupled to a sniffer or other device to provide for in-line sampling of gas and/or species in the gas within the microchannel of the microfluidic device 1625. In addition, by increasing the flow of gas provided by the midpoint pressure regulator to be greater than the flow rate to the column, the system 1600 can be backflushed or can be vented, e.g., can be operated in a MS vent mode.

[00149] In accordance with certain examples and referring to FIG. 17, a dual detector configuration is shown. The system 1700 includes an injector 1710 fluidically coupled to a pressure regulator 1705 through a supply line 1707. The injector 1710 is also fluidically coupled to a column 1720 through a supply line 1712. The column 1720 is fluidically coupled to a microfluidic device 1725 through a supply line 1717. The microfluidic device 1725 includes a column effluent port 1727 fluidically coupled to a midpoint pressure regulator 1730 through a port 1733. Gas is provided from the midpoint pressure regulator 1730 to the port 1733 through a supply line 1732. The microfluidic device 1725 includes ports 1735, 1740, 1745 and 1750. In the embodiment of FIG. 17, ports 1735 and 1740 are closed or plugged such that no gas flows into them. The ports 1745 and 1750 are each fluidically coupled to a detector 1770 and 1760, respectively, through a restrictor 1765 and 1755, respectively. In operation, a sample is introduced into the injector 1710 and species in the sample can be separated using the column 1720. Species elute from the column 1720 and are provided to one or both of the detectors 1760 and 1770 through the microfluidic device 1725. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 1700. The detectors 1760 and 1770 may be the same or may be different. In addition different peaks can be provided to different detectors by including suitable valving in the supply lines and/or by actuating one of more of the ports of the microfluidic device 1725 to be in a closed position, e.g., using a switching valve.

[00150] In accordance with certain examples and referring to FIG. 18, a three detector configuration is shown. The system 1800 includes an injector 1810 fluidically coupled to a pressure regulator 1805 through a supply line 1807. The

injector 1810 is also fluidically coupled to a column 1820 through a supply line 1812. The column 1820 is fluidically coupled to a microfluidic device 1825 through a supply line 1822. The microfluidic device 1825 includes a column effluent port 1827 fluidically coupled to a midpoint pressure regulator 1830 through a port 1833. Gas is provided from the midpoint pressure regulator 1830 to the port 1833 through a supply line 1832. The microfluidic device 1825 includes ports 1835, 1840, 1845 and 1850. In the embodiment of FIG. 18, port 1835 is closed or plugged such that no gas flows into it. The ports 1840, 1845 and 1850 are each fluidically coupled to a detector 1880, 1870 and 1860, respectively, through a restrictor 1875, 1865 and 1855, respectively. In operation, a sample is introduced into the injector 1810 and species in the sample can be separated using the column 1820. Species elute from the column 1820 and are provided to one or more of the detectors 1860, 1870 and 1880 through the microfluidic device 1825. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 1800. The detectors 1860, 1870 and 1880 may be the same or may be different or two of the detector 1860, 1870 and 1880 may be the same. In addition different peaks can be provided to different detectors by including suitable valving in the supply lines and/or by actuating one of more of the ports of the microfluidic device 1825 to be in a closed position, e.g., using a switching valve.

[00151] In accordance with certain examples and referring to FIG. 19, a system 1900 including four detectors is shown. The system 1900 includes an injector 1910 fluidically coupled to a pressure regulator 1905 through a supply line 1907. The injector 1910 is also fluidically coupled to a column 1920 through a supply line 1912. The column 1920 is fluidically coupled to a microfluidic device 1925 through a supply line 1922. The microfluidic device 1925 includes a column effluent port 1927 fluidically coupled to a midpoint pressure regulator 1930 through a port 1933. Gas is provided from the midpoint pressure regulator 1930 to the port 1933 through a supply line 1932. The microfluidic device 1925 includes ports 1935, 1940, 1945 and 1950. In the embodiment of FIG. 19, the ports 1935, 1940, 1945 and 1950 are each fluidically coupled to a detector 1990, 1980 and 1970 and 1960, respectively, through a restrictor 1985, 1975, 1965 and 1955, respectively. In operation, a sample is introduced into the injector 1910 and species in the sample can be separated using the column 1920. Species elute from the column 1920 and are provided to one or more of the detectors 1960, 1970, 1980 and 1990 through the microfluidic device 1925. Flow

control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 1900. The detectors 1960, 1970, 1980 and 1990 may be the same or may be different or any two or three of the detectors 1960, 1970, 1980 and 1990 may be the same. In addition different peaks can be provided to different detectors by including suitable valving in the supply lines and/or by actuating one of more of the ports of the microfluidic device 1925 to be in a closed position, e.g., using a switching valve.

[00152] In accordance with certain examples and referring to FIG. 20, a system 2000 including a single detector 2070 and a sniffer port 2060 is shown. The system 2000 includes an injector 2010 fluidically coupled to a pressure regulator 2005 through a supply line 2007. The injector 2010 is also fluidically coupled to a column 2020 through a supply line 2012. The column 2020 is fluidically coupled to a microfluidic device 2025 through a supply line 2022. The microfluidic device 2025 includes a column effluent port 2027 fluidically coupled to a midpoint pressure regulator 2030 through a port 2033. Gas is provided from the midpoint pressure regulator 2030 to the port 2033 through a supply line 2032. The microfluidic device 2025 includes ports 2035, 2040, 2045 and 2050. In the embodiment of FIG. 20, the ports 2035 and 2040 are closed or plugged such that no gas flows into them. The port 2045 is fluidically coupled to the detector 2070 through a restrictor 2065. The port 2050 is fluidically coupled to the sniffer port 2060, which can be used for in-line sampling or monitoring of species in the effluent or generally provides a port from which species in the fluid path can be withdrawn, if desired, through a restrictor 2055. In operation, a sample is introduced into the injector 2010 and species in the sample can be separated using the column 2020. Species elute from the column 2020 and are provided to one or more of the detector 2070 or the sniffer port 2060 through the microfluidic device 2025. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 2000. The sniffer port 2060 may typically remain in a closed position until the user desires to sample from that port. Suitable valving in the supply lines and/or by actuating the sniffer port 2060 can open the sniffer port, as desired.

[00153] In accordance with certain examples and referring to FIG. 21, a system 2100 that is configured for backflushing or venting in an MS system is provided. The system 2100 is similar to that shown in FIG. 17, but the flow rates of the various gases are altered to perform the backflushing or venting. Referring to FIG. 21, system

2100 includes an injector 2110 fluidically coupled to a pressure regulator 2105 through a supply line 2107. The injector 2110 is also fluidically coupled to a column 2120 through a supply line 2112. The column 2120 is fluidically coupled to a microfluidic device 2125 through a supply line 2122. The microfluidic device 2125 includes a column effluent port 2127 fluidically coupled to a midpoint pressure regulator 2130 through a port 2133. Gas is provided from the midpoint pressure regulator 2130 to the port 2133 through a supply line 2132. The microfluidic device 2125 includes ports 2135, 2140, 2145 and 2150. In the embodiment of FIG. 21, ports 2135 and 2140 are closed or plugged such that no gas flows into them. The ports 2145 and 2150 are each fluidically coupled to a detector 2170 and a detector 2160, respectively, through a restrictor 2165 and 2155, respectively. In operation, a sample is introduced into the injector 2110 and species in the sample can be separated using the column 2120. Species elute from the column 2120 and are provided to one or both of the detectors 2160 and 2170 through the microfluidic device 2125. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 2100 in this backflushing configuration. The detectors 2160 and 2170 may be the same or may be different. In the backflushing or venting mode, the flow rate of gas from the midpoint pressure regulator 2130 is greater than the flow of gas from the pressure regulator 2105, e.g., p_2 is greater than p_1. The result of this differential flow is that gas is flushed back through the column 2120 and can be vented from the system, for example, through outlet 2102. Where an MS detector is present, the differential fluid flow can be used to maintain the MS detector at its operating temperature while the system can be flushed. Such an advantage can provide a substantial time savings.

[00154] In accordance with certain examples and referring to FIG. 22, a dual column backflush configuration is shown. The system 2200 includes an injector 2210 fluidically coupled to a pressure regulator 2205 through a supply line 2207. The injector 2210 is also fluidically coupled to a first column 2220 through a supply line 2212. The first column 2220 is fluidically coupled to a microfluidic device 2225 through a supply line 2222. The microfluidic device 2225 includes a column effluent port 2227 fluidically coupled to a midpoint pressure regulator 2230 through a port 2233. Gas is provided from the midpoint pressure regulator 2230 to the port 2233 through a supply line 2232. The microfluidic device 2225 includes ports 2235, 2240, 2245 and 2250. In the embodiment of FIG. 22, ports 2235, 2240 and 2245 are closed

or plugged such that no gas flows into them. The port 2250 is fluidically coupled to a second column 2255. The second column 2255 is fluidically coupled to a detector 2260. In operation, a sample is introduced into the injector 2210 and species in the sample can be separated using the first column 2220. Species elute from the first column 2220 and are provided to the second column 2255 through the microfluidic device 2225. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 2200. The configuration of system 2200 permits backflushing of the first column 2220 once the effluent enters into the microfluidic device 2225. By increasing the pressure p_2 to be larger than the pressure p_1, e.g., so the flow rate from the midpoint pressure regulator 2230 is greater than the flow rate from the pressure regulator 2205, gas will flow into the first column 2220 to backflush it, and will also pass the effluent from the microfluidic device 2225 to the second column 2255 for continued or additional separation using the second column 2255. Polarity tuning methods may also be performed using a system as shown in FIG. 22.

[00155] In accordance with certain examples, a dual column backflush configuration with a midpoint monitoring detector is shown in FIG. 23. The system 2300 includes an injector 2310 fluidically coupled to a pressure regulator 2305 through a supply line 2307. The injector 2310 is also fluidically coupled to a first column 2320 through a supply line 2312. The first column 2320 is fluidically coupled to a microfluidic device 2325 through a supply line 2322. The microfluidic device 2325 includes a column effluent port 2327 fluidically coupled to a midpoint pressure regulator 2330 through a port 2333. Gas is provided from the midpoint pressure regulator 2330 to the port 2333 through a supply line 2332. The microfluidic device 2325 includes ports 2335, 2340, 2345 and 2350. In the embodiment of FIG. 23, ports 2335 and 2340 are closed or plugged such that no gas flows into them. The port 2350 is fluidically coupled to a second column 2355. The second column 2355 is fluidically coupled to a detector 2360. The port 2345 is fluidically coupled to a detector 2370 through a restrictor 2365. In operation, a sample is introduced into the injector 2310 and species in the sample can be separated using the first column 2320. Species elute from the first column 2320 and are provided to the second column 2355 through the microfluidic device 2325. In addition, species can be detected by using the detector 2370. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system

2300. The configuration of system 2300 permits backflushing of the first column 2320 once the effluent enters into the microfluidic device 2325. By increasing the pressure p_2 to be larger than the pressure p_1, gas will flow into the first column 2320 to backflush it, and will also pass the effluent from the microfluidic device 2325 to the second column 2355 for continued or additional separation using the second column 2355. Effluent can also be provided to the detector 2370 without any further separation. Polarity tuning methods may also be performed using a system as shown in FIG. 23.

[00156] In accordance with certain examples, a three column backflush configuration is shown in FIG. 24. The system 2400 includes an injector 2410 fluidically coupled to a pressure regulator 2405 through a supply line 2407. The injector 2410 is also fluidically coupled to a first column 2420 through a supply line 2412. The first column 2420 is fluidically coupled to a microfluidic device 2425 through a supply line 2422. The microfluidic device 2425 includes a column effluent port 2427 fluidically coupled to a midpoint pressure regulator 2430 through a port 2433. Gas is provided from the midpoint pressure regulator 2430 to the port 2433 through a supply line 2432. The microfluidic device 2425 includes ports 2435, 2440, 2445 and 2450. In the embodiment of FIG. 24, ports 2435 and 2340 are closed or plugged such that no gas flows into them. The port 2450 is fluidically coupled to a second column 2455. The second column 2455 is fluidically coupled to a detector 2460. The port 2445 is fluidically coupled to a third column 2465 that is fluidically coupled to a detector 2470. In operation, a sample is introduced into the injector 2410 and species in the sample can be separated using the first column 2420. Species elute from the first column 2420 and are provided to the second column 2455 and third column 2465 through the microfluidic device 2425. In addition, species can be detected by using the detectors 2460 and 2470. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 2400. The configuration of system 2400 permits backflushing of the first column 2420 once the effluent enters into the microfluidic device 2425. By increasing the pressure p_2 to be larger than the pressure p_1, gas will flow into the first column 2420 to backflush it, and will also pass the effluent from the microfluidic device 2425 to the second column 2455 and third column 2465 for continued or additional separation using these additional columns. Polarity tuning methods may also be performed using a system as shown in FIG. 24.

While not shown, the system of FIG. 24 may include a detector fluidically coupled to one of the ports 2435 or 2440.

[00157] In certain examples and referring to FIG. 25, a system may be configured with a microfluidic device to split the injector flow into two or more columns. The system 2500 includes an injector 2510 fluidically coupled to a pressure regulator 2505 through a supply line 2507. The injector 2510 is also fluidically coupled to a microfluidic device 2525 through a supply line 2512. The microfluidic device 2525 includes a port 2527 fluidically coupled to a midpoint pressure regulator 2530 through a port 2533. Gas is provided from the midpoint pressure regulator 2530 to the port 2533 through a supply line 2532. The microfluidic device 2525 includes ports 2535, 2540, 2545 and 2550. In the embodiment of FIG. 25, ports 2535 and 2540 are closed or plugged such that no gas flows into them. The port 2550 is fluidically coupled to a first column 2555. The port 2545 is fluidically coupled to a second column 2565. Each of the columns 2555 and 2565 is coupled to a detector 2560 and 2570, respectively. In operation, a sample is introduced into the injector 2510 and species in the sample can be separated using the first column 2555 and the second column 2565. The column media in columns 2555 and 2565 can be the same or can be different. Species elute from the columns and are passed to their respective detectors for detection. Flow control of the overall system may be performed as described herein or using other suitable algorithms. The arrows show the general gas flow in the system 2500. While not shown, the system of FIG. 25 may include a detector fluidically coupled to one of the ports 2535 or 2540. A restrictor or another column may be positioned between port 2535 or port 2540 and a detector.

[00158] In certain examples, while the systems described above include a microfluidic device that is designed to be coupled to a midpoint pressure regulator, there are applications that are cost sensitive or do not need any gas added to the column effluent. When such applications are performed, a different microfluidic device can be used. Or in the alternative, the midpoint pressure port of the wafers shown in FIGS. 11-15B can be blocked or capped such that no gas flow can enter. One such configuration where the midpoint pressure port is omitted is shown in FIG. 26A. The microfluidic device 2600 includes serial ports 2610, 2615, 2620, 2625, 2630 and 2635. The microfluidic device is scalable in that all or fewer than all of the ports can be used. Apertures 2640 and 2645 may be used to attach the microfluidic device 2600 to a holder or other device. The port 2615 is downstream of where the

column effluent enters the microfluidic device at the port 2610. The port 2615 may be connected to a gas inlet or this port can be capped or blocked. Each of the ports 2620, 2625, 2630 and 2635 may be fluidically coupled to a column, restrictor, detector and various combinations thereof. Where fewer than four couplings are desired, any one or more of the ports can be capped or plugged to shut that port off. In addition, a microfluidic device having fewer than six ports can be designed. One such example is shown in FIG 26B. The microfluidic device 2650 includes ports 2655, 2660, 2665 and 2670. Apertures 2675 and 2680 may be used to attach the wafer 2650 to a holder or other device. The ports 2660, 2665 and 2670 can be fluidically coupled to a column, restrictor, detector and various combinations thereof. In one alternative, the port 2660 can be fluidically coupled to a gas source to provide additional gas through the wafer 2650. Other port numbers, configuration and geometries consistent with the microfluidic devices 2600 and 2650 may also be used depending on the desired number of detectors to be used in the system or the particular desired configuration of the system.

[00159] In accordance with certain examples, the microfluidic devices described herein include one or more microchannels in the wafer. The exact configuration of the microchannel and how such microchannels are produced can vary depending on the particular material selected for use as a wafer. For example, the microchannel can be chemically etched, laser etched, drilled, grinded or molded into the wafer during production. The widths and overall geometry of the microchannels may vary. In one embodiment, the width of the microchannels can vary from about 10 microns to about 750 microns, for example, 50 microns to about 500 microns, for example, about 10 microns to about 100 microns, about 100 microns to about 300 microns, or about 300 microns to about 500 microns. The cross-sectional geometry of the microchannel may be circular, elliptical, triangular or other geometries. As discussed herein, it is desirable, but not required, that the microchannels have smooth transitions, e.g., elbows and the like, to facilitate gas flow through the microchannels.

[00160] In certain examples, the microfluidic device can be used in a multilayer device or a multicomponent device. For example, the microfluidic device can be sandwiched between two or more other devices to provide for a substantially fluid tight seal to prevent leaks. One or more gaskets or gasket materials can be used to further enhance the seal if desired. Additionally, gaskets, tapes or other materials can be used at the ports of the device to provide additional sealing, if desired. In some

examples, the microfluidic device can be a multi-layer structure itself, e.g., a laminated wafer, with sequential additions of layers being added to form the microchannels. One example of a microfluidic device and two plates used to hold the wafer is shown in FIG. 27. The wafer 2710 can be sandwiched between a first plate 2720 and a second plate 2730. A ferrule or fitting 2740 can be attached to the assembly to hold the microfluidic device 2710 and plates 2720, 2730 together during use of the microfluidic device.

[00161] In certain examples where the microfluidic device is configured as a wafer, the wafer can be produced from various materials including metals, plastics, composites, polymers, steels, stainless steels, alloys, and other materials that can be assembled to provide microchannels. For example, various layers of the wafer can be produced using stainless steel plates that can be laminated or welded together to form an overall microchannel structure within the wafer. In certain embodiments, layers of polyethertherketone or other polymers having a desired channel portion etched, drilled or otherwise carved into it can be laser or solvent welded to each other to provide the wafer. Regardless of the particular material selected for use in the wafer, the material desirably is inert such that no unwanted chemical reactions will occur between the sample and the wafer. In examples where the wafer material may be reactive, the microchannels (or the entire wafer surface) can be coated with an inert material such as, for example, polytetrafluoroethylene or other generally inert materials. Where the sample to be analyzed is corrosive, the microchannels (or the entire wafer surface) can be coated with yttria, alumina, or other materials that are resistant to corrosion and can protect the underlying wafer structure from damage. If a coating is used, the coating should be thick enough and robust enough to avoid leaching off, flaking or desorbing, which could lead to interference with the sample measurements. In addition, the materials used in the microfluidic device are desirably heat tolerant such that they do not melt or experience any substantial thermal deformation when used in a hot oven, such as those ovens and temperatures commonly encountered and used in chromatography system separations.

[00162] In accordance with certain examples, the restrictors that can be used with the devices and systems disclosed herein may vary in configuration and design. In certain examples, the microfluidic devices described herein can include a by-pass restrictor or other comparable device to reduce or restrict flow of gas and/or sample into unused areas of the microfluidic device. One such example is shown in FIG. 28.

The microfluidic device 2800 includes a plurality of ports 2810, 2815, 2820, 2825, 2830, and 2835. The port 2810 provides effluent from the column. The port 2815 can be fluidically coupled to a first detector (optionally with an in-line restrictor). The port 2820 is fluidically coupled to a switching gas source. The port 2825 is not used in this configuration. The port 2830 is fluidically coupled to another switching gas source. The port 2835 is fluidically coupled to a second detector (optionally with an in-line restrictor). A fluid connection 2850, e.g., internal or external, may be provided between the ports 2820 and 2830 to by-pass the port 2825. The fluid connection 2850 may include an external needle valve or may include a valve such as, for example, a switching valve such as a solenoid valve. The bypass dimensions can be adjusted to ensure that substantially no diffusion of gas occurs into an unused portion of the wafer. In operation, sufficient gas flow is provided to the by-pass restrictor to prevent sample diffusion along the switching gas inlet channel not in use at a particular point in time. The flow rate is desirably low to avoid or reduce the volume of gas entering into the GC column, which can dilute the sample.

[00163] In certain examples, to reduce the flow rate through the microchannels, the switching gas channels can be narrowed, tapered or constricted near the ends to increase the gas velocity as it enters into the sample flow path. For example and referring to FIG. 28, at or near the union of microchannel sections 2852 and 2854, the diameter of the microchannel section 2854 may be less than that of the microchannel section 2852. For example, if the microchannel section 2852 is about 600 microns in diameter, the microchannel section 2854 can be reduced to 300 microns in diameter. It will be recognized by the person of ordinary skill in the art, given the benefit of this disclosure, that a 50% reduction is not required. Other percentages and ratios may be used. For example, the ratio of the unconstricted microchannel section diameter:constricted microchannel section diameter can be about 5:1 to about 1.1:1, e.g., 4:1, 3.5:1, 3:1, 2.5:1, 2:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, more particularly about 3:1 to about 1.1:1, for example about 2.5:1 to about 1.2:1 or about 2:1 to about 1.5:1. In some examples, the overall diameter of the microchannel can be about 400-500 microns and constricted portions of the microchannel can have diameters of about 100-200 microns. The exact length of the bypass restrictor channel can also vary with illustrative lengths of about 5 mm to about 30 mm, more particularly about 10 mm to about 20 mm, e.g., about 11, 12, 13, 14, 15, 16, 17, 18, 19 mm or any value in between these specific lengths.

[00164] In accordance with certain examples, in assembly and use of the microfluidic devices described herein, the microfluidic device is typically sandwiched or encased in a multicomponent device to provide a microfluidic device that can be coupled to pneumatic tubing in a GC system. As described herein, these systems can be used in many different configurations and in multi-dimensional chromatographic analyses. In addition, while certain embodiments of the microfluidic devices are described herein, it will be recognized by the person of ordinary skill in the art, given the benefit of this disclosure, that the microfluidic devices can be used in combination with each other, e.g., by mounting them back to back in the same system. Suitable fluid connections to desired ports may be provided using pneumatic tubing and other connectors. In addition, crossover channels, e.g., either within one microfluidic device or between two or more different microfluidic devices can be provided. In-line valves or actuators can be used to control the gas flow to a desired port and/or to a desired microfluidic device. For example, a solenoid valve can be modulated, e.g., at about 10-100 Hz, e.g., about 50 Hz, to permit flow of one species to a desired port or detector. The solenoid valve may be closed or switched to stop such flow to a particular port. Some of these configurations are described in detail below.

[00165] In certain examples and referring to FIG. 29, one example of a system including a microfluidic device with a crossover switch is shown. Use of a crossover switch may be particularly desirable where, for example, column switching, automated screening, backflushing, large volume injections, multi-dimensional chromatography or multiplexing operations are desired. For example, one MS detector can simultaneously receive sample from two different columns. The system 2900 includes an injector (not shown) fluidically coupled to a first column 2905. The first column 2905 is fluidically coupled to a microfluidic device 2910 through a fluid flow path, e.g., pneumatic tubing. The system also includes a plurality of restrictors 2915, 2920, 2925 and 2930 each fluidically coupled to the microfluidic device 2910. As discussed herein, the restrictors can be used to balance the pressure in the system. The microfluidic device 2910 includes a switching valve that is operative to provide a crossover path such that species from the first column 2905 and a second column 2940, which can be fluidically coupled to its own injector (not shown), can selectively be provided to a detector 2935 or a detector 2945. For example, by modulating the switching valve, a fluid flow path can be provided between two or more desired components of the system and can provide different species eluting from either

column to one or both of the detectors. In addition, the sample flow can be split such that effluent from one column is provided to both of the detectors 2935 and 2945, as described herein. The various pressures in the system can be balanced as described herein or using other suitable configurations. It is desirable that the columns have the same internal diameter but the lengths can be different. The injectors can be liquid injectors or other suitable injectors, e.g., high speed injectors, automatic thermal desorption injectors and other injectors commonly used with GC devices and systems. The detectors 2935 and 2945 may be the same or may be different. Desirably, one of the detectors can be a MS detector and the other may be a different detector such as, for example, those described herein.

[00166] In certain embodiments, two or more microfluidic devices can be used in the illustrative embodiment of FIG. 29. For example, a crossover connection can be provided between two or more microfluidic devices each fluidically coupled to at least one column. For example, a first microfluidic device can be fluidically coupled to the first column 2905, and a second microfluidic device can be fluidically coupled to the second column 2940. The switching valve can be actuated to provide desired flow to a particular port of the microfluidic device or to provide flow between the microfluidic devices.

[00167] Various possible connections between the components of FIG. 29 are shown in more detail in FIGS. 30A and 30B. Referring to FIG. 30A, the switching valve of the microfluidic device 3015 may be configured such that effluent from a column 3010 is provided to a detector 3025. An injector 3005 is fluidically coupled to the column 3010. An in-line restrictor 3020 is between the microfluidic device 3015 and the detector 3025. An injector 3030 provides sample to a second column 3035. The microfluidic device 3015 is also fluidically coupled to the second column 3035. The microfluidic device 3015 is fluidically coupled to a second detector 3045 through a restrictor 3040. In the configuration shown in FIG. 30A, the microfluidic device 3015 is configured such that effluent from the column 3005 is provided to the detector 3025 and effluent from the column 3030 is provided to the detector 3045. In the crossover configuration shown in FIG. 30B, effluent from the column 3030 is provided to the detector 3025 and effluent from the column 3030 is provided to the detector 3045. In operation of the system, the microfluidic device may be operated between these two different states such that certain species from the column 3010 can be provided to the detector 3025 and other species from the column 3010 can be provided to the detector

3045. Similar operations may be performed for species exiting the column 3035. If desired, sample from the different columns 3010, 3035 can be provided to the same detector using the microfluidic device. In addition, more than one microfluidic device can be used in the system of FIG. 30, if desired.

[00168] In certain examples, the microfluidic device 3015 can also be used to backflush one or both of the columns 3010 and 3035. For example, by lowering the gas flow from pressure regulators p_1 and p_2 to be less than the flow from pressure regulator p_3, both of the column 3010 and 3035 can be backflushed for cleaning. In an alternative configuration, only the pressure p_1 or p_2 can be lowered to be less than p_3 such that only one of the columns is backflushed and separation may continue using the other column.

[00169] In certain examples, one of the detectors shown in FIG. 30A and 30B may be omitted or substituted with another device such as another column, a vent or other components commonly used in chromatography systems. One configuration is shown in FIG. 31. The system 3100 includes an injector 3105 fluidically coupled to a first column 3110. The column 3110 is fluidically coupled to a microfluidic device 3115. A vent 3125 is in fluid communication with the microfluidic device 3115 through a restrictor 3120. A second injector 3130 is fluidically coupled to a second column 3135. The second column 3135 is fluidically coupled to the microfluidic device 3115. The microfluidic device 3115 is also fluidically coupled to a detector 3145 through a restrictor 3140. In operation of the system 3100, the microfluidic device can be used to provide column effluent from both the column 3110 and the column 3135 to the detector 3145. Where species eluting from the column are not desired for analysis, the microfluidic device can be used to divert those species to the vent 3125. In an alternative configuration, the vent 3125 permits venting of the system while permitting the detector 3145 to be maintained at normal operating temperature and pressure. This feature is particularly desirable where the detector 3145 is a mass spectrometer.

[00170] In accordance with certain examples, one illustration of a microfluidic device configured as a wafer and including a crossover flow path is shown in FIGS. 32A-32D. The overall wafer construct is shown in FIG. 32A with various layers shown exploded in FIGS. 32B-32D. Referring to FIG. 32A, the wafer 3200 generally includes a multilayer substrate 3205 having one or more microchannels therein. In this embodiment, the microchannel has six ports 3210, 3215, 3220, 3225, 3230, and

3235. The port 3210 can be an inlet port from a first column, the port 3230 can be a port for a first switching gas, the port 3220 can be a port for a second switching gas, the port 3235 can be an outlet port to a first detector, the port 3225 can be an inlet port from a second column, and the port 3215 can be an outlet port to a second detector (or vent). The device includes crossover channels 3242 and 3244 and constricted channels 3246 and 3247. In producing the wafer, different layers can be assembled to provide the various flow paths. Referring to FIG. 32B, one layer can provide the crossover path 3242, which provides fluidic coupling between ports 3215 and 3230. The layer shown in FIG. 32B can be the bottom layer of the laminate or a layer on an external surface of the laminate depending on the exact orientation of the microfluidic device. Another layer (see FIG. 32D), can provide the crossover path 3244 and the constricted channels 3246 and 3247. The layer shown in FIG. 32D can be the top layer of the laminate or a layer on an external surface of the laminate opposite to the layer shown in FIG. 32B with the middle layer positioned between the top layer and the bottom layer. The crossover path 3244 can provide for fluidic coupling between the ports 3220 and 3235. The constricted channel 3246 provides fluidic coupling between the ports 3230 and 3235. The constricted channel 3247 provides fluidic coupling between the ports 3215 and 3220. The middle layer (FIG. 32C) may include suitable flow paths to complete the microchannel and to provide fluid flow between desired ports of the microfluidic device. When the various layers are laminated together to provide a wafer, the fluid flow paths will be produced and can be used to control the direction of species in the chromatographic system. The overall wafer can be mounted to a sample holder or other suitable device using apertures 3250 and 3255 and suitable fittings, e.g., screws, nuts, bolts, ferrules, etc. Each of the various layers shown in FIGS. 32B-32D may itself be a multilayer structure or laminate or may be a generally solid body having respective microchannels etched or otherwise included therein. Gaskets, sealants or other materials may be added between the layers to facilitate a fluid tight seal to avoid or reduce the likelihood that internal leaks may occur.

[00171] In certain examples and as discussed herein, the microfluidic device may include one or more actuators or switching valves that can couple or decouple two or more fluid flow paths. The position of the actuator provides for fluid flow between two or more ports or prevents fluid flow between two or more ports. The microfluidic device may include a low cost solenoid valve that can be opened, closed or modulated

at a desired frequency to connect two or more flow paths or to stop flow between two or more flow paths. In some examples, the solenoid valve can be actuated between a fully open and a fully closed position. The frequency with which the solenoid is actuated depends on the particular type of chromatography being performed, e.g., heartcut or solvent dump, the particular ports to be connected and the desired effect on pressure that can be accomplished by opening and closing the valve, and illustrative frequencies include, but are not limited to 5-200 Hz, 10-100 Hz, 20-90 Hz, 30-80 Hz, 40-70 Hz, 45-65 Hz, and 50-60 Hz. The solenoid valves are typically external to the wafer and coupled to a desired port through pneumatic tubing or other suitable connections. In some examples, the switching valve may be integrated into the port of the wafer to provide for fewer components for the end user to connect.

[00172] In certain embodiments, two or more serially connected switching valves can be used which are the same or are different. For example, a solenoid valve in-line with a proportional valve can be fluidically coupled to a port of the microfluidic device. The system may include gas flow monitors, pressure transducers or other devices to ensure that the pressure in the system is balanced.

[00173] In certain examples, the switching valve can be controlled using a controller, processor or other suitable electrical components. One configuration that can be used to modulate the valve is shown in FIGS. 33A and 33B. Referring to FIG. 33A, a function generator 3310 is electrically coupled to a transistor driver 3320 and is operative to provide a desired waveform to the transistor driver 3320. The transistor driver 3320 is electrically coupled to a solenoid valve 3330 and is operative to modulate the solenoid valve 3330 at a frequency corresponding to the particular waveform provided by the function generator 3310. The waveform provided by the function generator 3310 can vary during the course of a separation depending, for example, on the desired cycle frequency. In certain examples, a square wave can be provided by the function generator 3310 such that the solenoid valve 3330 will cycle between an open and a closed position, with respect to a given outlet. For example, where a 3-way solenoid valve is used, it can switch the inlet flow between two different outlets and thus can be "on" with respect to one of the outlets and can be "off" with respect to the other outlet. Other waveforms, e.g., triangular, sinusoidal, sawtooth and the like, can also be used depending in the particular type of switching valve fluidically coupled to the wafer. In certain embodiments, each port of the microfluidic device may include an individually controllable solenoid valve

electrically coupled to a controller and a gas source such that fluid flow through each port can be individually controlled.

[00174] In certain embodiments, the microfluidic devices disclosed herein can permit simultaneous analysis of two chromatograms. Referring to FIG. 36 (FIGS. 34 and 35 are described below), a system 3600 is shown that includes a first injector 3610 fluidically coupled to a first column 3615. The system 3600 also includes a second injector 3620 fluidically coupled to a second column 3625. Each of the first column 3615 and the second column 3625 are fluidically coupled to a microfluidic device 3630. The microfluidic device 3630 is fluidically coupled to a vent 3640 through a restrictor 3635. The microfluidic device 3640 is also fluidically coupled to a MS detector 3650 through a restrictor 3645. The MS detector 3650 is electrically coupled to a solenoid valve (not shown) between a gas source 3655 and the microfluidic device 3630. The MS detector 3650 drives the midpoint solenoid valve modulation to synchronize the microfluidic device 3630 with the scanning of the MS detector 3650. Species eluting from both the first column 3615 and the second column 3625 can be directed to the MS detector 3650 using the microfluidic device 3630. Such direction permits simultaneous processing of two chromatograms. The exact configuration of the microfluidic device 3630 may be any of the illustrative configurations described herein or other suitable configurations.

[00175] In certain embodiments, a system configured to perform simultaneous confirmatory chromatography is shown in FIG. 37. The system 3700 includes an injector 3710 fluidically coupled to each of a first column 3715 and a second column 3720. The injector 3710 splits the sample flow such that a portion is provided to the first column 3715 and a portion is provided to the second column 3720. Each of the first column 3715 and the second column 3720 is fluidically coupled to a microfluidic device 3725. The microfluidic device 3725 is fluidically coupled to a vent 3735 through a restrictor 3730 and to a detector 3745 through a restrictor 3740. If the separation media in the columns and column internal diameters are the same, then the separation by each of the columns should be substantially the same. The switch 3725 can provide peaks from both the first column 3715 and the second column 3720 to the detector 3745 such as, for example, a MS detector. The system can be vented through the vent 3735 while the detector 3745 is kept at an operating temperature and pressure

[00176] In certain examples, an illustrative system configured for multidimensional separations and multiplexed detection is shown in FIG. 38. The system 3800 includes

an injector 3810 fluidically coupled to a column 3815 and a carrier gas source 3805. The column 3815 is fluidically coupled to a first microfluidic device 3820 which is coupled to a gas source 3822. The first microfluidic device 3820 is also fluidically coupled to a second column 3825 and a second microfluidic device 3830. A restrictor 3827 is between the first microfluidic device 3815 and the second microfluidic device 3830. The second microfluidic device 3830 is fluidically coupled to a gas source 3835. The second microfluidic device 3830 is also fluidically coupled to a vent 3845 and a detector 3855. Peaks can elute from the first column 3815 and can be provided to the second column 3825 or can be provided to the detector 3855 or the vent 3845 using the first microfluidic device 3815 and the second microfluidic device 3830. Peaks may be selectively provided to a desired component based on the particular ports fluidically coupled within each of the first and second microfluidic devices 3815 and 3830, respectively. The first microfluidic device 3820 and second microfluidic device 3830 need not be the same but they may be the same. In some examples, the first and second microfluidic devices are selected to be different to provide for increased control of sample eluting from the column or columns.

[00177] In certain embodiments and referring to FIG. 39, a dual column, single detector configuration is shown. The system 3900 includes a first column 3910 and a second column 3920 each fluidically coupled to a gas source 3905 and 3915, respectively. Each of the first and second columns is also fluidically coupled to a microfluidic device 3925. The microfluidic device 3925 includes a first buffer 3932 and a second buffer 3934. The term buffer is used interchangeably in certain instances with the term charging chamber. The first buffer 3932 and the second buffer 3934 can each be used to retain peaks from the columns. For example, species can elute from the column 3910 and be collected in the first buffer 3932. Once collected, the species can be directed to the detector 3945 through a restrictor 3940 using a switching gas from a gas source 3930 and a switching valve 3927. Simultaneously, species eluting from the second column 3920 can be collected in the second buffer 3934. After the species from the first buffer 3932 have been provided to the detector 3945, the valve 3927 can be switched such that the sample in the second buffer 3934 is now directed to the detector 3945 using the switching gas. The capacity of the buffers 3932, 3934 is desirably matched and of a sufficient volume for the column effluent flow rates and for the multiplexing frequency. For example, the buffers may each be from about 80 microliters to about 150 microliters, for example

about 120 microliters, which is suitable for use with a column effluent rate of about 1 milliliter per minute at a multiplexing frequency of about 10 Hz. Other suitable column effluent rates and multiplexing frequencies will be selected by the person of ordinary skill in the art, given the benefit of this disclosure.

[00178] In certain examples, a wafer that includes a buffer is shown in more detail in FIG. 40. The wafer 4000 includes a plurality of ports and two buffers. A port 4010 is fluidically coupled to a first buffer 4012 and to a second port 4015. The buffer 4012 is inline between the port 4010 and a port 4020. A second buffer 4022 is inline between the ports 4025 and 4030. The buffers 4012 and 4022 can be part of the microchannel but are larger in diameter. In particular, the diameters and lengths of the buffers can be selected to be of sufficient capacity to accommodate the effluent flowing from a column, at the applied mid-point pressure, during one cycle of the modulated switching valve. In certain examples, the length and width of the buffer can be selected so that the buffer fits inside the wafer and still provides a desired fluid capacity. In operation, the port 4010 can be fluidically coupled to a switching gas source, the port 4015 can be fluidically coupled to a first column to receive effluent from the first column, the port 4020 can be fluidically coupled to a detector to provide sample to the detector, the port 4025 can be fluidically coupled to another column to receive effluent from the second column, and the port 4030 can be fluidically coupled to another switching gas source. As discussed in more detail below, the buffers can be used along with flow control to reduce diffusional broadening of peaks.

[00179] In certain embodiments, the devices described herein can be used to provide flow modulation. Flow modulation can provide substantial benefits including improved peak detection. A typical chromatogram is shown in FIG. 41, in which the response is related to either the flow rate (for mass flow detectors) or the concentration (concentration dependent detectors) of analytes flowing through a detector. When flow modulation is used for the same sample used in FIG. 41, the signal should appear closer to that shown in FIG. 42. The flow modulation allows the column effluent which is normally flowing at a slow rate (e.g. 1mL/min) to flow into a chamber. For example, referring to FIG. 43A, column effluent 4305 can flow from a column to a fluid flow path 4310 and into a buffer or charging chamber 4320. When a switching valve 4315 is in a first position, the column effluent 4305 will have a tendency to build up in the charging chamber 4320, though some may exit the charging chamber and be provided to a detector in the direction of arrow 4325.

Referring now to FIG. 43B, when the switching valve 4315 is actuated to a second position, a modulating gas will be provided in the direction of arrow 4330. The flow rate of the modulating gas exceeds the flow rate of column effluent. This large difference in flow rate acts to push any column effluent 4305 in the charging chamber 4320 to the detector in a single large pulse or bolus. This process results in a large, narrow peak as shown in FIG. 42. In particular, this modulation has the effect of introducing narrow bands of column effluent into the detector separated by the clean modulating carrier gas as shown in FIG. 42. The signal processing system desirably synchronizes discrete detector readings with the flow modulation so that these readings are taken at the apexes of the pulses. Detector readings can also be taken in between the pulses to obtain a background signal. Several advantages can be obtained using flow modulation optionally along with the microfluidic devices described herein. For example, with mass-flow sensitive detectors, the mass flow of analyte may be increased by a factor of 50x or more giving rise to greater sensitivity and, improved detection limits. With concentration dependent detectors, the modulation may enable the column effluent to travel through the detector cell at a high rate but with little dilution. This result may enable relatively large cell detectors to be used with narrow bore columns without the loss in sensitivity normally associated with the use of a make-up gas. The ability to monitor the detector background signal between the pulses may help eliminate the effects of detector drift and improve detection limits and analytical stability. There may be opportunities to use a modulator with a conventional flame photometric detector to enable time-gating of the optical emissions from the flame subsequent to each pulse to improve selectivity and reduce noise – in a similar way as achieved using, for example, certain pulsed flame photometric detectors. Also, a good flow modulator may form the basis of a GCxGC system.

[00180] In certain embodiments, the charging chamber shown in FIGS. 43A and 43B may have some limitations. In FIG. 43A, the switching valve prevents flow of a modulating gas into the charging chamber 4340 and so the flow rate of gas into the detector will be that of the column effluent. When the switching valve is actuated to a second position, there is a sudden increase in the flow rate entering the detector. This slow-fast-slow flow rate of carrier gas can lead to noise and instability in the detector. If such noise is present, a 3-way switching valve can be used as shown in FIGS. 44A and 44B to provide a more stable flow rate of gas into the detector. Referring to FIGS.

44A and 44B, a device includes a charging chamber 4420 fluidically coupled to a column (not shown) through a fluid flow path 4405. The device also includes a fluid flow path 4410 between a 3-way switching valve 4415 and the charging chamber 4420. A by-pass flow path 4430 is also fluidically coupled to the 3-way switching valve 4415. When the 3-way switching valve 4415 fluidically couples a modulating gas from fluid flow path 4435 to the by-pass flow path 4430 (FIG. 44A), modulating gas travels into the by-pass flow path and out to a detector through fluid flow path 4425. In this state, effluent from the column can build up in the charging chamber 4420. When the 3-way switching valve is actuated to a different position (FIG. 44B), the modulating gas can be provided to the charging chamber 4420 through the fluid flow path 4410 and can act to force the accumulated effluent from the charging chamber to the detector along the fluid flow path 4425. One cycle of the 3-way switching valve will produce one pulse into the detector. Thus to generate, for example, fifty pulses each second, the 3-way switching valve must oscillate at 50 Hz. This high level of cycling can place significant stress on the switching valve. In addition, while the chamber is being flushed as shown in FIGS. 43B and 44B, column effluent will continue to enter the chamber. This material will be diluted and effectively lost from the analysis.

[00181] In certain embodiments, more than one charging chamber can be used in the flow modulation methods described herein and the microfluidic devices described herein. An illustration of this configuration is shown in FIGS. 45A and 45B. The device includes a 3-way switching valve 4515 fluidically coupled to a modulating gas through a fluid flow path 4510. The switching valve 4515 is fluidically coupled to a first chamber 4525 through a fluid flow path 4520 and to a second chamber 4540 through a fluid flow path 4535. A column (not shown) is fluidically coupled to each of the first chamber 4525 and the second chamber 4540 through an inlet 4505. The chamber 4525 is fluidically coupled to a detector (not shown) through fluid flow paths 4530 and 4550, and the chamber 4540 is fluidically coupled to the detector through fluid flow paths 4545 and 4550. While one of the chambers 4525, 4540 is being charged, the other chamber is being flushed with the modulating gas. This arrangement should generate two pulses for each cycle of the switching valve, which permits the switching valve to oscillate at half the speed as the single chamber design to achieve the same performance. In addition, none of the column effluent is wasted – it is always charging one of the chambers. A small flow of the modulating gas

between the chambers can prevent or reduce diffusion of sample vapor into the chamber being swept. The flow rate to the detector will be the same in either position of the switching valve.

[00182] In certain examples, the microfluidic devices described herein can be configured with one or more of the charging chambers. FIG. 46 shows one illustration of a microfluidic device with at least one charging chamber. The microfluidic device 4600 includes a first chamber 4610 and a second chamber 4615. A column effluent port 4640 is fluidically coupled to each of the first chamber 4610 and the second chamber 4615. Modulating gas may be introduced at a port 4630 or a port 4650 depending on which of the chambers 4610, 4615 is swept or flushed. A switching valve (not shown) is fluidically coupled to each of the ports 4630 and 3650 to control which port receives the modulating gas. One or more restrictors may be present to balance the flow in the system. When the modulating gas is introduced into the port 4630 the modulating gas will sweep chamber 4615 into the detector, and the column effluent will be charging the chamber 4610. When the modulating gas is switched to the port 4650, the chamber 4610 will be swept and the chamber 4610 will be charged. The chambers can be designed to be long and narrow to minimize dilution and dispersion of the sample as it is flushed.

[00183] In certain embodiments where a charging chamber is present, the chamber geometry can be selected to suit the operating conditions. For example, the following variables can be considered when selecting the chamber geometry and dimensions: column flow rate, modulating gas flow rate, pressure inside the microfluidic device and switching valve modulation frequency. In one illustration, if the column flow rate is in the range 0.5 to 3 mL/min (e.g., columns with a maximum internal diameter of 0.32mm) and the flow rate into the detector is about 50mL/min, then these assumptions provide a compression factor in the range 17X to 100X. If the internal pressure of the microfluidic device is about 8 psig, then a piece of fused silica tubing can be connected to the detector to provide about 50mL/min at 8psig. The restrictor geometry will be dependent on the particular detector selected. The charging chamber is desirably large enough to hold all the column effluent eluting from the column before it is pulsed. At the pressure inside the microfluidic device, the maximum volumetric flow rate from the column will be

3 x (Ambient Pressure)/(Microfluidic device Pressure + Ambient Pressure) = 3 x 15/23 = ~2mL/min

Table II lists the chamber capacities desired for a range of switching valve modulation frequencies with a 2mL/min flow rate at 8 psig.

Table II. Predicted chamber capacity requirements

Valve Frequency (Hz)	Charge Time (milliseconds)	Chamber Capacity (µL)
5	100	13.33
10	50	6.67
20	25	3.33
50	10	1.33

If the channels of the microfluidic device are 80 microns in height and the chamber length is about 30 mm, the chamber widths can be selected as shown in Table III.

Table III. Predicted chamber dimensions

Chamber Capacity (µL)	Chamber Height (µm)	Chamber Length (mm)	Chamber Width (µm)
13.33	80	30	444
6.67	80	30	222
3.33	80	30	111
1.33	80	30	44

Tables II and III are provided as a guide, but any of the assumptions can be varied which would change the exact dimensions selected.

[00184] In certain embodiments, the internal chamber channel geometry can also be selected to provide desired flow properties. For example, the geometry of the microchannels between the column port and each of the two chambers can alter the fluid flow. If these are too wide, the modulating gas will be able to cross between the two chambers and flush them both simultaneously. If they are too narrow, then the column port will increase in pressure from the column effluent and so the effluent will split into both chambers. The flow of modulating gas into the chamber being charged should be kept very low (e.g., < 50µL/min). FIG. 47 shows one illustration of how this process can be controlled using a microfluidic device. The microfluidic device 4700 includes a first chamber 4710 and a second chamber 4715. The microfluidic device 4700 also includes a plurality of ports. A column effluent port 4740 is fluidically coupled to the first chamber 4710 and the second chamber 4715. A 3-way switching valve (not shown) is fluidically coupled to a port 4730 and 4750 and can provide a modulating gas to either of the ports 4730, 4750 depending on the position of the valve. If the modulating gas is switched to the port 4730, then 50 mL/min of

gas should flow through the chamber 4715 and out to a detector through a fluid flow path connected to port 4720. The chamber 4715 should not impose any significant restriction but the fluid flow path 4755 to the port 4720 may provide restricted flow. The flow rate of the modulating gas from the port 4730 through the chamber 4710 will be controlled by a fluid flow path 4760. It is the relative impedance of the flow paths 4755 and 4760 that will dictate the flow rate of the modulating gas through the chamber 4710 and the chamber 4715. Thus, the dimensions of the fluid flow paths 4755 and 4760 can be restricted or expanded, relative to the dimensions of other portions of the microchannel, to provide a desired flow rate through the microfluidic device.

[00185] In certain embodiments, the microfluidic devices described herein can be used, for example, to split peaks. For example, individual peaks can be cut and provided to different detectors (or different components) or a single peak may be split and provided to two different components. For example, where a particular species in the sample is highly concentrated, it may be desirable to split that sample peak and send a portion of it to a vent rather than send the entire peak to a detector. Such splitting can overcome the dynamic range limitations of a column and/or a detector. In addition, large injection volumes can be used and the solvent peak can be split (or removed) entirely to avoid overloading the detector. One configuration of a system that is configured for peak splitting is shown in FIG. 48. The system 4800 includes an injector 4810 fluidically coupled to a carrier gas source 4805 and a first column 4815. The first column 4815 is fluidically coupled to a microfluidic device 4820, which itself is fluidically coupled to a switching valve 4825. A second column 4830 is fluidically coupled to the microfluidic device 4820 and a detector 4835. The microfluidic device 4820 is also fluidically coupled to a restrictor 4840 and a vent 4845. Depending on the position of the switching valve 4845, peaks eluting from the first column 4815 can be cut and provided to the second column 4830 or to the vent 4845. For example, contamination peaks or solvent peaks can be selectively provided to the vent 4845 so that they do not interfere with detection of sample peaks, which can be provided to the second column 4830 and to the detector 4835. In some examples, a portion of a peak can be cut and provided to the vent. For example, where one component in a sample (or a contaminant in a sample) is present at a substantially higher concentration than the other components, the highly concentrated component may be present at a concentration higher than the dynamic range of the

detector, which can result in a flat top peak if the signal exceeds the maximum detector signal. By splitting the peak into two or more portions, the concentration may fall within the detector range to provide a more accurate assessment of how much of that component is present in the sample. Different speaks can be split different amounts, e.g., 25%, 50%, 75% or other splitting percentages. The system of FIG. 48 also permits venting through the vent 4845. Such venting can overcome issues resulting from large solvent amounts, which can permit larger injection volumes to be used. In addition, backflushing and MS vent functionalities can be performed as described herein.

[00186] In certain embodiments, a system can be configured to split different peaks or provide different peaks to two or more detectors. Referring to FIG. 49, a system 4900 includes an injector 4905 fluidically coupled to a carrier gas source 4910 and a column 4915. The column 4915 is fluidically coupled to a microfluidic device 4975. A midpoint pressure regulator 4920 may optionally be fluidically coupled to the microfluidic device 4975. The microfluidic device 4975 is fluidically coupled to a first detector 4940 through a restrictor 4925, fluidically coupled to a second detector 4945 through a restrictor 4930, and fluidically coupled to a MS device 4950 through a restrictor 4935. In operation of the system, peaks may be cut and a portion can be provided to the first detector 4940 and the remainder of the cut peak can be provided to the second detector 4945. In an alternative, a portion of the peak can be provided to the MS device 4950. In some embodiments, entire peaks may be provided to the different detectors. Other uses of the system 4900 will be readily selected by the person of ordinary skill in the art, given the benefit of this disclosure.

[00187] In certain embodiments, the sample can be split prior to any separation. One configuration of such a system is shown in FIG. 50. The system 5000 includes an injector 5005 fluidically coupled to a carrier gas source 5010. The injector 5005 is fluidically coupled to a microfluidic device 5075. A midpoint pressure regulator 5020 may optionally be fluidically coupled to the microfluidic device 5075 through a restrictor 5015. A switching gas source 5020 can be fluidically coupled to the microfluidic device 5075. The microfluidic device 5075 is fluidically coupled to a first detector 5040 through a first column 5025, fluidically coupled to a second detector 5045 through a second column 5030, and fluidically coupled to a MS device 5050 through a third column 5035. Sample can be injected into the system using the injector 5005 and can be split to the different components using the microfluidic

device 5075. Separation can be performed using the columns 5025, 5030 and 5035 and the peaks can be provided to the corresponding detector. The configuration shown in FIG. 50 permits simultaneous analysis of a sample using different types of detectors and/or different types of column materials.

[00188] In certain embodiments, splitting of the peaks can permit use of different carrier gases. One configuration of such a system is shown in FIG. 51. The system 5100 includes an injector 5105 fluidically coupled to a first carrier gas source 5110. The injector 5105 is fluidically coupled to a first column 5115. A switching valve 5125 can be fluidically coupled to a microfluidic device 5155, a splitter 5160 and a second carrier gas source 5120, which may be the same as the first carrier gas source or may be different. The microfluidic device 5155 is also fluidically coupled to a third gas source 5145, which may be the same or may be different from the first and second carrier gas sources 5110, 5120. The microfluidic device 5155 is further fluidically coupled to a first detector 5135 through a second column 5140 and to a third detector 5165 through a third column 5150. In one scheme using the system of FIG. 51, the first gas source 5110 can be nitrogen which is used at a flow rate of 10 cm/sec. The second gas source 5120 may also be nitrogen, which can be introduced at a sufficient flow rate to provide a flow rate through the second column 5140 of about 40 cm/sec. The second gas source can be provided, for example, to sweep effluent from a charging chamber 5130. The third gas source can be hydrogen and can be provided at a flow rate of about 40 cm/sec to the third column 5150. In this configuration, the different carrier gases can provide different separation using the second and third columns 5140, 5150. Such different carrier gases may be desirable where, for example, a single type of carrier gas does not provide suitable separation of all the components in the sample.

[00189] In certain embodiments, the systems described herein can be used for multidimensional separations. One illustration is shown in FIGS. 52A and 52B. The system 5200 includes an injector 5210 fluidically coupled to a carrier gas source 5205 and a first column 5215. The first column 5215 is fluidically coupled to a microfluidic device 5220, which itself is fluidically coupled to a modulating gas source 5225. The microfluidic device 5220 is also fluidically coupled to a first detector 5235 through a restrictor 5230 and to a second detector 5245 through a first column 5240. The microfluidic device 5220 is also typically in fluid communication with a switching valve (not shown), which can permit fluid flow from the first column

5215 to the second column 5240 and second detector 5245 in one position (FIG. 52A) and can permit fluid flow to the second detector 5235 through the restrictor 5230 in another position (FIG. 52B). The position of the switching valve may be changed to control which components of the system receive column effluent. Such direction of flow provides for different data sets which can be used to provide a better analysis of components in the sample and can be, if desired, provided on the same chromatogram for easier analysis.

[00190] In some embodiments, the multidimensional separation can occur after column effluent is split but before any separation has occurred. One configuration of a system that uses a split flow for multidimensional analysis is shown in FIG. 53. The system 5300 includes an injector 5310 fluidically coupled to a carrier gas source 5305 and a restrictor 5315. The restrictor 5315 is fluidically coupled to a microfluidic device 5320, which itself is fluidically coupled to a modulating gas source 5325. The microfluidic device 5320 is also fluidically coupled to a first detector 5335 through a first column 5330 and to a second detector 5345 through a second column 5340. The microfluidic device 5320 is also typically in fluid communication with a switching valve (not shown), which can split the fluid flow from the injector 5310 and provide the split flow to the different columns of the system 5300. Such splitting permits the use of different separation media in the two columns to provide different data sets and different separations using a single system.

[00191] In certain examples, a system for use in a multidimensional separation, e.g., GCxGC, can include three or more columns. One system that includes three columns is shown in FIG. 54. The system 5400 includes an injector 5410 fluidically coupled to a carrier gas source 5405 and a first column 5415. The first column 5415 is fluidically coupled to a microfluidic device 5420, which itself is fluidically coupled to a modulating gas source 5425. The microfluidic device 5420 is also fluidically coupled to a first detector 5435 through a second column 5430 and to a second detector 5445 through a third column 5440. The microfluidic device 5420 is also typically in fluid communication with a switching valve (not shown), which can split the column effluent flow (or particular peaks if desired) from the first column 5415 and provide the split flow to the two other columns of the system 5400. Such splitting permits the use of different separation media in the three columns, if desired, to provide different data sets and different separations using a single system.

[00192] In certain examples, the microfluidic devices described herein can be used to switch a single input between a plurality of outputs or to switch a plurality of inputs to a single output. For example, a single output may be switched between one of three outputs or one of three inputs may be switched to a single output. Such microfluidic devices operative to provide three-way switching are referred to in certain instances as three-way switched microfluidic devices. It may be desirable to use two or more switching valves, e.g., solenoid valves and the like. Where two or more switching valves are used, additional tubing, fluidic couplings and the like may be used to facilitate fluid flow in the overall system.

[00193] Referring to FIG. 58, a microfluidic device 5800 including seven ports 5810, 5820, 5830, 5840, 5850, 5860 and 5870 is shown. The microfluidic device also includes a plurality of bypass restrictors such as bypass restrictors 5875, 5880 and 5885 that loop around the ports 5820, 5840 and 5860. In producing the microfluidic device 5800, two laminated wafer layers can be joined together. FIG. 59 shows a first wafer layer 5900 and FIG. 60 shows a second wafer layer 6000. The first wafer layer 5900 includes the ports 5810, 5820, 5830, 5840, 5850, 5860 and 5870 that are fluidically coupled to each other through fluid flow paths. The second wafer layer 6000 includes the bypass restrictors 5875, 5880 and 5885 that become coupled to their respective ports when the layer 5900 is laminated or otherwise joined to the layer 6000. If is it desired to provide more room between the ports and the edges for the bypass restrictors, then three or more wafer layers could be used, for example. By using three or more wafer layers, the bypass restrictor flow paths could cross over other fluid flow paths such that the bypass restrictor flow paths are not positioned along the edges of the microfluidic devices.

[00194] Where the device shown in FIG. 58 is used, the dimensions of the bypass restrictors can be selected such that desired flow rates are provided. For example, with switching gas provided to two of the three switching ports at any time, the by-pass (sweep) gas flow will pass through two restrictors. The restrictor geometry calculations noted above may be used. To maintain the same total flow rate in the device of FIG. 58 as compared to the total flow rate using the single bypass restrictor device (or similar devices), each restrictor in FIG. 58 would desirably provide about half the flow rate. For illustration purposes only, that means that each of the restrictors shown in FIG. 58 would need to be about twice the length of those noted in the calculations above, or would be about 0.84 times its internal channel width. These

calculations are merely illustrative and each set of circumstances and each configuration can be considered using the equations noted herein.

[00195] In certain embodiments, the microfluidic device shown in FIG. 58 can be used with two or more switching valves, e.g., solenoid valves. An illustrative configuration is shown in FIG. 60. In this configuration, a first 3-way solenoid valve (SV1) 6110 and a second 3-way solenoid valve (SV2) 6120 are present. When solenoid valves 6110 and 6120 are in a first position (off, closed or inactive), carrier gat at the midpoint pressure will be directed to ports 5810 and 5830 in the wafer 5800. This configuration would drive any precolumn effluent, which would enter the microfluidic device 5800 at port 5870, out through port 5820. Where SV1 is on (second position) and SV2 is off (first position), the active switching ports would be ports 5830 and 5850, and precolumn effluent would be directed to port 5860. Where SV1 is off and SV2 is on, the active switching ports would be ports 5810 and 5830, and precolumn effluent would be directed to port 5840. Where both SV1 and SV2 are on, the active switching ports would be port 5830, and precolumn effluent would be directed to ports 5840 and 5860. The first three solenoid valve combinations provides the functionality to switch a single input between one of three outputs. The fourth combination (SV1 and SV2 both on) represents a state where two of the three outlet ports can be made active simultaneously.

[00196] FIG. 63-66 show a system 6300 that can be used for a three-column heartcut application using a single pre-column whose effluent can be switched between a restrictor connected directly to a detector and two independent analytical columns. The system 6300 includes an injector 6305 fluidically coupled to a carrier gas 6302. The injector 6305 is fluidically coupled to a column 6310, which is fluidically coupled to a microfluidic device 6315. Where the microfluidic device 6315 takes the form shown in FIG. 58, the column 6310 can be fluidically coupled to the microfluidic device through the port 5870. A first solenoid valve 6320 and a second solenoid valve 6325 are each fluidically coupled to a switching gas source 6330 through a fluid line. The first solenoid valve 6320 is fluidically coupled to the ports 5810 and 5830. The second solenoid valve is fluidically coupled to the ports 5830 and 5850. A first detector 6350 is fluidically coupled to the port 5820 through a column 6355 (or restrictor). A second detector 6360 is fluidically coupled to the port 5860 through a column 6365 (or a restrictor). A third detector 6370 is fluidically coupled to the port 5840 optionally through a restrictor.

[00197] In the configuration shown in FIG. 63, both the solenoid valves 6320 and 6325 are off (first position). Gas from the switching source 6330 enters the microfluidic device 6315 through the ports 5310 and 5350. As analyte enters the microfluidic device 6315 from the column 6310, it is directed to the port 5820 and onto the column 6355 and the detector 6350.

[00198] In the configuration shown in FIG. 64, the solenoid valve 6320 is on (second position) and the solenoid valve 6325 is off. Gas from the switching source 6330 enters the microfluidic device 6315 through the ports 5330 and 5350. As analyte enters the microfluidic device 6315 from the column 6310, it is directed to the port 5860 and onto the column 6365 and the detector 6360.

[00199] In the configuration shown in FIG. 65, the solenoid valve 6320 is off and the solenoid valve 6325 is on. Gas from the switching source 6330 enters the microfluidic device 6315 through the ports 5310 and 5330. As analyte enters the microfluidic device 6315 from the column 6310, it is directed to the port 5840, through the restrictor, and onto the detector 6370.

[00200] In the configuration shown in FIG. 66, the solenoid valve 6320 is on and the solenoid valve 6325 is on. Gas from the switching source 6330 enters the microfluidic device 6315 through the port 5330. As analyte enters the microfluidic device 6315 from the column 6310, it is directed to the ports 5840 and 5860. Analyte will be provided to the detector 6370 through the port 5840 and to the column 6365 and the detector 6360 through the port 5860.

[00201] The configurations shown in FIGS 63-66 are merely illustrative. Microfluidic devices similar to the one shown in FIGS. 58-66 can be used for heartcutting, detector switching, column switching, inlet switching and other uses. For example, three-column heartcutting can be used along with a midpoint detector to enable the pre-column chromatography to be directly monitored. In other configurations, effluent from a single column can be switched between any one of three detectors, three columns or combinations thereof during a chromatography run. An illustrative combination would be an MS for one detector and an FID, ECD, NPD or FPD for the other two detectors. In another configuration, a three-way switched microfluidic device can be used to receive an input from one of three different columns, which may be the same or may be different. Such a configuration enables three different applications to be used and selected in a single system. In yet other configurations, the three-way switched microfluidic device can be used to select one

sample stream from three different potential sample streams that may be present. For example, a system may include two or more injectors or may include an autosampler with two or more injectors that can provide multiple sample streams.

[00202] While the three-way switching systems are shown as including a single microfluidic device, two or more separate microfluidic devices can also be used to provide three-way switching. The microfluidic devices can be arranged in series or in parallel depending on the desired output and use of the device. In some configuration, two or more two-way switched microfluidic devices may be used together to provide three-way or higher ordered switching. Each microfluidic device can include a respective switching valve that can be actuated in conjunction with other switching valves to provide a selected fluid flow in the overall system. In addition, the three-way switched microfluidic device need not be configured in wafer form, but instead can be assembled using tubing, unions, valves, ferrules and the like.

[00203] In certain embodiments, the switching devices described herein can be used in backflushing techniques and methods. One illustrative system is shown in FIG. 67. The system 6700 includes an injector 6710 fluidically coupled to a pressure source 6705. The injector is fluidically coupled to a switching device 6720, e.g., an S-swafer, D-swafer or T-swafer available from PerkinElmer Health Sciences, Inc., through a restrictor 6715. The switching device 6720 is fluidically coupled to a pressure source 6725, a first chromatography column 6730, and a second chromatography column 6735. The first chromatography column 6730 is fluidically coupled to a restrictor 6742, which itself is coupled to a pressure source 6740 and a detector 6745. The second chromatography column 6735 is fluidically coupled to a restrictor 6752, which itself is coupled to a pressure source 6750 and a detector 6755. Each of restrictors 6742 and 6752 can be fluidically coupled to components other than a detector, e.g., an additional switching device or the like. The pressure inside the injector 6710 is regulated by a carrier gas supply from the pressure source 6705. The restrictor 6715 may take many configurations such as a short piece of deactivated fused silica restrictor tubing. A supply of a carrier gas can be introduced using pressure sources 6725, 6740 and 6750 to control the overall pressure in the system 6700.

[00204] In certain examples, the restrictors 6742 and 6752 can be PerkinElmer PreVent adapters, which are unions with built-in restrictors that can be coupled to a detector port or port of another device. An exploded view of a suitable restrictor is shown in FIG. 68. The restrictor 6800 includes an inlet port 6810, a mid port 6820

where a carrier gas can be provided, and an outlet port 6830 which can be fluidically coupled to a detector or other component of the system. In many instances, the restrictors would be fluidically coupled to a capillary column such that effluent from the capillary column can enter the inlet port 6810 of the restrictor 6800 if the various pressure are selected properly, as discussed in more detail below.

[00205] In certain embodiments, to control the direction of sample flow in the system 6700 shown in FIG. 67, the various pressures from the pressure sources 6705, 6725, 6740 and 6750 can be altered. In many instances, the pressure source 6725 can be held constant, and alteration of the pressures using pressure sources 6705, 6740 and 6750 can be used to direct sample flow in the system.

[00206] In certain examples, the pressure from pressure sources can be implemented such that pressure is highest from pressure source 6705, next highest at pressure source 6725 and lower at pressure sources 6740 and 6750, with the pressures from pressure sources 6740 and 6750 being substantially the same. The resulting fluid flow in the system 6700 is that sample enters the microfluidic device 6720 and is split into the fluid flow paths and enters the columns 6730 and 6735.

[00207] In other examples, the pressure from pressure sources can be implemented such that pressure is highest from pressure source 6705, next highest from pressure source 6750, next highest from pressure source 6725 and lowest from pressure sources 6740. The resulting fluid flow in the system 6700 is that sample enters the microfluidic device 6720 and enters the fluid flow path fluidically coupled to column 6730, with little or substantially no sample entering the fluid flow path fluidically coupled to the column 6735.

[00208] In another example, the pressure from pressure sources can be implemented such that pressure is highest from pressure source 6705, next highest from pressure source 6740, next highest from pressure source 6725 and lowest from pressure source 6750. The resulting fluid flow in the system 6700 is that sample enters the microfluidic device 6720 and enters the fluid flow path fluidically coupled to column 6735, with little or substantially no sample entering the fluid flow path fluidically coupled to the column 6730.

[00209] In another configuration, the pressure from pressure sources can be implemented such that pressure is highest from pressure source 6740, next highest from pressure source 6725, and lowest from pressure source 6750. Pressure source 6705 can be at any pressure. The resulting fluid flow in the system 6700 is that fluid

is backflushed from the fluid flow path comprising the column 6730 into the fluid flow path comprising the column 6735.

[00210] In yet another configuration, the pressure from pressure sources can be implemented such that pressure is highest from pressure source 6750, next highest from pressure source 6725, and lowest from pressure source 6740. Pressure source 6705 can be at any pressure. The resulting fluid flow in the system 6700 is that fluid is backflushed from the fluid flow path comprising the column 6735 into the fluid flow path comprising the column 6730.

[00211] In a different configuration, the pressure from pressure sources 6740 and 6750 can be highest, with the next highest from pressure source 6725 and the lowest pressure from the pressure source 6705. The resulting fluid flow in the system 6700 is that fluid is backflushed from both of the fluid flow paths including the columns 6730 and 6735 through the microfluidic device 6720 and to a vent.

[00212] While the illustrative fluid flows described above are provided in reference to using a splitting device as the microfluidic device 6720, additional fluid flows are possible where the microfluidic device 6720 includes additional inlet and outlet ports and is used in conjunction with one more valves as described elsewhere herein.

[00213] In certain examples, another illustrative configuration of a system that can be used for backflushing is shown in FIG. 69. The system 6900 includes an injector 6910 fluidically coupled to a pressure source 6905. The injector is fluidically coupled to a switching device 6920, e.g., an S-swafer, D-swafer or T-swafer available from PerkinElmer Health Sciences, Inc., through a restrictor 6915. The switching device 6920 is fluidically coupled to a pressure source 6925, a restrictor 6930, and a chromatography column 6935. The restrictor 6930 is fluidically coupled to a restrictor 6942, which can be omitted is restrictor 6930 is designed for suitable fluid flow as described herein. The restrictor 6942 is fluidically coupled to a pressure source 6940 and a detector 6945. A chromatography column 6935 is fluidically coupled to a restrictor 6952, which itself is coupled to a pressure source 6950 and a detector 6955. Each of restrictors 6942 and 6952 can be fluidically coupled to components other than a detector, e.g., an additional switching device or the like. The pressure inside the injector 6910 is regulated by a carrier gas supply from the pressure source 6905. The restrictor 6915 may take many configurations such as a short piece of deactivated fused silica restrictor tubing. A supply of a carrier gas can be

introduced using pressure sources 6795, 6940 and 6950 to control the overall pressure in the system 6900.

[00214] In certain embodiments, the switching device 6920 can be configured as an S-swafer, a D-swafer, a T-swafer or other suitable switching devices including, but not limited to, splitting devices, two-way switching devices, three-way switching devices and other devices that can be used to direct a fluid flow in a system. It will be within the ability of the person of ordinary skill in the art, given the benefit of this disclosure, to use with switching devices described herein in the backflushing methods and systems described herein.

[00215] In certain examples, the systems described herein can be used to modulate sample flow. For example, the fluid pressure of the system can be controlled to permit a sample to flow from the injector and into the microfluidic device and to a first fluid flow path and a second fluid flow path each fluidically coupled to the microfluidic device at a separate outlet port of the microfluidic device. The pressure of the system can ten be altered to reverse the flow of the sample in at least one of the first fluid flow path and the second fluid flow path to backflush sample in the fluid flow path where the pressure is reversed. If desired the pressure can be altered after a portion of the sample has eluted from the chromatography column in the first fluid flow path to reverse the sample flow or even after a portion of the sample has eluted and been detected. Depending on the desired components in the system, the microfluidic device can be a splitter, a two-way switching device, a three-way switching device, and the overall system can include more than a single microfluidic device.

[00216] In certain embodiments where backflushing is implemented, the pressure in one of the fluid flow paths to backflush column effluent in that fluid flow path to the other fluid flow path. Such alteration can be performed before all column effluent is detected or can be performed after at least some column effluent is detected. In other embodiments, altering the pressure in one of the fluid flow paths to reverse sample flow in that fluid flow path acts to direct the reversed sample flow to the other fluid flow path. For example, a user can select pressures in the system to permit sample flow into a first fluid flow path fluidically coupled to the microfluidic device. The pressures can be adjusted to reverse the sample flow in the first fluid flow path and direct the reversed sample flow into a second fluid flow path fluidically coupled to the microfluidic device.

[00217] In certain embodiments, the backflushing techniques and systems described herein can be used with existing analysis methods or may replace such existing analysis methods. Illustrative analytical methods includes but are not limited to ASTM-D4815 – 09. In ASTM Method D-4815, a TCEP precolumn is used to remove volatile hydrocarbons from an injected sample. The residual sample containing the oxygenated analytes is backflushed onto a methyl silicone. Chromatographic separation of these analytes occurs in this column and then any residual hydrocarbons left in the column are backflushed to vent. There is a need to backflush both columns and be able to transfer the backflushed effluent between columns. Method D-4815 specifies a 10-port mechanical valve to perform these functions. Mechanical valves are not well suited to high resolution capillary chromatography for a number of reasons: internal dead volumes – the fittings and internal channels inside a mechanical valve are not well suited to handle sample streams at low flow rates and peaks may broaden or tail as a result; active surfaces – the sample stream may be exposed to metallic or other active surfaces causing loss of sensitive analyte; thermal mass – mechanical valves are (in GC terms) large and heavy and will not track the column oven temperature. They have to be continually heated for many applications which may cause breakdown of temperature sensitive compounds; temperature limit – the rotor material in the valves will have a temperature limit which may be restrictive for some sample types; moving parts – the rotor rotates within the valve body. There is friction and wear associated with this and the rotor will eventually wear out and leak. A small leak would be highly disruptive in capillary chromatography; carrier gas pressure transients – operation of a mechanical valve will often cause a momentary change in the pressure (and flow rate) of the gas stream. On many systems this will manifest itself as detector baseline disturbance or step; change in column flow rate – switching the position of a mechanical valve normally applies carrier gas from a different source to columns. This affects peak retention times. For method development, this may mean that each time a valve timed event is modified, it will affect the timing of other timed events with would then need to be modified too. This may make method development time consuming and tedious. The systems and methods described herein can be used with the materials and methods of ASTM D-4815 to advantageously analyze samples. The backflushing techniques and system may also be used in ASTM D5580 -02 (2007). In this method, benzene, toluene, ethylbenzene, the xylenes, C9 and heavier aromatics, and total aromatics in finished

motor gasoline by gas chromatography are determined. Other ASTM methods can also be implemented using the microfluidic devices described herein either alone or with the backflushing techniques and systems described herein.

[00218] In certain examples, the devices, methods and systems described herein (or portions thereof) can be implemented or controlled using a computer or other device that includes a processor, or the devices and systems described herein can be electrically coupled to a computer system or processor. Such computer implemented methods can provide for more user friendly implementation of the methods by permitting control using a graphical user interface or the like. In addition, the computer can be used to monitor flow rates, receive data from one or more detectors and to store or recall separation routines for subsequent use. The computer system typically includes at least one processor optionally electrically coupled to one or more memory units. The computer system may be, for example, a general-purpose computer such as those based on Unix, Intel PENTIUM-type processor, Motorola PowerPC, Sun UltraSPARC, Hewlett-Packard PA-RISC processors, or any other type of processor. In some examples, the processor may be an inexpensive processor that may be programmable to receive inputs and output treatment parameters based on the received inputs. It should be appreciated that one or more of any type computer system may be used according to various embodiments of the technology. Further, the system may be located on a single computer or may be distributed among a plurality of computers attached by a communications network. A general-purpose computer system may be configured, for example, to perform any of the described functions including but not limited to: restrictor length and diameter calculations, gas source control, switching valve control, temperature control, run times, and the like. It should be appreciated that the system may perform other functions, including network communication, and the technology is not limited to having any particular function or set of functions.

[00219] For example, various aspects may be implemented as specialized software executing in a general-purpose computer system. The computer system may include a processor connected to one or more memory devices, such as a disk drive, memory, or other device for storing data. Memory is typically used for storing programs and data during operation of the computer system. Components of the computer system may be coupled by an interconnection device, which may include one or more buses (e.g., between components that are integrated within a same machine) and/or a network

(e.g., between components that reside on separate discrete machines). The interconnection device provides for communications (e.g., signals, data, instructions) to be exchanged between components of the system. The computer system typically is electrically coupled to the detector such that electrical signals may be provided to and from the detector to the computer to receive data for storage and/or processing. The computer system may also include one or more input devices, for example, a keyboard, mouse, trackball, microphone, touch screen, manual switch (e.g., override switch) and one or more output devices, for example, a printing device, display screen, speaker. In addition, the computer system may contain one or more interfaces (not shown) that connect the computer system to a communication network (in addition or as an alternative to the interconnection device).

[00220] The storage system typically includes a computer readable and writeable nonvolatile recording medium in which signals are stored that define a program to be executed by the processor or information stored on or in the medium to be processed by the program. For example, the oven temperatures, flow rates, switching valve position and modulation frequencies and the like for a particular separation may be stored on the medium. The medium may, for example, be a disk or flash memory. Typically, in operation, the processor causes data to be read from the nonvolatile recording medium into another memory that allows for faster access to the information by the processor than does the medium. This memory is typically a volatile, random access memory such as a dynamic random access memory (DRAM) or static memory (SRAM). It may be located in the storage system or in the memory system. The processor generally manipulates the data within the integrated circuit memory and then copies the data to the medium after processing is completed. A variety of mechanisms are known for managing data movement between the medium and the integrated circuit memory element and the technology is not limited thereto. The technology is also not limited to a particular memory system or storage system.

[00221] In certain examples, the computer system may also include specially-programmed, special-purpose hardware, for example, an application-specific integrated circuit (ASIC). Aspects of the technology may be implemented in software, hardware or firmware, or any combination thereof. Further, such methods, acts, systems, system elements and components thereof may be implemented as part of the computer system described above or as an independent component.

[00222] Although a computer system is described by way of example as one type of computer system upon which various aspects of the technology may be practiced, it should be appreciated that aspects are not limited to being implemented on the illustrated computer system. Various aspects may be practiced on one or more computers having a different architecture or components. The computer system may be a general-purpose computer system that is programmable using a high-level computer programming language. The computer system may be also implemented using specially programmed, special purpose hardware. In the computer system, the processor is typically a commercially available processor such as the well-known Pentium class processor available from the Intel Corporation. Many other processors are available. Such a processor usually executes an operating system which may be, for example, the Windows 95, Windows 98, Windows NT, Windows 2000 (Windows ME), Windows XP or Windows Vista operating systems available from the Microsoft Corporation, MAC OS System X operating system available from Apple Computer, the Solaris operating system available from Sun Microsystems, or UNIX or Linux operating systems available from various sources. Many other operating systems may be used, and in certain embodiments a simple set of commands or instructions may function as the operating system.

[00223] In accordance with certain examples, the processor and operating system may together define a computer platform for which application programs in high-level programming languages may be written. It should be understood that the technology is not limited to a particular computer system platform, processor, operating system, or network. Also, it should be apparent to those skilled in the art, given the benefit of this disclosure, that the present technology is not limited to a specific programming language or computer system. Further, it should be appreciated that other appropriate programming languages and other appropriate computer systems could also be used. In certain examples, the hardware or software is configured to implement cognitive architecture, neural networks or other suitable implementations.

[00224] One or more portions of the computer system may be distributed across one or more computer systems coupled to a communications network. These computer systems also may be general-purpose computer systems. For example, various aspects may be distributed among one or more computer systems configured to provide a service (e.g., servers) to one or more client computers, or to perform an overall task as part of a distributed system. For example, various aspects may be

performed on a client-server or multi-tier system that includes components distributed among one or more server systems that perform various functions according to various embodiments. These components may be executable, intermediate (e.g., IL) or interpreted (e.g., Java) code which communicate over a communication network (e.g., the Internet) using a communication protocol (e.g., TCP/IP). It should also be appreciated that the technology is not limited to executing on any particular system or group of systems. Also, it should be appreciated that the technology is not limited to any particular distributed architecture, network, or communication protocol.

[00225] In accordance with certain examples, various embodiments may be programmed using an object-oriented programming language, such as SmallTalk, Basic, Java, C++, Ada, or C# (C-Sharp). Other object-oriented programming languages may also be used. Alternatively, functional, scripting, and/or logical programming languages may be used. Various configurations may be implemented in a non-programmed environment (e.g., documents created in HTML, XML or other format that, when viewed in a window of a browser program, render aspects of a graphical-user interface (GUI) or perform other functions). Certain configurations may be implemented as programmed or non-programmed elements, or any combination thereof.

[00226] In certain examples, a user interface may be provided such that a user may enter desired flow rates, tubing lengths and diameters, column types, solvent gradient runs and other information commonly entered prior to a gas or liquid chromatography separation is commenced. Other features for inclusion in a user interface will be readily selected by the person of ordinary skill in the art, given the benefit of this disclosure.

[00227] In certain embodiments, the microfluidic devices described herein may be packaged in a kit optionally with instructions for using the microfluidic device. In some examples, the kit may further include a computer readable medium that contains algorithms suitable for implementing flow control or modulation as described herein. The kit may further include fittings, tubing, restrictors or the like of a desired length or diameter to facilitate a desired flow rate in the system. In some examples, one or more separation columns may also be included in the kit.

[00228] Certain specific examples are described below to illustrate further some of the new and useful features of the technology described herein.

Example 1

[00229] To validate the tubing diameter algorithms, a length of fused silica tubing (listed as having an internal diameter of 150 microns) was tested with helium and nitrogen carrier gases. A least squares linear fit was applied to the flow rate versus the square of the absolute applied pressure to establish the value of the constant b in Equation (17) and d_c was calculated from Equation (18). The ambient pressure was determined from a digital barometer at the location and the viscosity at the ambient temperature was taken from tables. The results are given in Tables IV (helium gas) and V (nitrogen gas) and are listed in order of decrementing length L.

Table IV

	Measured Flow Rate (mL/min)										
L (cm)	80 (psig)	70 (psig)	60 (psig)	50 (psig)	40 (psig)	30 (psig)	20 (psig)	10 (psig)	5 (psig)	Fit r^2	d_c (µm)
200	42.40	33.70	25.90	19.30	13.50	8.74	4.68	1.87		1.0000	152.9
190	44.20	35.40	27.10	20.30	14.30	9.16	4.91	2.01		0.9999	152.6
180	46.90	37.40	28.70	21.50	15.10	9.64	5.20	2.15		1.0000	152.8
170	49.30	39.00	30.30	22.60	15.90	10.10	5.48	2.29		0.9999	152.4
160	53.10	42.30	32.60	24.20	16.90	10.80	5.87	2.44		1.0000	153.1
150	55.50	44.30	34.40	25.50	18.00	11.40	6.25	2.61		1.0000	152.3
140	60.70	48.40	37.20	27.40	19.50	12.50	6.82	2.84		1.0000	153.0
130	65.40	52.10	40.00	29.60	21.00	13.50	7.31	3.04		1.0000	153.0
120	70.50	56.40	43.60	32.30	22.60	14.60	8.01	3.20		1.0000	152.9
110	76.30	61.20	47.40	35.10	24.60	15.90	8.75	3.56		1.0000	152.6
100	84.00	67.00	52.00	38.40	27.00	17.40	9.60	3.86		1.0000	152.6
90	93.00	74.20	57.80	42.80	29.80	19.40	10.60	4.25	1.90	1.0000	152.5
80	104.00	82.90	64.70	48.00	33.60	21.60	12.00	4.65	2.10	0.9999	152.3
70	118.00	94.10	73.20	54.40	38.20	24.60	13.80	5.28	2.35	0.9999	152.0
60	136.00	109.00	84.80	63.10	44.20	28.30	15.90	6.16	2.74	1.0000	151.6
50	163.00	130.00	101.00	75.00	52.80	34.00	19.00	7.42	3.30	1.0000	151.4
40	201.00	162.00	125.00	93.60	66.10	42.70	23.70	9.48	4.14	0.9999	151.0
30	265.00	211.00	166.00	123.00	87.10	56.50	31.50	12.60	5.43	0.9998	150.4

Table V

	Measured Flow Rate (mL/min)										
L (cm)	80 (psig)	70 (psig)	60 (psig)	50 (psig)	40 (psig)	30 (psig)	20 (psig)	10 (psig)	5 (psig)	Fit r^2	d_c (µm)
200		37.60	28.90	21.70	15.30	9.77	5.19	2.21		0.9999	153.5
190		38.70	30.00	22.40	15.80	10.20	5.47	2.25		0.9999	152.7
180		40.30	31.50	23.50	16.50	10.60	5.74	2.37		0.9999	152.3
170		43.80	33.80	25.20	17.80	11.40	6.19	2.55		1.0000	153.1
160		46.30	35.90	26.90	18.90	12.10	6.64	2.74		0.9999	153.0
150		47.60	37.20	27.80	19.90	12.80	7.02	2.90		0.9998	151.5
140		52.70	41.20	30.40	21.50	14.00	7.64	3.11		0.9999	152.8
130		56.10	44.00	32.80	23.20	15.10	8.23	3.37		0.9998	152.4
120		59.60	47.00	35.00	24.80	16.10	8.80	3.60		0.9998	151.7
110			52.10	38.40	27.10	17.50	9.76	3.94		1.0000	152.9
100			56.90	42.50	29.70	19.30	10.70	4.26		1.0000	152.8

Tables IV and V show that a highly consistent value for the internal diameter is achieved as the restrictor tubing is progressively shortened. The mean values (152.3µm for helium and 152.6µm for nitrogen) are very close and the precision in the calculations is excellent (0.49% RSD for helium and 0.40 % RSD for nitrogen).

[00230] To validate the accuracy of these results, the sections of fused silica tubing removed during the flow measurement tests were examined under a 500x magnification microscope and the true diameters determined by photomicrography. FIG. 55 shows a photomicrograph of the end of one section of the fused silica tubing with an overlaid graticule with 10 microns scale divisions. Table VI lists the results of the manual measurements taken through the microscope. These measurements agree very closely with the calculated values in Tables IV and V.

Table VI

Section Taken (cm)	Measured Bore (µm)
200	153
180	153
160	152
140	152
120	153
100	153

Thus, to better determine the true size of tubing used in fluid chromatography systems, a calibration protocol can be implemented to accurately assess the true internal diameter of tubing, e.g., columns, restrictors, etc. used in the systems.

Example 2

[00231] The results from a thermal desorption system that uses algorithms based on the equations described above to control the flow rate of gas through a transfer line and a column are shown in FIG. 56. The carrier gas was doped with a fixed concentration of methane so that the mass flow rate of gas eluting from the column could be directly monitored by a flame ionization detector. FIG. 56 shows plots of the detector signal during a column temperature program with constant pressure control and then with constant flow control. As can be seen from these plots, the mass flow rate is reasonably constant during the program when constant flow control is used.

[00232] In FIG. 56, the flow rates were controlled as the column temperature was increased. The methane was added to the column and the resulting signal was used to monitor the detector response as a function of the flow rate. The oven was heated to 40 °C and maintained at this temperature for 1 minute. The temperature was then increased by 10 °C/minute up to a final temperature of 300 °C. The final temperature was maintained for 10 minutes. Curve 5610 represents the actual flow rate, curve 5620 represents the expected flow rate, and curve 5630 represents the flow rate without use of the flow control (where constant pressure control was used). Where pressure control was used, the flow rate differed markedly from the desired flow rate. Where the flow control algorithms described herein are implemented, the flow rate closely tracked that of the desired flow rate.

Example 3

[00233] FIGS. 34 and 35A-35C show illustrations of chromatography peaks where modulation was performed, as described herein. Referring to FIG. 34, a single peak is shown. The modulation breaks the peak into a plurality of individual modulated portions. Modulation was performed at 10 Hz using the controller of FIGS. 33A and 33B and the microfluidic device of FIG. 11. The column used was a 30 m x 0.25 mm x 0.25 micron methyl silicone column. Helium was used as the mobile phase. The inlet pressure was 40 psig, and the midpoint pressure was 30 psig (helium gas). The oven was heated to 35 °C and maintained at this temperature for 1 minute. The temperature was then increased by 10 °C per minute up to a temperature of 300 °C. A fast FID detector at 275 °C was used to detect the peaks. A 100 mL/min split injector was used to introduce the sample, which was 1 microgram/microliter NIOSH aromatics (0.2 microliters was injected).

[00234] Referring to FIGS. 35A-35C, three different traces are shown. In FIG. 35A, two peaks 3510, 3520 representative of sample effluent from a first column are shown. In FIG. 35B, a single peak 3530 representative of sample effluent from a second column is shown. These peaks can be analyzed simultaneously using the modulation techniques described herein. Referring to FIG. 35C, the modulated output of the different samples is shown where, for example, the sample from the first and second columns can be provided to a single output. The peaks 3510 and 3530 overlap or are interlaced in the modulated output as shown in the modulated signal group 3540. The

peak 3520 is shown as a modulated peak 3550. In this manner, sample peaks from different columns can be analyzed simultaneously.

Example 4

[00235] A microfluidic device that included an internal bypass restrictor is shown in FIG. 57. The resulting microfluidic device (FIG. 57) included a plurality of ports 5705, 5710, 5715, 5720, 5725 and 5730. The port 5705 is designed to receive effluent from a column. A switching gas from a solenoid valve can be connected to each of the ports 5715 and 5725. Outlet ports 5710 and 5730 can be connected to columns or restrictors or other devices. The internal bypass restrictor 5750 has a diameter that is less than that of other portions 5760 of the internal microfluidic channel. The particular diameter and length selected for this internal bypass restrictor can provide for flow control using the microfluidic device.

Example 5

[00236] A Clarus 680 was equipped with a PSS injector, 2x FIDs, an S-Swafer system and two PreVent detector adapters; all with supporting PPC modules. Two bleed regulators for the PreVent PPC modules were attached to a bracket (that is included in the PreVent installation kits) and mounted under the GC top cover as shown in the photo in FIG. 70. FIG. 71 shows a photograph of the installed system in the GC oven.

[00237] The examples that follow used the chromatography conditions and settings shown in Table VII.

Table VII

Gas Chromatograph	PerkinElmer Clarus 680
Oven	60°C isothermal
Injector	Programmable Split/Splitless. 100mL/min Split at 200°C
Detector	Flame Ionization at 200°C Air 450mL/min, Hydrogen 45mL/min Range x1, Attenuation x16
Splitting Device	S-Swafer
Backflush Devices	PreVent detector adapters with 137mm x 75µm restrictors
Precolumn	30m x 0.25mm x 0.25µm Elite-1
Analytical Column	a) 50m x 0.25mm x 0.4µm TCEP b) 60m x 0.32mm x 5.0µm methyl silicone
Injector-Swafer Restrictor	Fused silica, 60cm x 0.100mm

Carrier Gas	Hydrogen
Carrier Gas Pressure Programming	As shown in the Figures in each example below
Injection	0.3μL by Autosampler in fast mode. 4 solvent washes following injection.
Sample	D-4815 calibration mixture (ex. AccuStandard) containing alcohols and ethers at concentrations ranging from 4 to 7.3%.

Example 6

[00238] FIG. 72 shows how gas pressures were applied to enable the injected sample to be simultaneously chromatographed on both columns with this system. Carrier gas flows forward from the Swafer 7230 through both columns 7240, 7250 and through their PreVent adapters 7242, 7252, respectively, and into their detectors 7260, 7270, respectively. The applied pressures (and those in the figures following) were calculated using the Swafer Utility Software available from Perkin Elmer Health Sciences, Inc. The resultant chromatography using the D-4815 calibration mixture is shown in FIGS. 73A and 73B.

Example 7

[00239] FIG. 74 shows how the gas pressure at the PreVent adapter at the exit of the methyl silicone column may be re-adjusted upward, e.g., from 20 psig to 31 psig, to give a slight backflow so that the whole sample is now directed into the TCEP column. All other conditions remained the same as shown in FIG. 72. The resultant chromatography is shown in FIG. 75B. No chromatography peaks were detected by the detector connected to the methyl silicone column (FIG. 75A).

Example 8

[00240] In the same way as for FIG. 74, the injected sample may be directed to the methyl silicone column by adjusting the pressure at the PreVent adapter at the exit to the TCEP column as shown in FIG. 76. All other conditions remained the same as shown in FIG. 72. The resultant chromatography is shown in FIG. 77A. No chromatography peaks were detected by the detector connected to the TCEP column (FIG. 77B).

Example 9

[00241] By changing the applied pressures during a run, part or whole of the sample may be moved between columns or vented. For example, if the conditions shown in FIG. FIG. 74 are applied to enable injection into only the TCEP column and then, after 3.70 minutes, the conditions shown in FIG. 78 are applied to backflush this column and direct the backflushed effluent to the methyl silicone column. This result is achieved by raising the pressure at the PreVent adapter at the end of the TCEP column to reverse the direction of the carrier gas flow through that column and decrease the pressure at the PreVent adapter at the end of the methyl silicone column to allow gas (including the backflushed vapor) to flow into it from the switching device. During the backflushing, the pressure at the injector was reduced so that only a small flow rate flowed into the switching device to prevent the backflushed vapor from being lost into the injector.

[00242] FIGS. 79A and 79B shows the chromatography of the early-eluting components on the TCEP column with (FIG. 79A) and without (FIG. 79B) the backflush conditions shown in FIG. 78 applied at 3.70 minutes. The first 5 peaks are effectively eliminated from the backflushed effluent that enters the second column. These 5 peaks are the ethers contained in the standard mixture.

[00243] With the backflush settings applied, all sample left in the column after 3.70 minutes will be redirected to the methyl silicone column chromatographed on that column. The resultant chromatography on the methyl silicone column is shown in FIG. 80. The positions of the 5 'missing' ethers are highlighted in red text. This chromatogram was produced in the same run as the chromatogram shown in FIG. 79B.

Example 10

[00244] The chromatography in the methyl silicone column as shown in FIG. 80 may be further processed by backflushing. For instance, if only the first 5 peaks are of interest (i.e. the lighter alcohols), then the remainder of the sample left in the methyl silicone column may be backflushed to vent by applying the conditions shown in FIG. 81 after the last peak of interest has eluted at 23.6 minutes. This backflushing is enabled by raising the pressure at the PreVent adapter at the exit of the methyl silicone column, adjusting the pressure at the PreVent adapter at the TCEP column exit to deliver a low reverse flow and reducing the pressure at the injector to accept the backflushed effluent. The injector split vent is open during this process.

[00245] FIG. 82 shows a chromatogram of the sample which has been introduced into the TCEP column, part of it was backflushed into the methyl silicone column where it was chromatographed; finally, some further backflushing was performed to remove unwanted later eluting columns. Portions of this sample have effectively traveled 3 times along the lengths of these columns and been backflushed twice. Even so, better than satisfactory chromatography performance was obtained.

[00246] When introducing elements of the examples disclosed herein, the articles "a," "an," and "the" are intended to mean that there are one or more of the elements. The terms "comprising," "including" and "having" are intended to be open ended and mean that there may be additional elements other than the listed elements. It will be recognized by the person of ordinary skill in the art, given the benefit of this disclosure, that various components of the examples can be interchanged or substituted with various components in other examples.

[00247] Although certain features, aspects, examples and embodiments have been described above, additions, substitutions, modifications, and alterations of the disclosed illustrative features, aspects, examples and embodiments will be readily recognized by the person of ordinary skill in the art, given the benefit of this disclosure.

CLAIMS:

1. A method of modulating flow of a fluid in a chromatography system including a microfluidic device comprising a laminated wafer body fluidically coupled to an injector, the method comprising:

controlling fluid pressure of the system to permit a sample to flow from the injector and into the laminated wafer body of the microfluidic device and to a first fluid flow path and a second fluid flow path each fluidically coupled to the laminated wafer body of the microfluidic device at a separate outlet port of the laminated wafer body of the microfluidic device; and

altering the pressure of the system to reverse the flow of the sample in at least one of the first fluid flow path and the second fluid flow path to backflush sample in the fluid flow path where the pressure is reversed.

2. The method of claim 1, further comprising configuring each of the first fluid flow path and the second fluid with a chromatography column.

3. The method of claim 2, further comprising altering the pressure of the system to reverse flow of the sample after a portion of the sample has eluted from the chromatography column in the first fluid flow path to reverse the sample flow.

4. The method of claim 3, further comprising controlling the pressure of the system to direct the reversed sample flow to the chromatography column in the second fluid flow path.

5. The method of claim 2, further comprising altering the pressure of the system to reverse flow of the sample after a portion of the sample has eluted from the chromatography column in the first fluid flow path and been detected by a detector fluidically coupled to the chromatography column in the first fluid flow path to reverse the sample flow.

6. The method of claim 5, further comprising controlling the pressure of the system to direct the reversed sample flow to the chromatography column in the second fluid flow path.

7. The method of claim 1, further comprising configuring the laminated wafer body of the microfluidic device with an inlet port and two outlet ports each fluidically coupled to the inlet port through an internal microchannel in the laminated wafer body.

8. A system comprising:

a microfluidic device comprising a laminated wafer body comprising an input port and at least two outlet ports each fluidically coupled to the inlet port;

a first fluid flow path fluidically coupled to one outlet port of the laminated wafer body of the microfluidic device;

a second fluid flow path fluidically coupled to the other outlet port of laminated wafer body of the microfluidic device;

a first restrictor in the first fluid flow path, the first restrictor configured to be fluidically coupled to a first pressure source; and

a second restrictor in the second fluid flow path, the second restrictor configured to be fluidically coupled to a second pressure source.

9. The system of claim 8, in which the laminated wafer body of the microfluidic device is configured to be fluidically coupled to a pressure source through an additional port of the laminated wafer body of the microfluidic device, in which the additional port is fluidically coupled to the inlet port and the at least two outlet ports of the laminated wafer body of the microfluidic device.

10. The system of claim 9, in which the pressure sources are configured to control flow of sample in the system.

11. The system of claim 10, in which the second pressure source is configured with a higher pressure to direct sample from the laminated wafer body of the microfluidic device to the first fluid flow path.

12. The system of claim 10, in which the first pressure source is configured with a higher pressure to direct sample from the laminated wafer body of the microfluidic device to the second fluid flow path.

13. The system of claim 10, in which the first and second pressure sources are operative in a first condition to direct sample from the laminated wafer body of the microfluidic device to both the first and second fluid flow paths and are operative in a second condition to direct sample to only one of the first and second fluid flow paths.

14. The system of claim 13, in which the system is configured to permit sample flow during the first condition followed by sample flow during the second condition after a single injection of sample into the system.

15. A method of backflushing column effluent in a chromatography system including two different fluid flow paths each fluidically coupled to a laminated wafer body microfluidic device, the method comprising altering pressure in the laminated wafer body microfluidic device to alter pressure in one of the fluid flow paths to backflush column effluent in that fluid flow path to the other fluid flow path.

16. The method of claim 15, further comprising performing the backflushing step before all column effluent is detected by a detector fluidically coupled to an outlet port of the laminated wafer body microfluidic device.

17. The method of claim 15, further comprising performing the backflushing step after at least some column effluent is detected by a detector fluidically coupled to an outlet port of the laminated wafer body microfluidic device.

18. A method of backflushing in a chromatography system including two different fluid flow paths each fluidically coupled to a laminated wafer body microfluidic device, the method comprising:

selecting pressures in the system to permit sample flow into a first fluid flow path fluidically coupled to the laminated wafer body microfluidic device, the first fluid flow path comprising a first chromatography column fluidically coupled to the laminated wafer microfluidic device and a first restrictor fluidically coupled to the first chromatography column; and

adjusting pressures in the system to reverse the sample flow in the first fluid flow path and direct the reversed sample flow into a second fluid flow path fluidically coupled to the laminated wafer body microfluidic device, the second fluid flow path comprising a second chromatography column fluidically coupled to the microfluidic device and a second restrictor fluidically coupled to the second chromatography column.

19. The method of claim 18, further comprising adjusting the pressures in the system to reverse the sample flow after a portion of the sample has eluted from the first chromatography column and been detected with a detector fluidically coupled to the first restrictor.

20. The method of claim 19, further comprising adjusting the pressure in the system to reverse the reversed sample flow in the second fluid flow path to direct twice-reversed flow into the first fluid flow path.

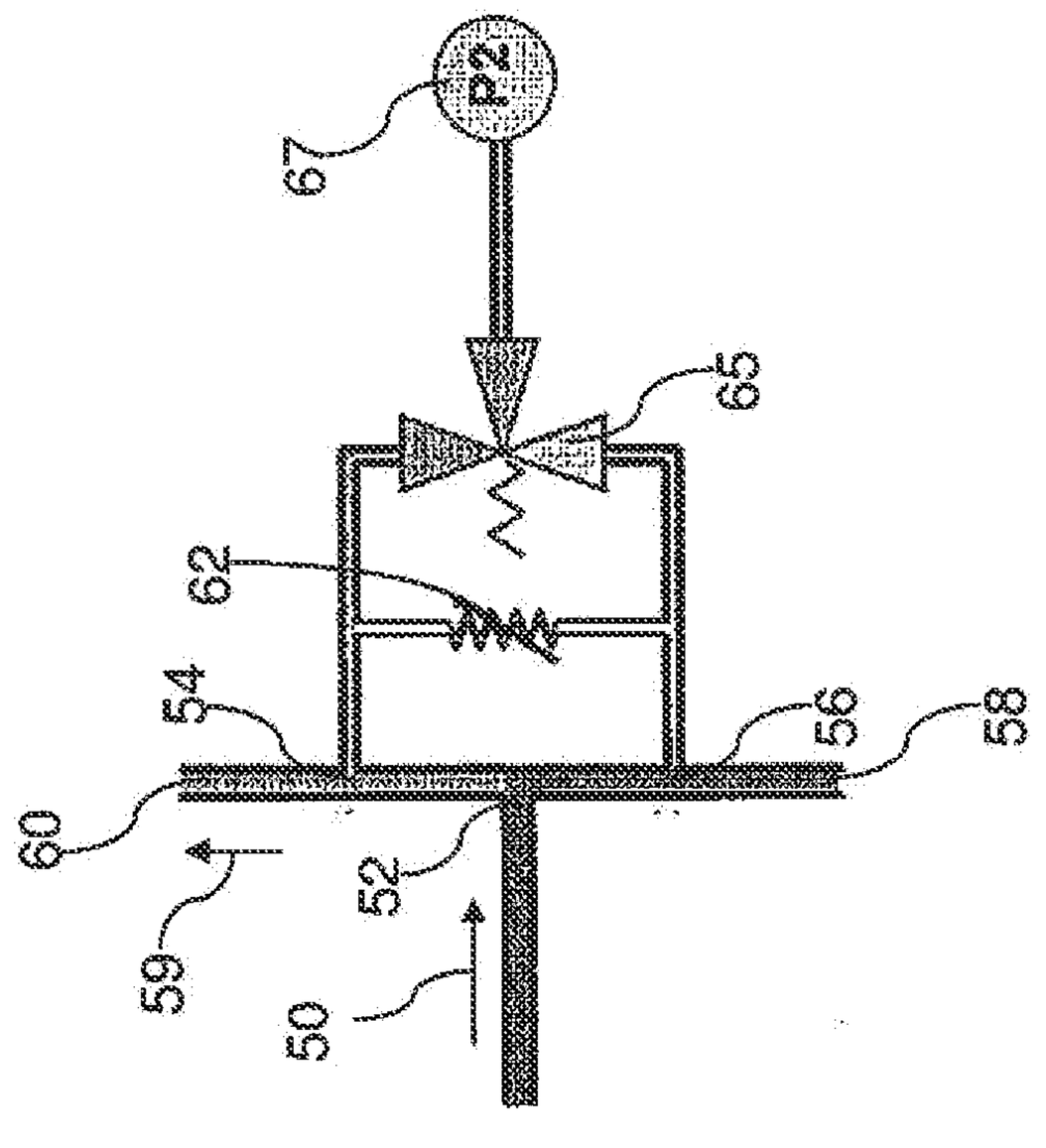


FIG. 1B

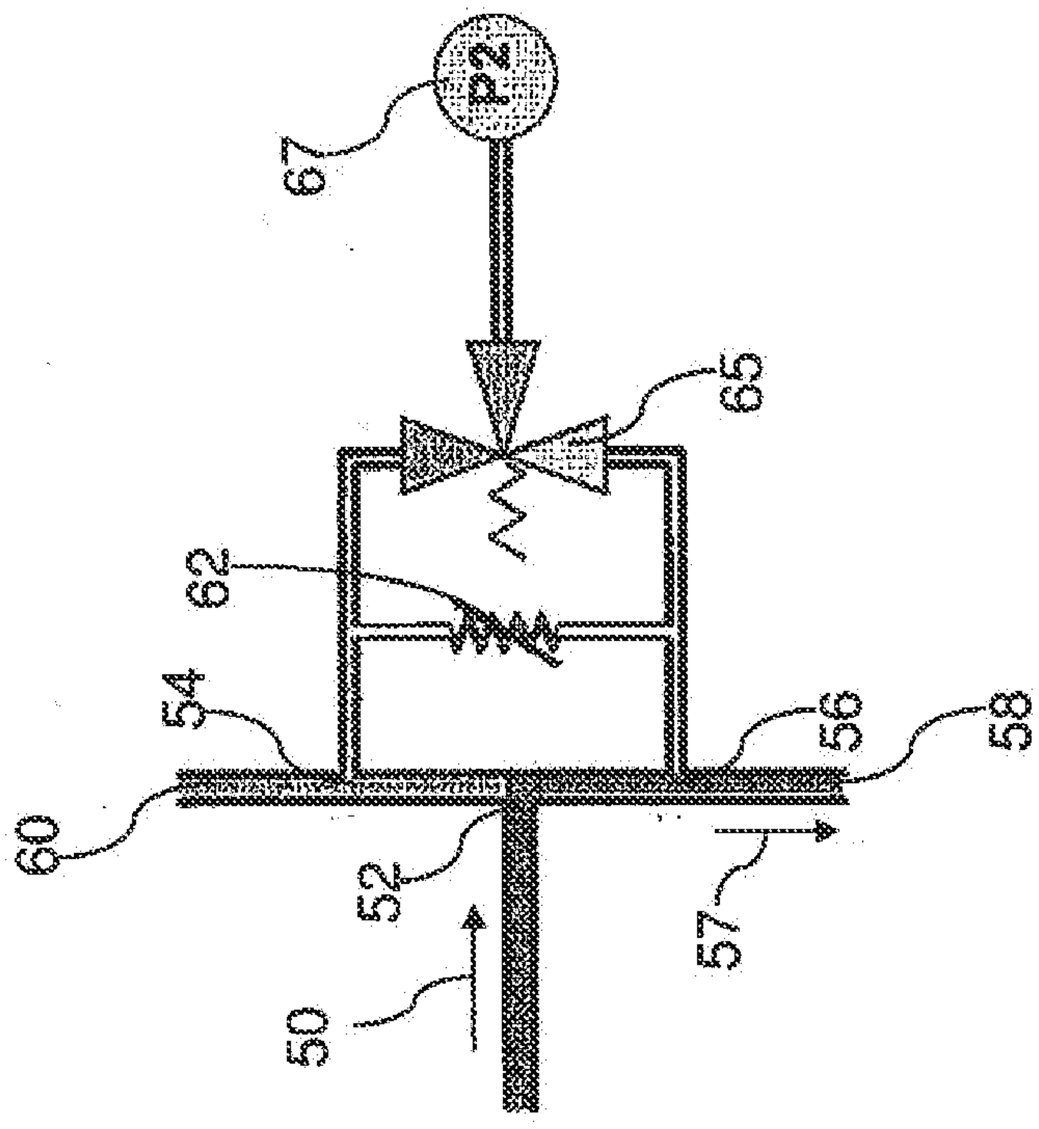


FIG. 1A

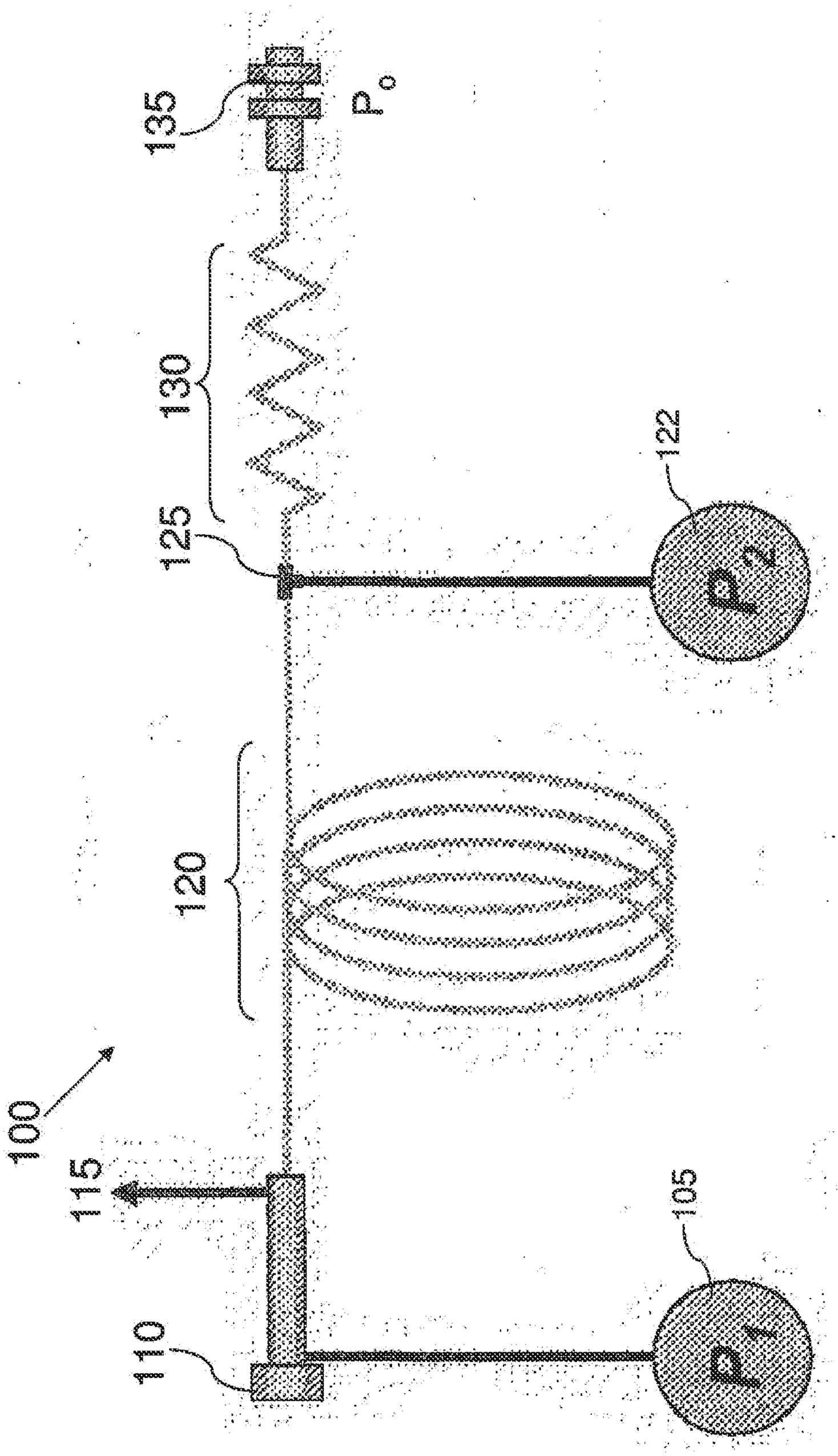


FIG. 2

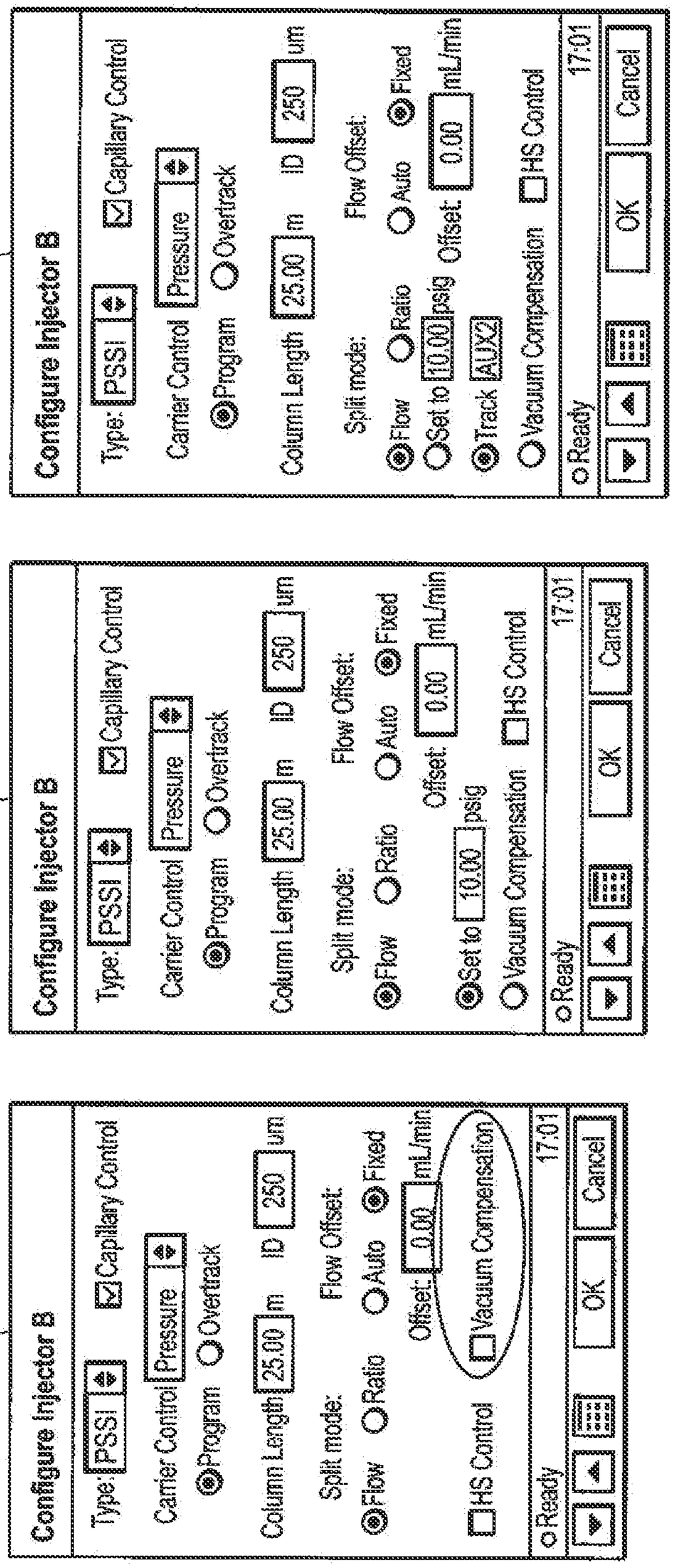


FIG. 3C

FIG. 3B

FIG. 3A

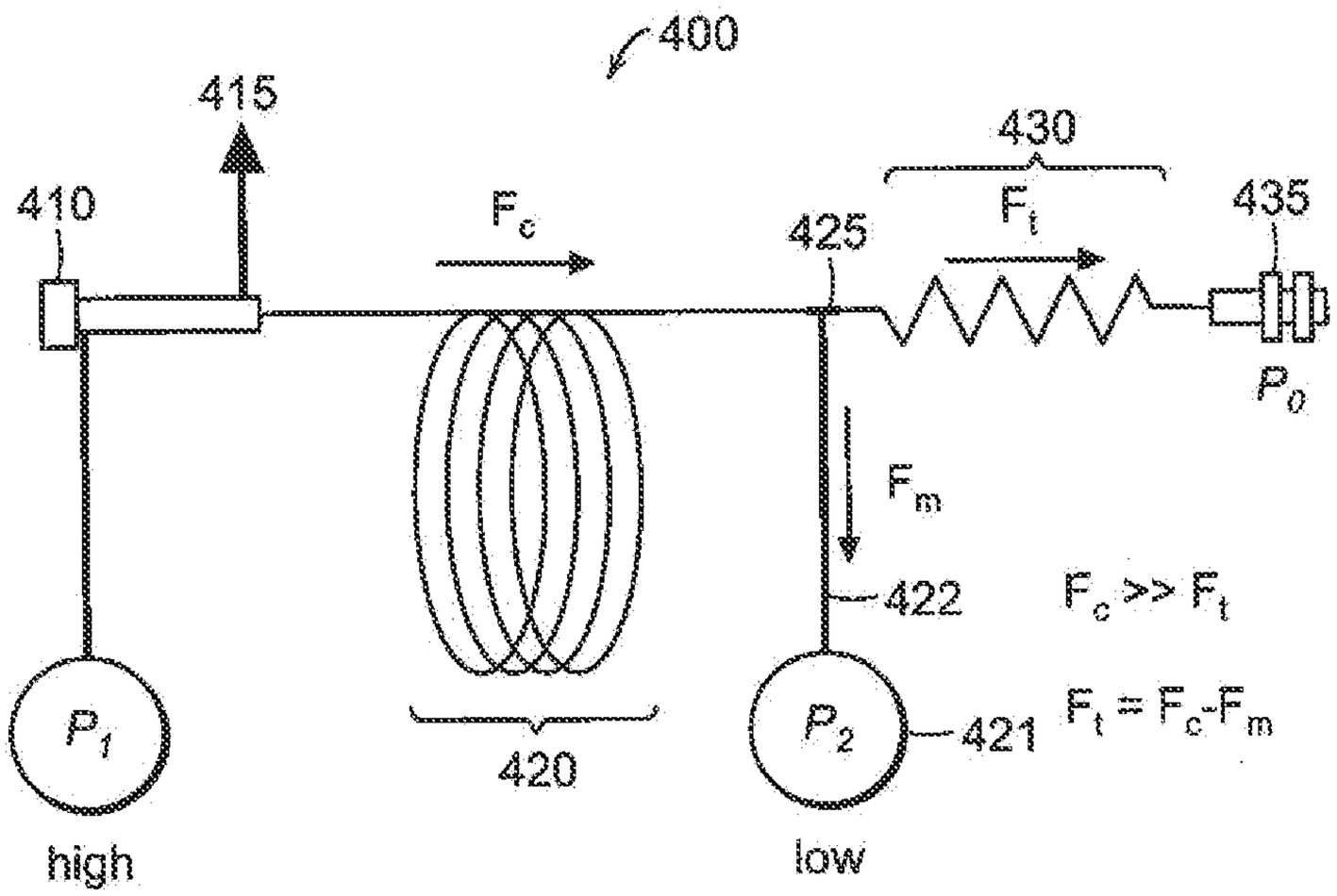

400
415
410
F_c
425
430
F_t
435
P_0
F_m
422
$F_c >> F_t$
$F_t = F_c - F_m$
P_1
420
P_2
421
high
low

FIG. 4A

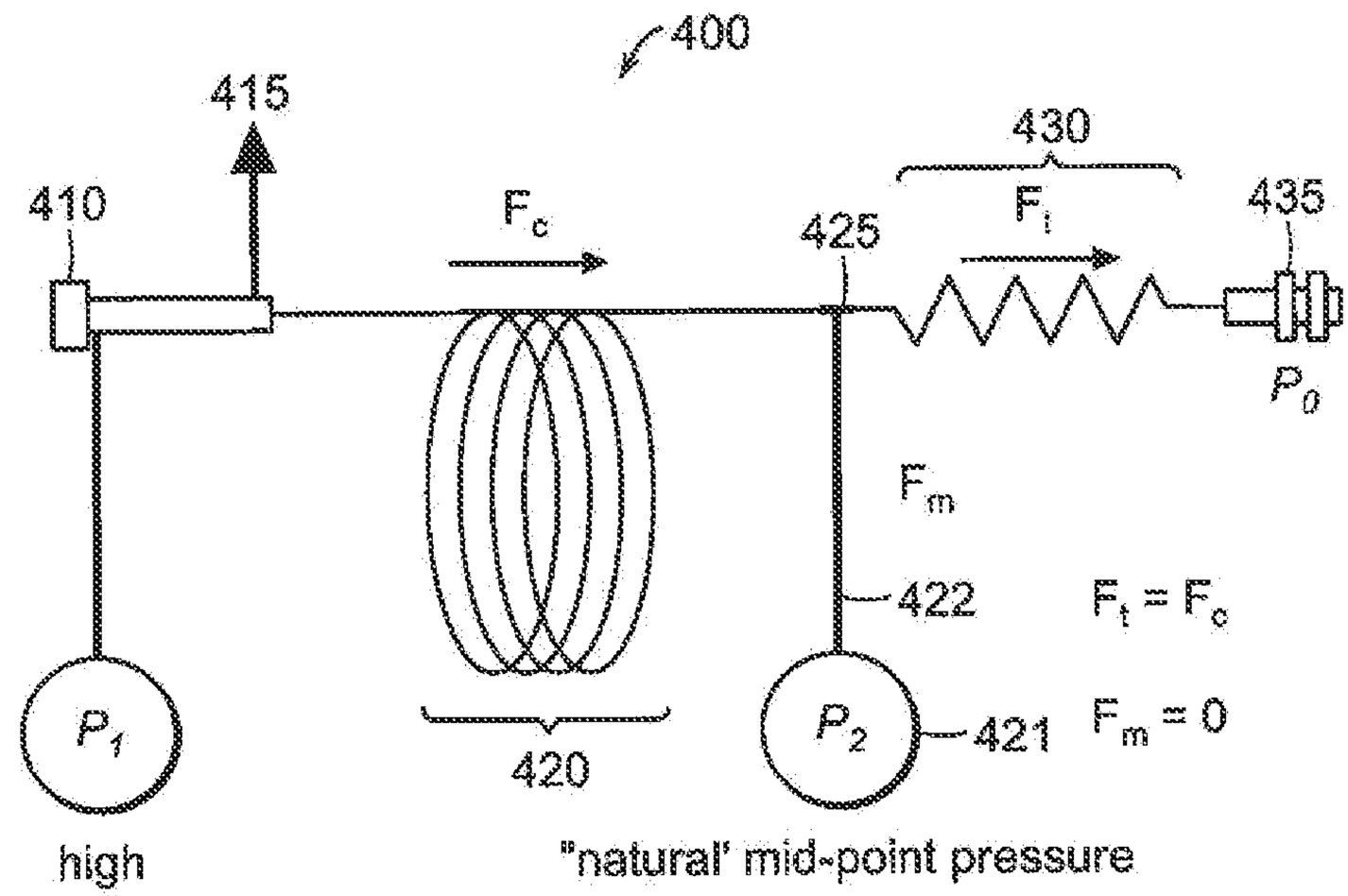

400
415
410
F_c
425
430
F_t
435
P_0
F_m
422
$F_t = F_c$
P_1
420
P_2
421
$F_m = 0$
high
"natural' mid-point pressure

FIG. 4B

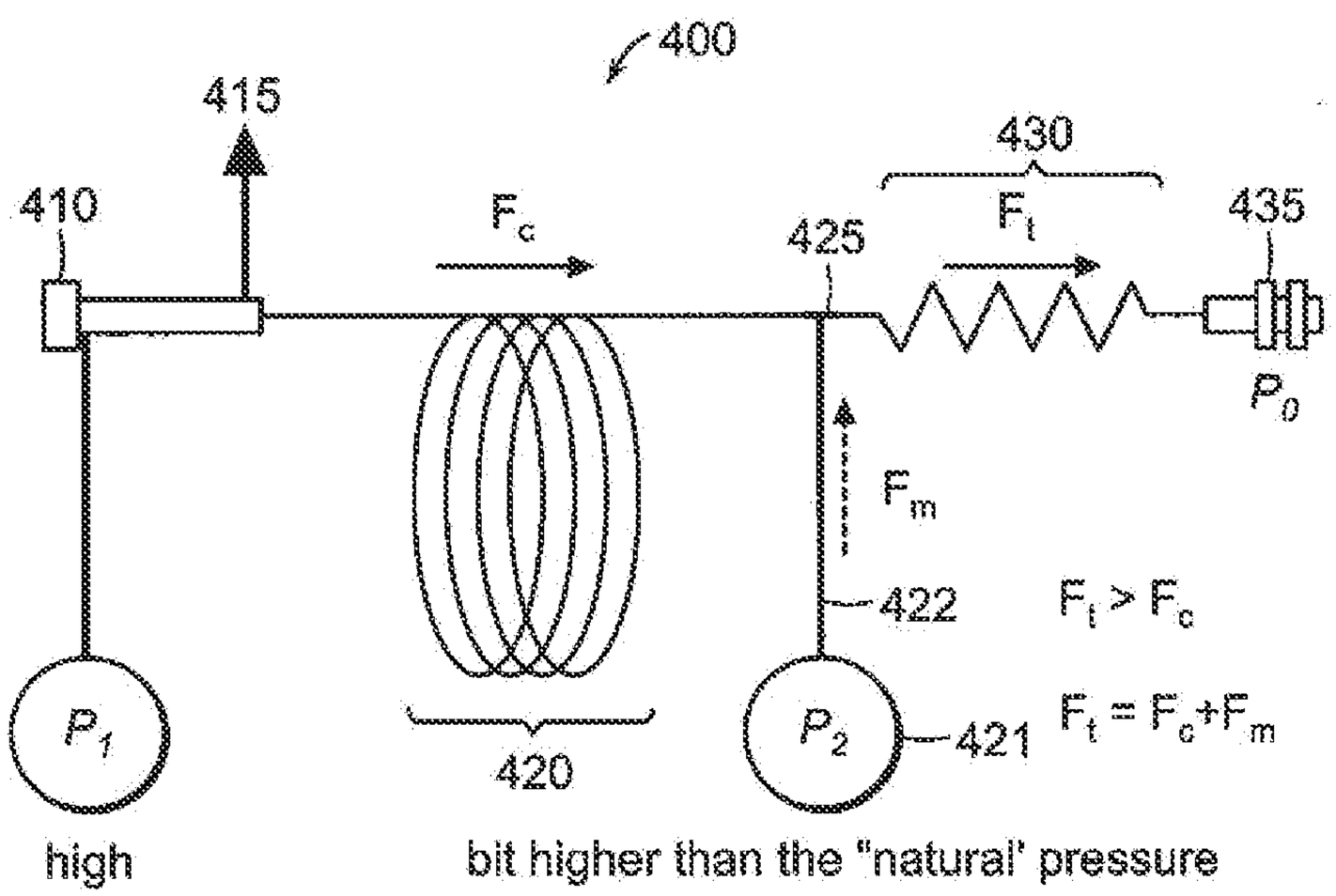

400
415
410
F_c
425
430
F_t
435
P_0
F_m
422
$F_t > F_c$
$F_t = F_c + F_m$
P_1
420
P_2
421
high
bit higher than the "natural' pressure

FIG. 4C

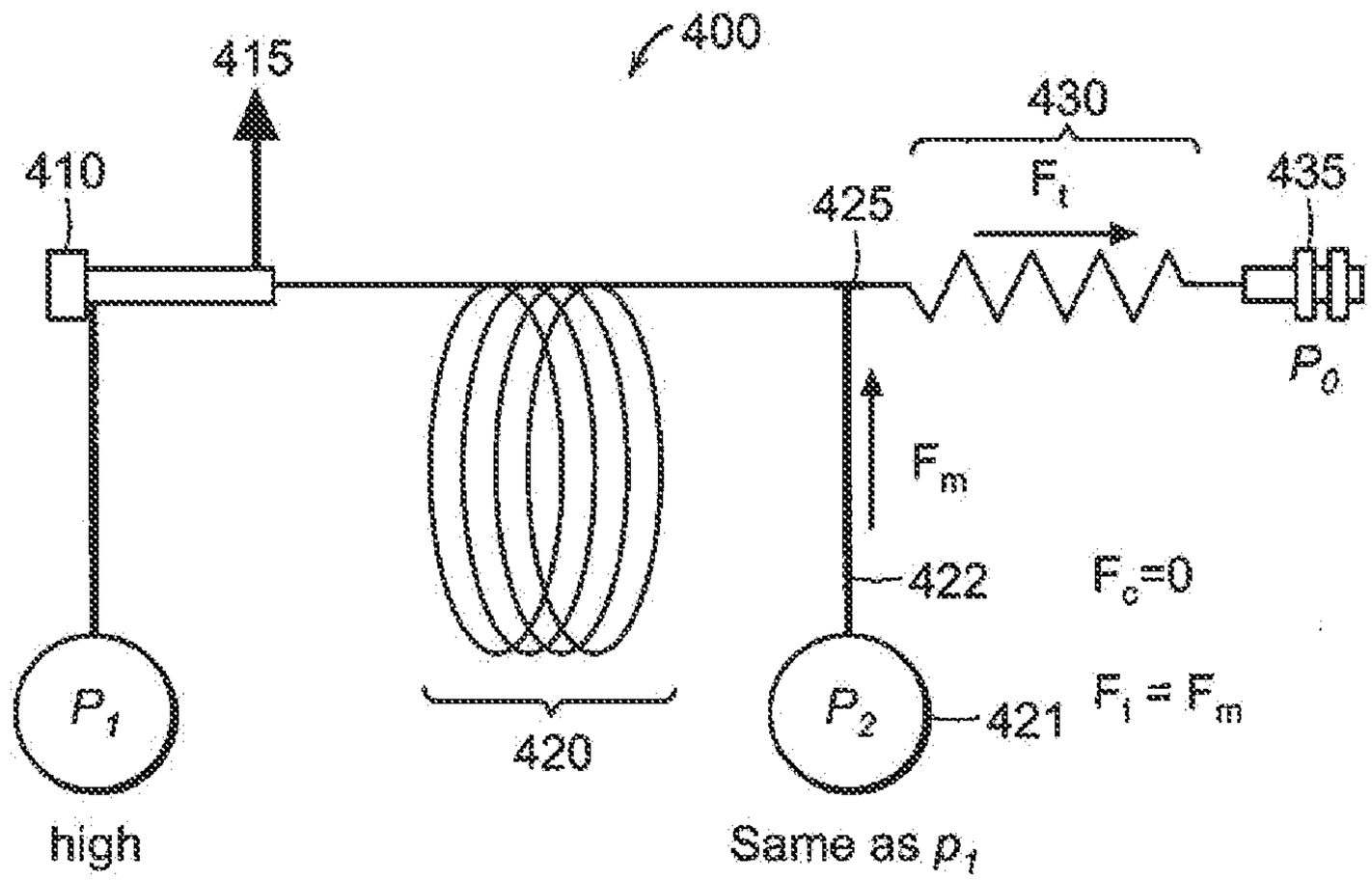

400
415
410
430
F_t
425
435
P_0
F_m
422
$F_c=0$
$F_t = F_m$
P_1
420
P_2
421
high
Same as p_1

FIG. 4D

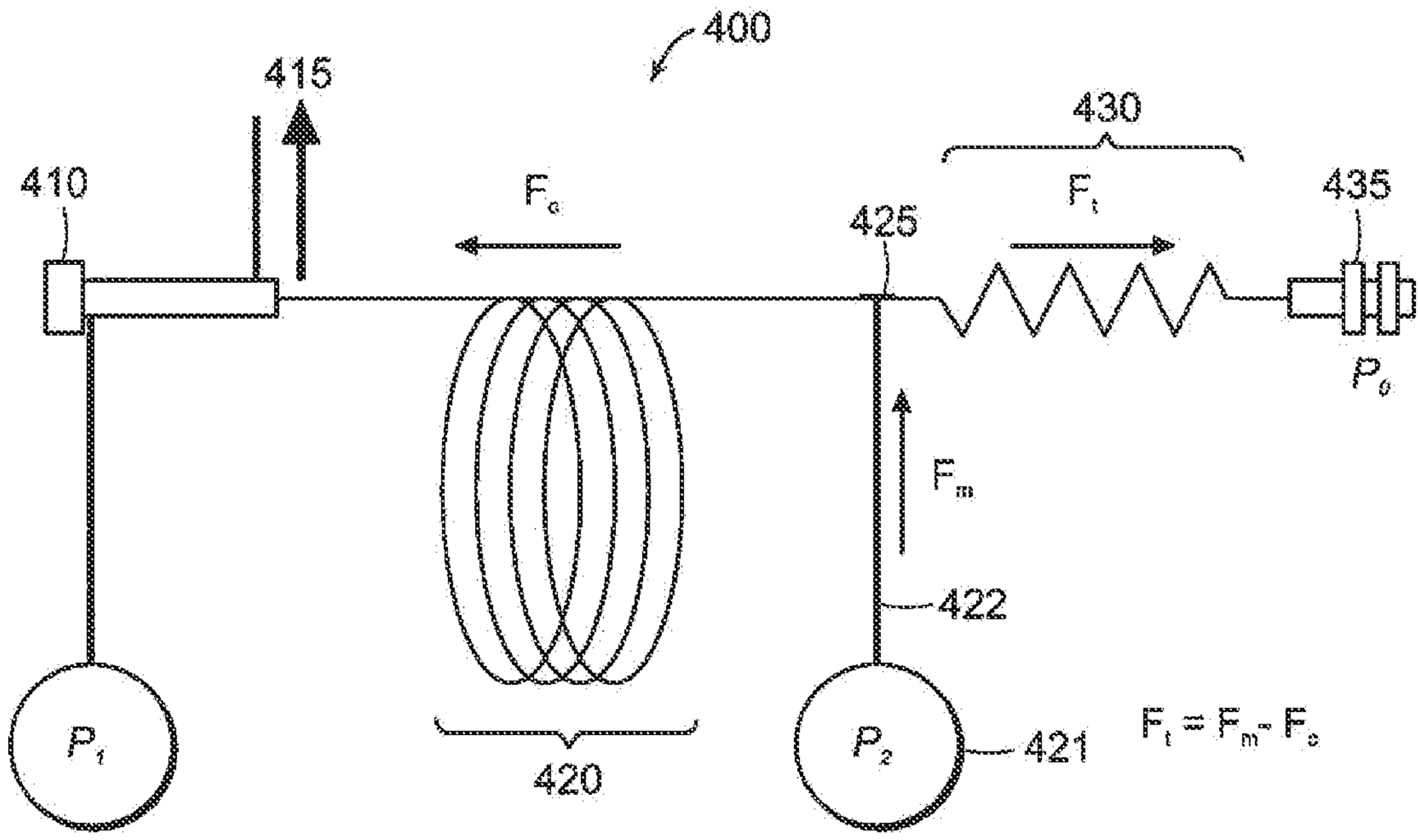

400
415
410
F_o
430
F_t
425
435
P_o
F_m
422
P_1
420
P_2
421
$F_t = F_m - F_o$
high
Higher than p_1

FIG. 4E

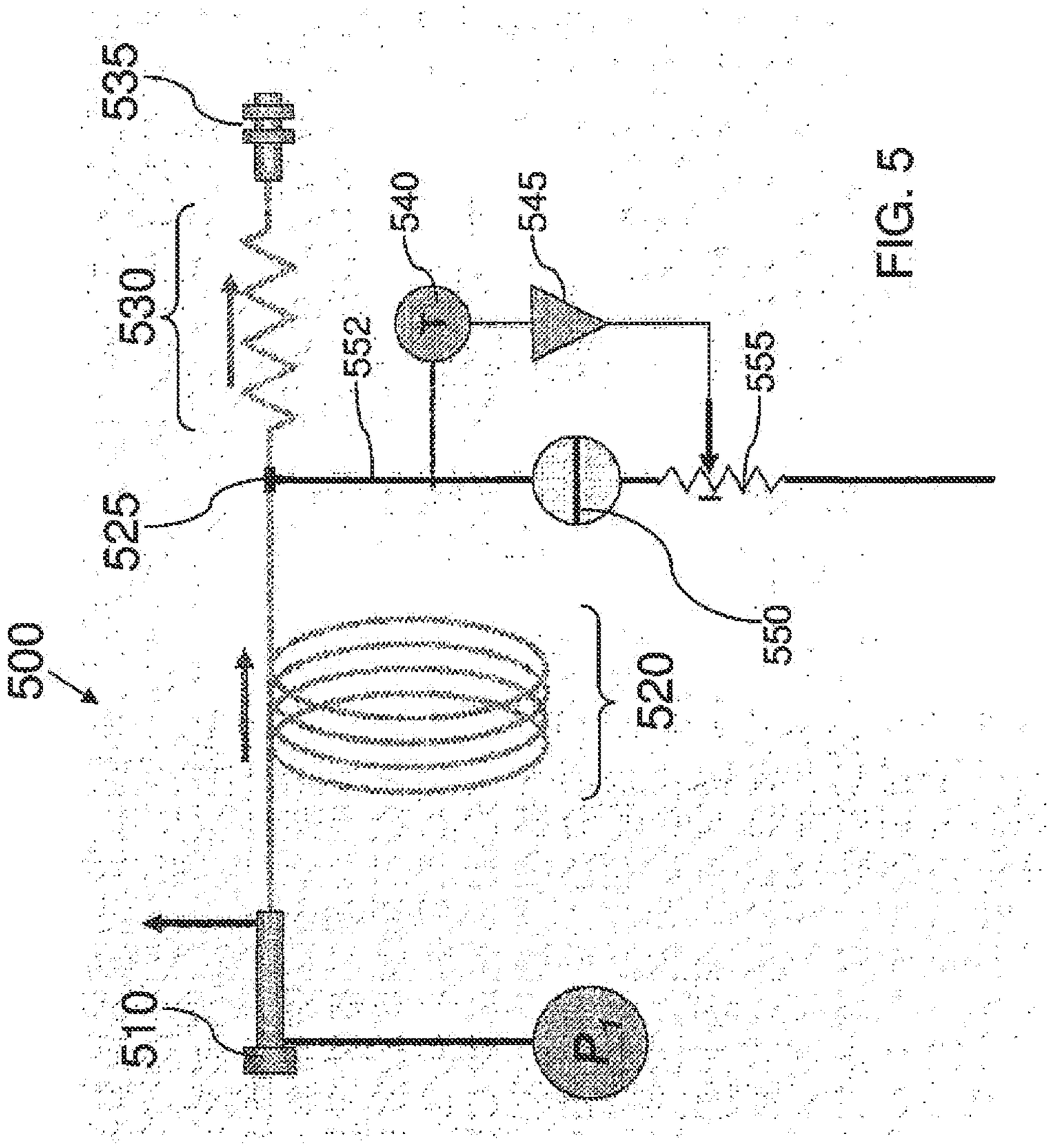


FIG. 5

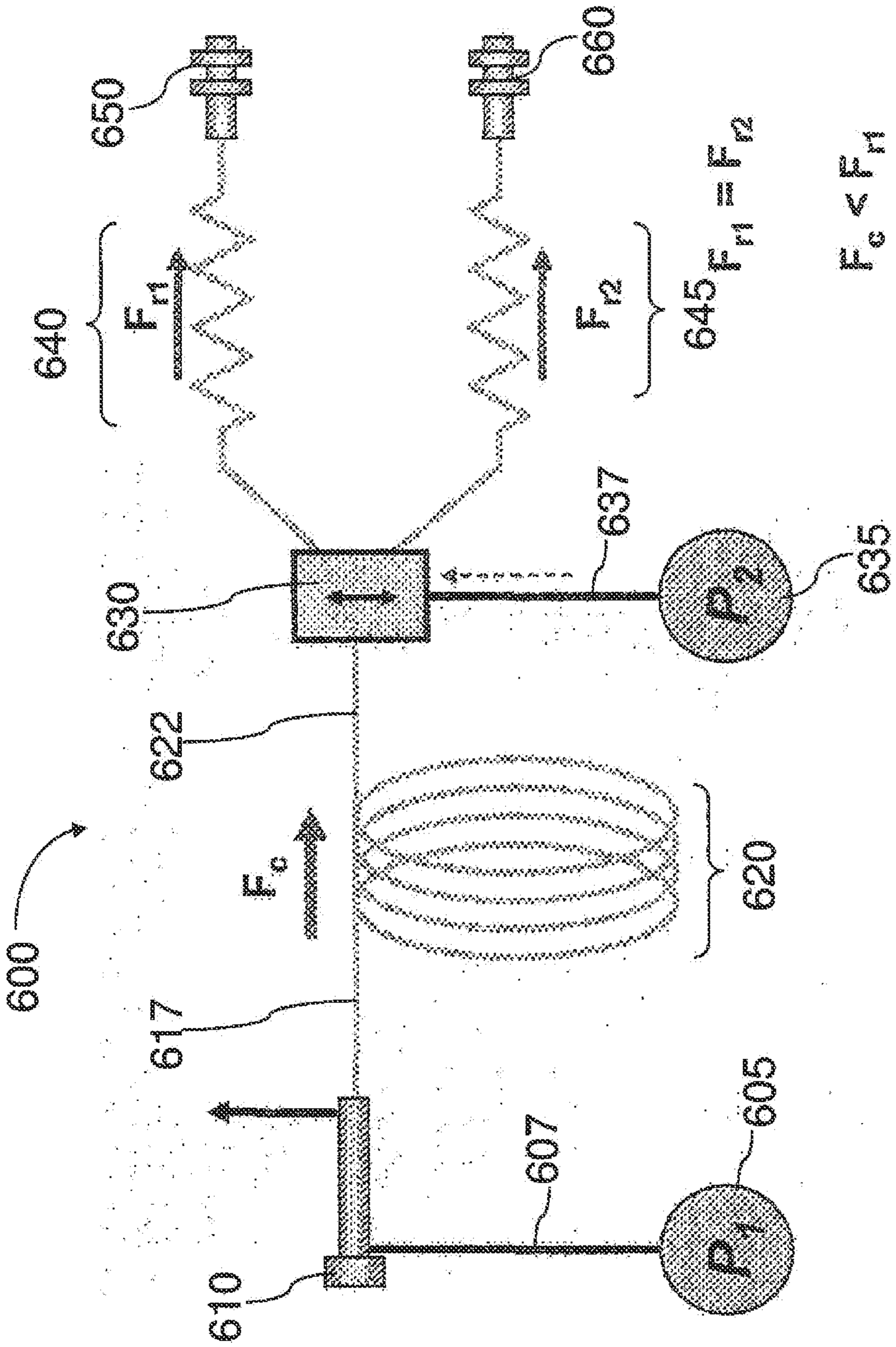

600
610
615
617
620
622
625
630
635
637
640
645
650
660
605
607
F_{r1}
F_{r2}
F_c
Q_1
Q_2
$E_{r1} = E_{r2}$
E_c

FIG. 6

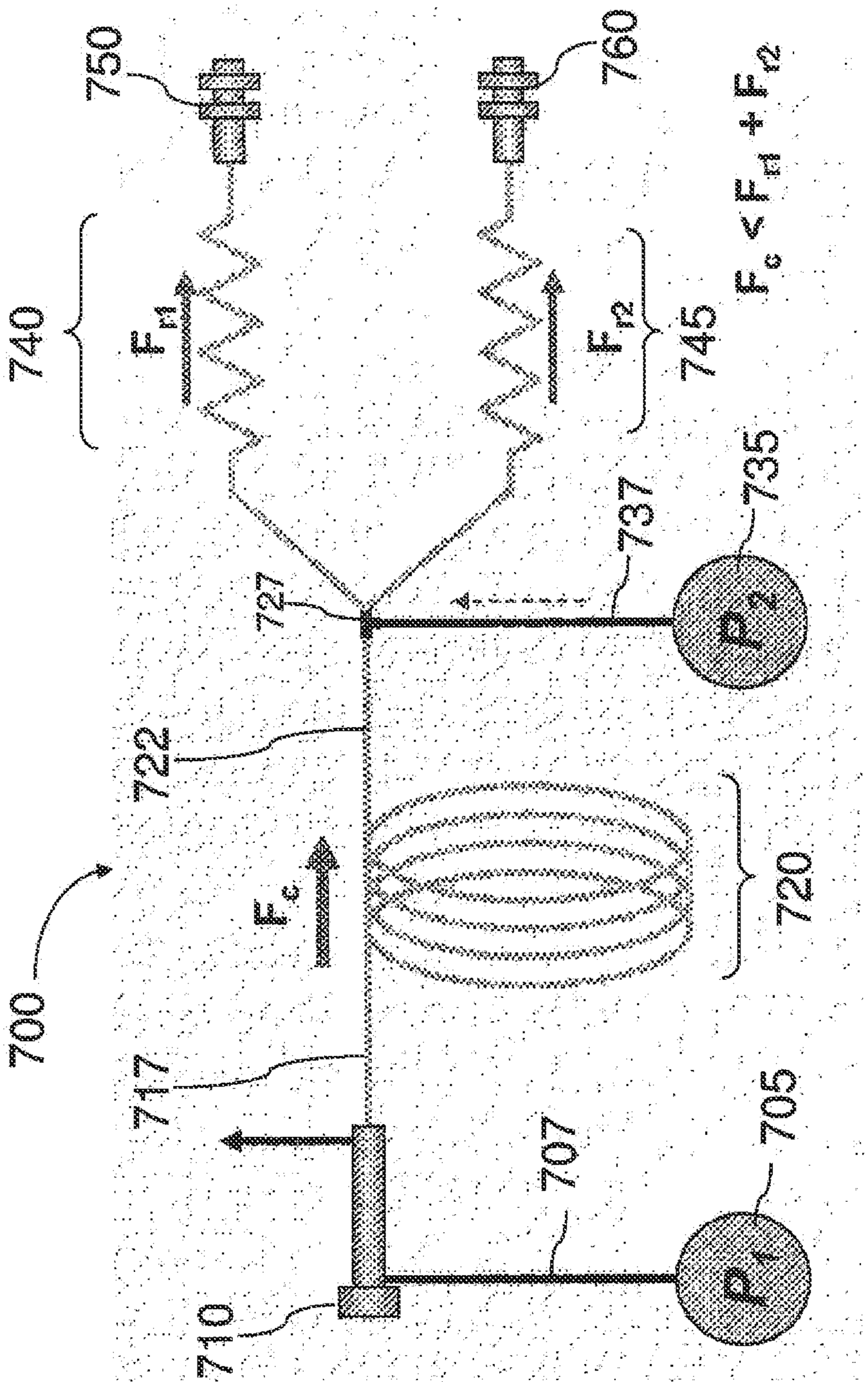

750
790
740
F_{r1}
F_{r2}
745
$F_c < F_{r1} + F_{r2}$
727
737
735
722
F_c
720
700
717
707
705
710

FIG. 7

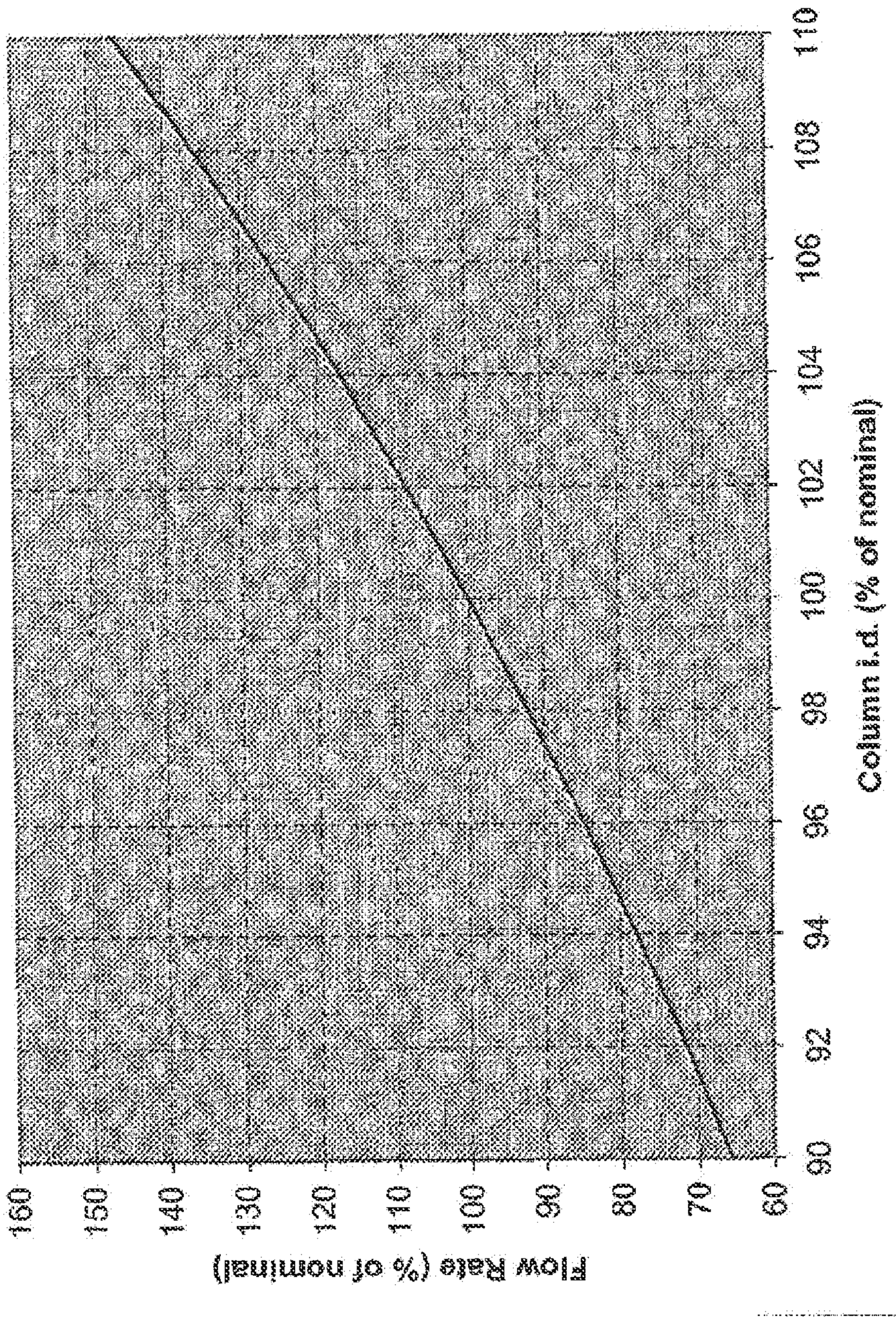

Flow Rate (% of nominal)
160
150
140
130
120
110
100
90
80
70
60
Column i.d. (% of nominal)
90
92
94
96
98
100
102
104
106
108

FIG. 8A

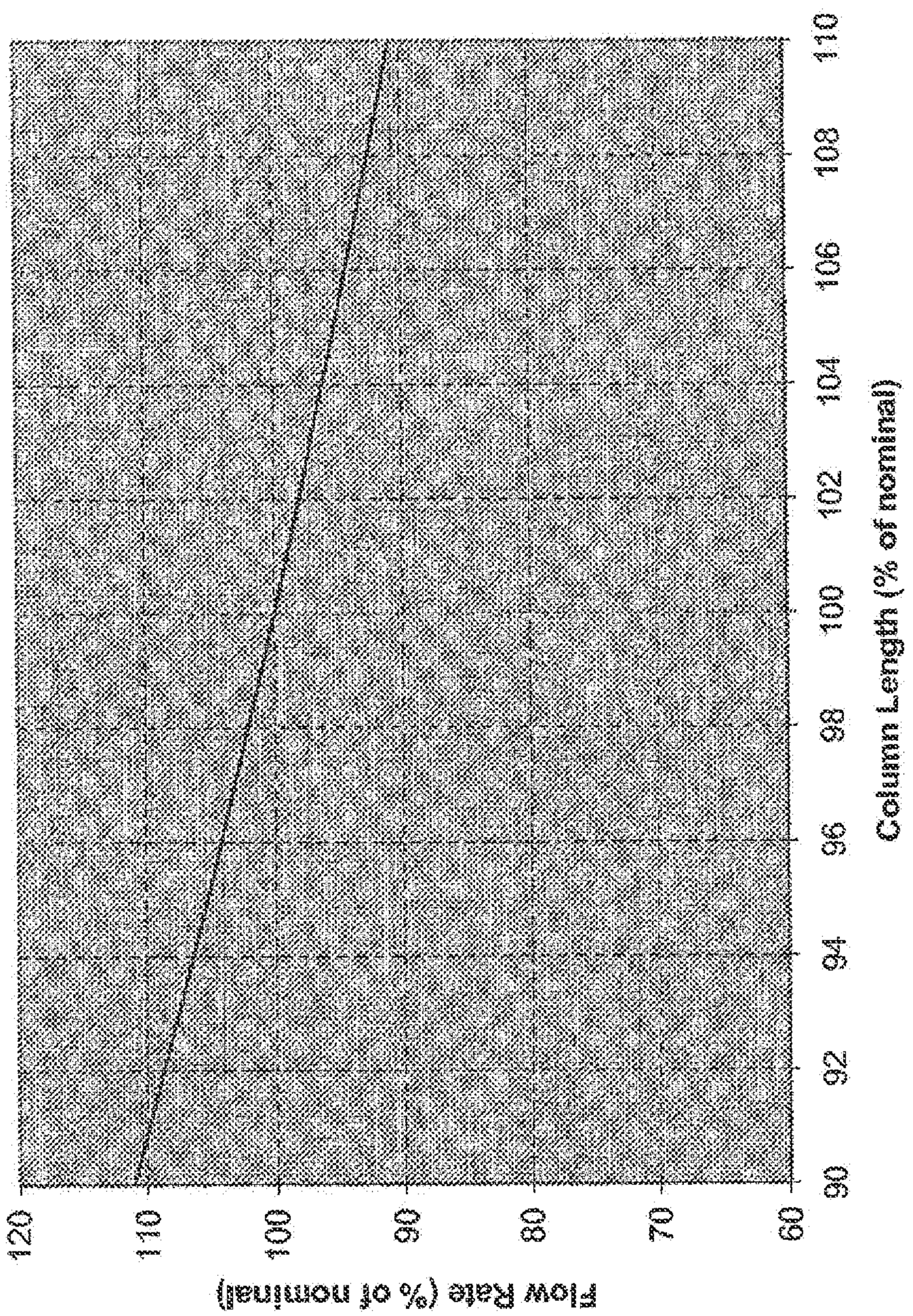

Flow Rate (% of nominal)
120
110
100
90
80
70
60
Column Length (% of nominal)
90
92
94
96
98
100
102
104
106
108
11

FIG. 8B

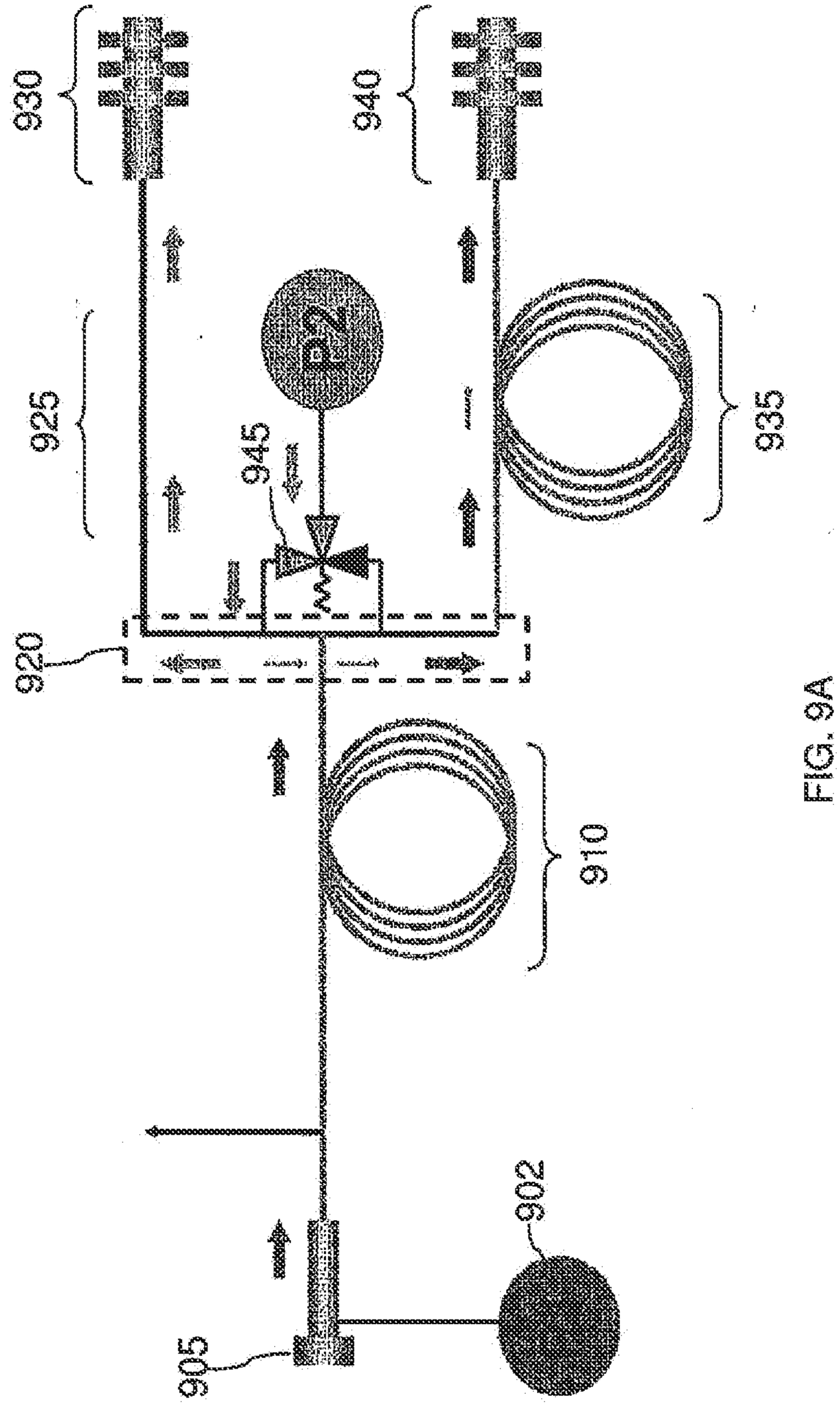


FIG. 9A

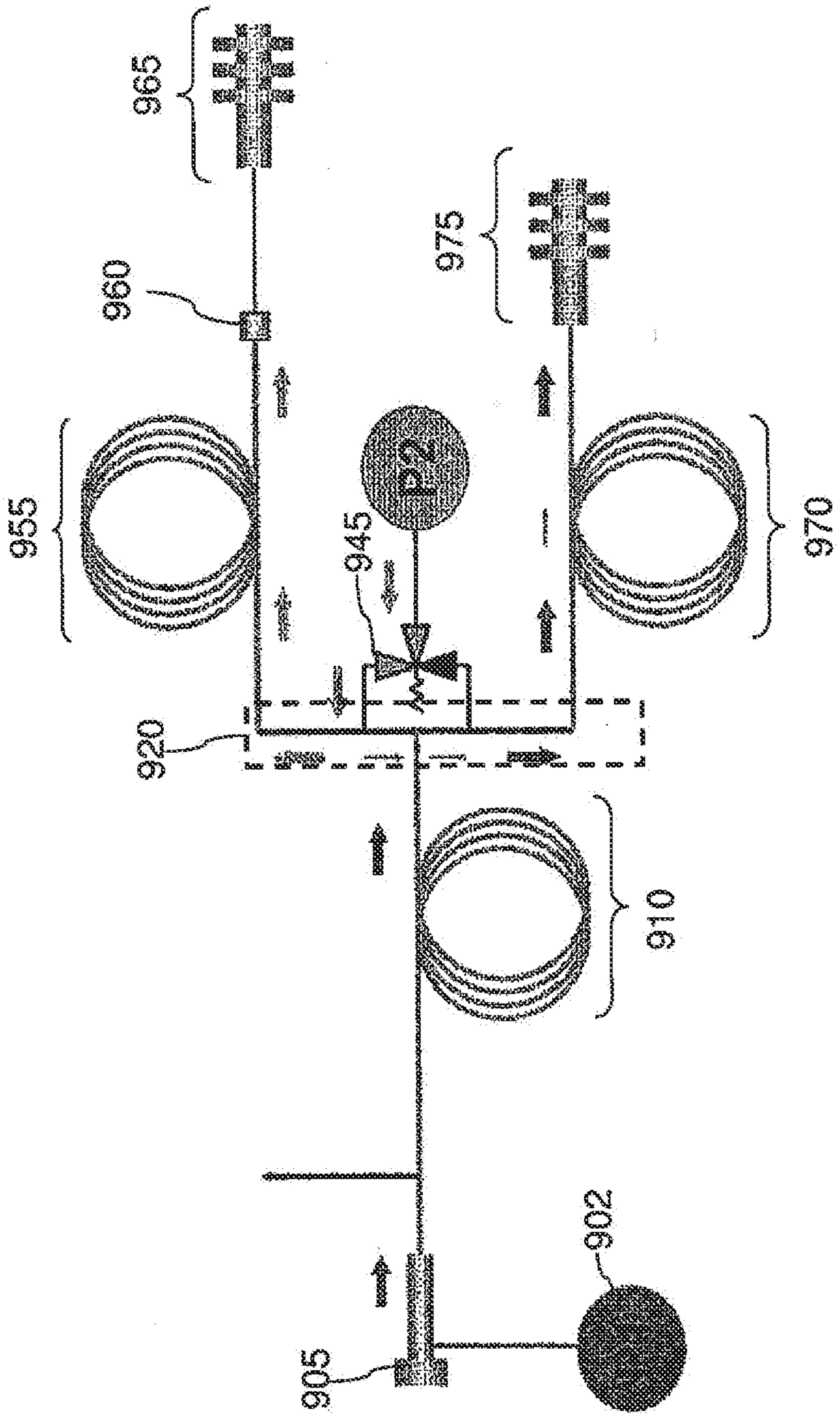


FIG. 9B

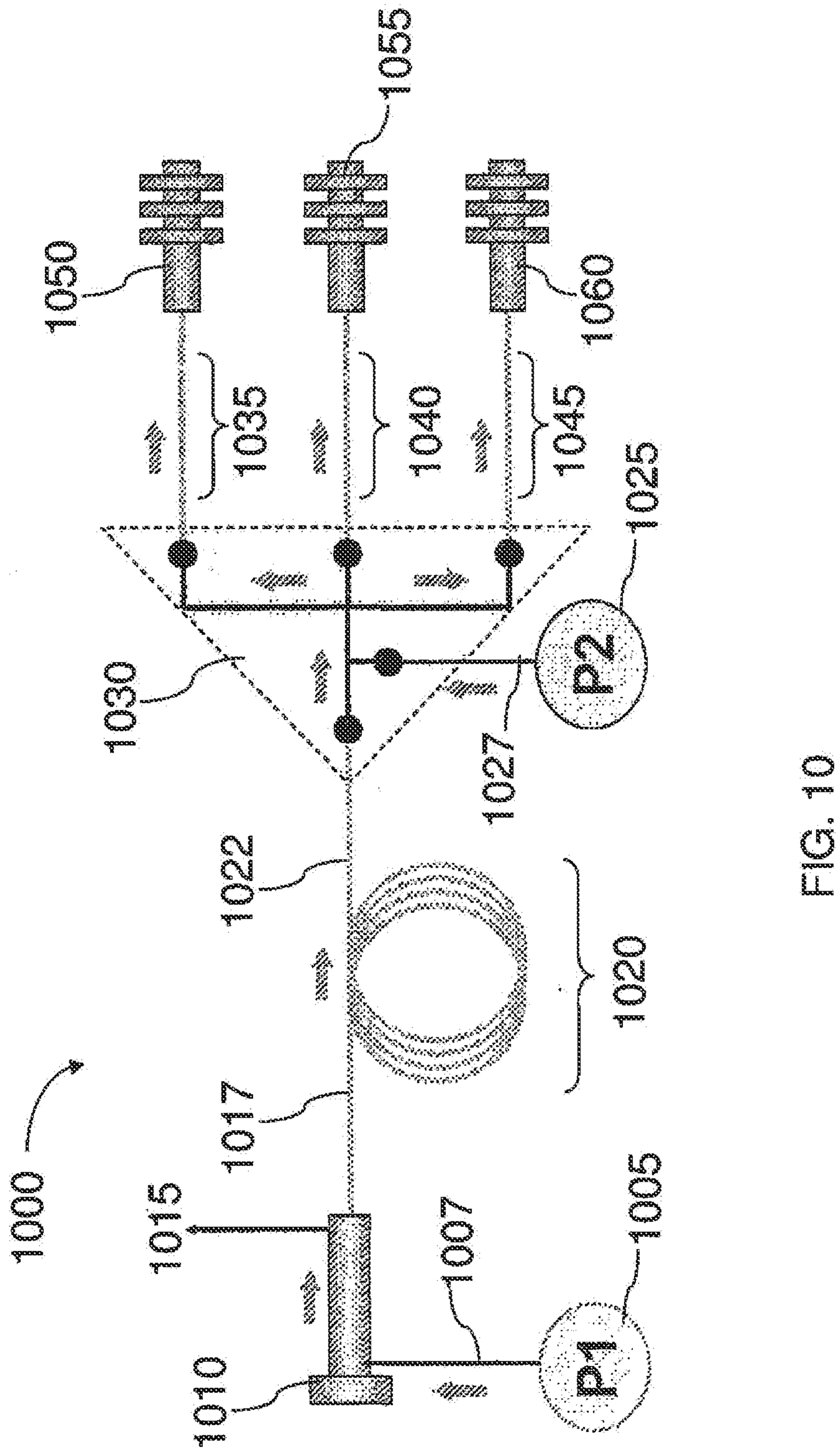

1055
1050
1060
1035
1040
1045
1025
1030
P2
027
10
1022
1020
1017
1015
1007
1005
1000
1010

FIG.

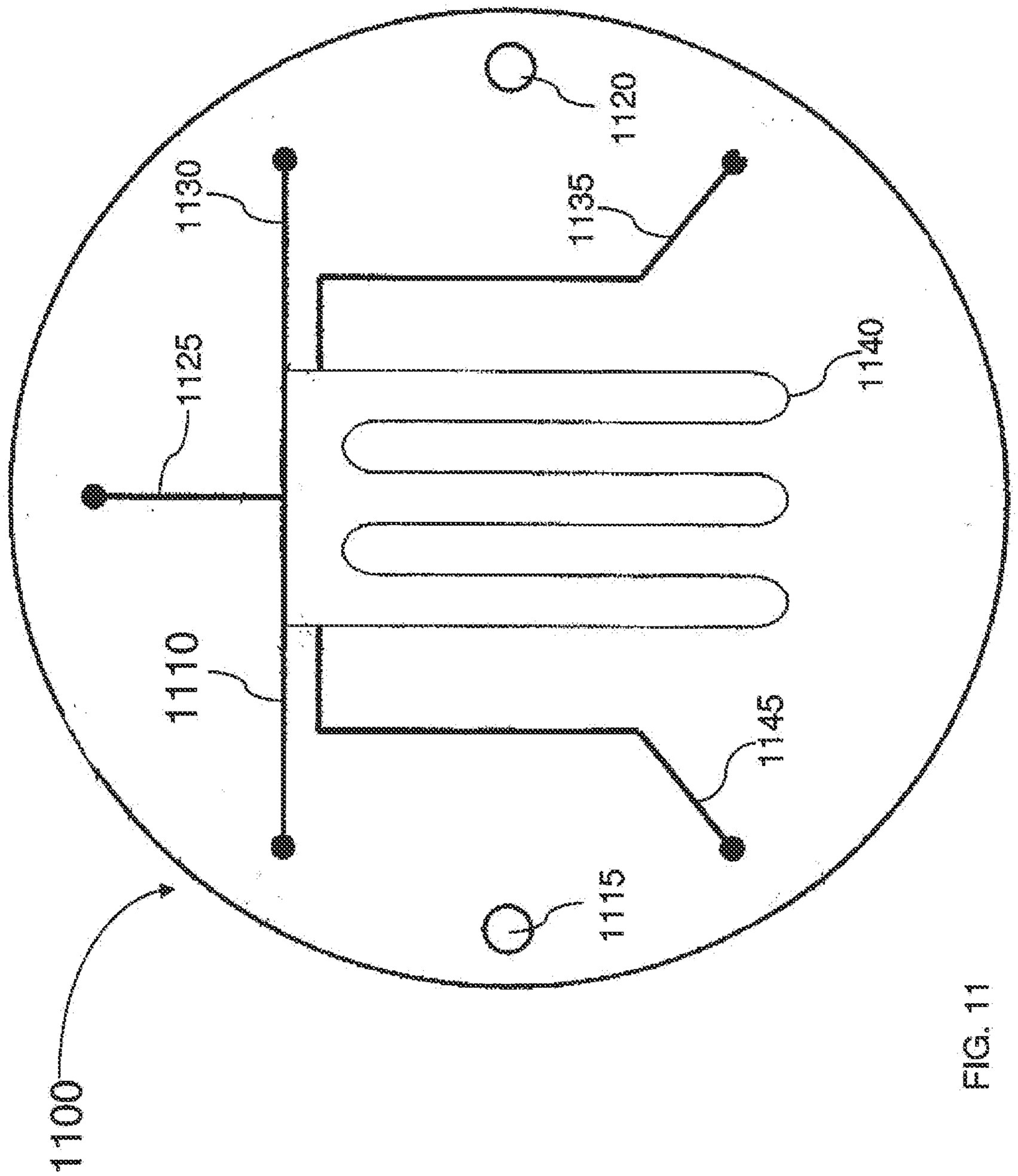


FIG. 11

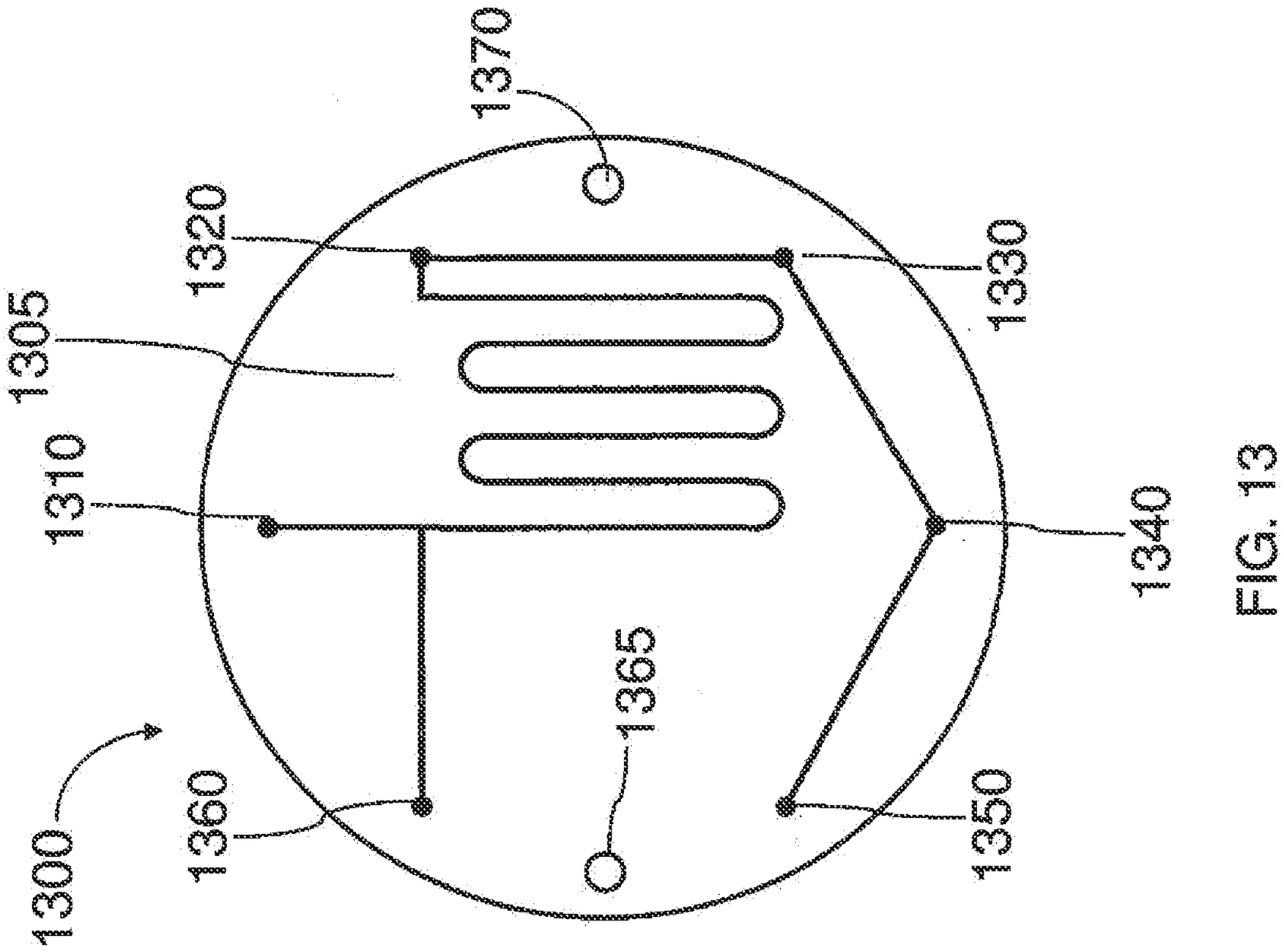

1370
1320
1330
1305
1310
1340
1365
1360
1350
1300

FIG. 13

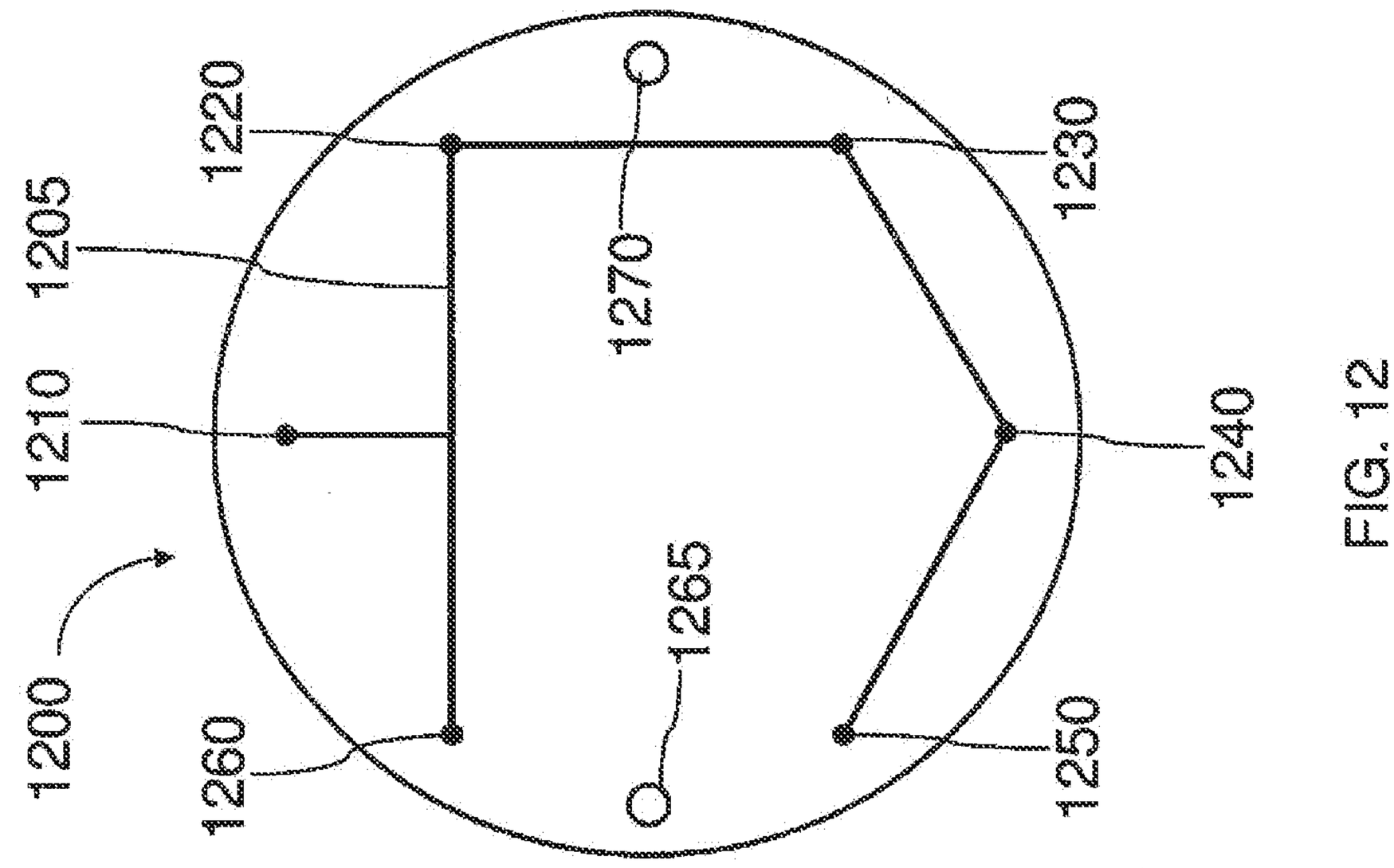

1220
1270
1230
1210
1205
1240
1265
1260
1250
1200

FIG. 12

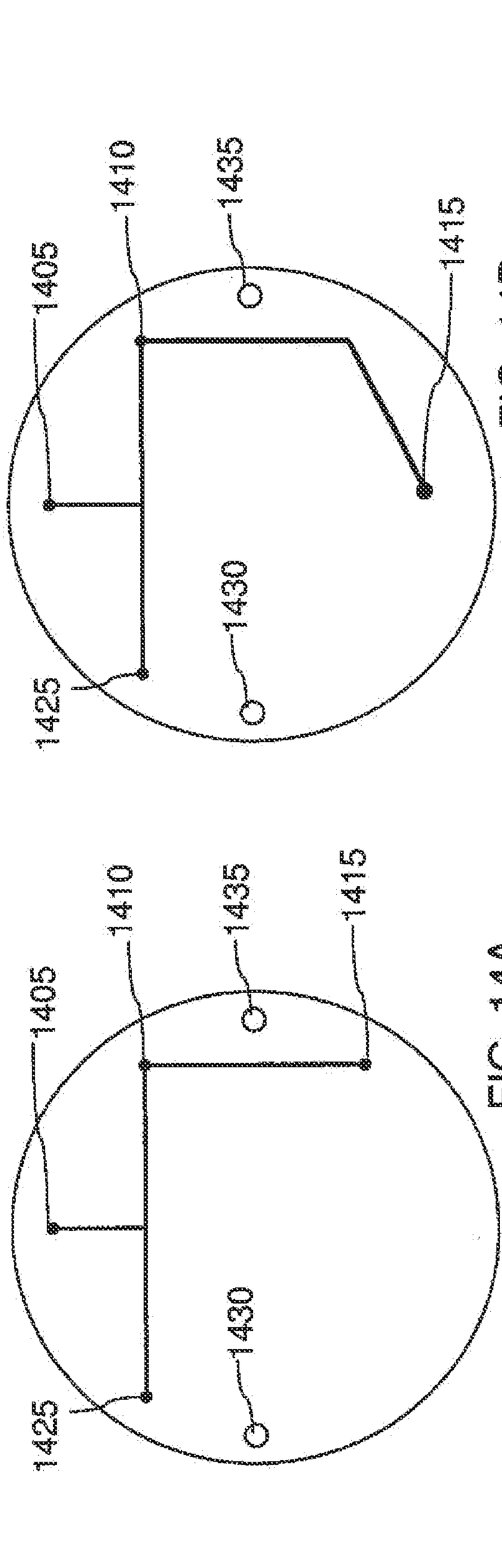


FIG. 14A

FIG. 14B

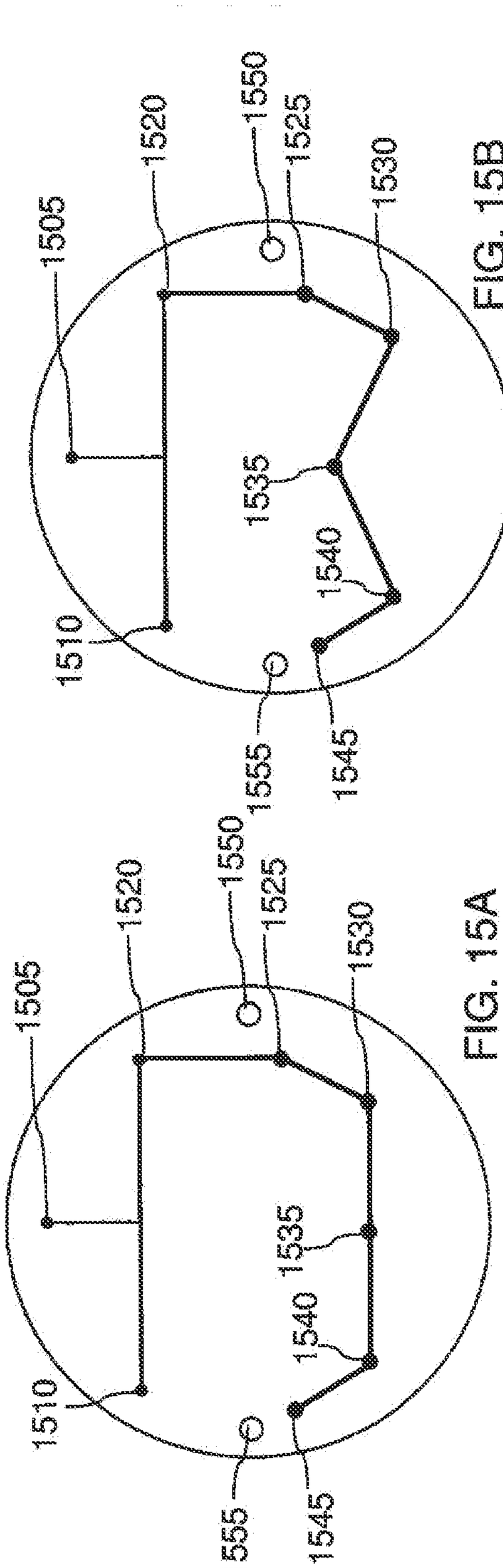


FIG. 15A

FIG. 15B

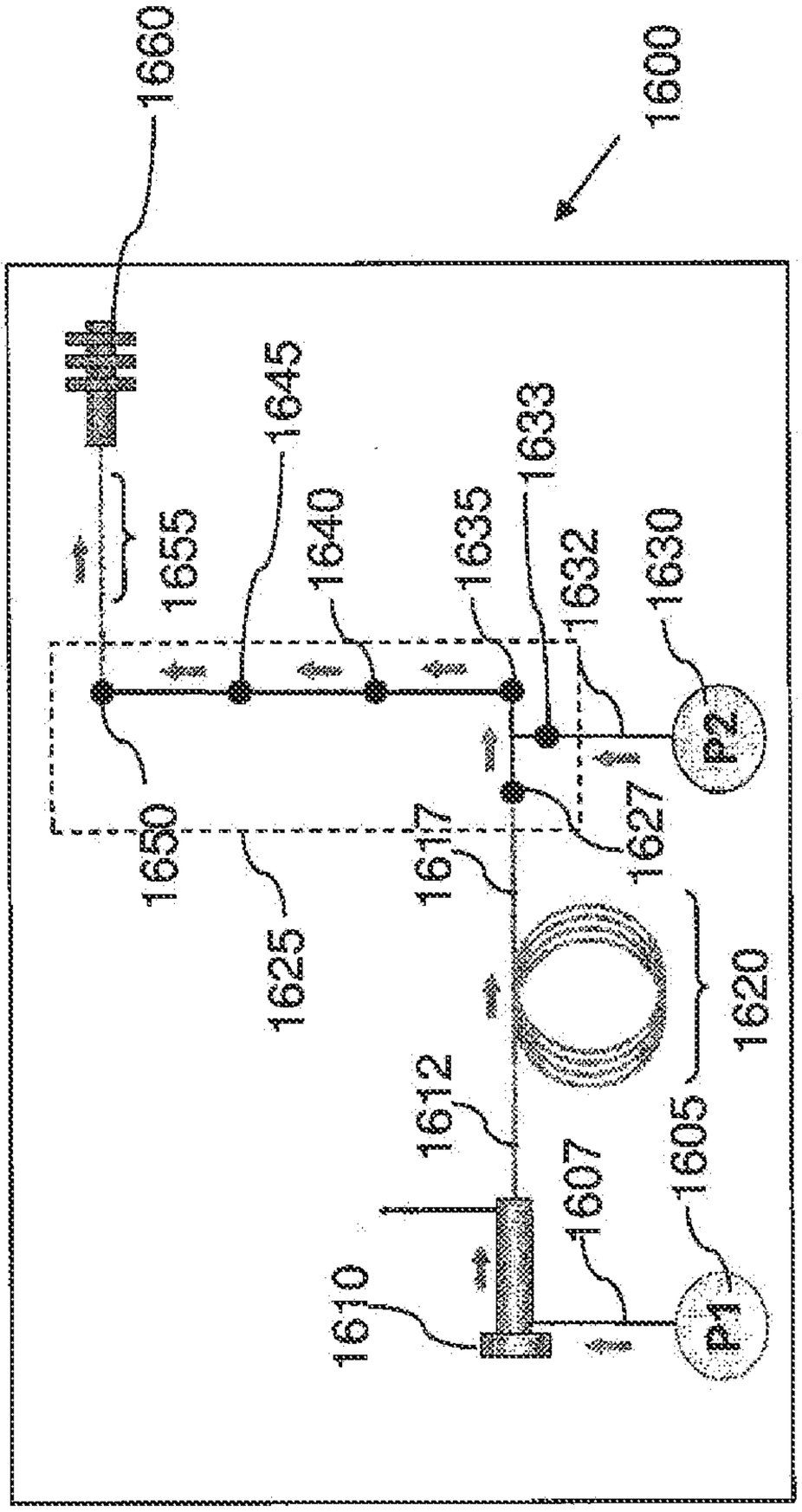

1600
1660
1655
1645
1640
1635
1633
1632
1630
1650
1625
1617
1627
P2
1620
1612
1607
1605
1610
P1

FIG. 16

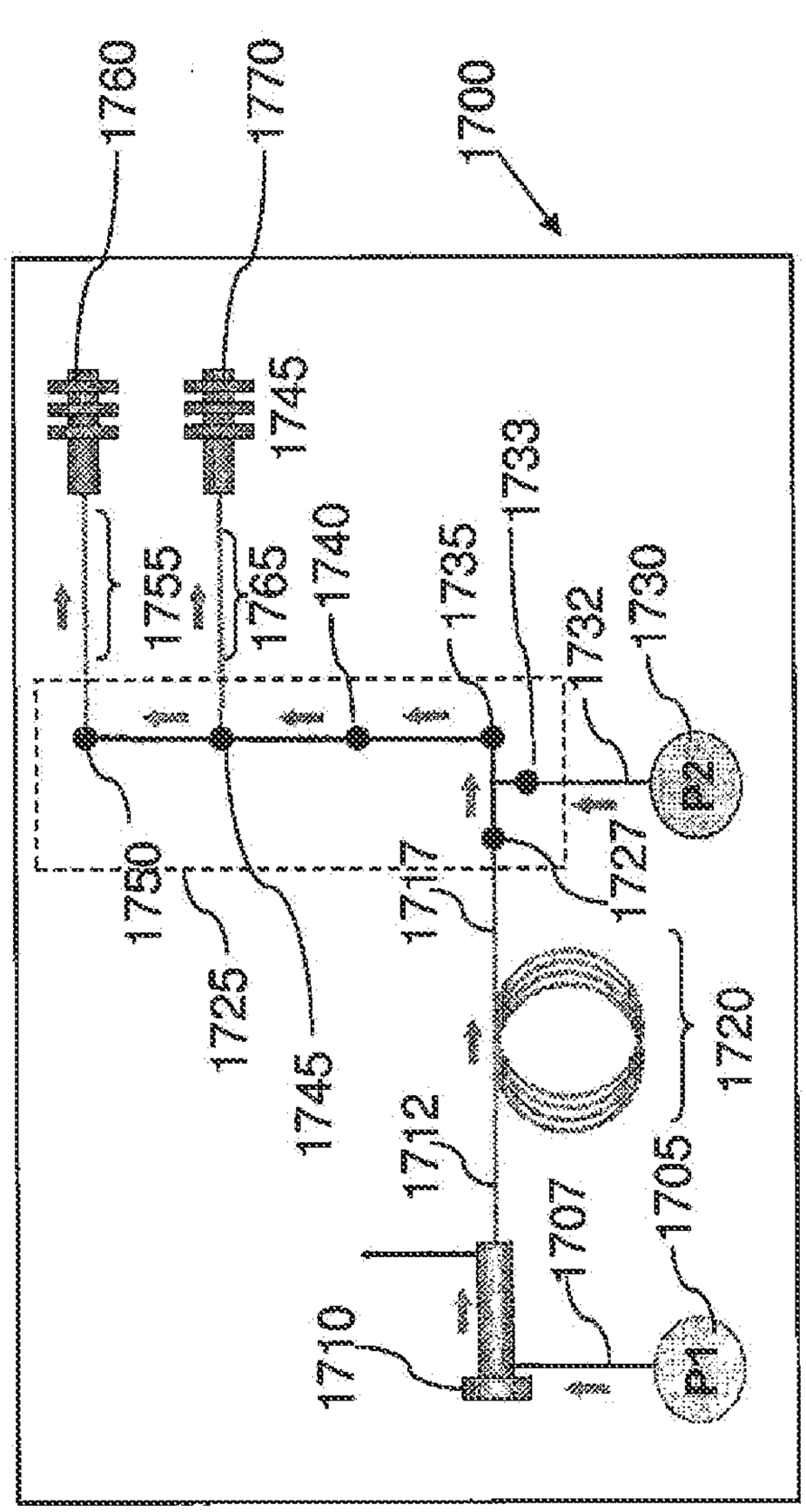

1700
1760
1770
1755
1745
1765
1740
1735
1733
1732
1730
1750
1725
1745
1717
1727
P2
1720
1712
1707
1705
1710
P1

FIG. 17

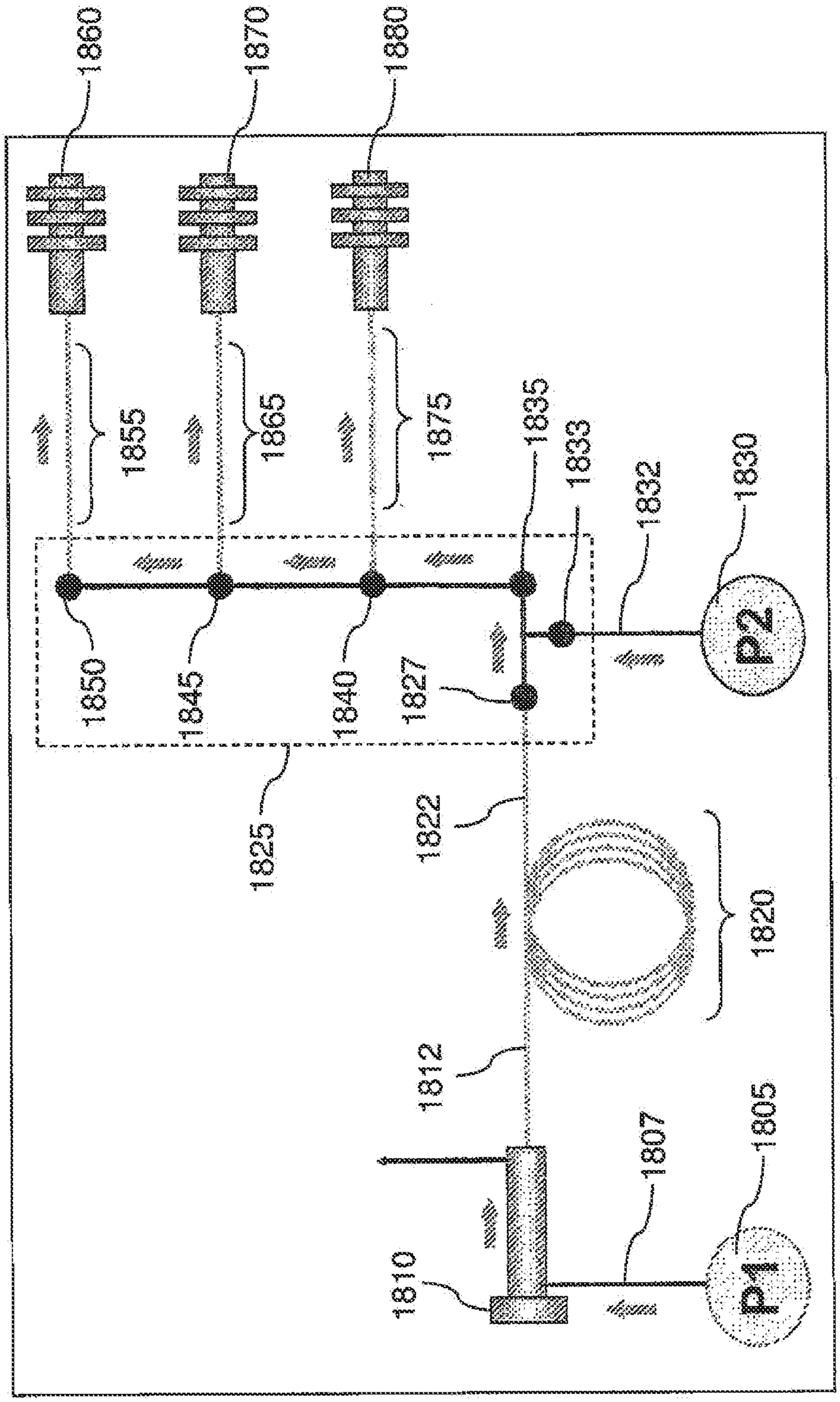

1860
1870
1880
1855
1865
1875
1835
1833
1832
1830
P2
1850
1845
1840
1827
1825
1822
1820
1812
1807
1805
1810
P1
1800

FIG. 18

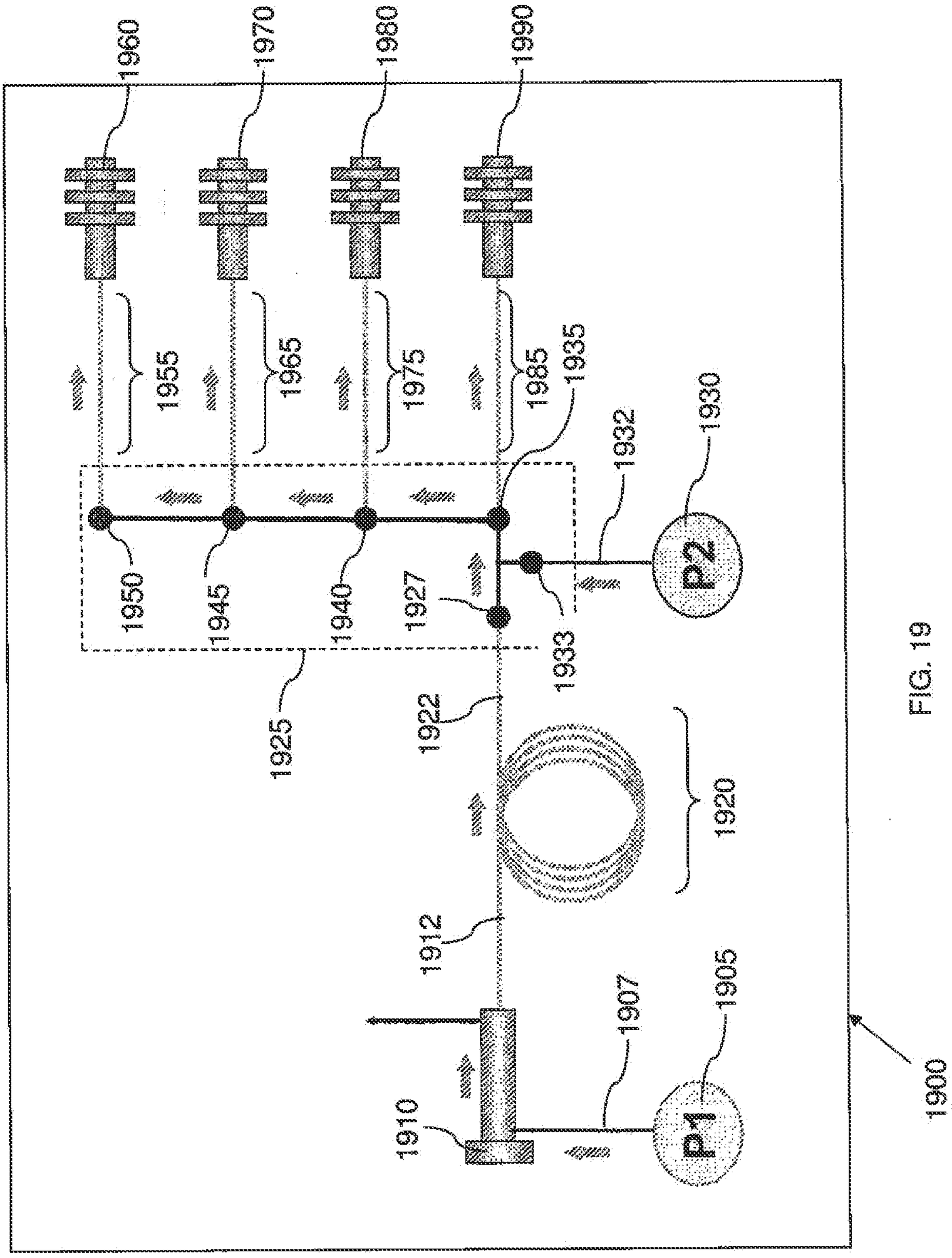

1960
1970
1980
1990
1955
1965
1975
1985
1935
1932
1930
P2
1950
1945
1940
1927
1933
1925
1922
1912
1907
1905
1920
1910
1902
P1
1900

FIG. 19

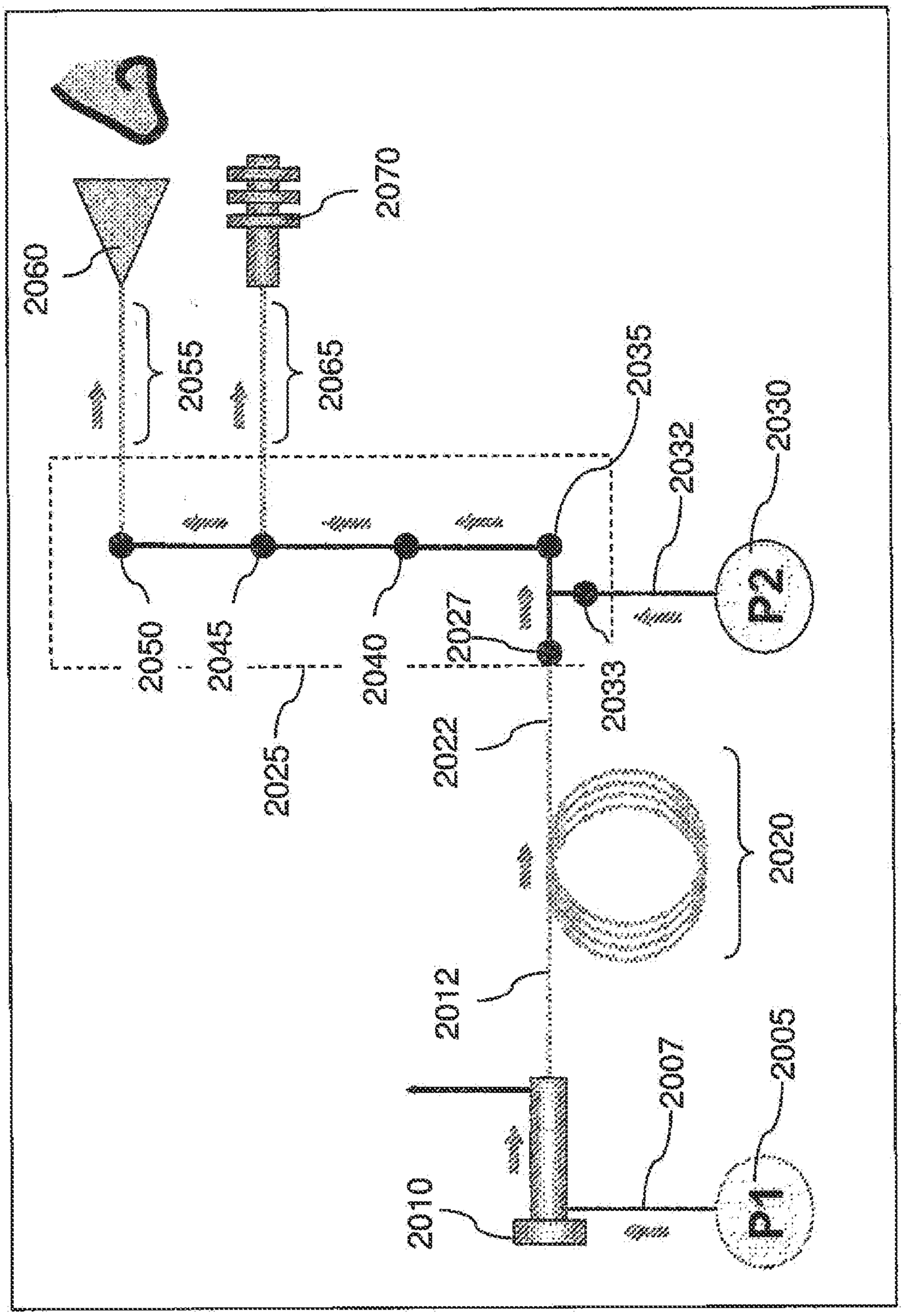

2060
2070
2055
2065
2035
2032
2030
2050
2045
2040
2027
2033
2025
2022
2020
2012
2007
2005
2010
P2
P1
2000

FIG. 20

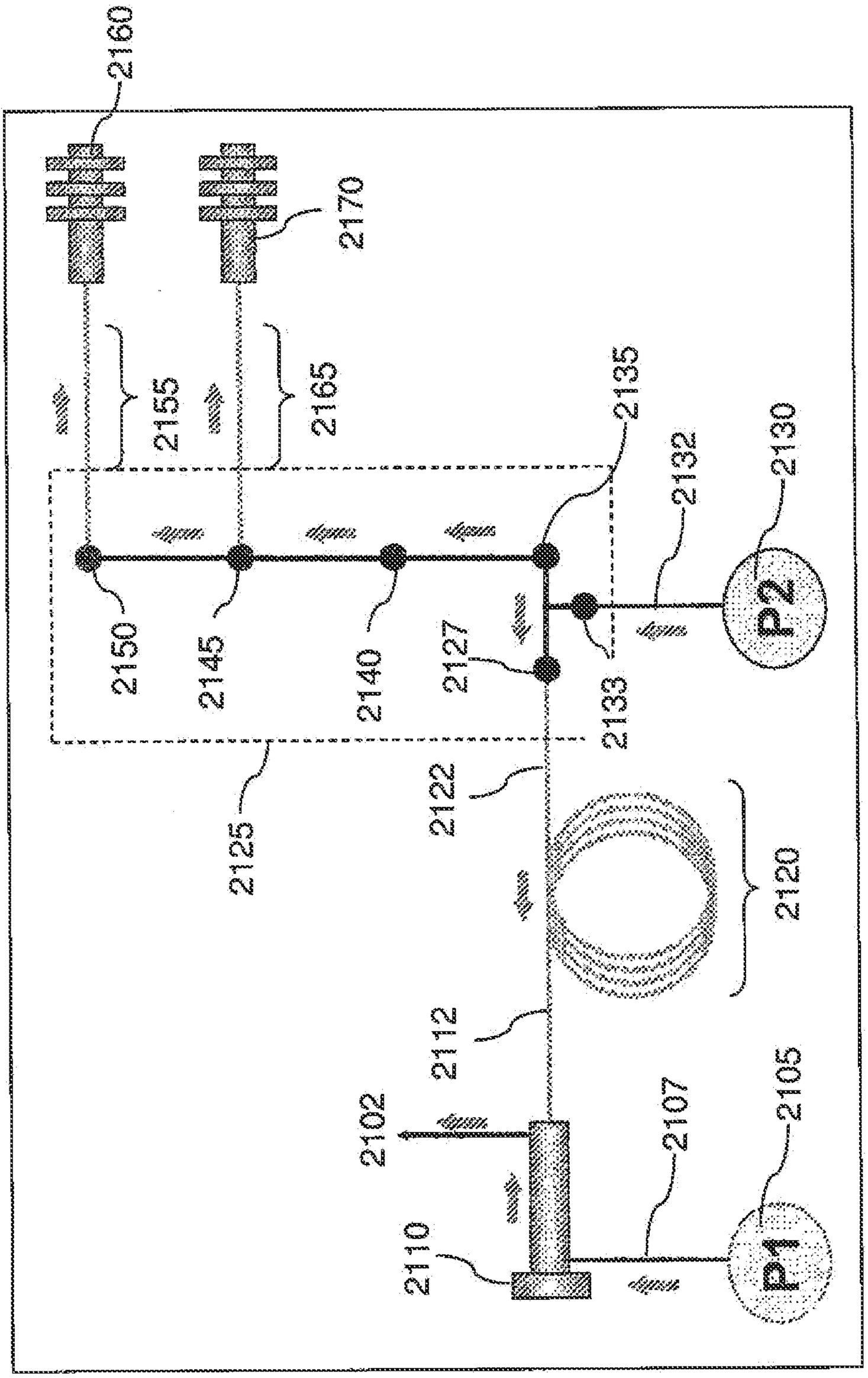

2160
2155
2165
2170
2135
2132
2130
2150
2145
2140
2127
2133
P2
2125
2122
2120
2112
2102
2107
2105
2110
P1
2100

FIG. 21

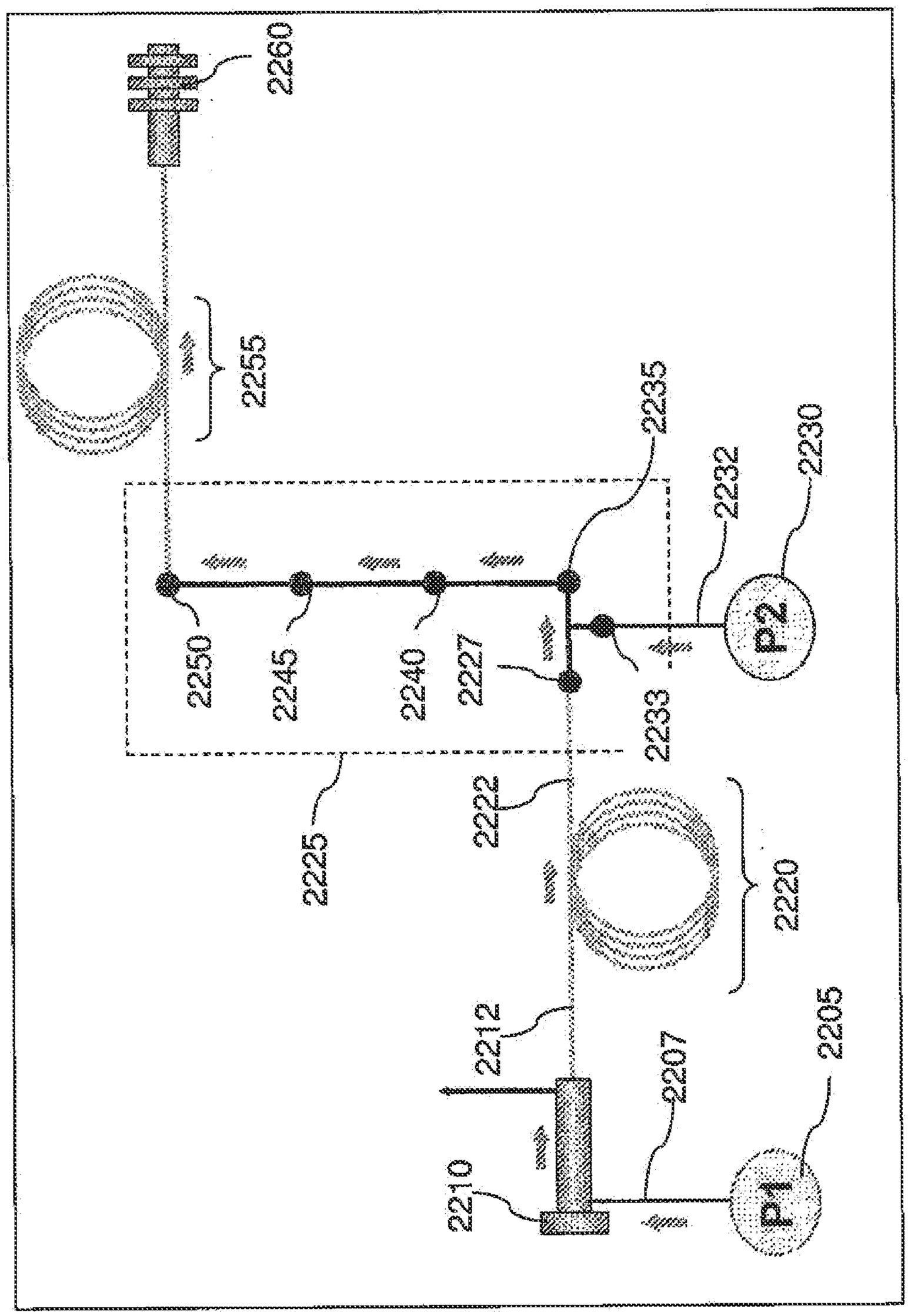

2260
2255
2235
2232
2230
P2
2250
2245
2240
2227
2233
2225
2222
2220
2212
2207
2205
P1
2210
2200

FIG. 22

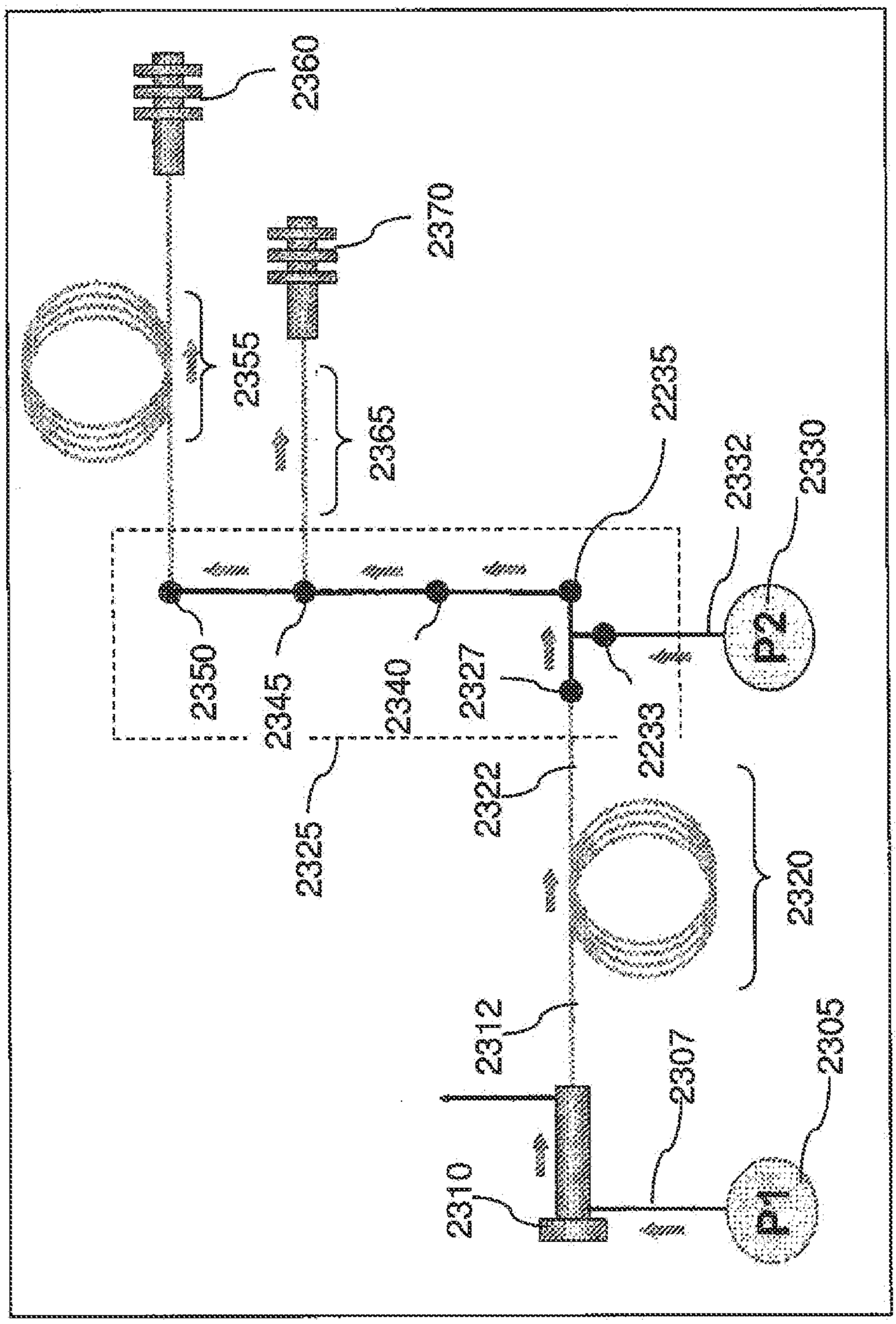

2360
2370
2355
2365
2235
2332
2330
2350
2345
2340
2327
2233
2325
2322
2320
2312
2307
2305
2310
P2
P1
2300

FIG. 23

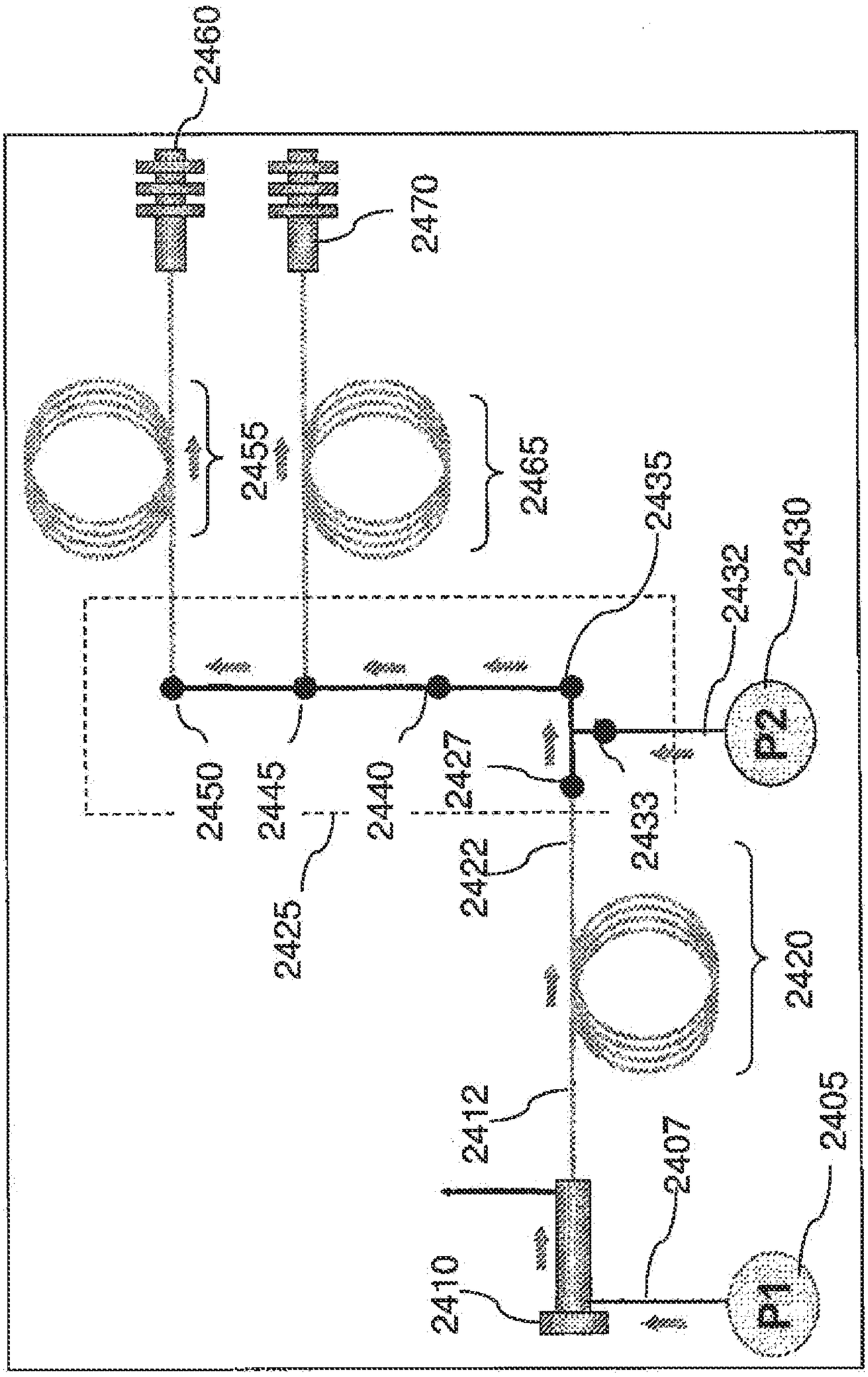

2460
2470
2455
2465
2435
2432
2430
2450
2445
2440
2427
2433
2425
2422
2420
2412
2407
2405
2410
P1
P2
2400

FIG. 24

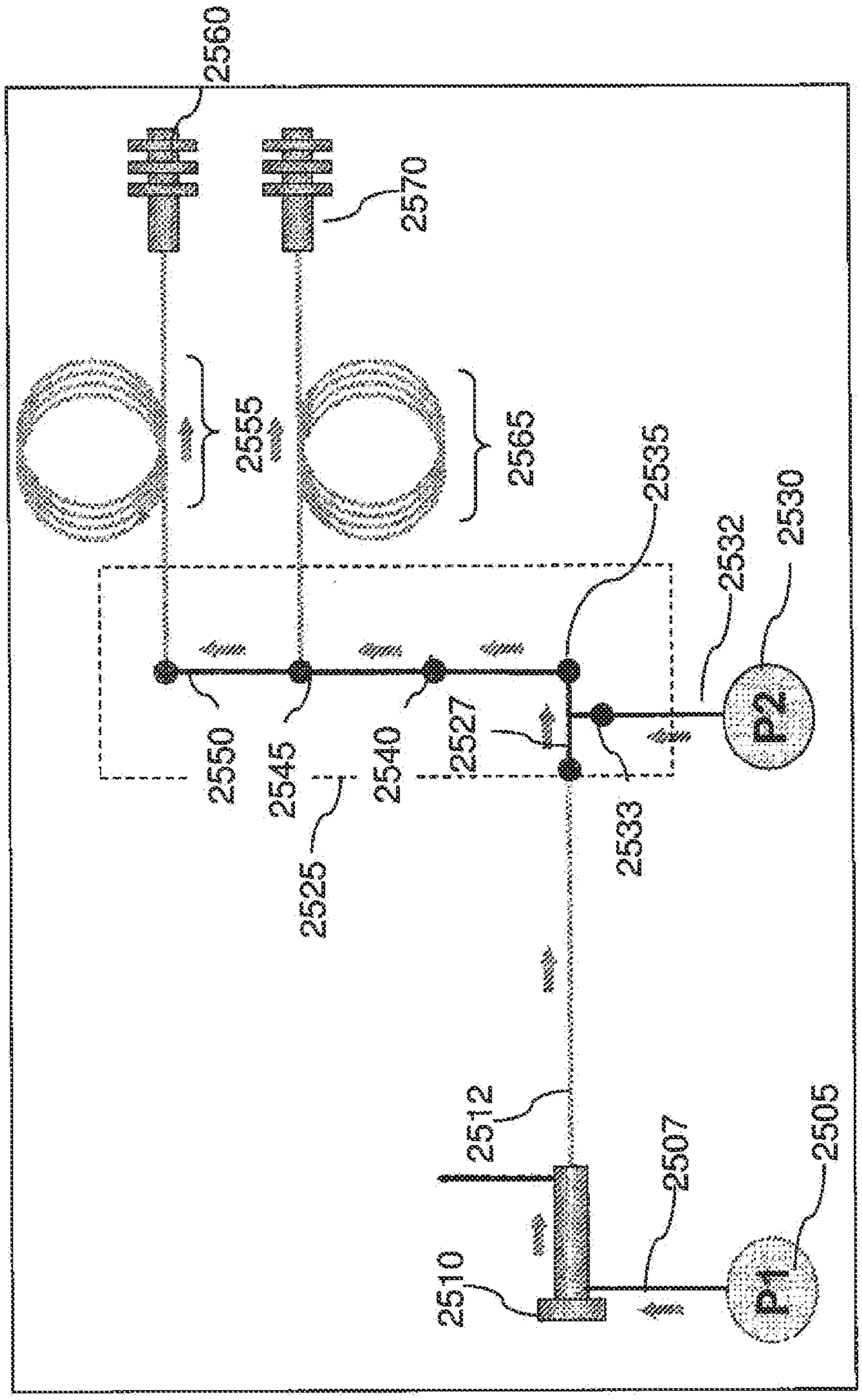

2560
2570
2555
2565
2535
2532
2530
2550
2545
2540
2527
2525
2533
P2
2512
2507
2505
2510
P1
2500

FIG. 25

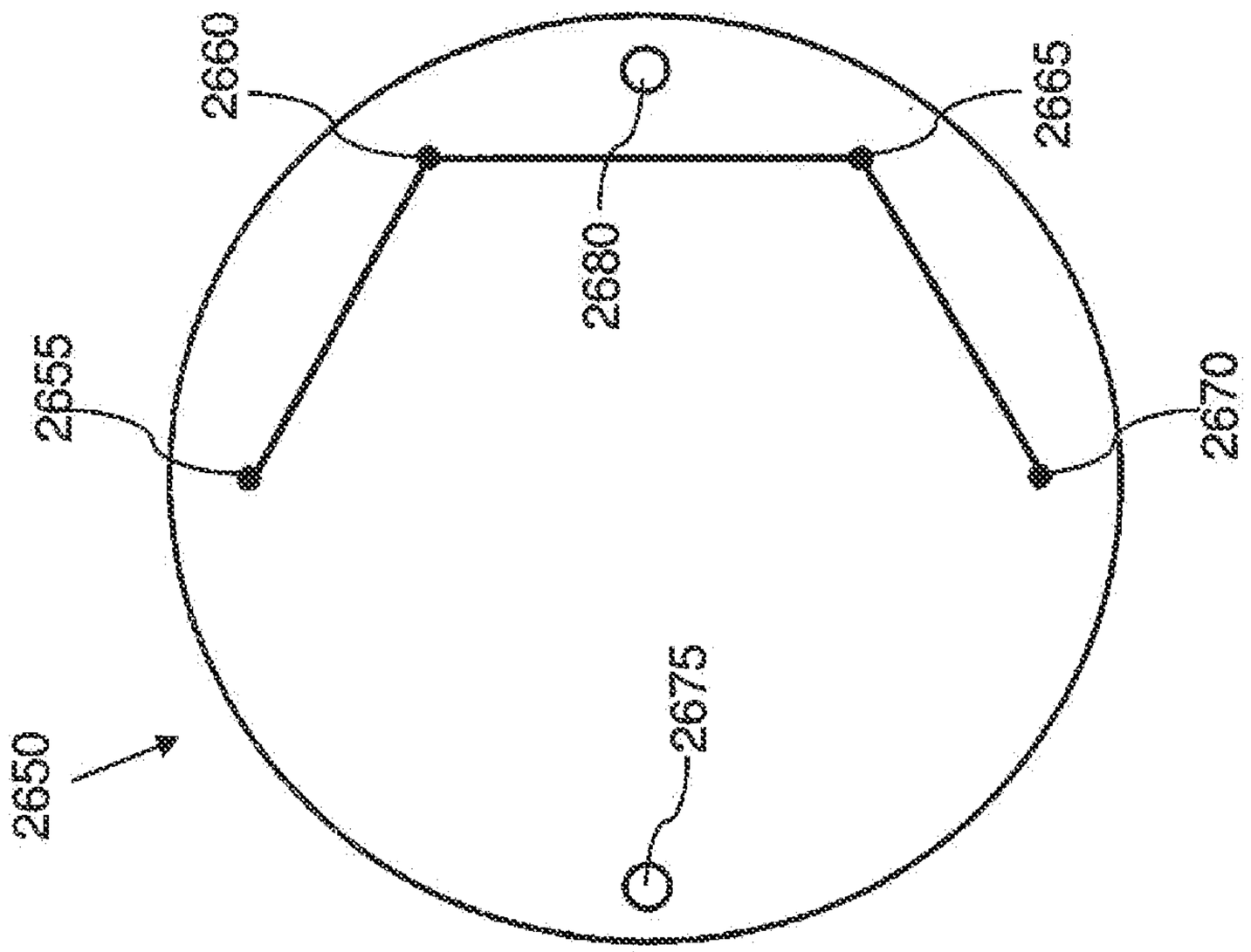


FIG. 26A

FIG. 26B

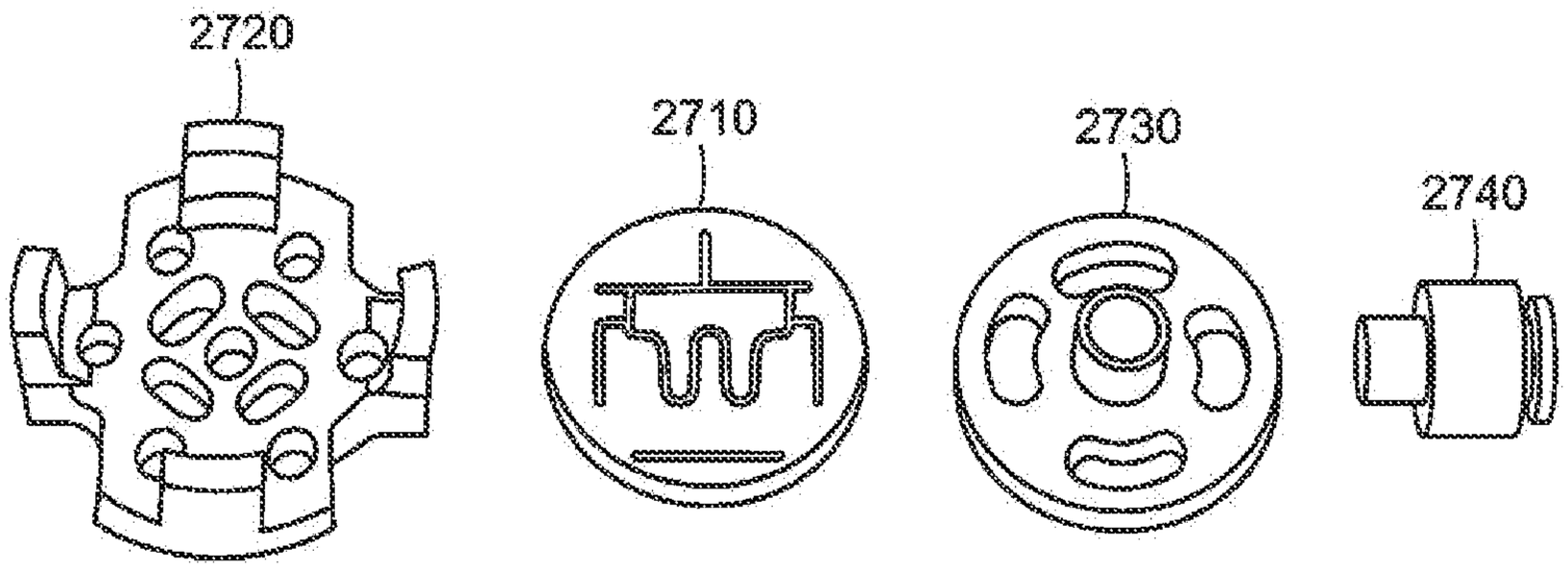


FIG. 27

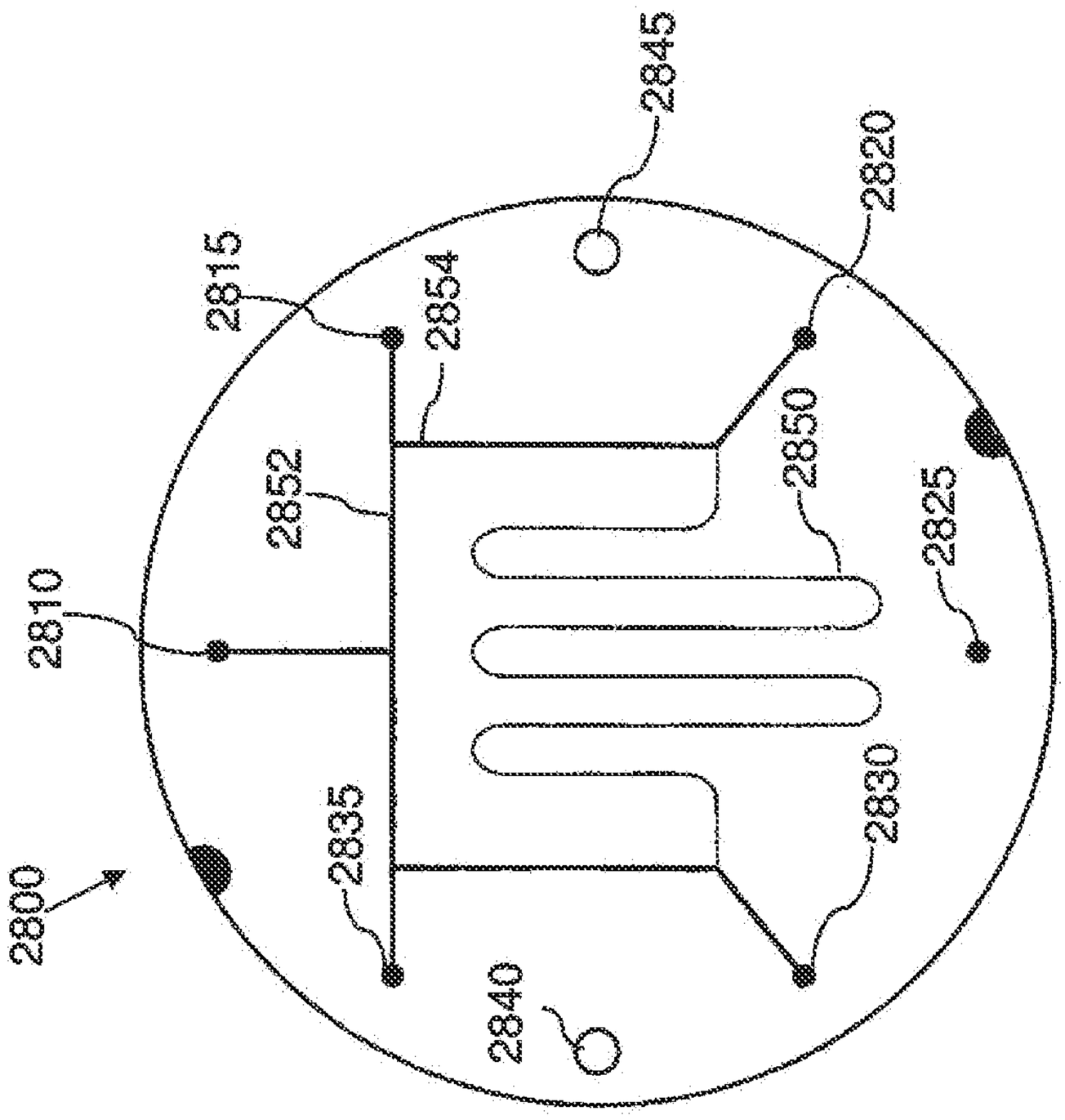


FIG. 28

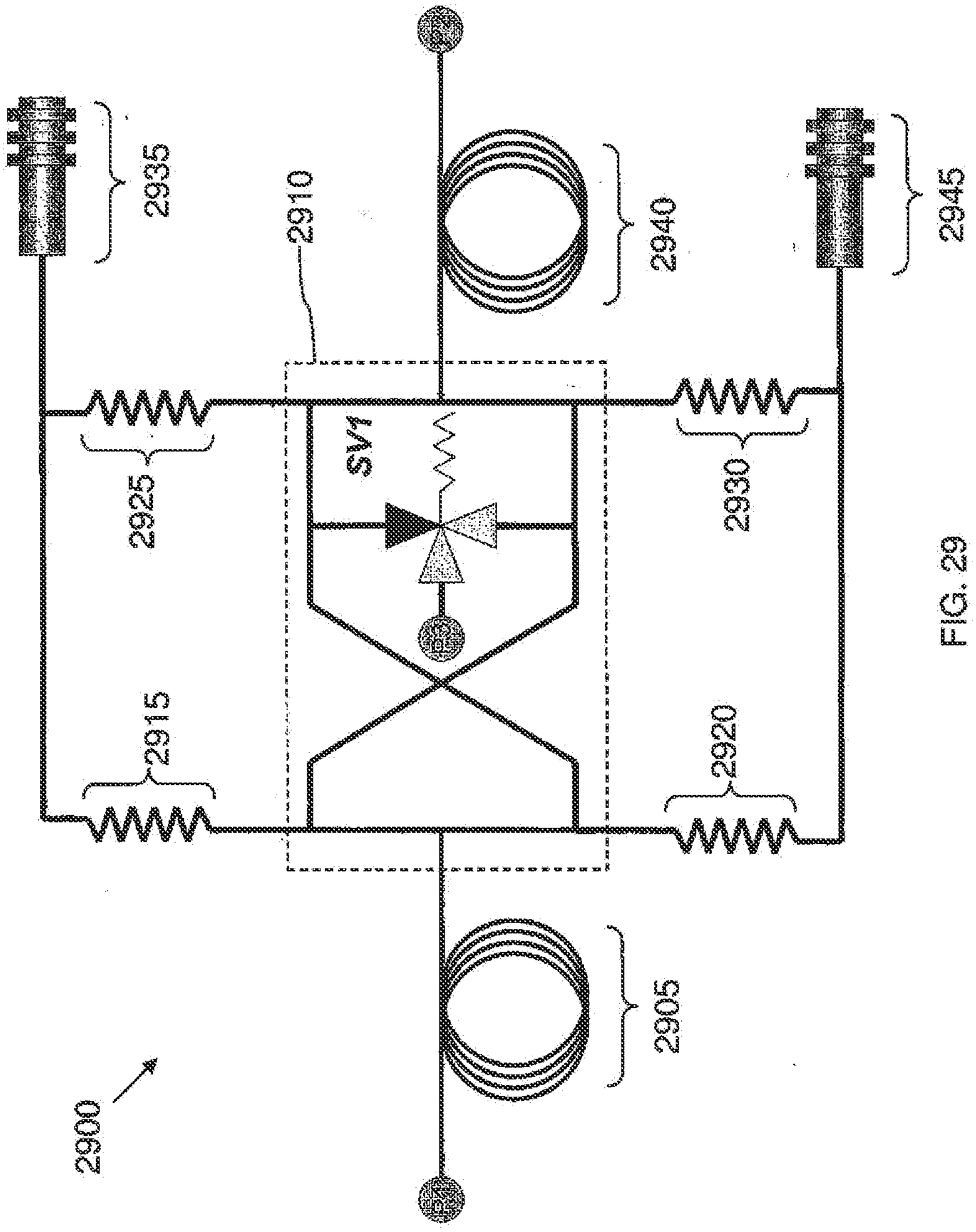


FIG. 29

FIG. 30A

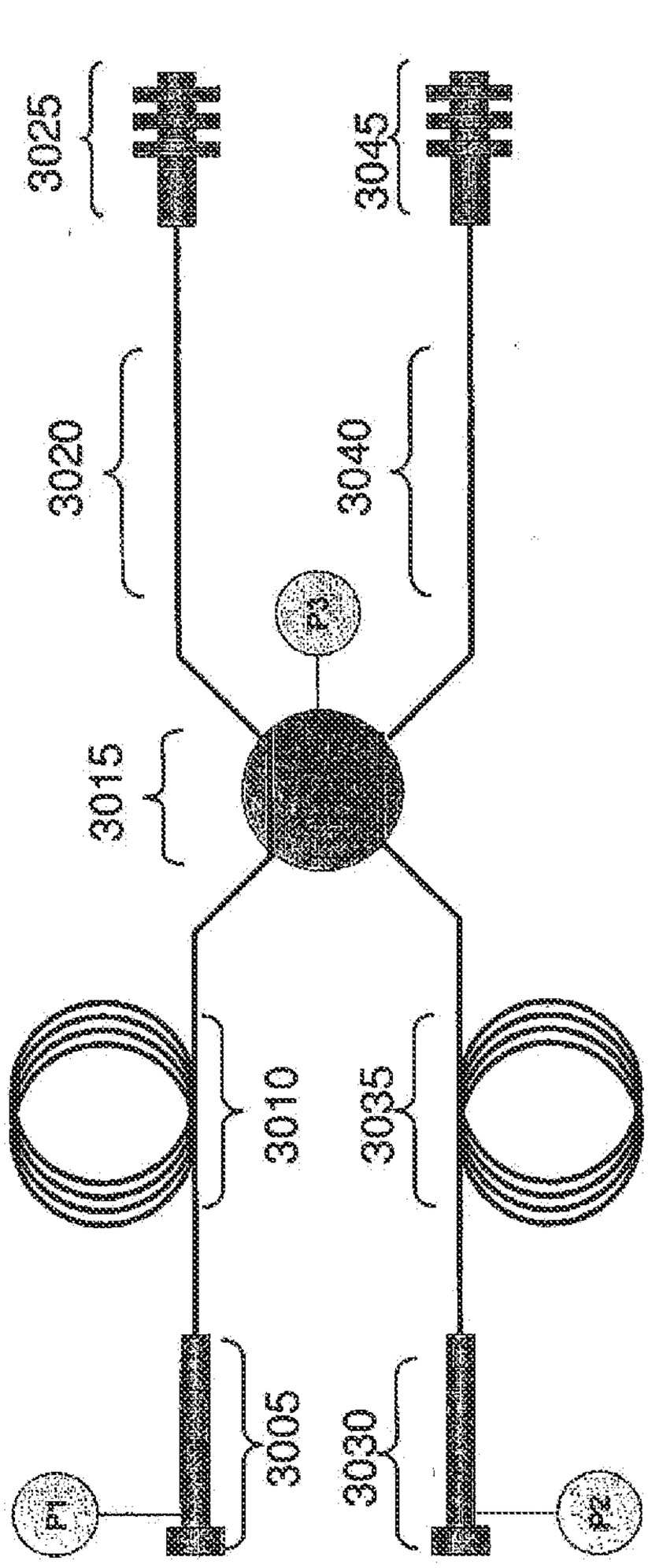
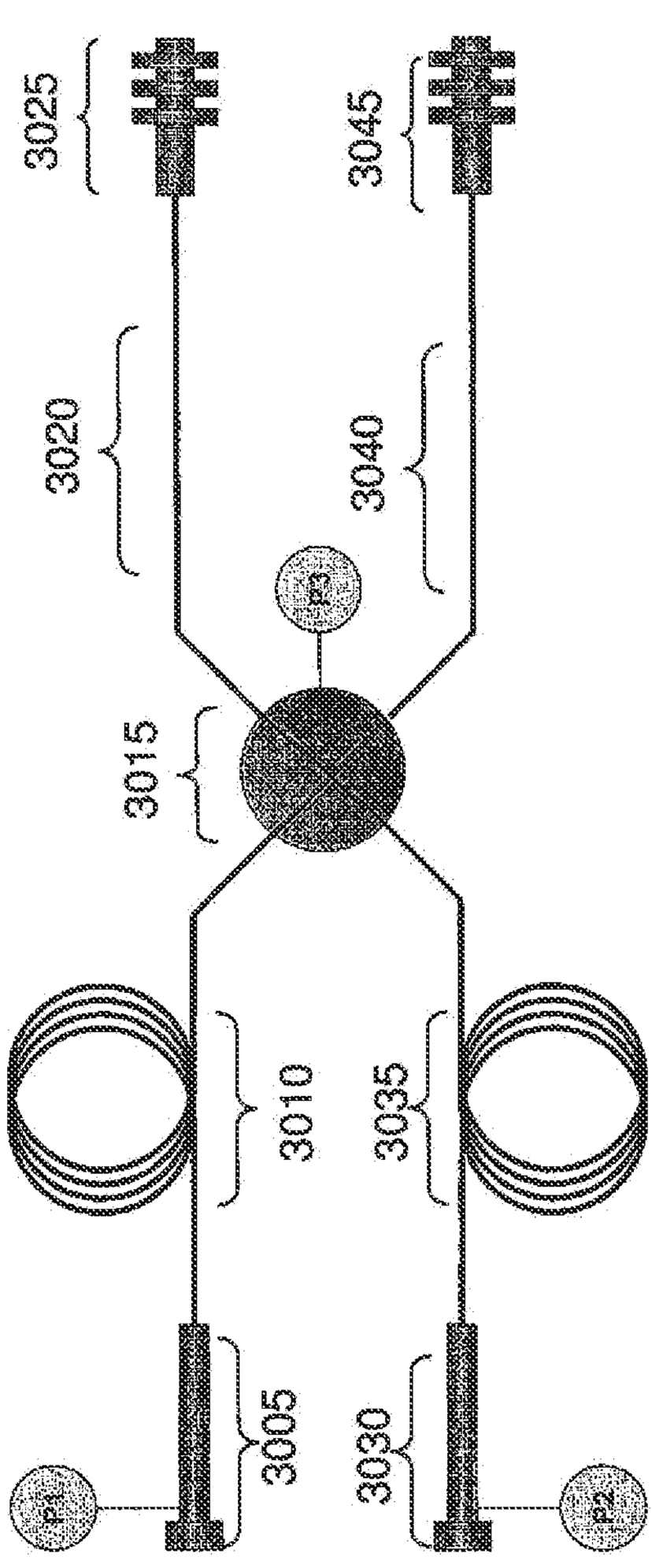


FIG. 30B



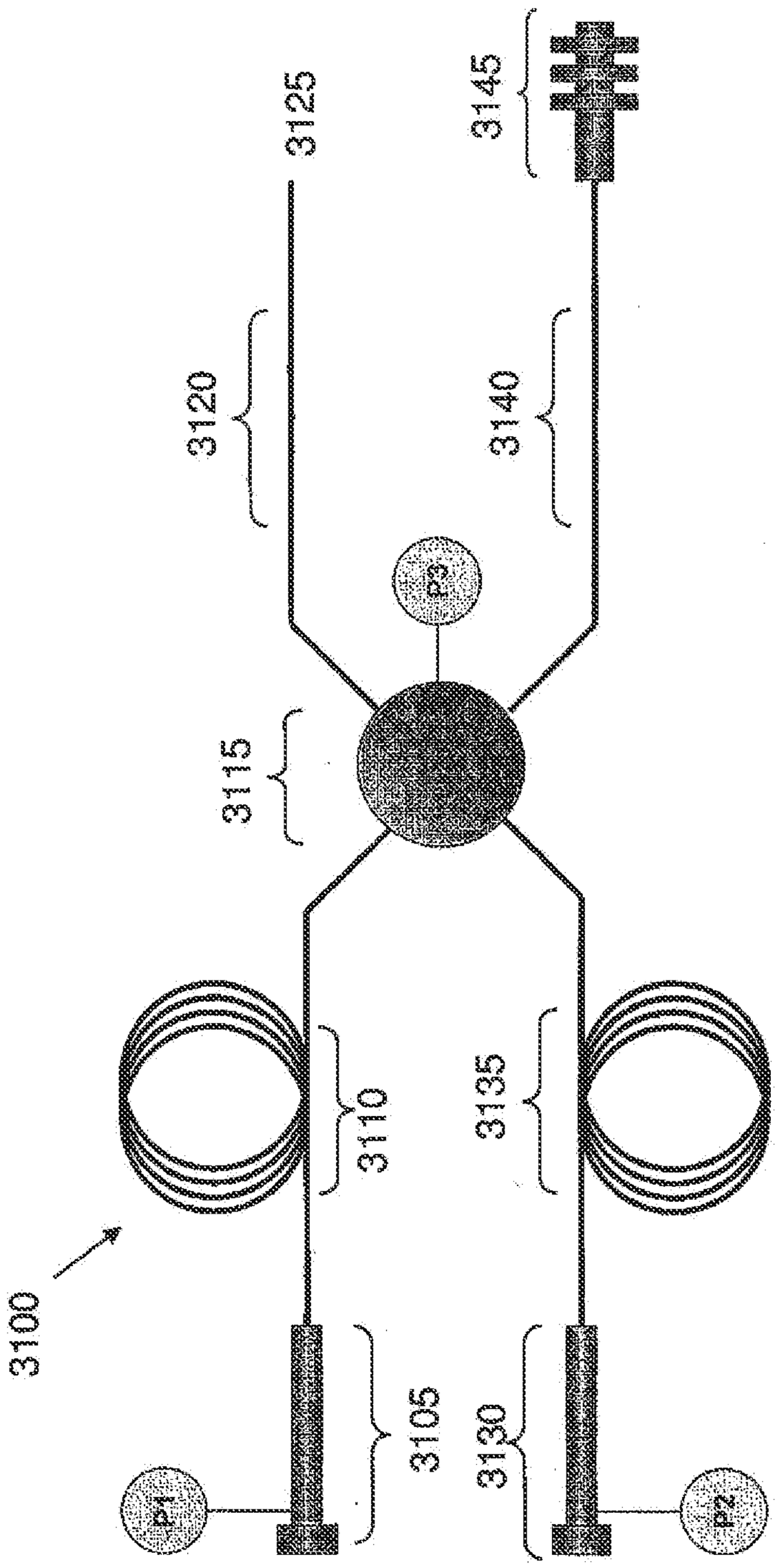


FIG. 31

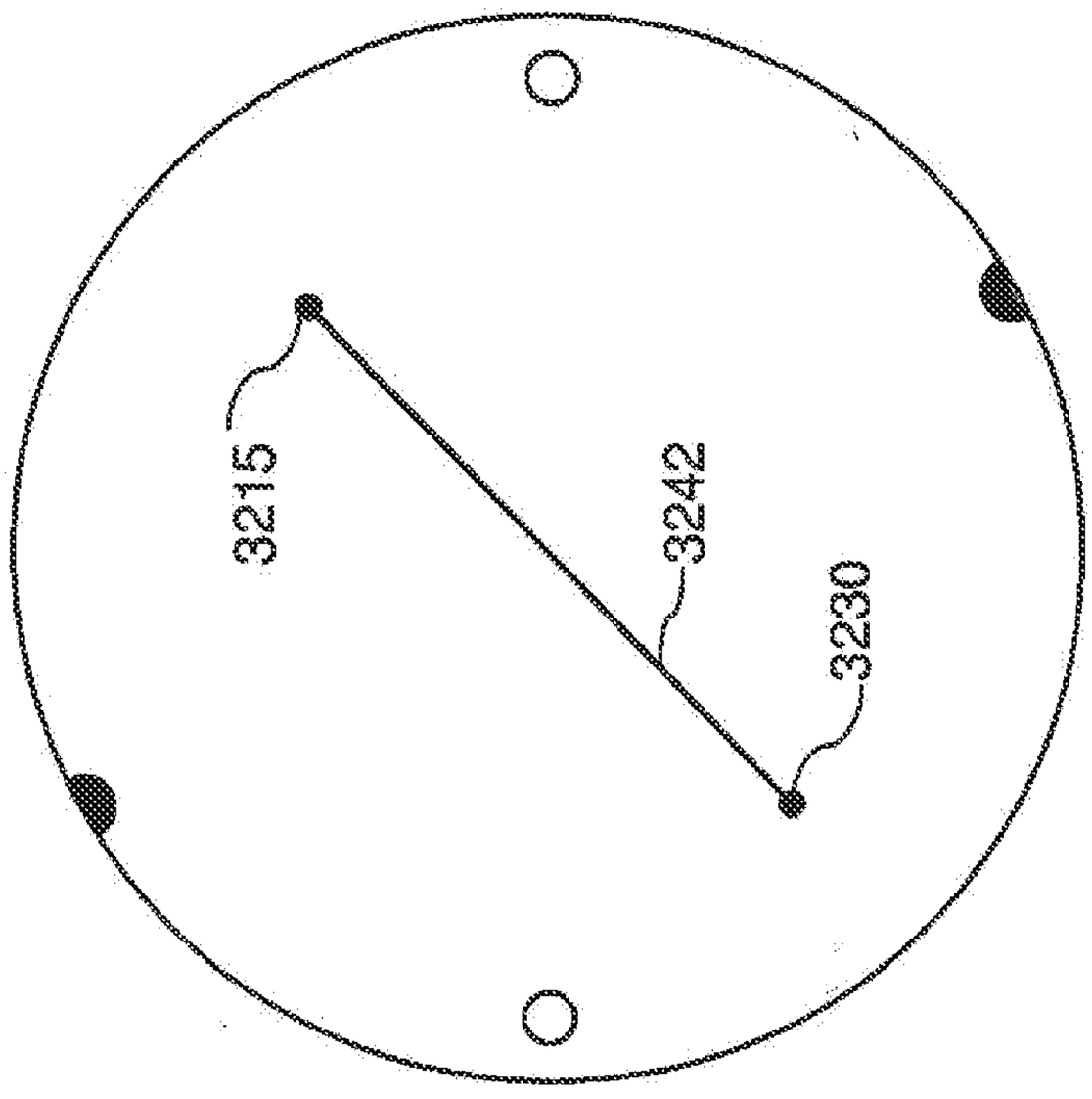


FIG. 32B

FIG. 32A

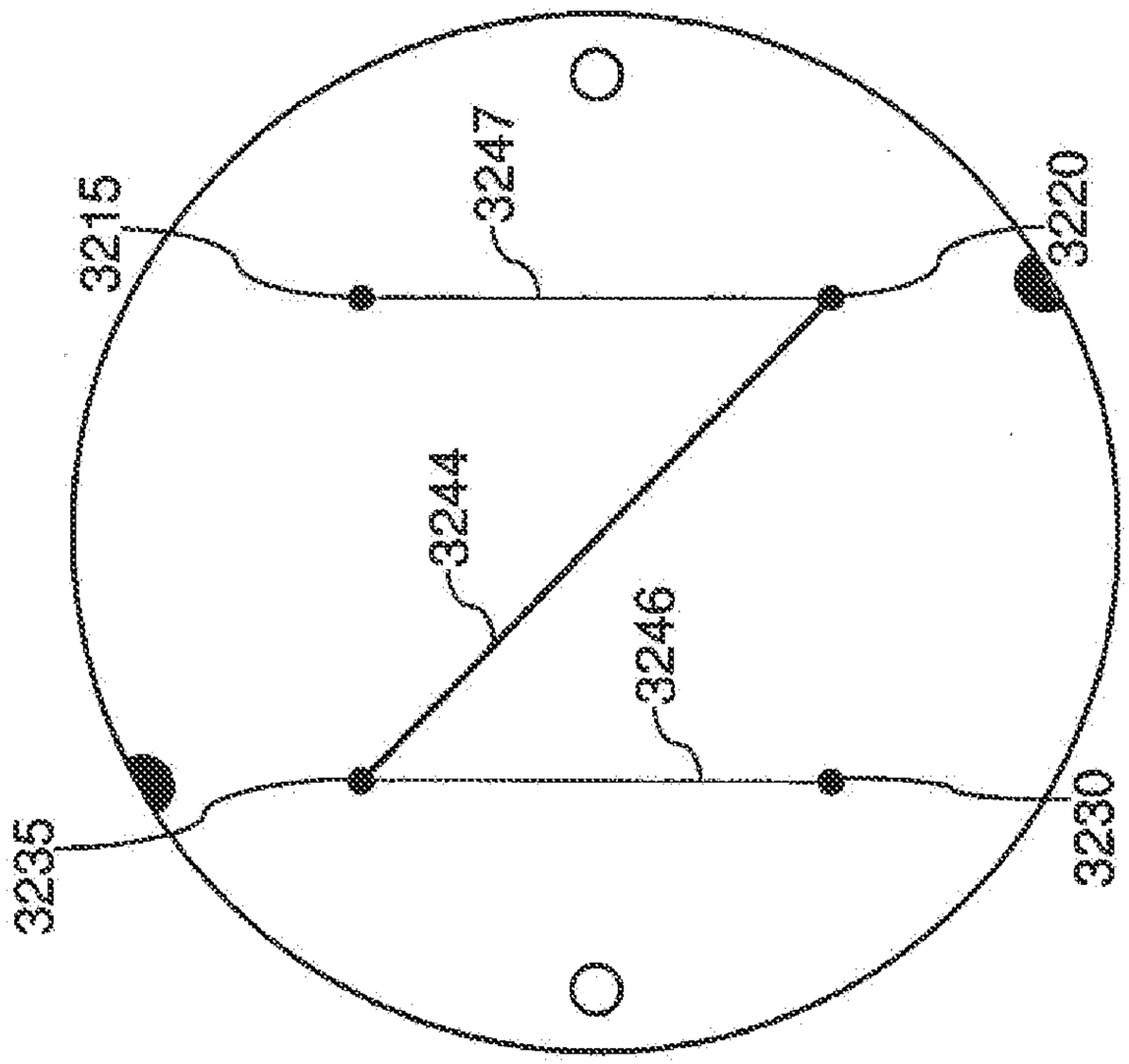


FIG. 32D

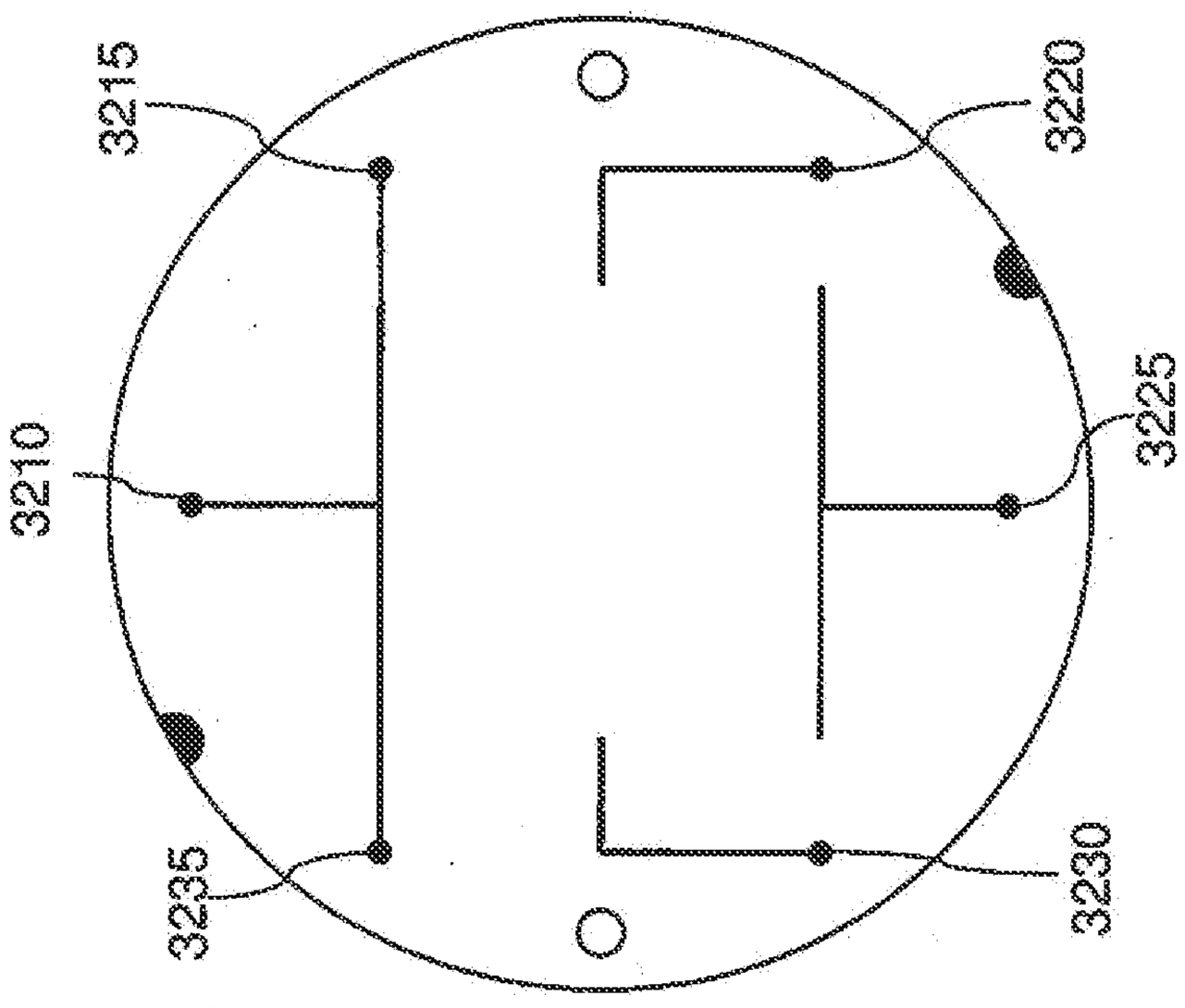


FIG. 32C

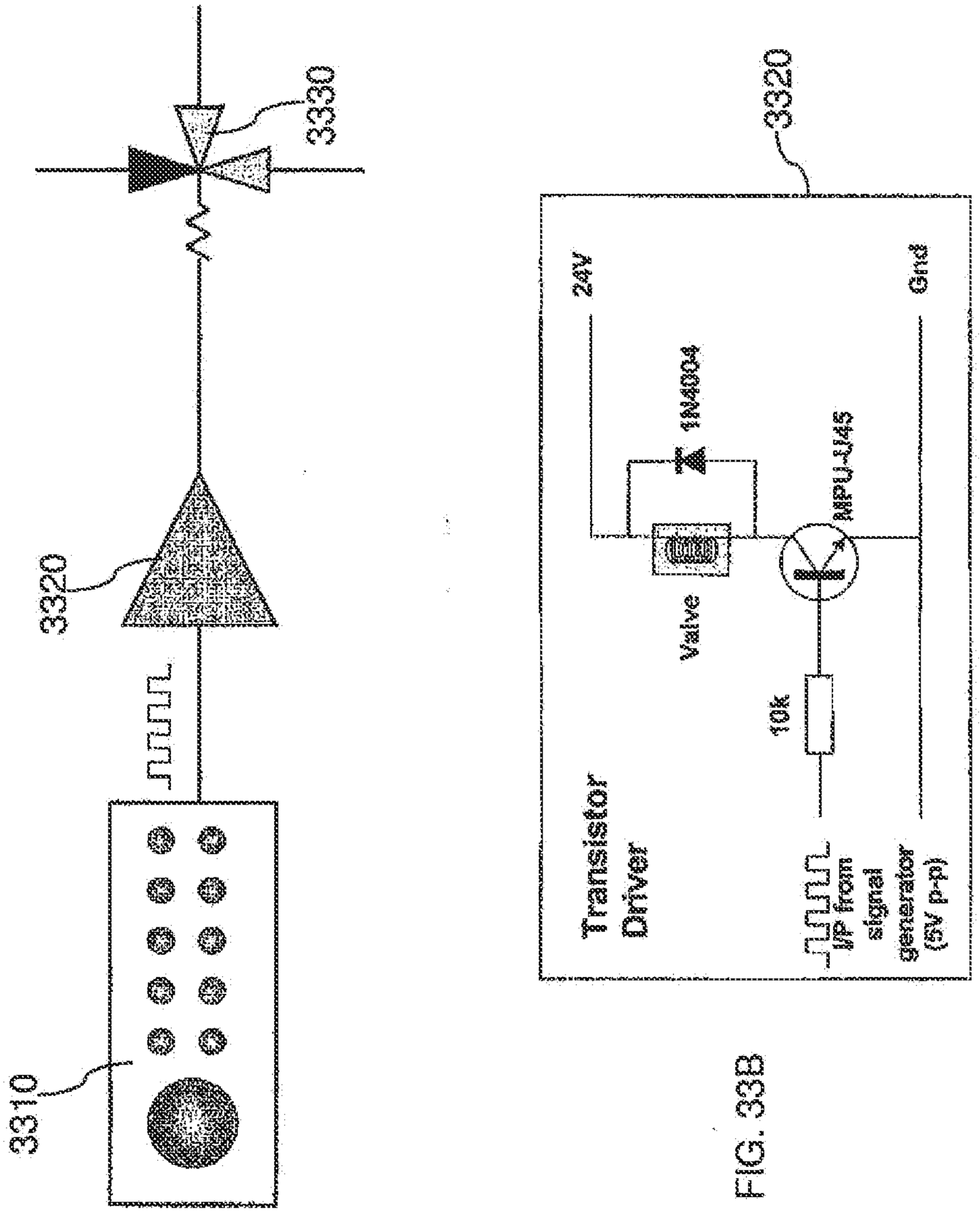


FIG. 33B

FIG. 33A

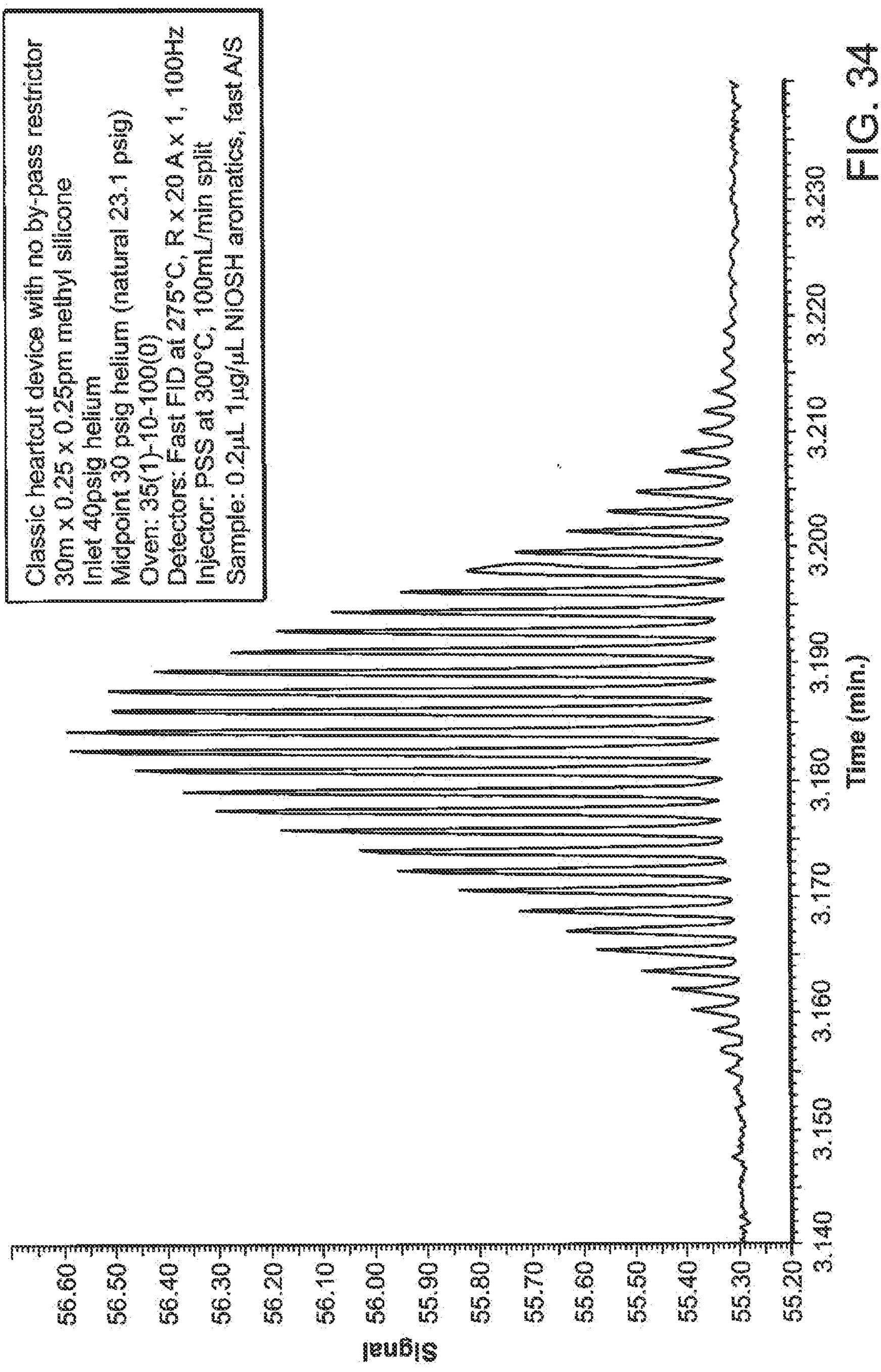

Classic heartcut device with no by-pass restrictor
30m x 0.25 x 0.25pm methyl silicone
Inlet 40psig helium
Midpoint 30 psig helium (natural 23.1 psig)
Oven: 35(1)-10-100(0)
Detectors: Fast FID at 275°C, R x 20 A x 1, 100Hz
Injector: PSS at 300°C, 100mL/min split
Sample: 0.2µL 1µg/µL NIOSH aromatics, fast A/S
signal
56.60
56.50
56.40
56.30
56.20
56.10
56.00
55.90
55.80
55.70
55.60
55.50
55.40
55.30
55.20
3.140
3.150
3.160
3.170
3.180
3.190
3.200
3.210
3.220
3.230
Time (min.)

FIG. 34

FIG. 35A

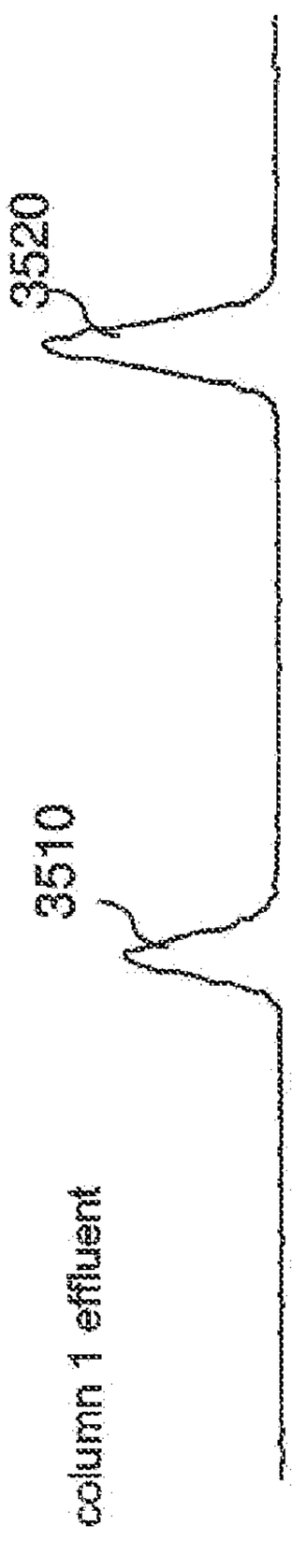


FIG. 35B

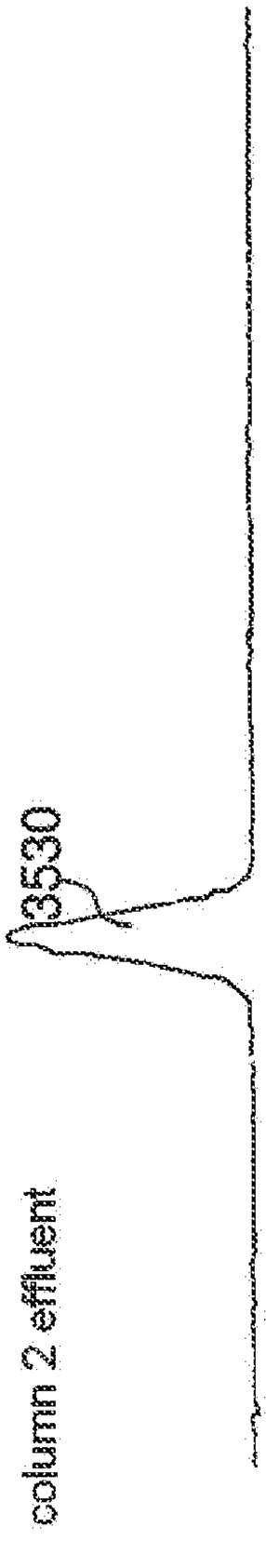


FIG. 35C

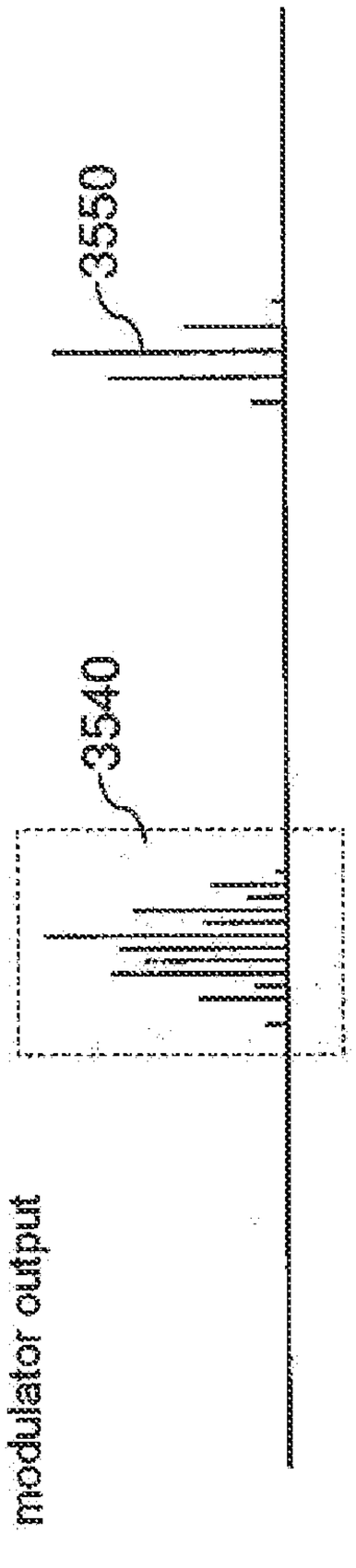


FIG. 36

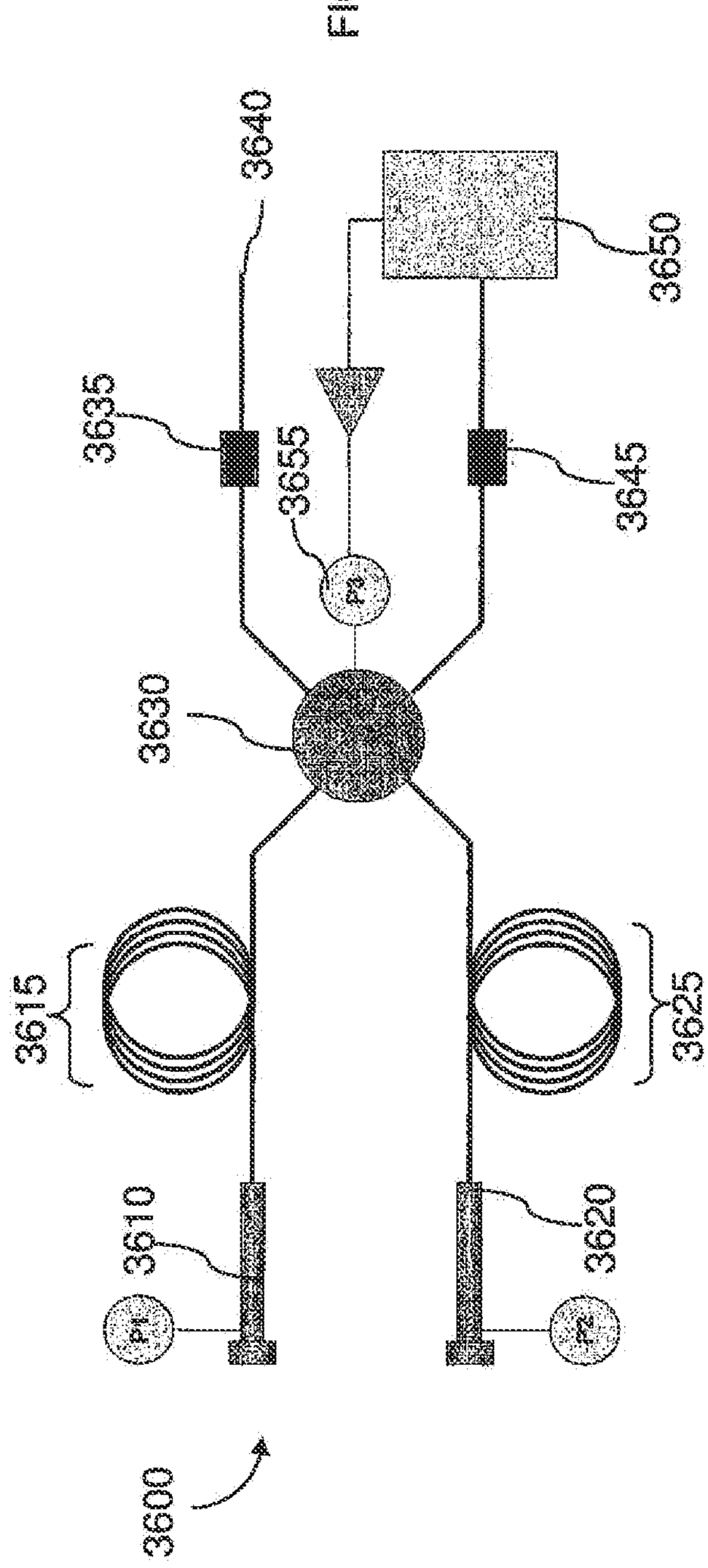

3600
3610
3620
3615
3625
3630
3635
3640
3645
3650
3655
P1
P2
P3

FIG. 37

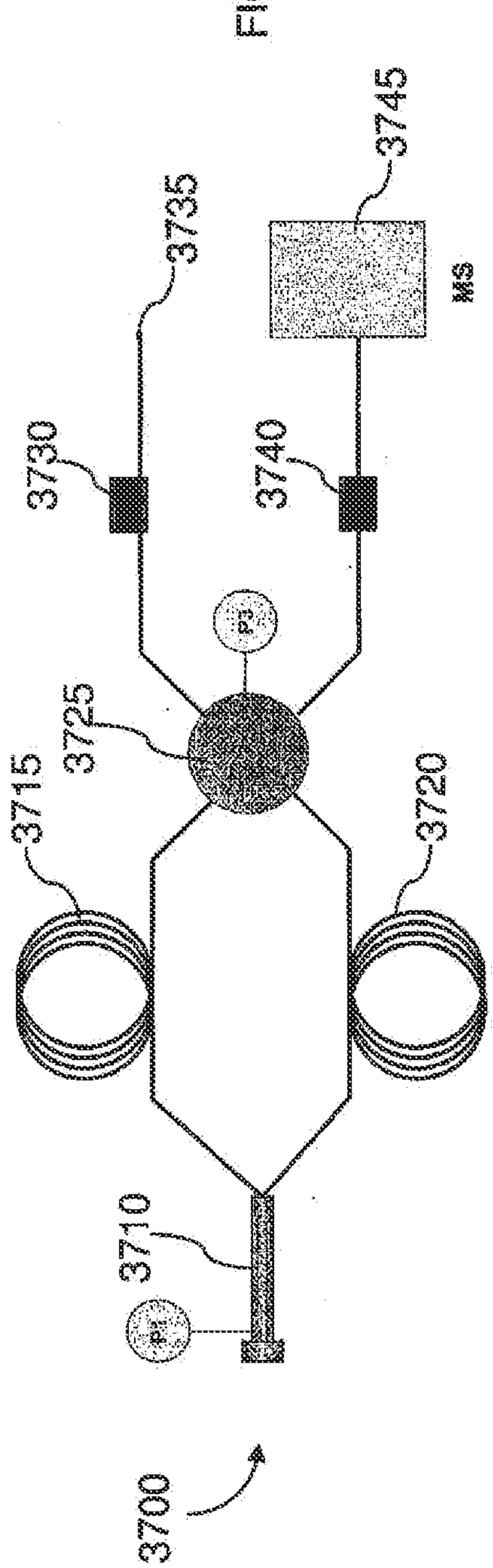

3700
3710
3715
3720
3725
3730
3735
3740
3745
MS
P1
P3

FIG. 38

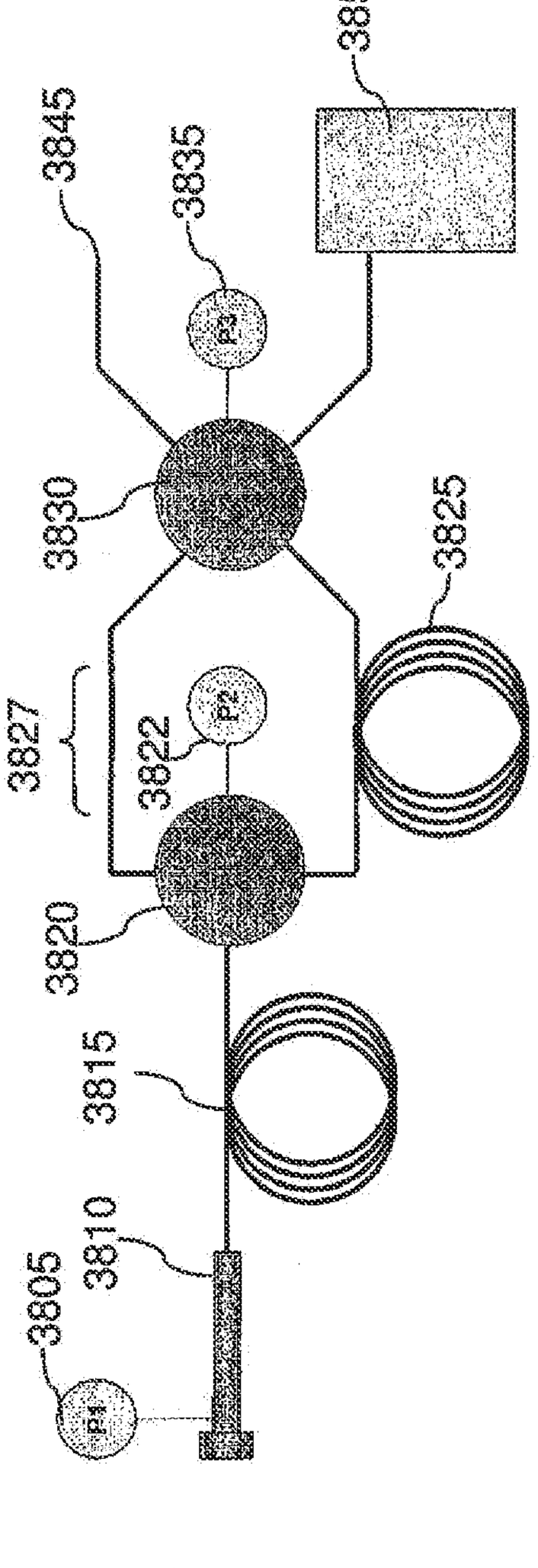
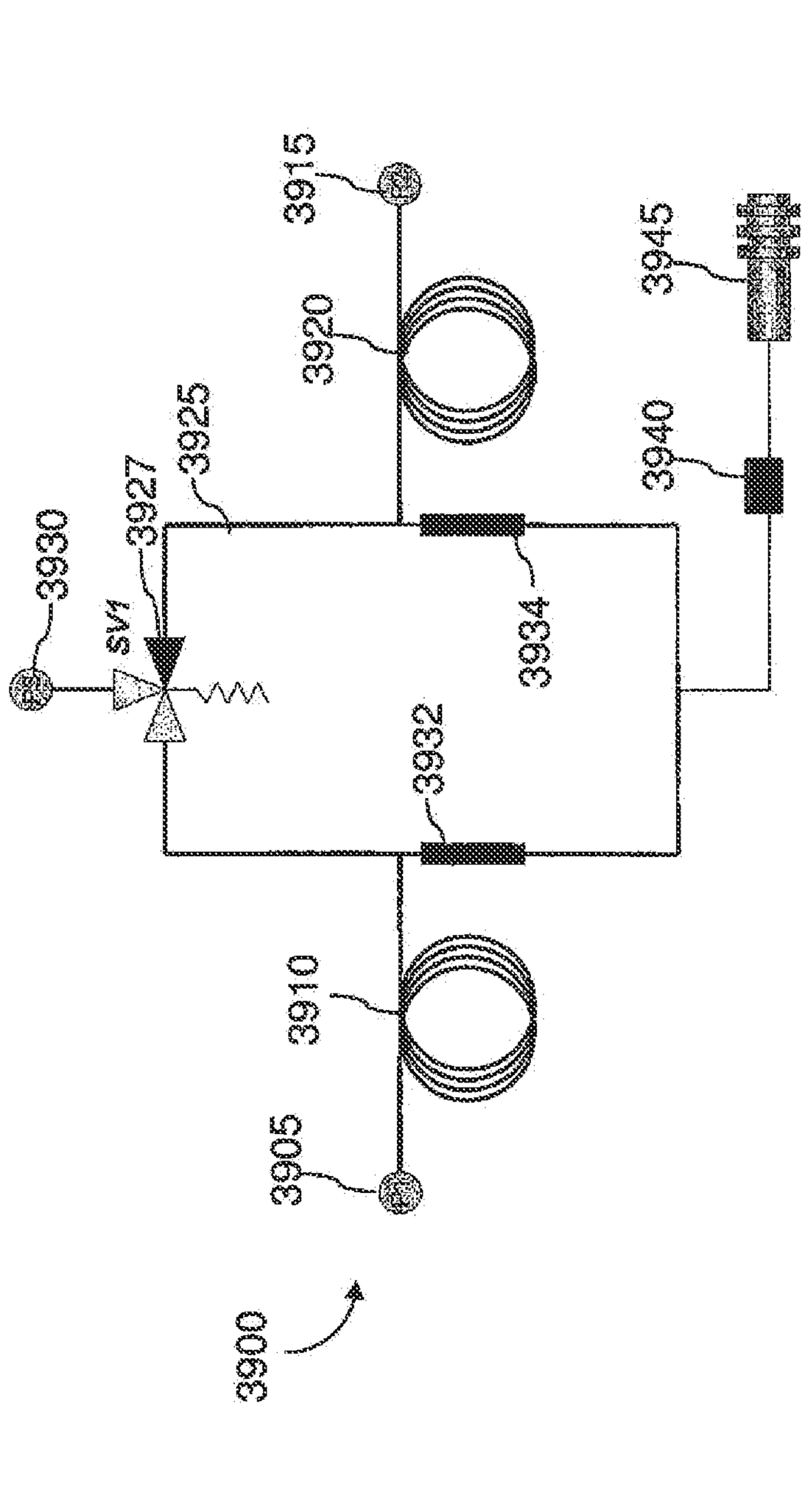
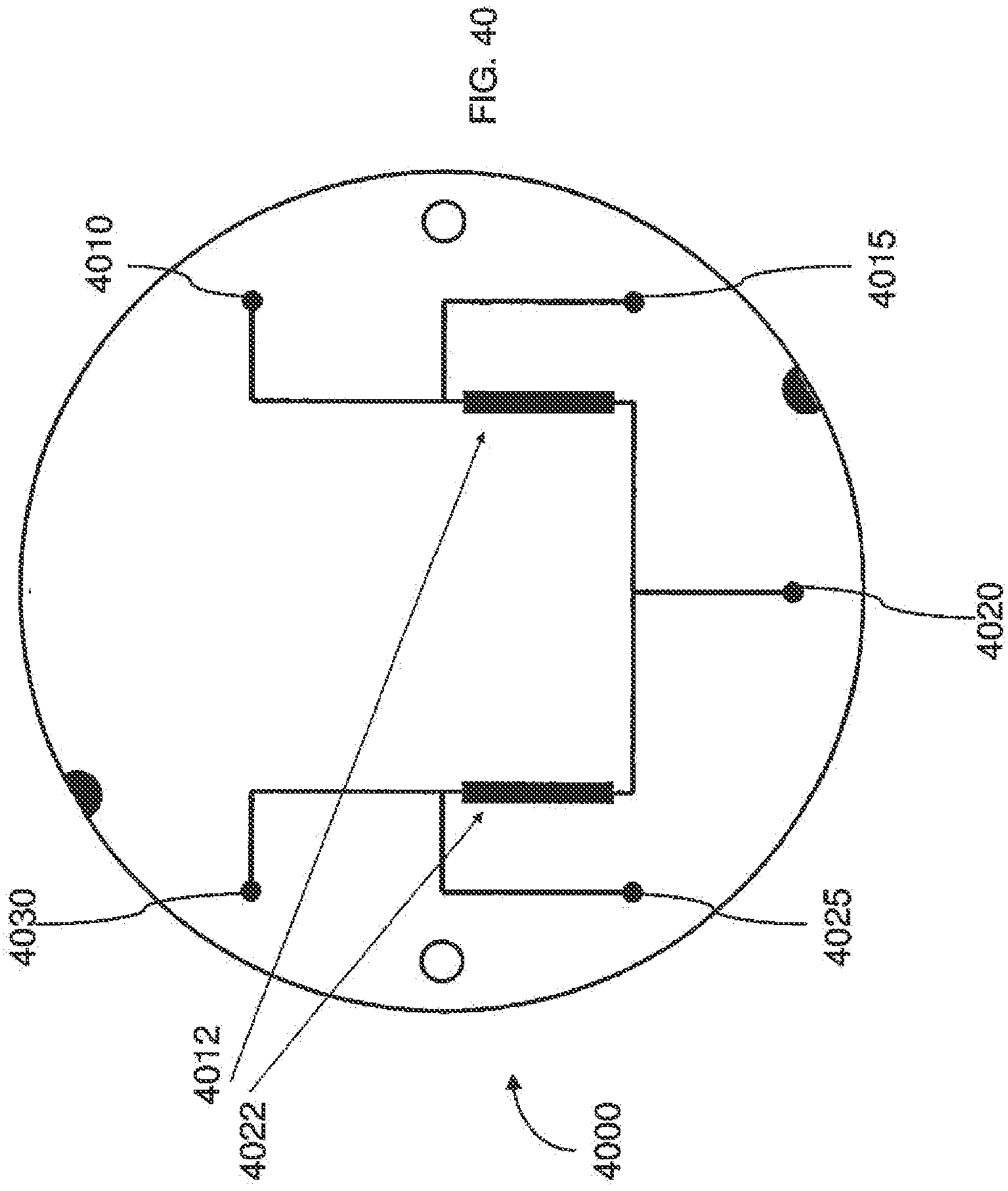

3800
3805
3810
3815
3820
3822
3825
3827
3830
3835
3845
38
P1
P2

FIG. 39


3900
3905
3910
3915
3920
3925
3927
3930
3932
3934
3940
3945
SV1
P3


FIG. 40
4010
4015
4020
4025
4030
4012
4022
4000

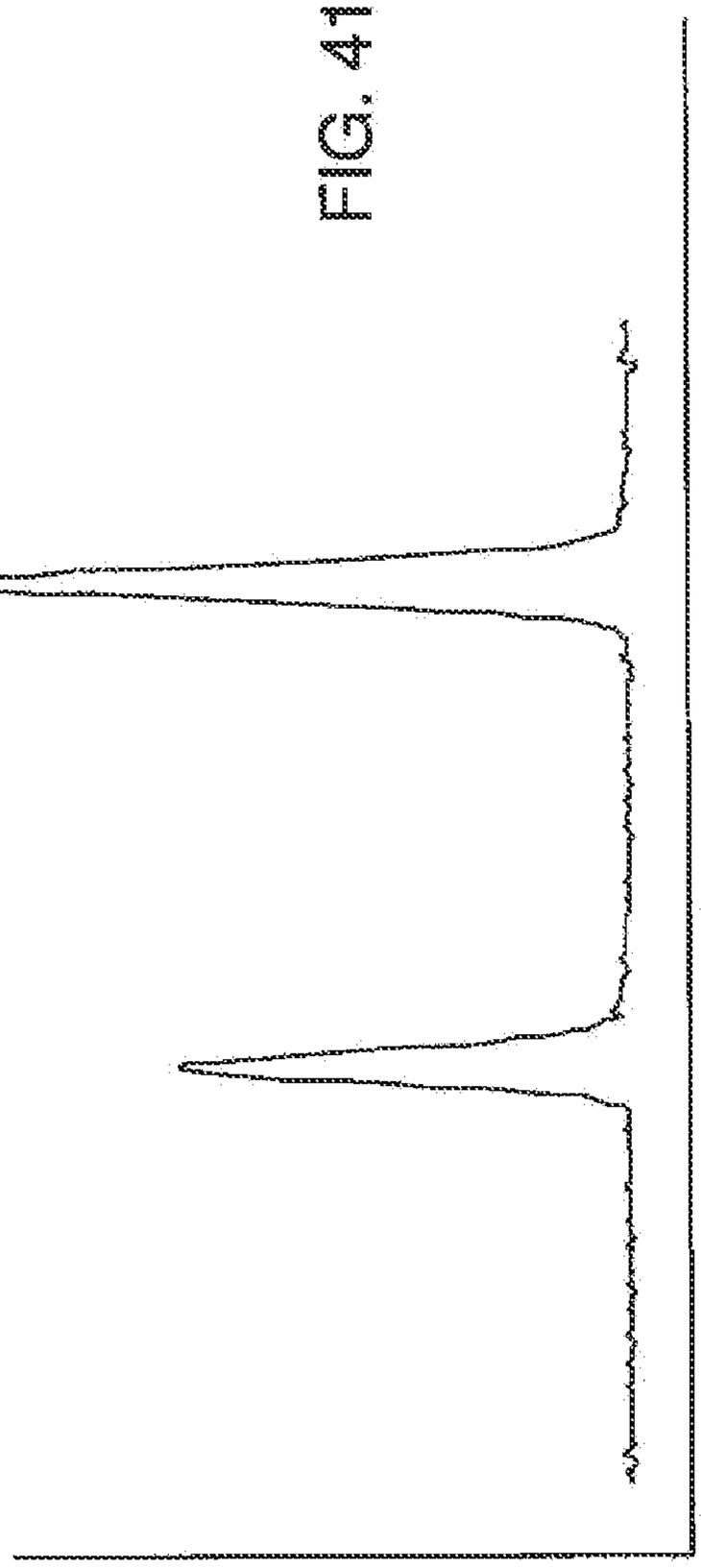


FIG. 41

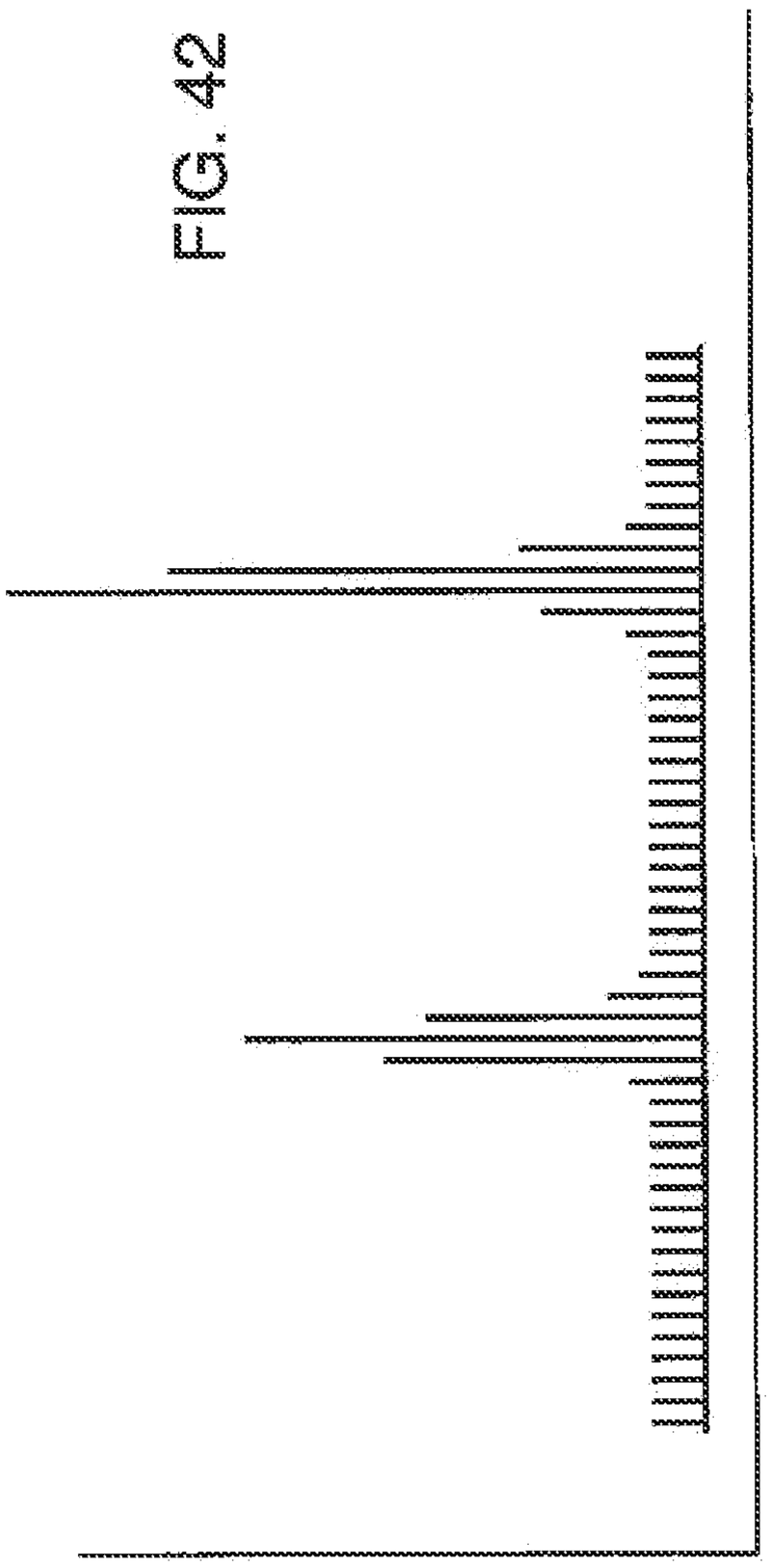


FIG. 42

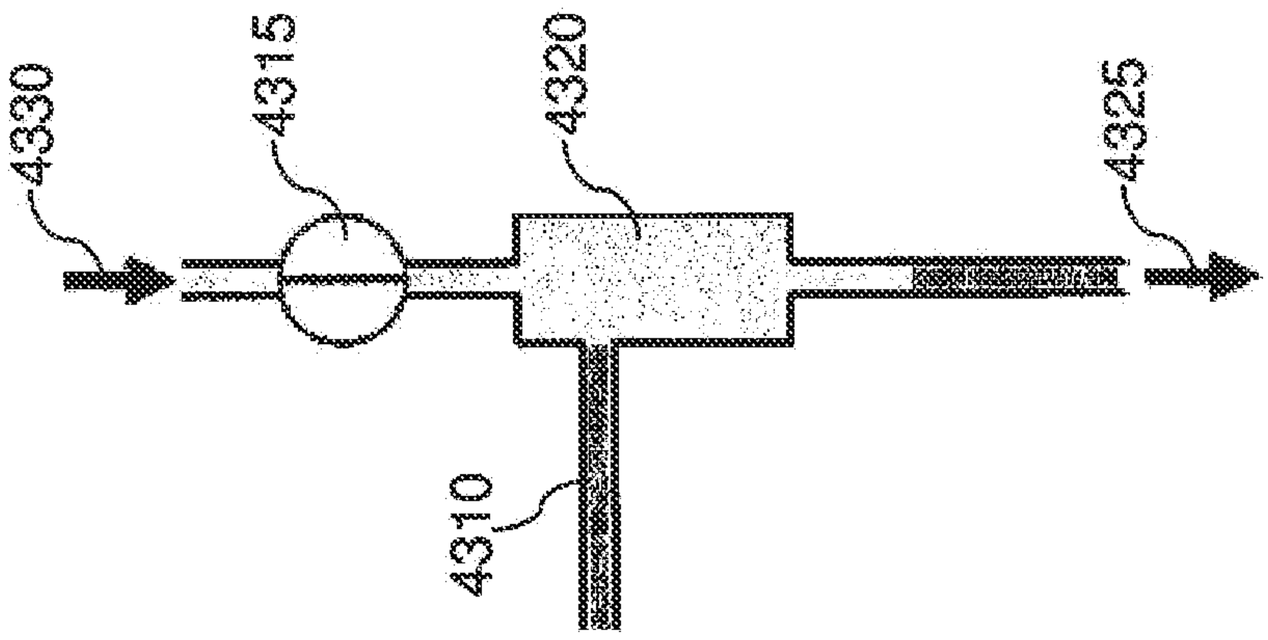


FIG. 43B

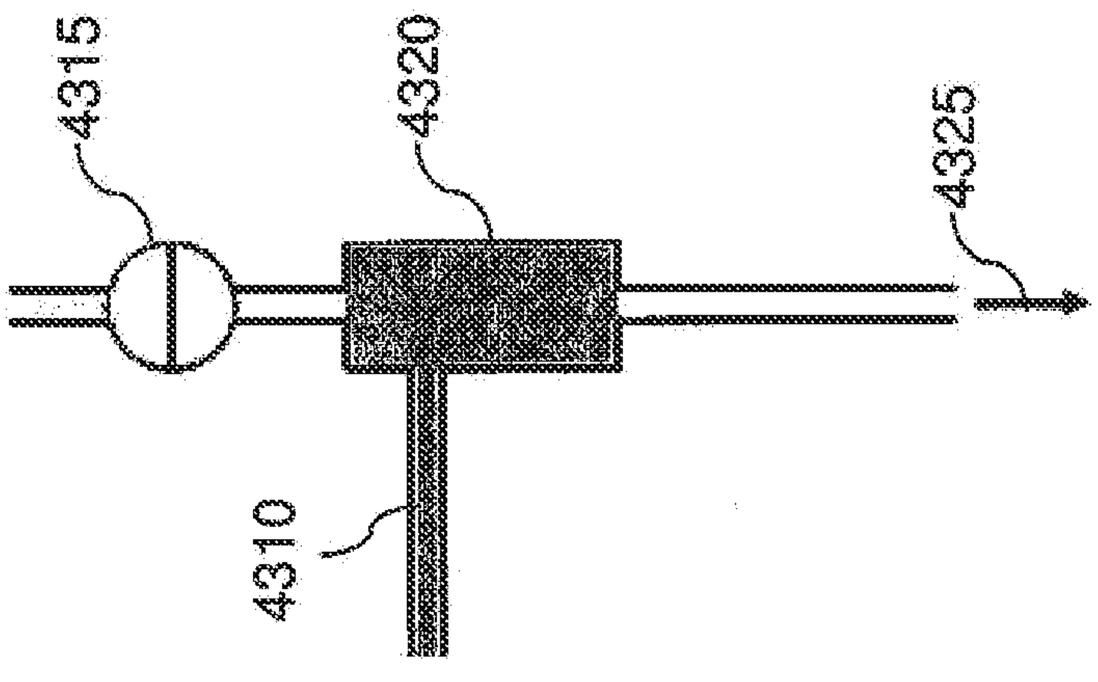


FIG. 43A

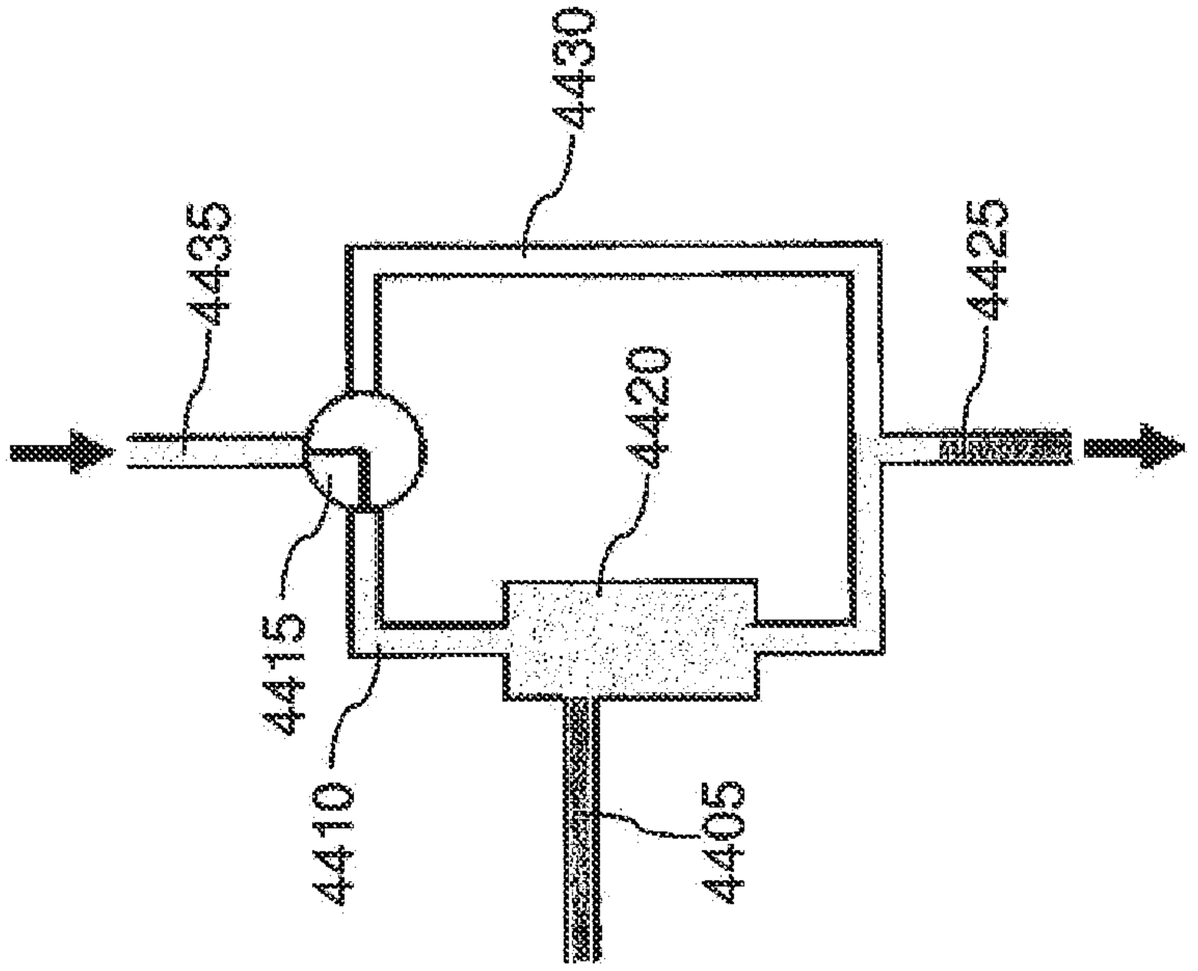


FIG. 44B

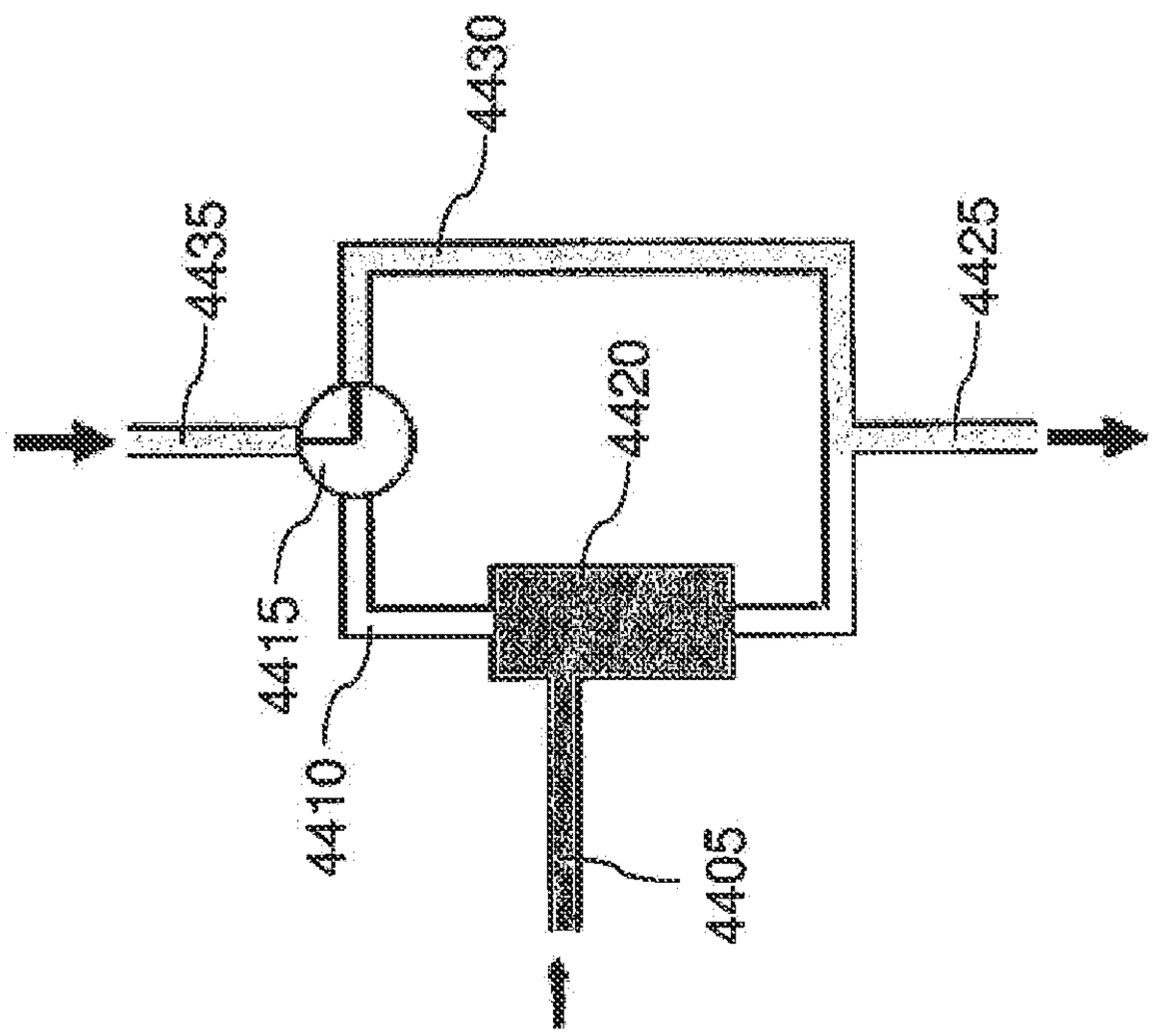


FIG. 44A

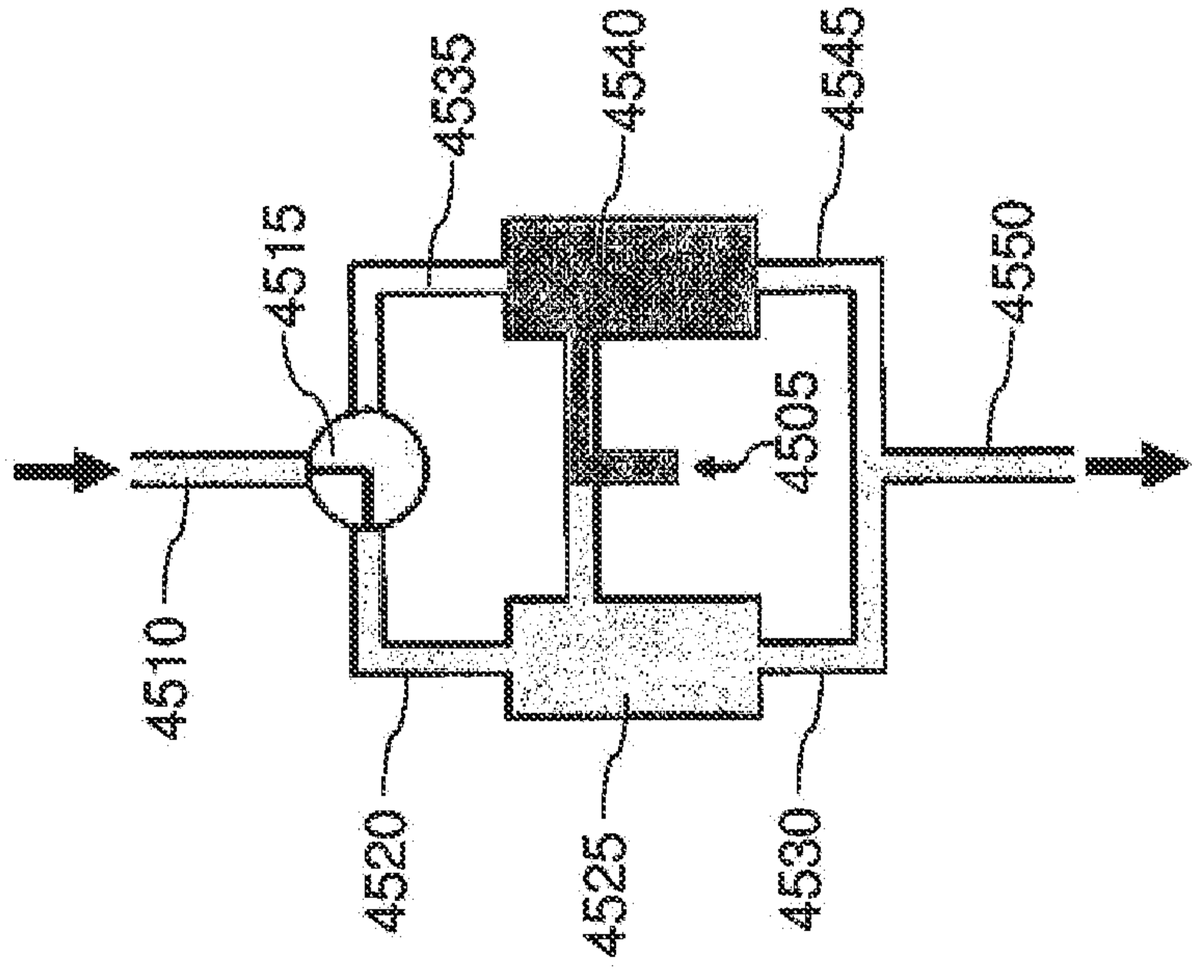


FIG. 45B

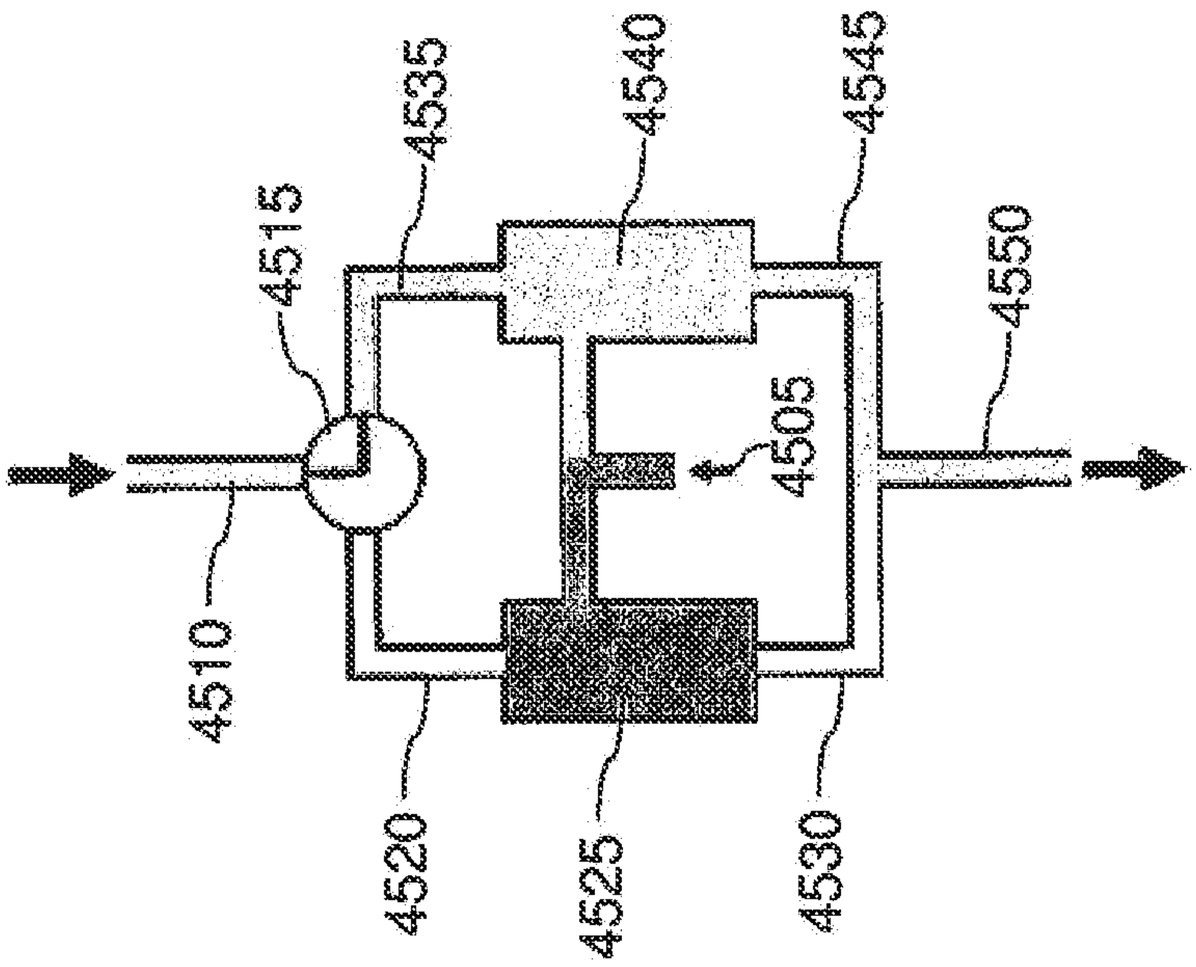


FIG. 45A

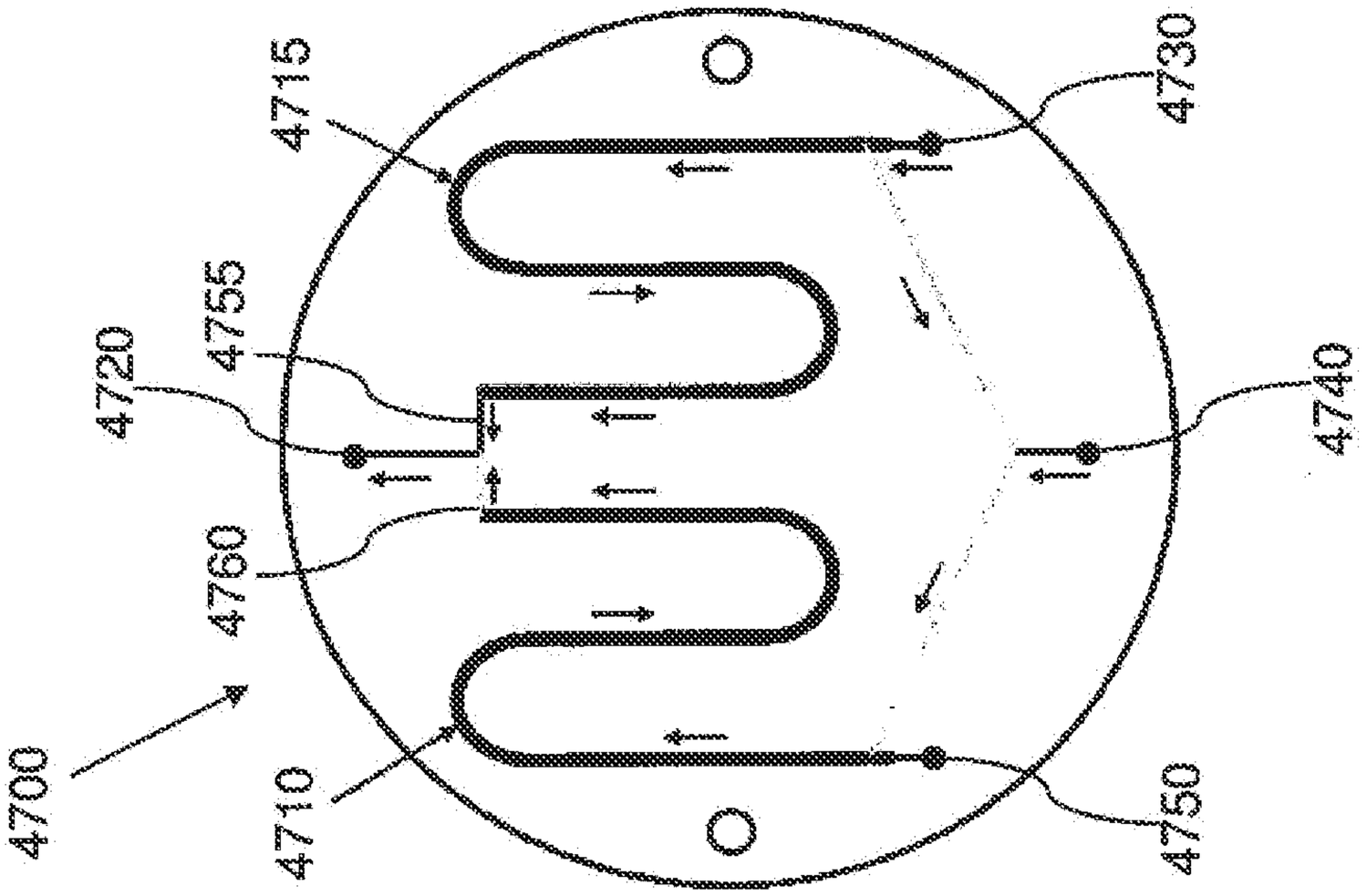

4700
4710
4715
4720
4730
4740
4750
4755
4760

FIG. 47

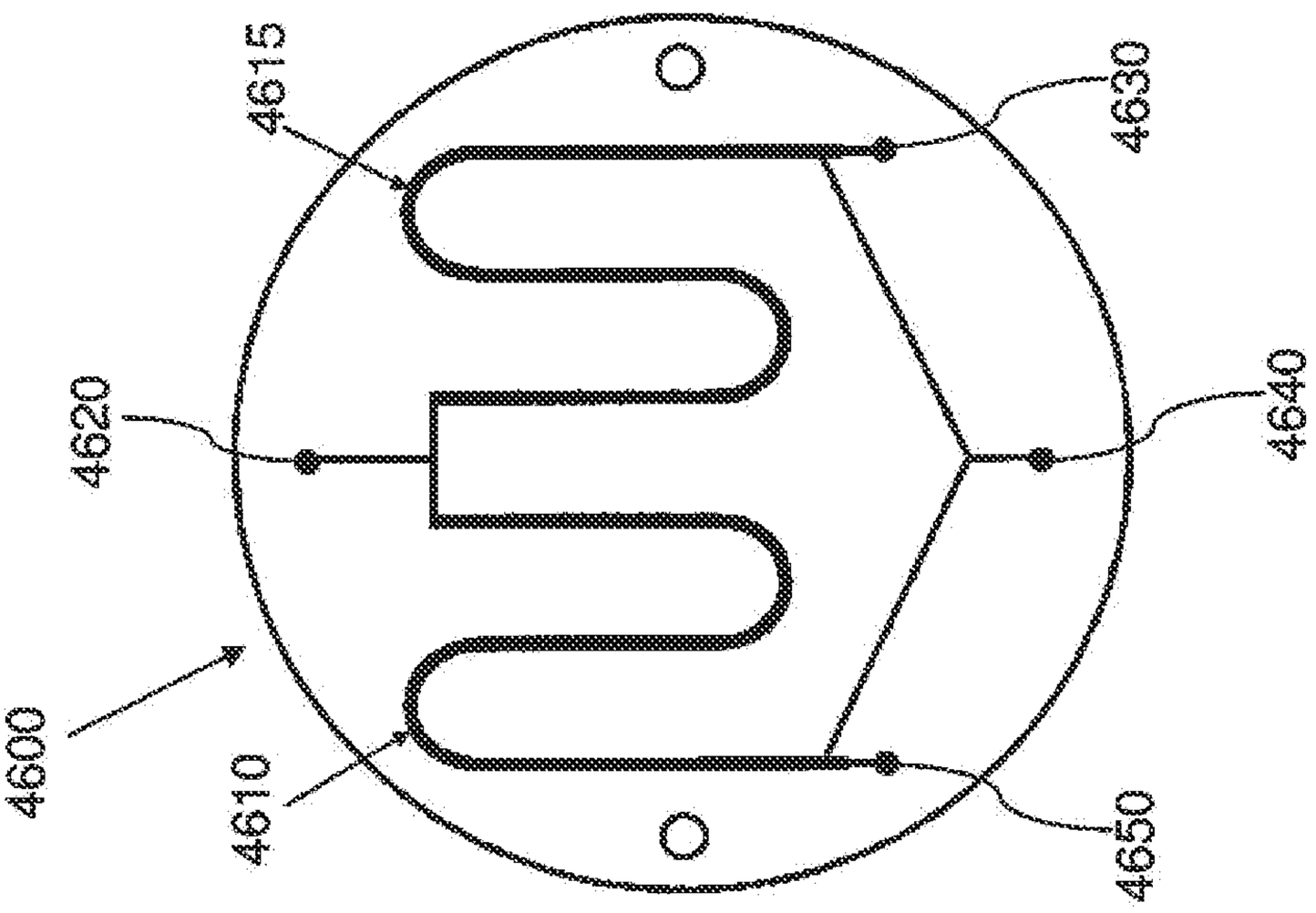

4600
4610
4615
4620
4630
4640
4650

FIG. 46

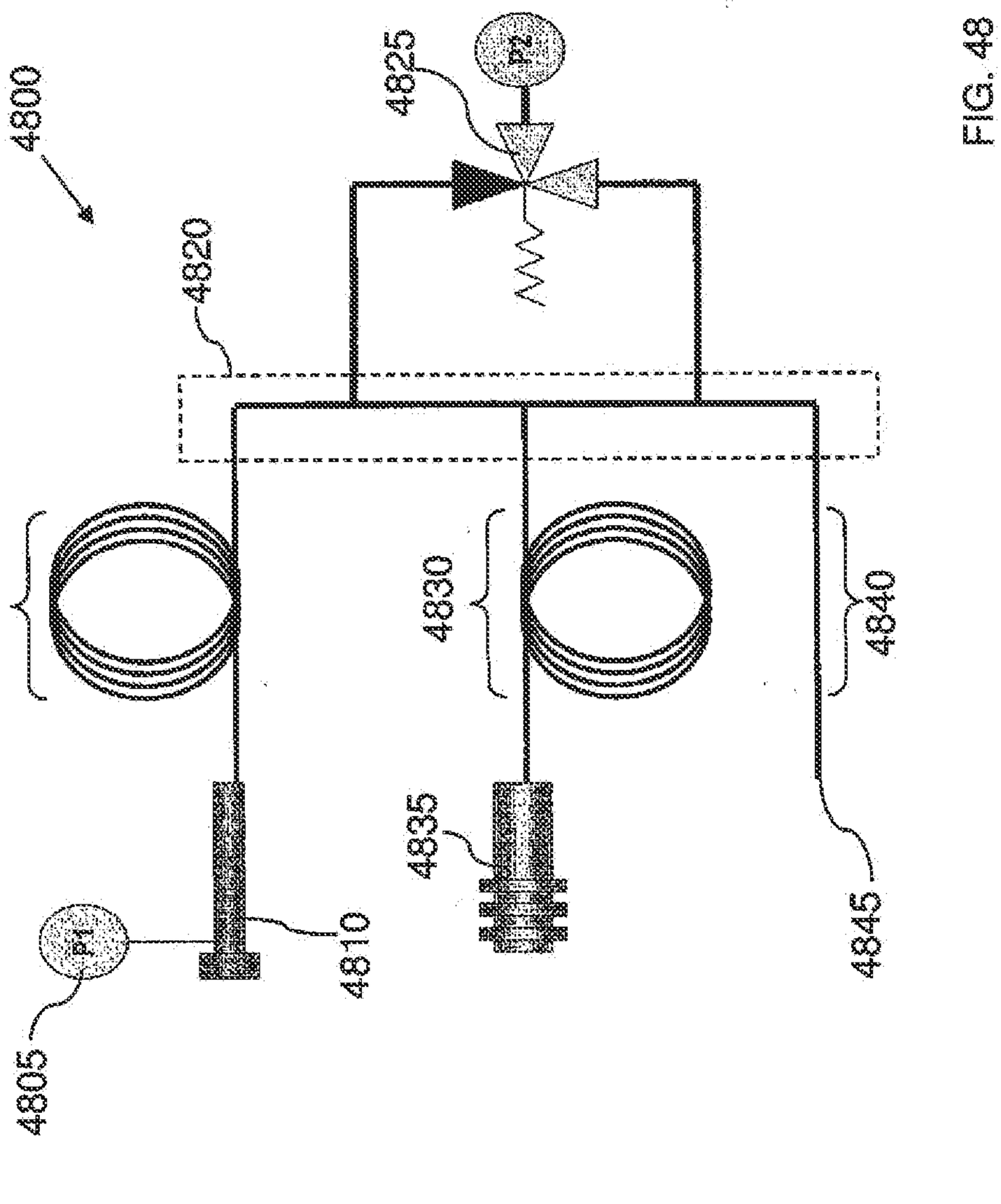


FIG. 48

FIG. 49

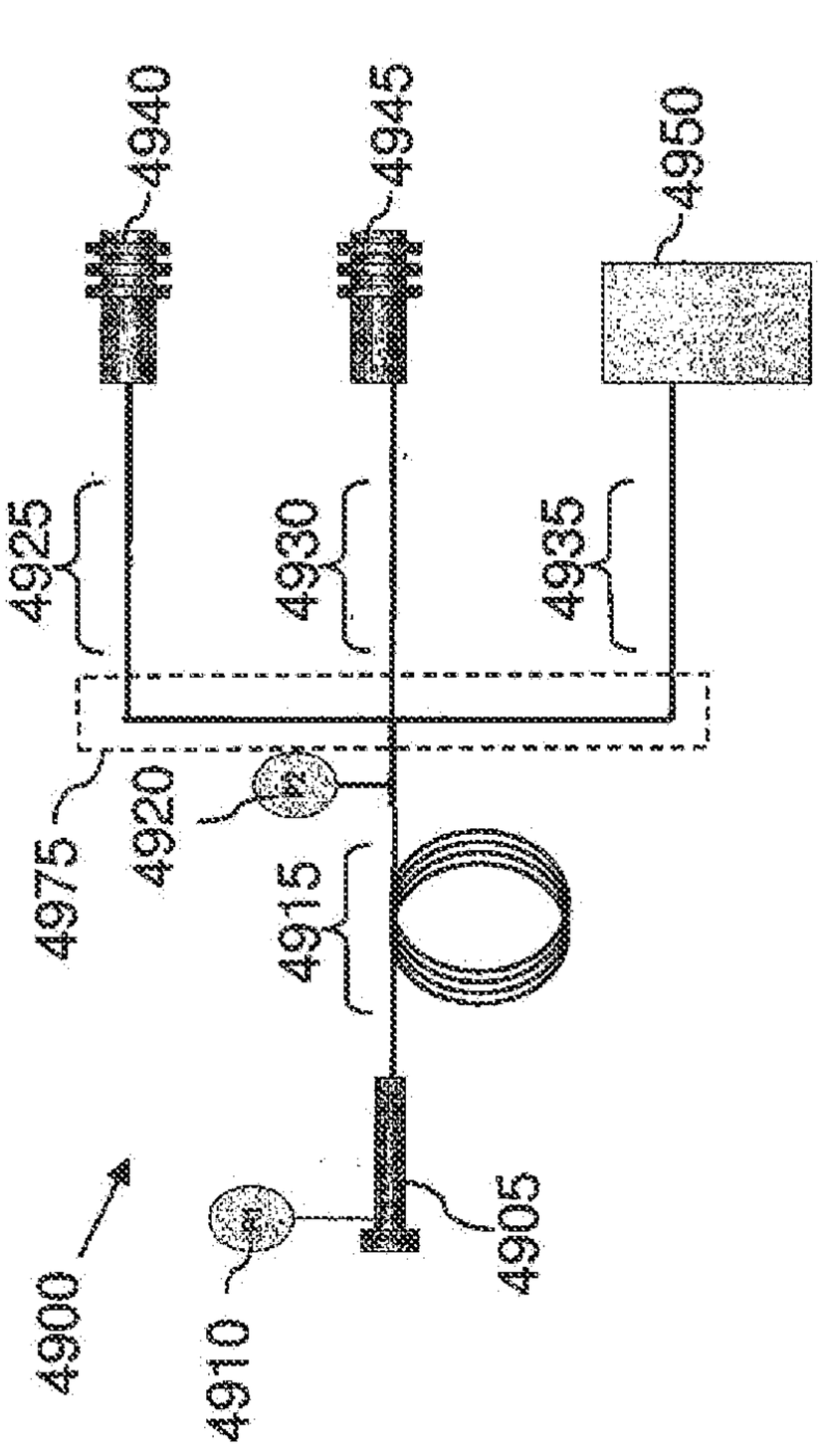
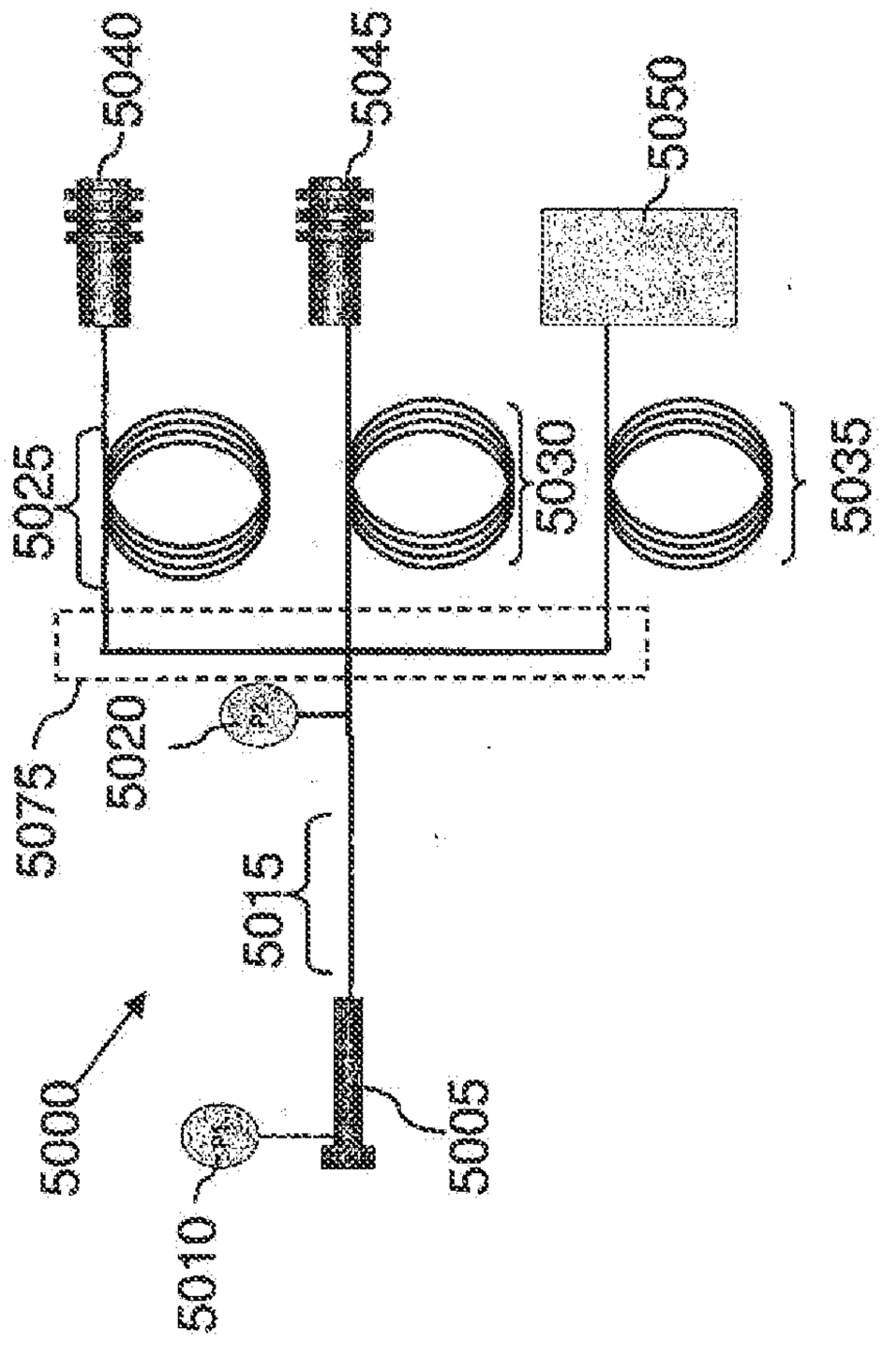

4940
4945
4950
4925
4930
4935
4975
4920
4915
4900
4910
4905

FIG. 50


5040
5045
5050
5025
5030
5035
5075
5020
5015
5000
5010
5005

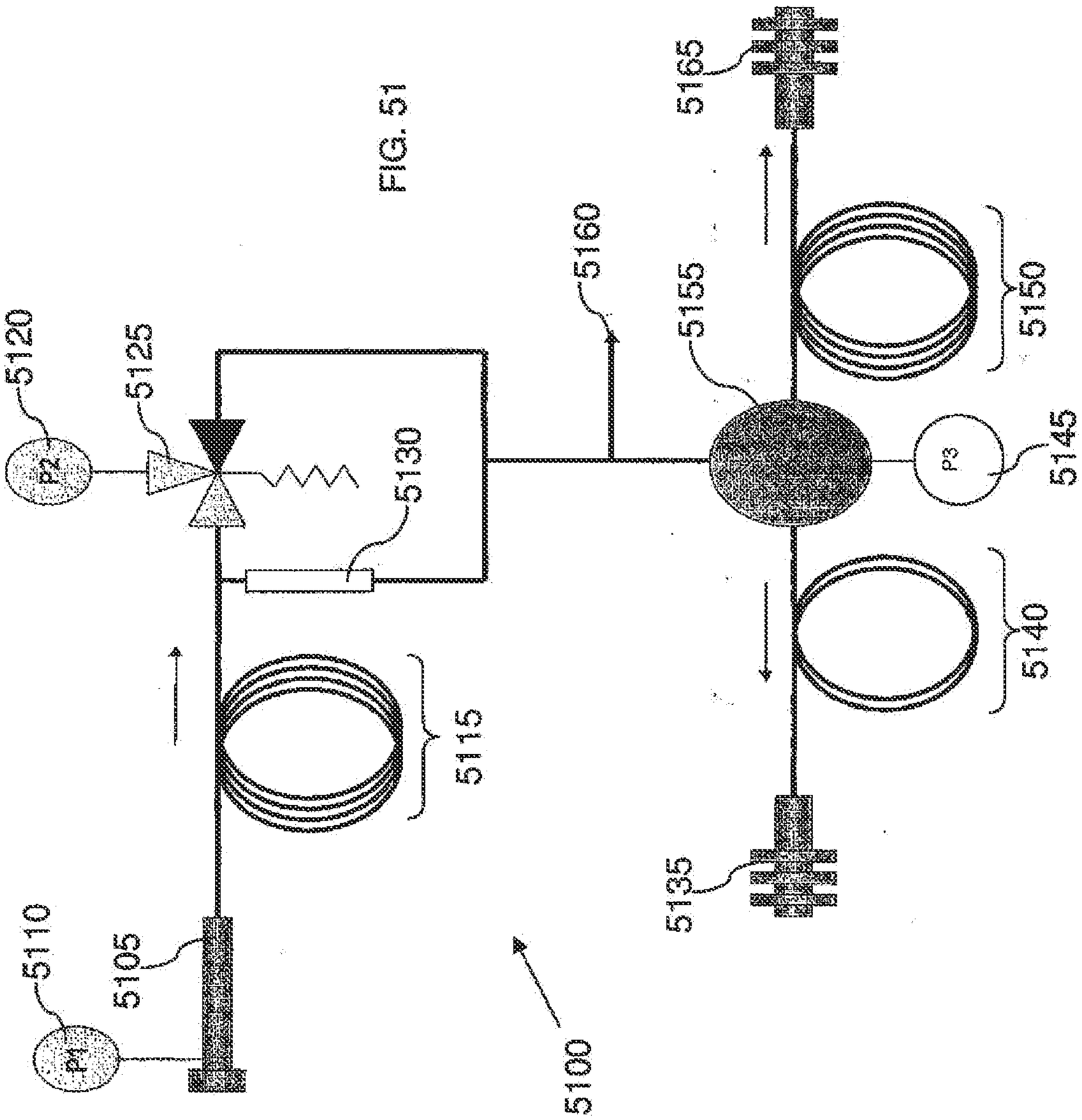

5120
P2
5125
5130
5160
5165
5155
5150
P3
5145
5115
5140
5135
5110
P1
5105
100

FIG. 51

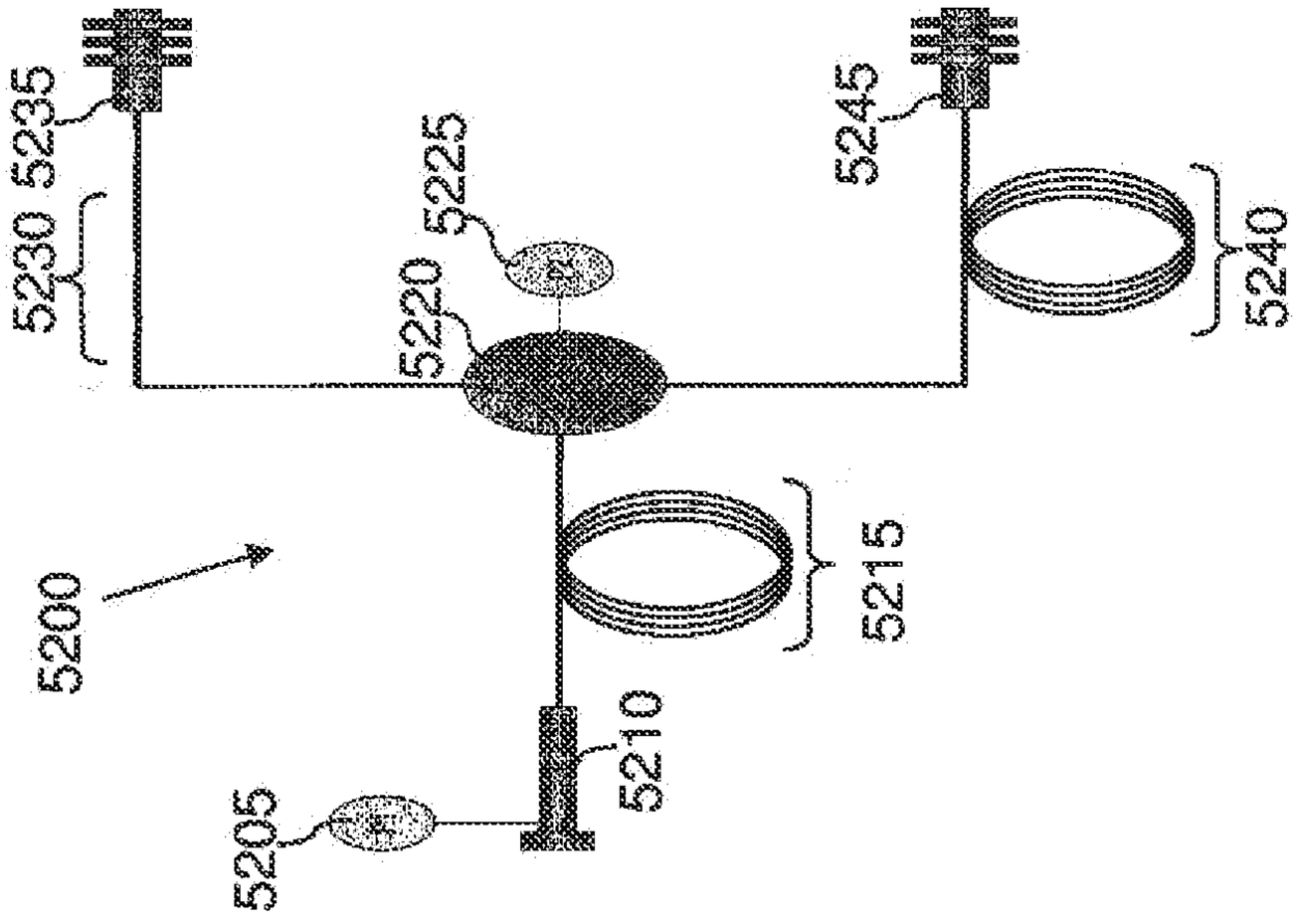


FIG. 52B

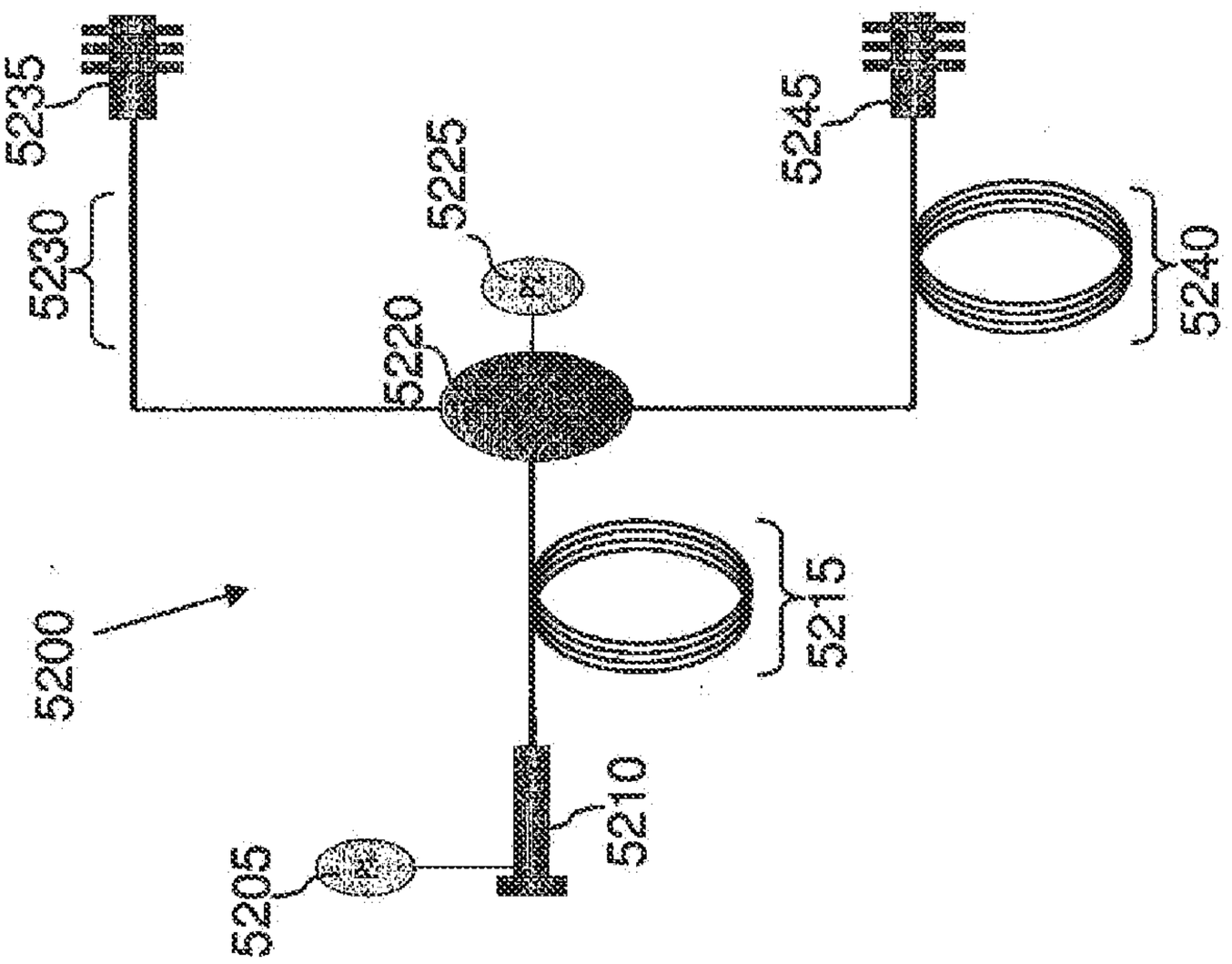


FIG. 52A

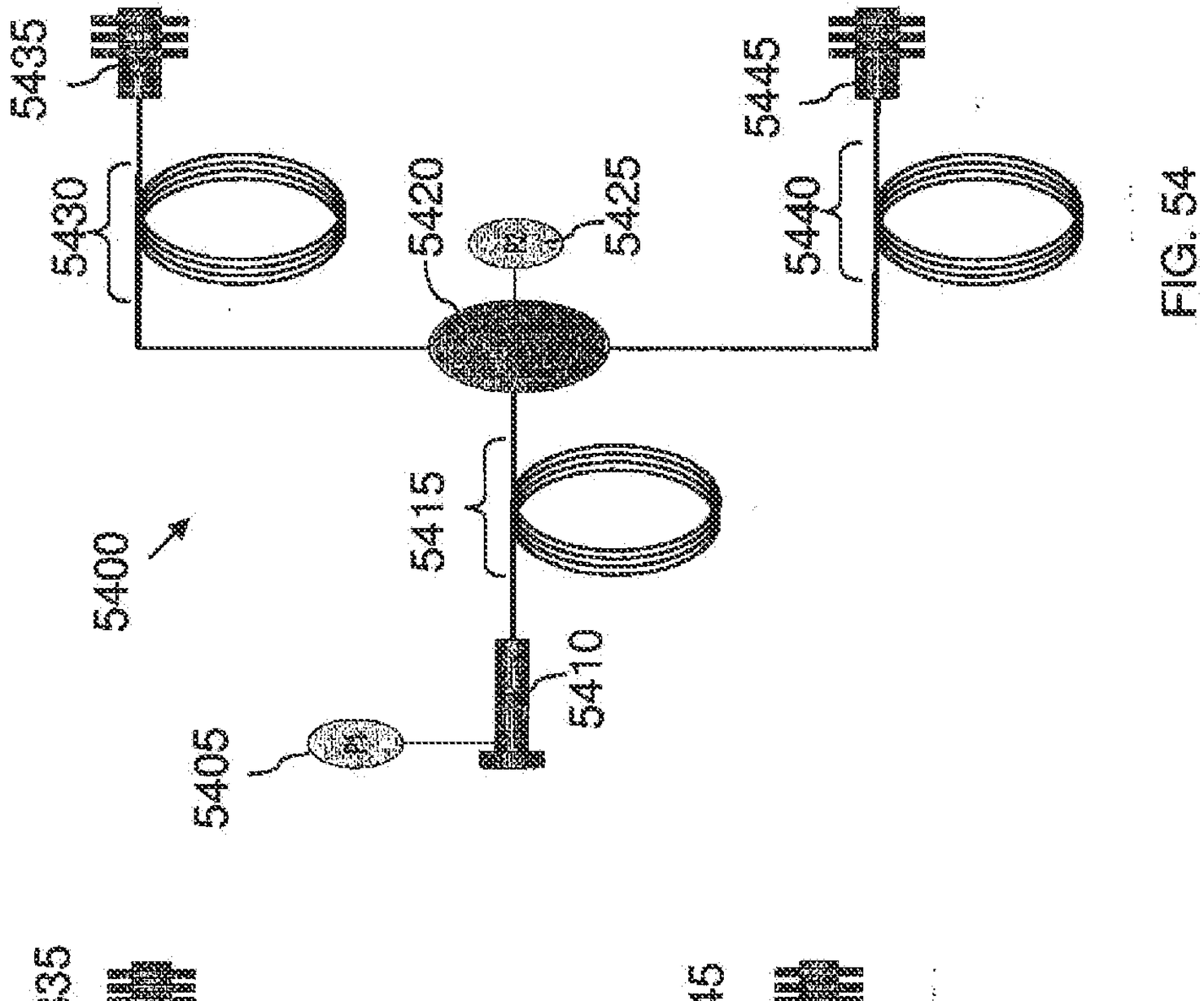

5435
5430
5420
5425
5445
5440
5415
5400
5410
05
54

FIG. 54

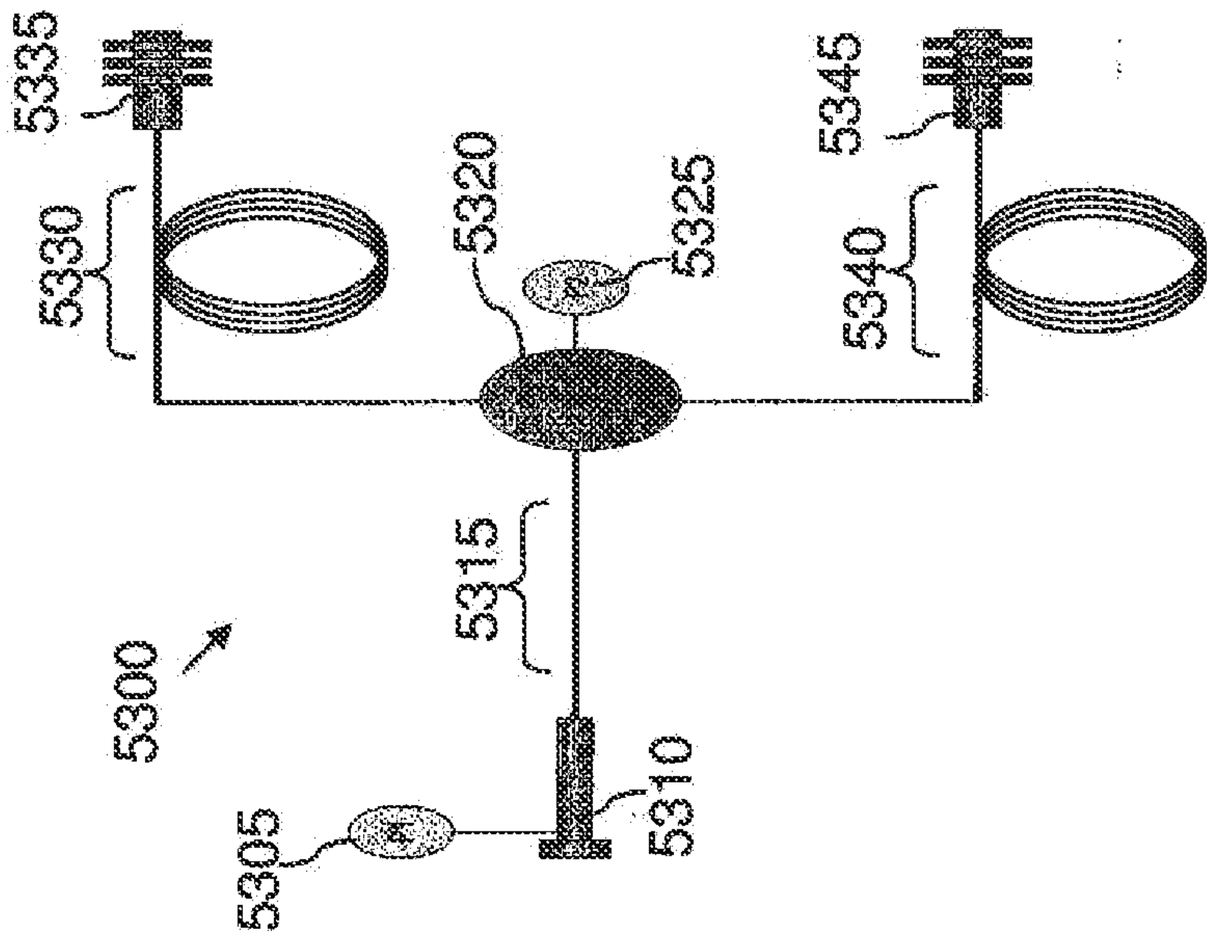

5335
5330
5320
5325
5345
5340
5315
300
5310
5305

FIG. 53

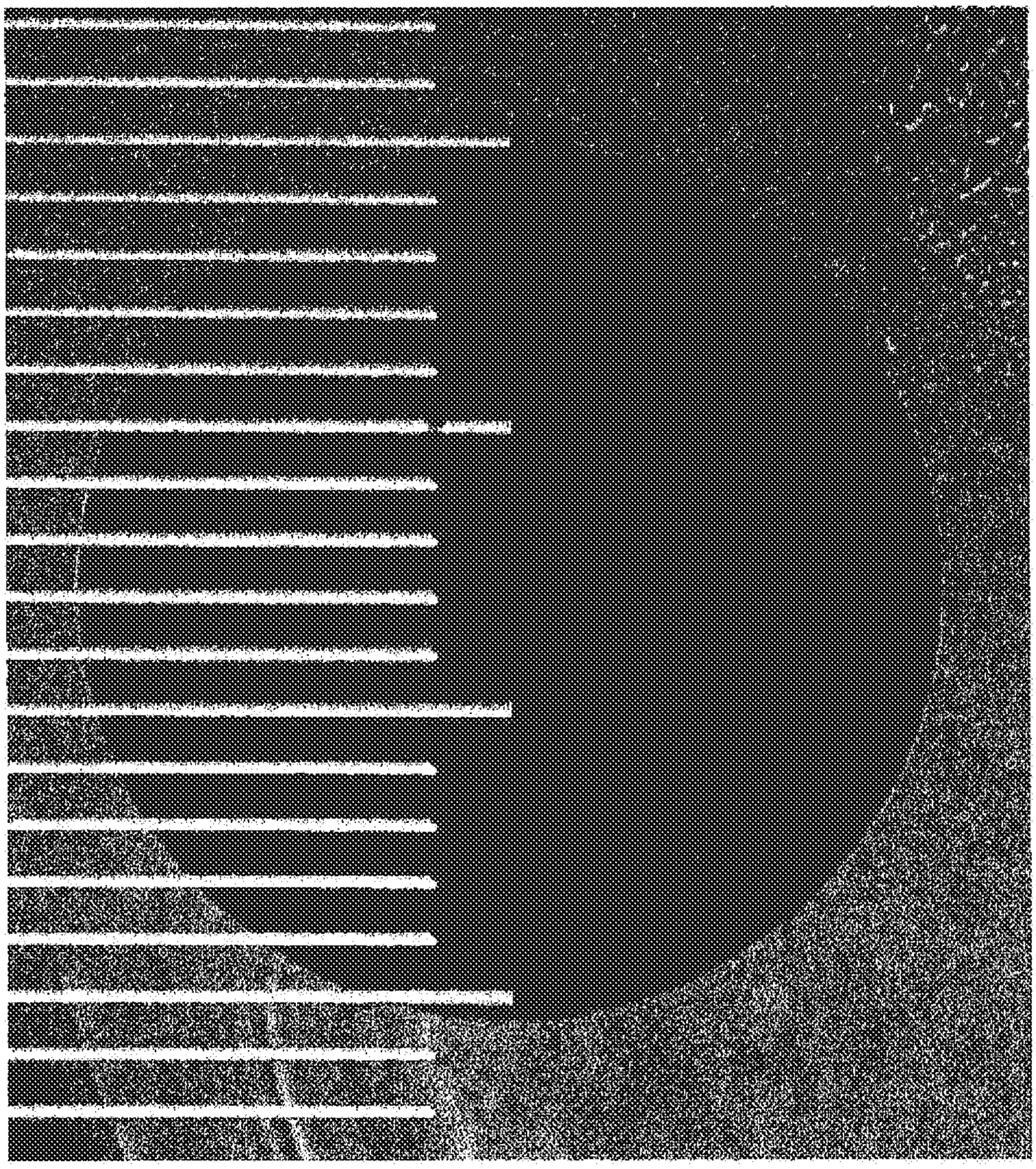


FIG. 55

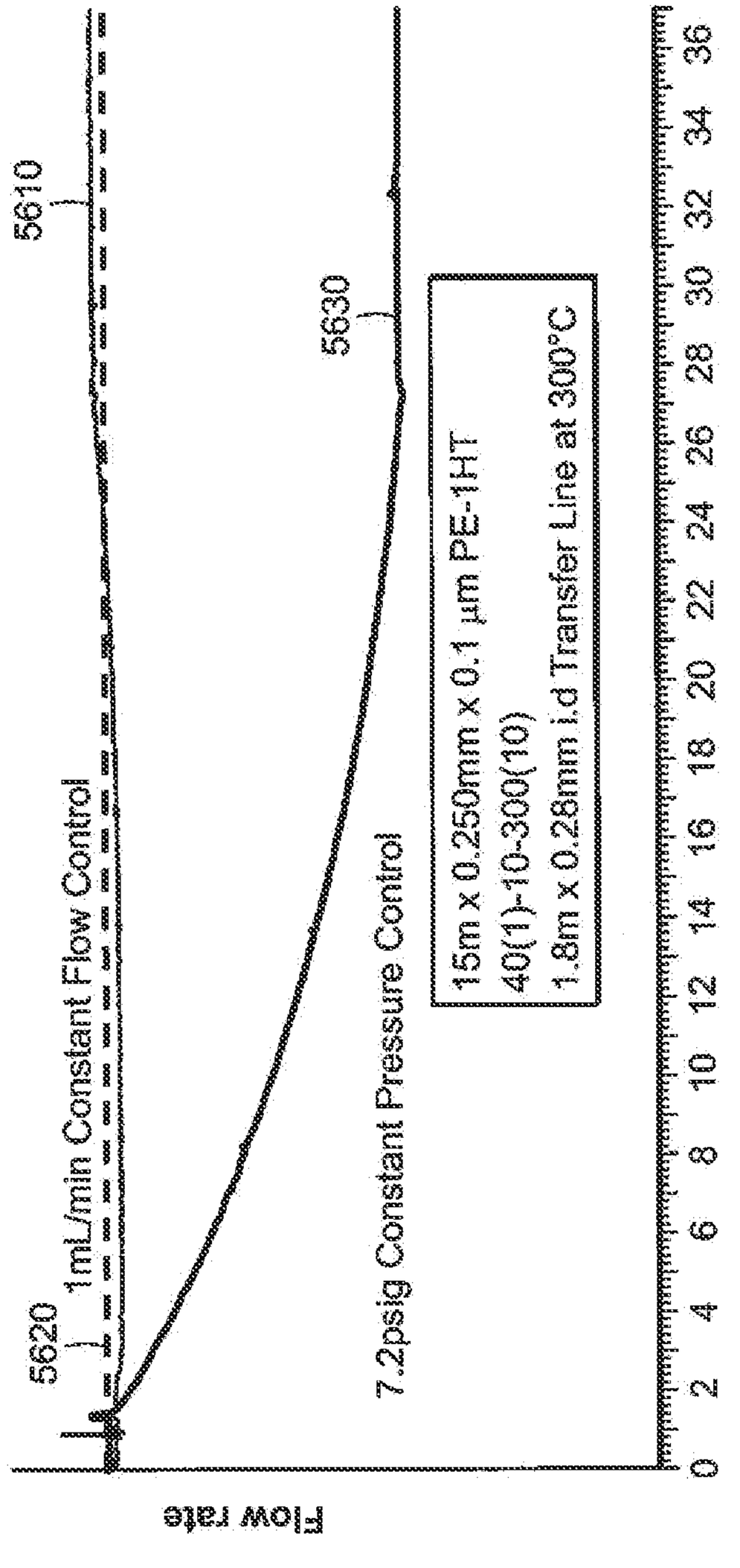

5610
5630
5620
1mL/min Constant Flow Control
7.2psig Constant Pressure Control
15m x 0.250mm x 0.1 µm PE-1HT
40(1)-10-300(10)
1.8m x 0.28mm i.d Transfer Line at 300°C
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Time (min)
Flow rate

FIG. 56

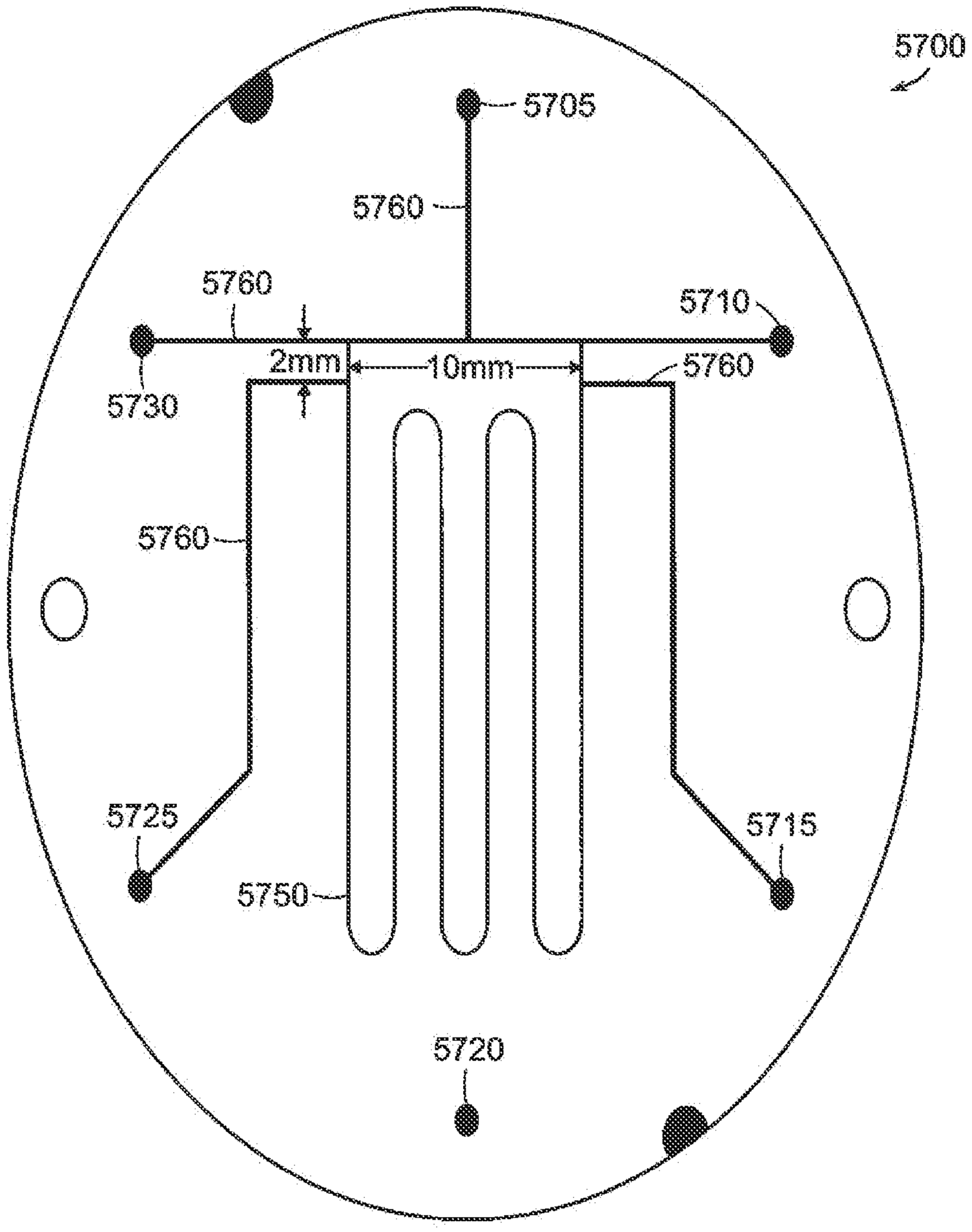

5700
5705
5760
5760
5710
2mm
10mm
5760
5730
5760
5725
5715
5750
5720

FIG. 57

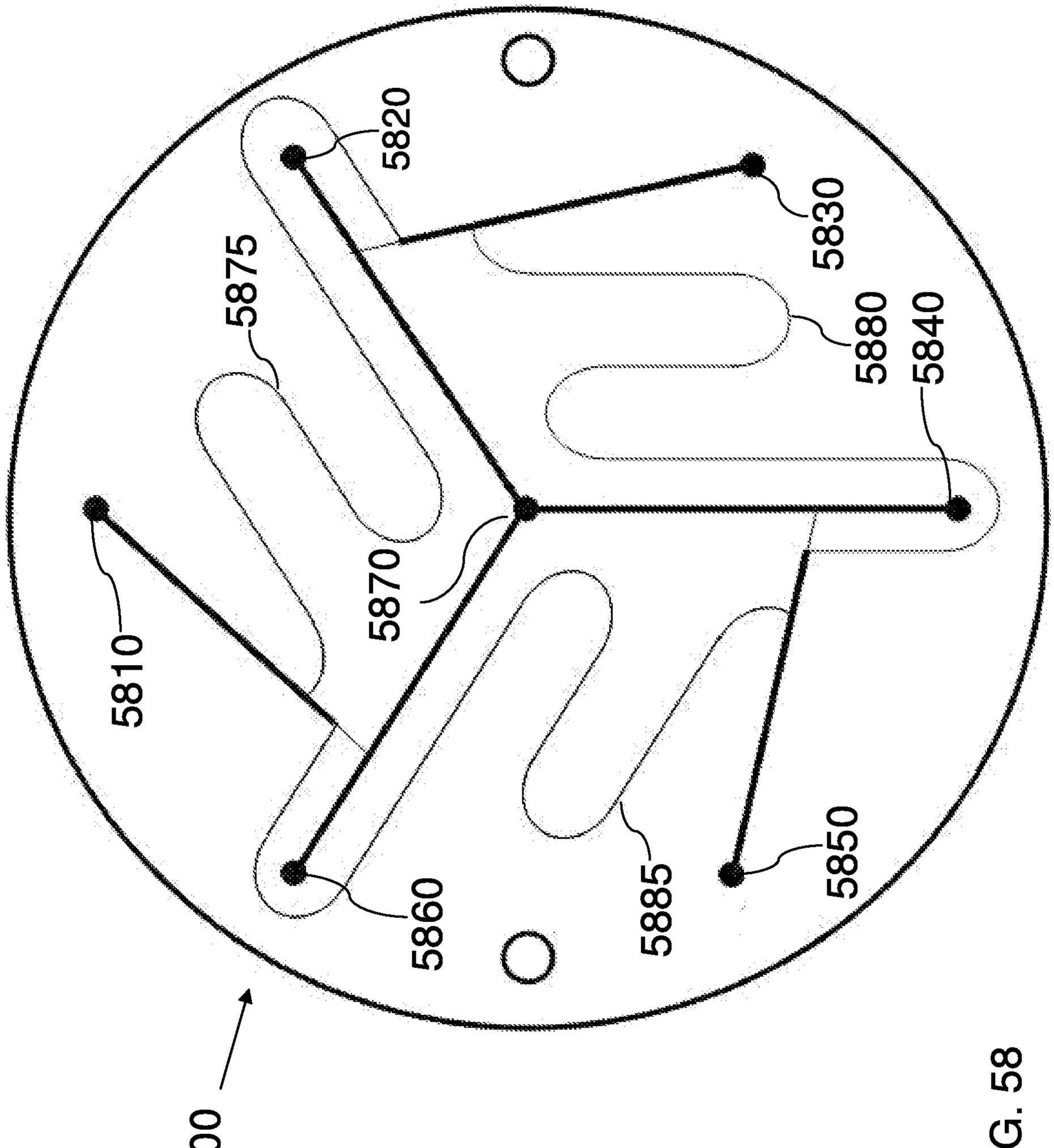


FIG. 58

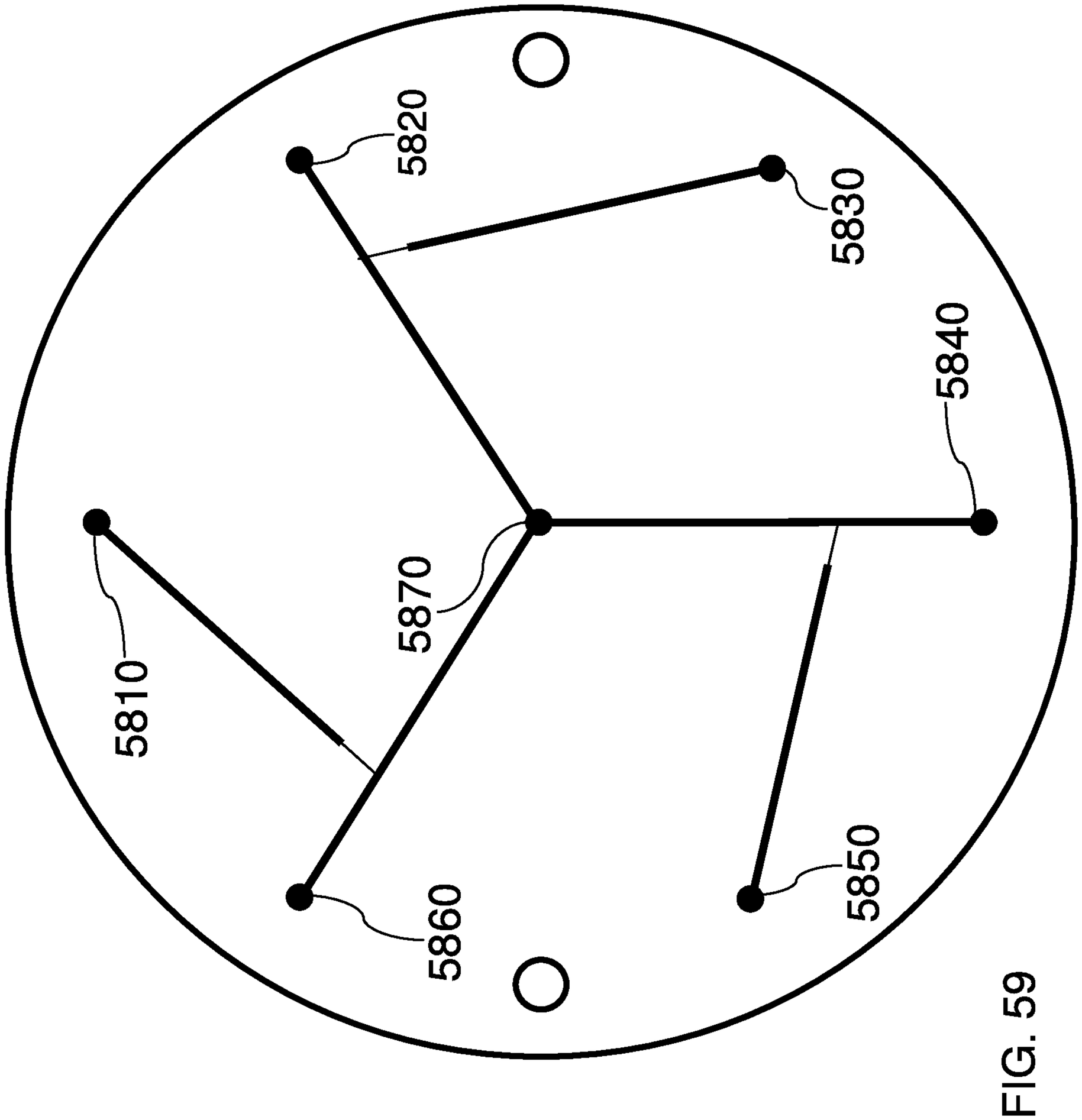


FIG. 59

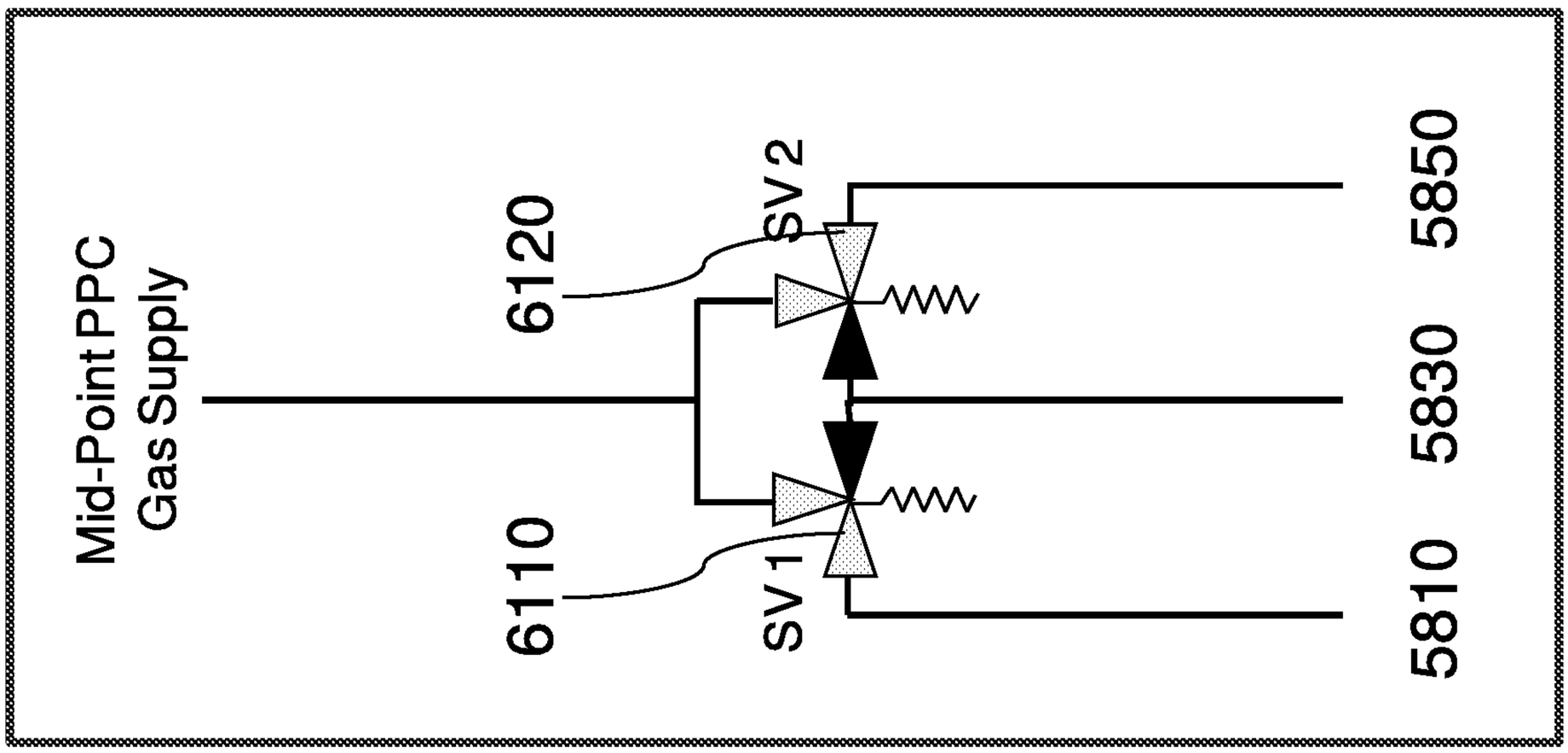

Mid-Point PPC Gas Supply
6120
6110
SV2
SV1
5810
5830
5850

FIG. 61

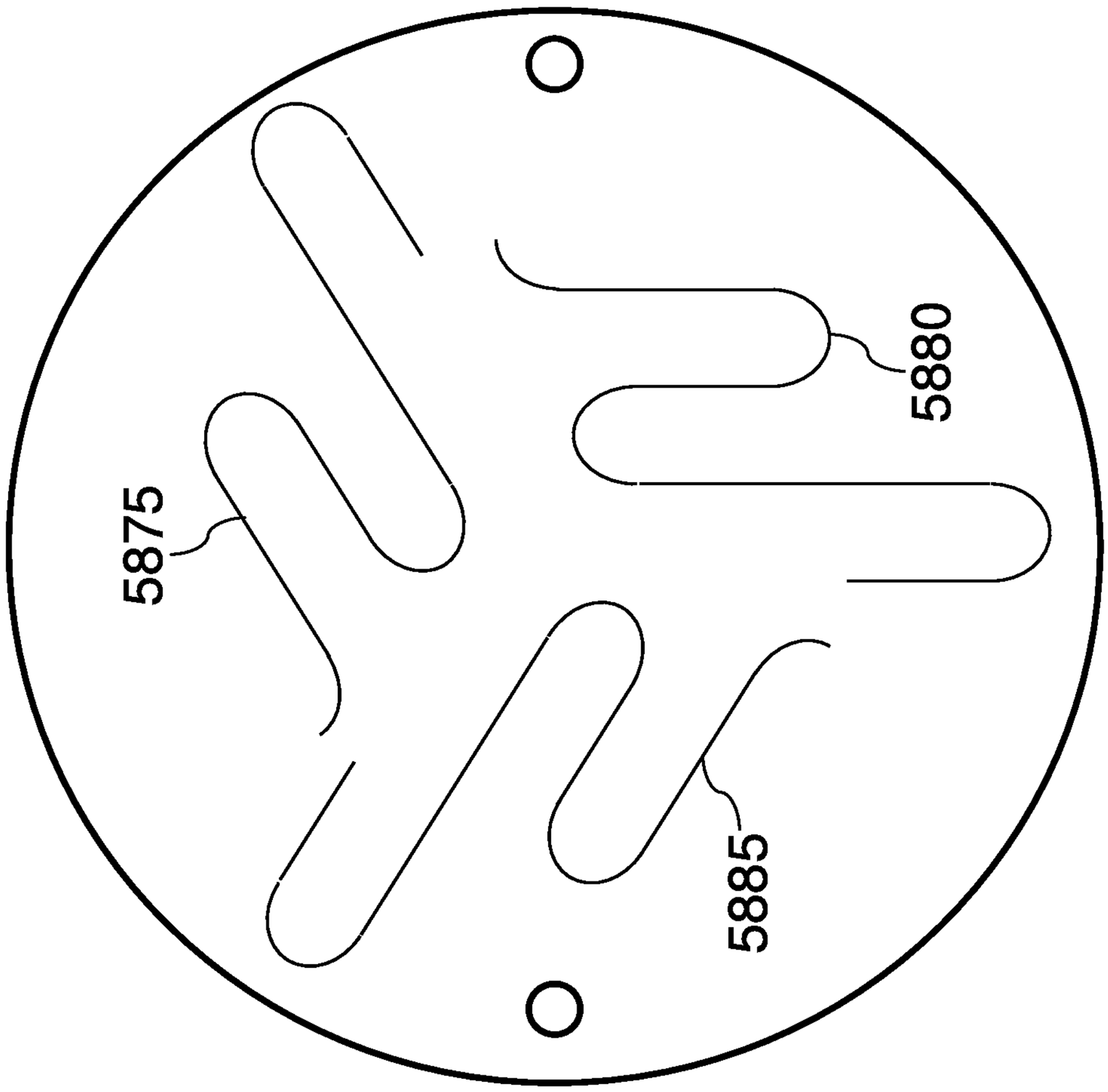

5875
5880
5885

FIG. 60

SV 1 (6110)	SV 2 (6120)	Active Switching Ports	Precolumn Effluent Directed to Port
OFF	OFF	5810 and 5850	5820
ON	OFF	5830 and 5850	5860
OFF	ON	5810 and 5830	5840
ON	ON	5830	5840 and 5860

FIG. 62

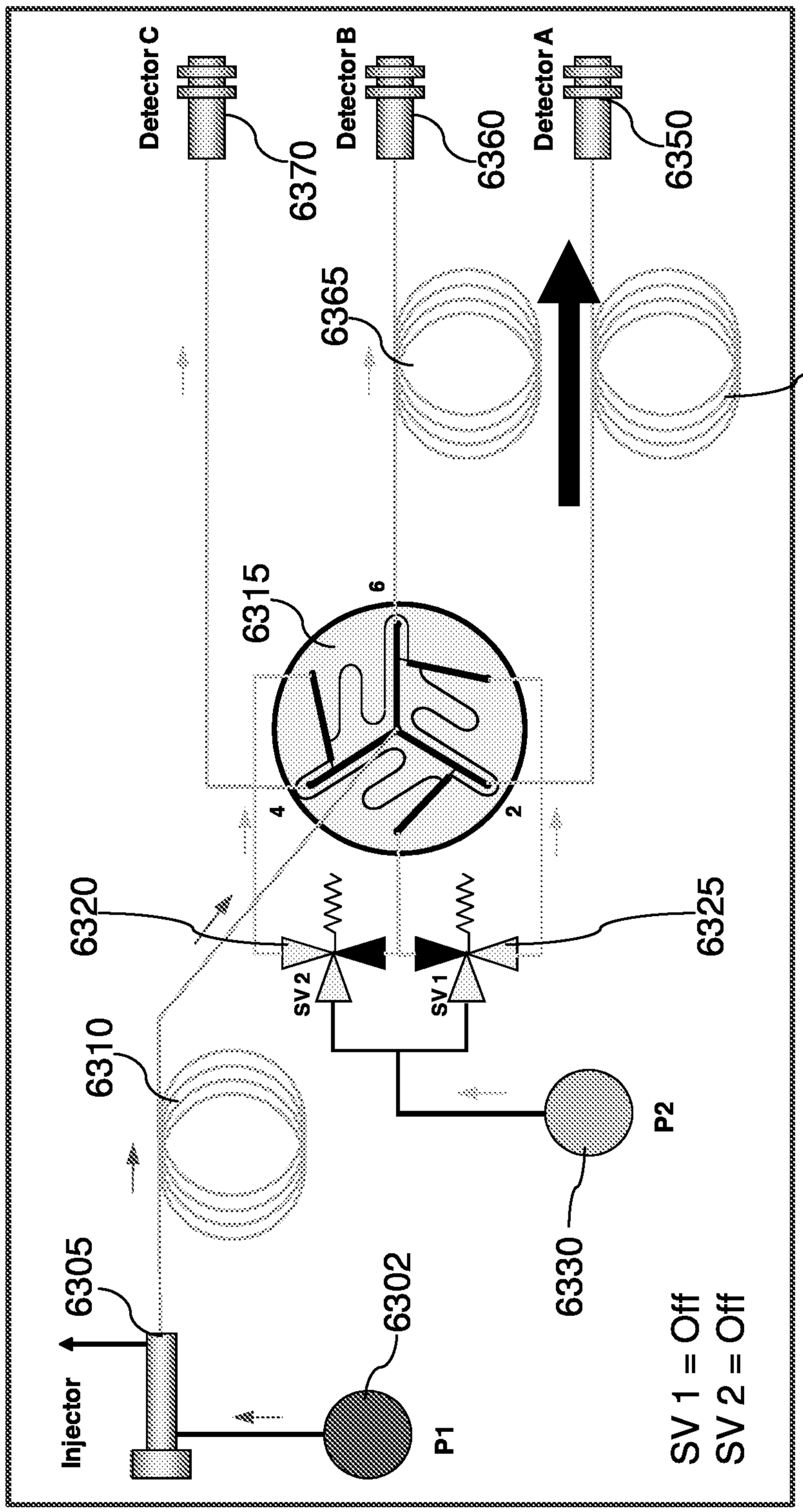

6300
Detector C
Detector B
Detector A
6370
6360
6350
6365
6355
6315
6
4
2
6320
6325
SV 2
SV 1
6310
P2
6330
6305
6302
Injector
P1
SV 1 = Off
SV 2 = Off

FIG. 63

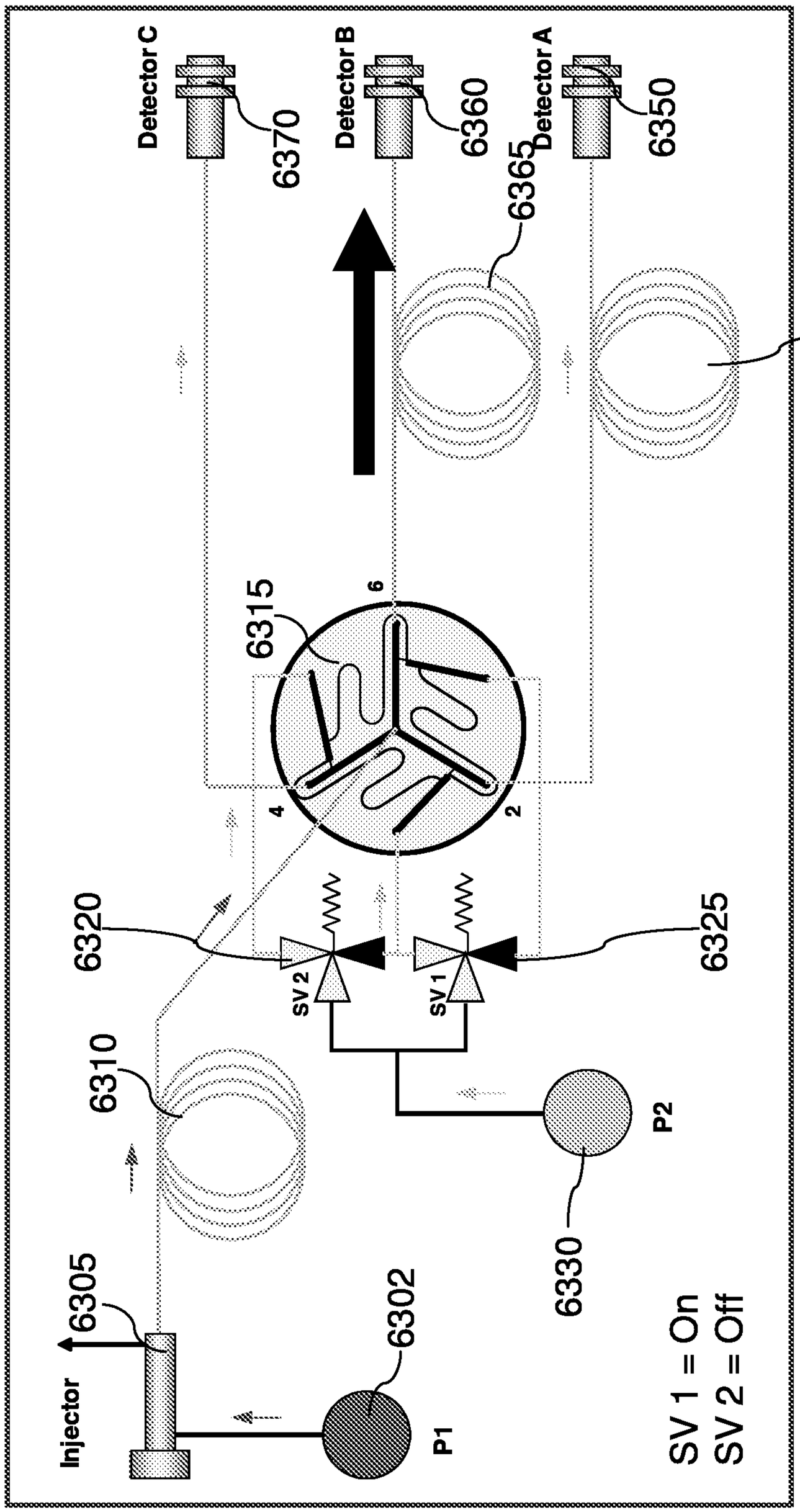

Detector C
6370
Detector B
6360
6365
Detector A
6350
6355
6315
6
4
2
6320
6325
SV2
SV1
6310
P2
Injector
6305
6302
P1
6330
SV 1 = On
SV 2 = Off

FIG. 64

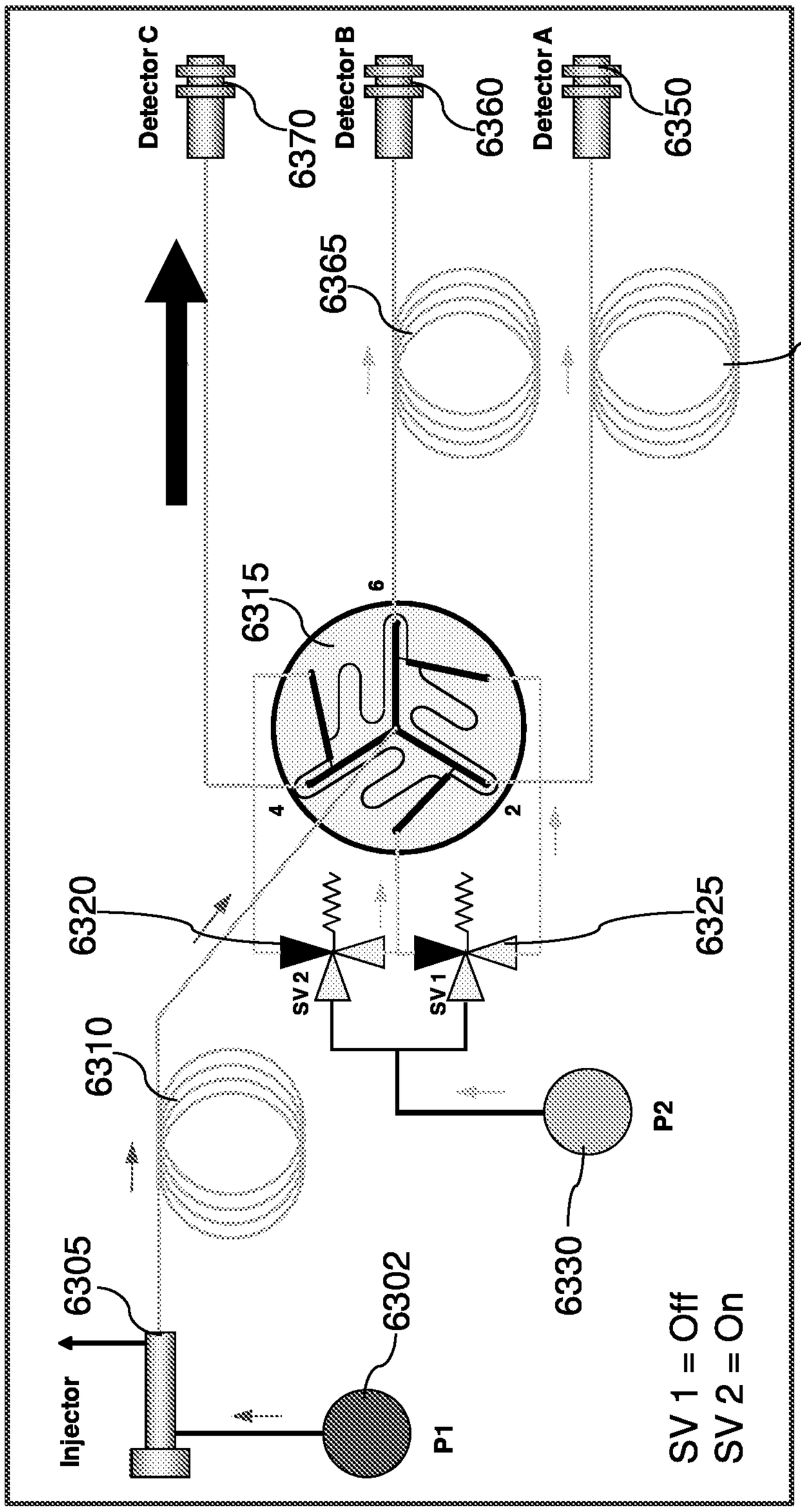

Detector C
6370
Detector B
6360
Detector A
6350
6365
6355
6315
6
4
2
6320
6325
SV2
SV1
6310
P2
6330
6305
6302
Injector
P1
SV 1 = Off
SV 2 = On

FIG. 65

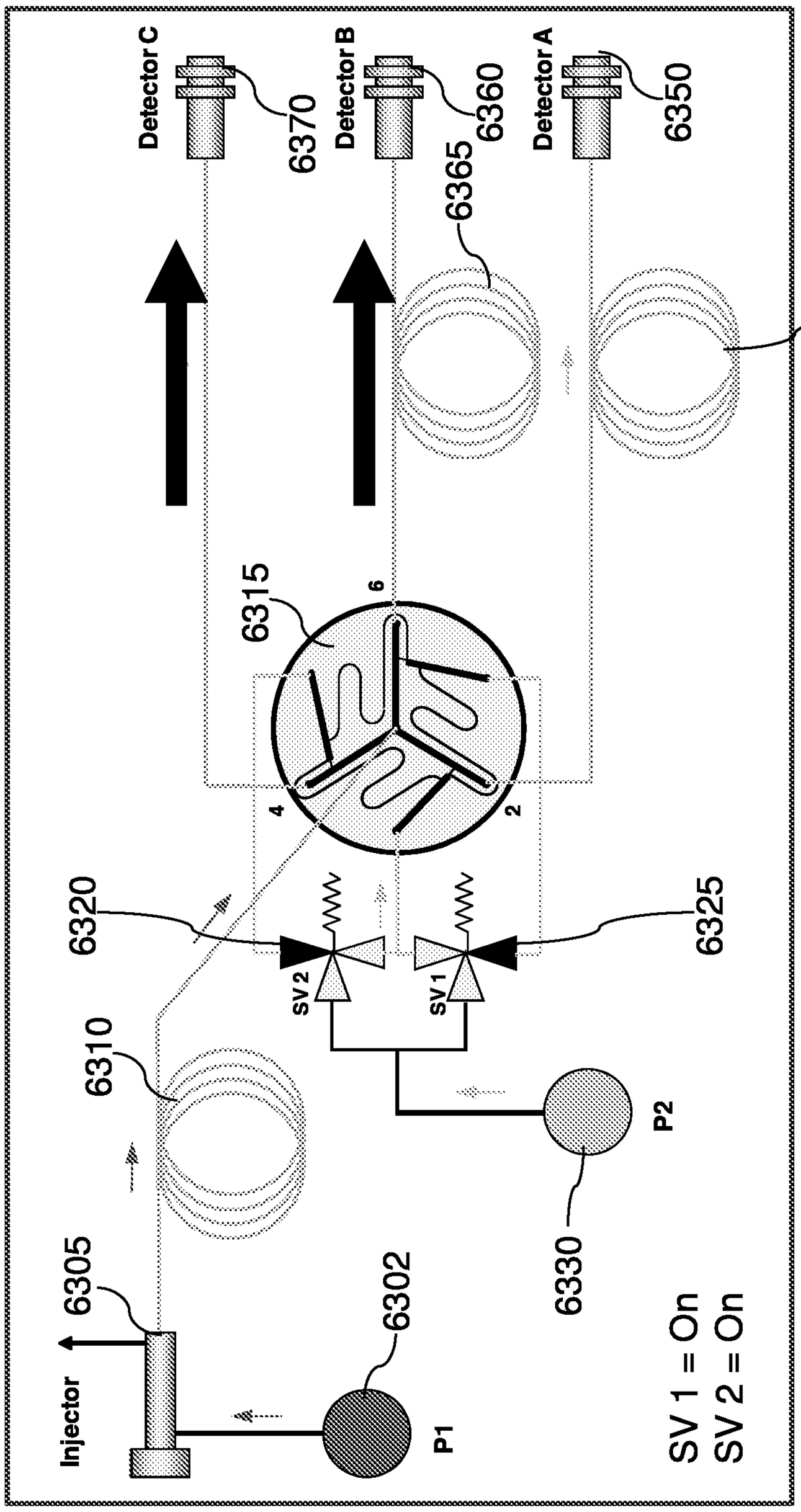

Detector C
Detector B
Detector A
6370
6360
6365
6350
6355
6315
6
4
2
6320
6325
SV2
SV1
6310
P2
6330
6305
6302
Injector
P1
SV 1 = On
SV 2 = On

FIG. 66

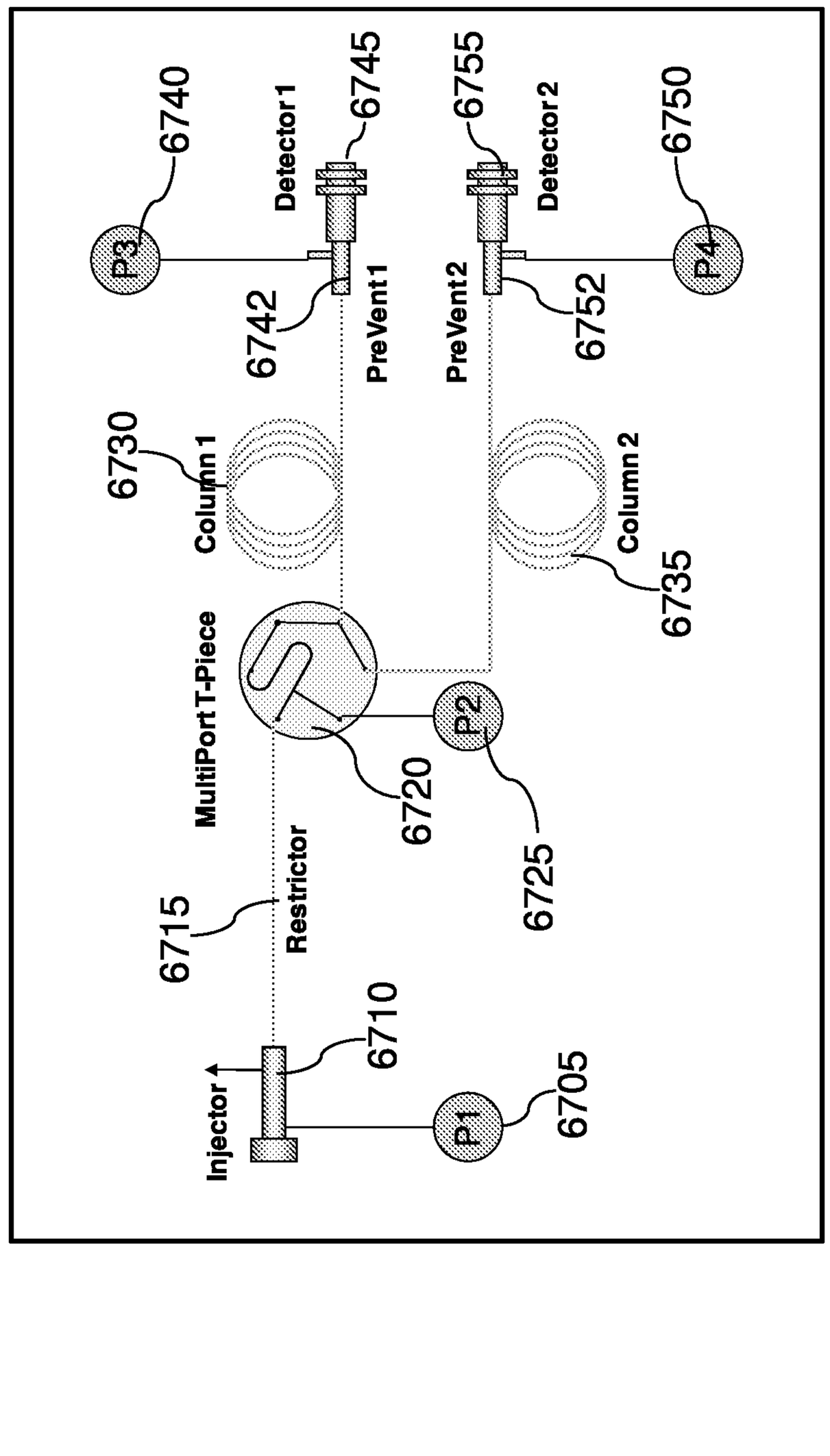

6700
Injector
6710
6705
P1
6715
Restrictor
MultiPort T-Piece
6720
P2
6725
6730
Column 1
6742
PreVent1
Detector 1
6740
P3
6745
6735
Column 2
6752
PreVent2
Detector 2
6755
P4
6750

FIG. 67

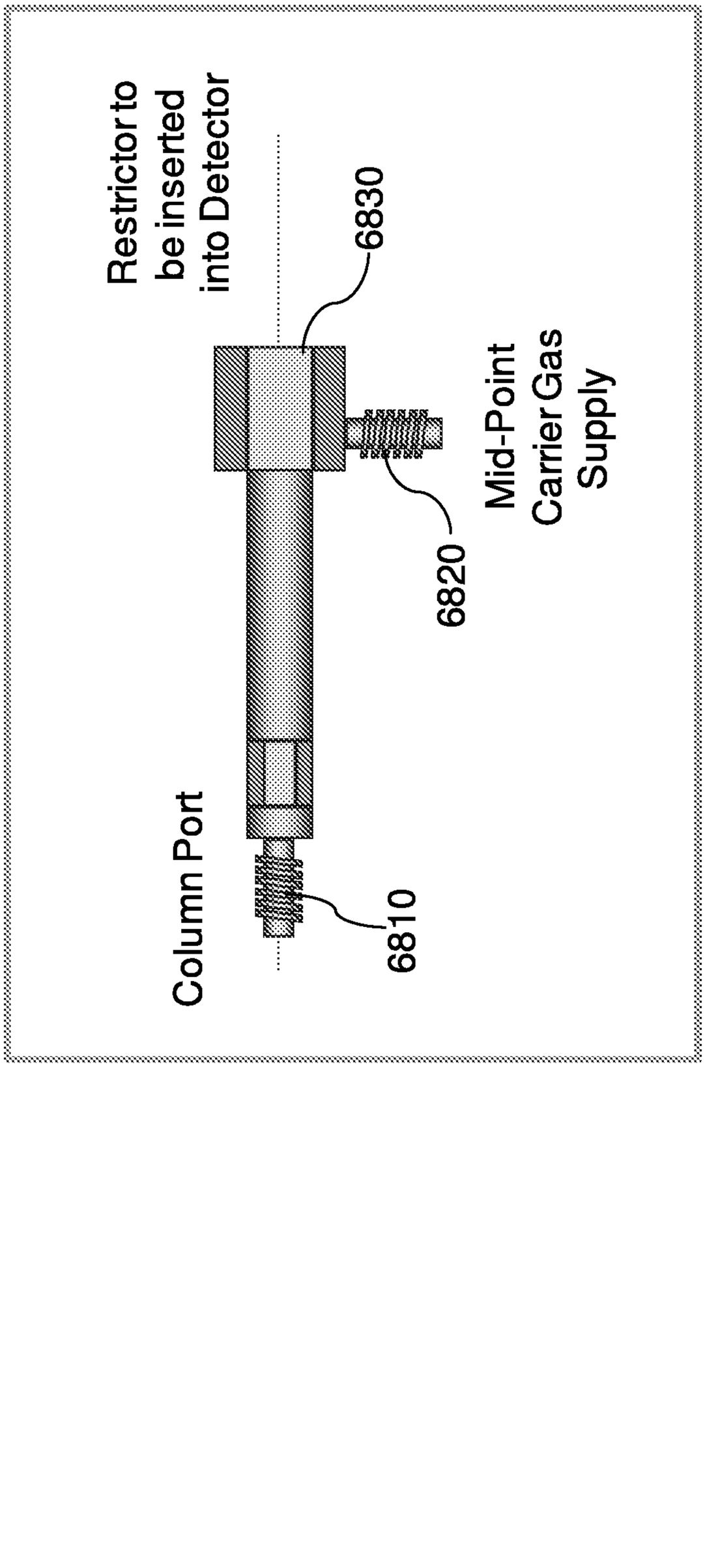

Restrictor to be inserted into Detector
6830
Mid-Point Carrier Gas Supply
6820
Column Port
6810
6800

FIG. 68

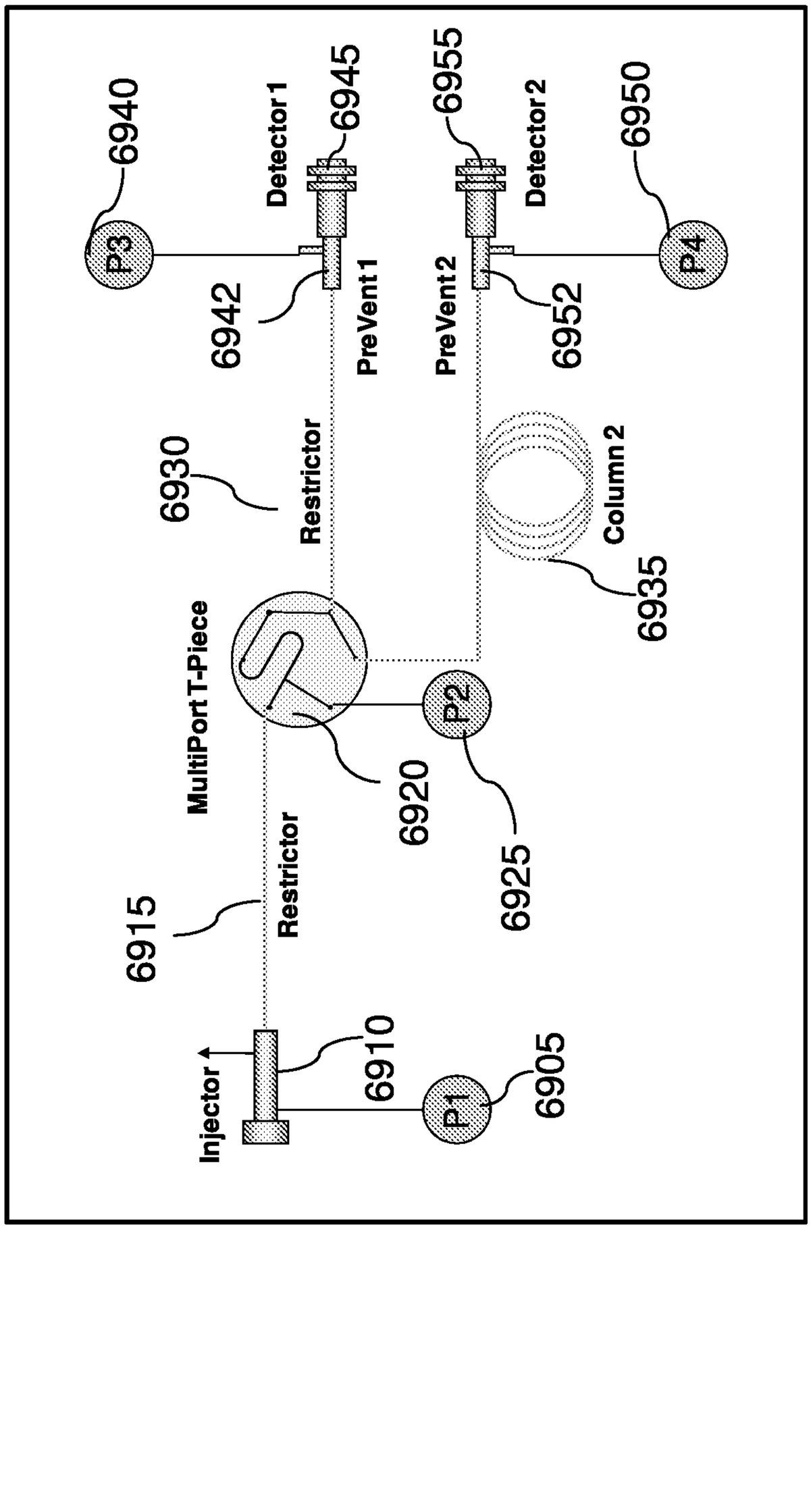

6900
6905
P1
6910
Injector
6915
Restrictor
MultiPort T-Piece
6920
P2
6925
Restrictor
6930
Column 2
6935
PreVent2
6952
Detector2
6955
P4
6950
PreVent1
6942
Detector1
6945
P3
6940

FIG. 69

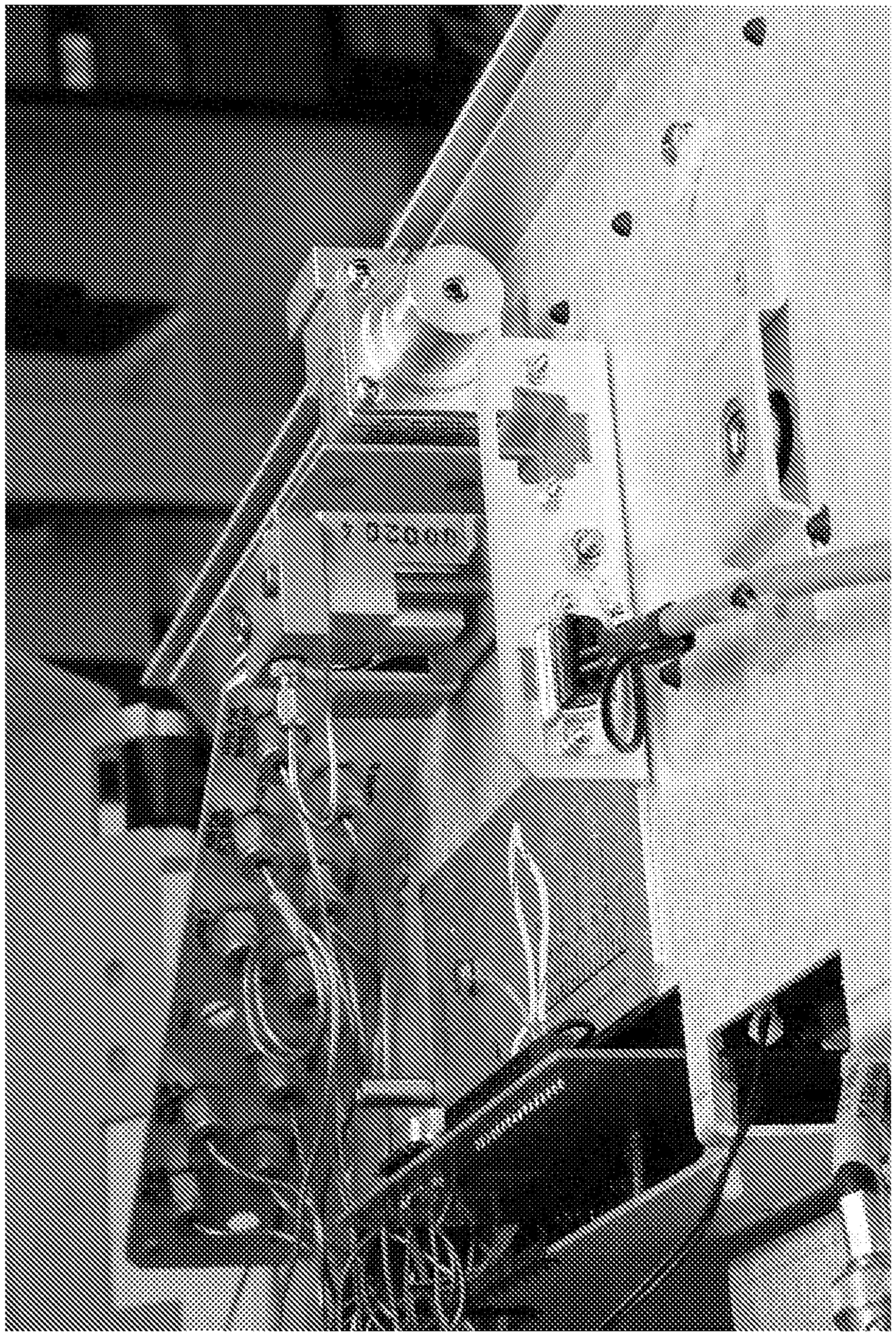


FIG. 70

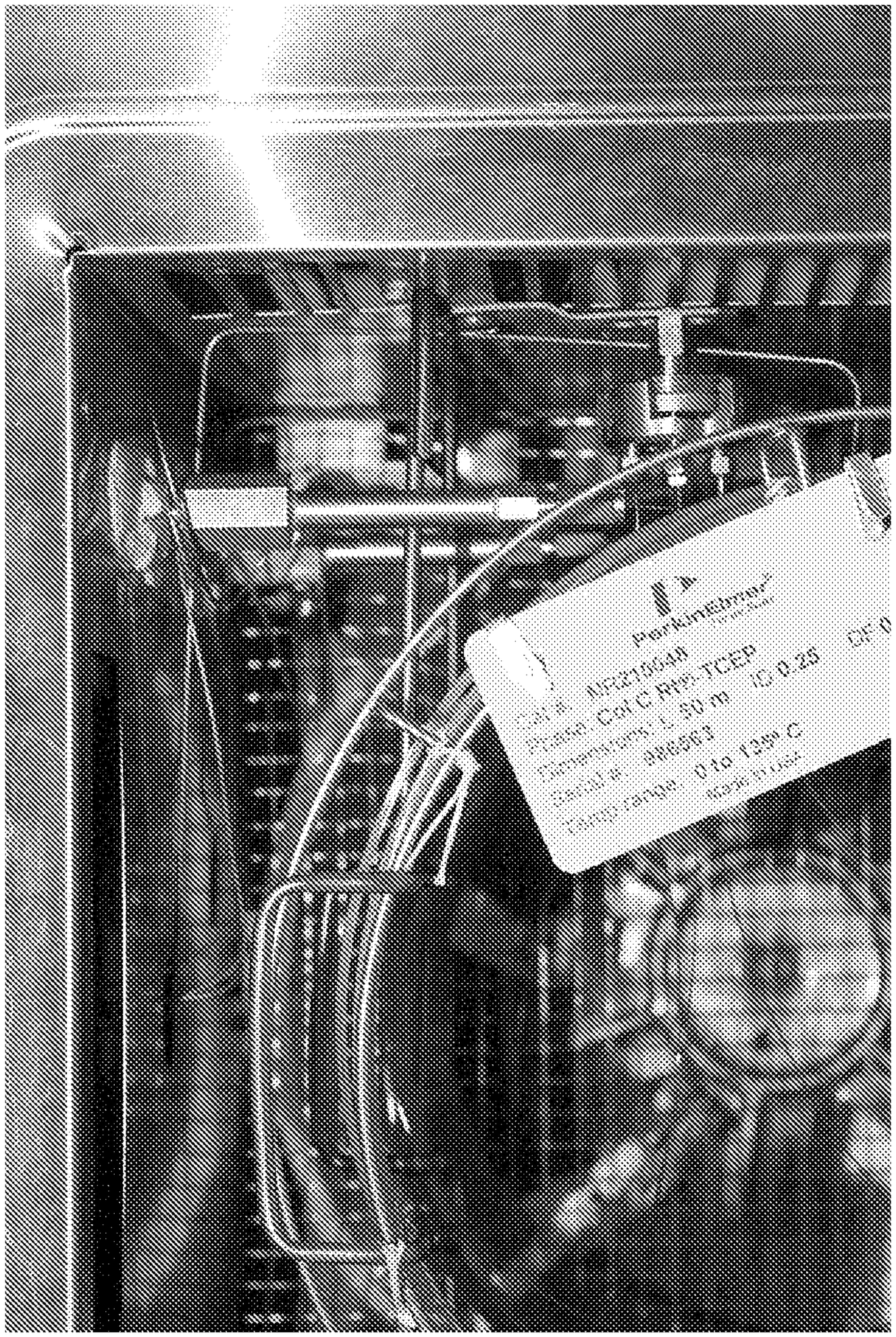


FIG. 71

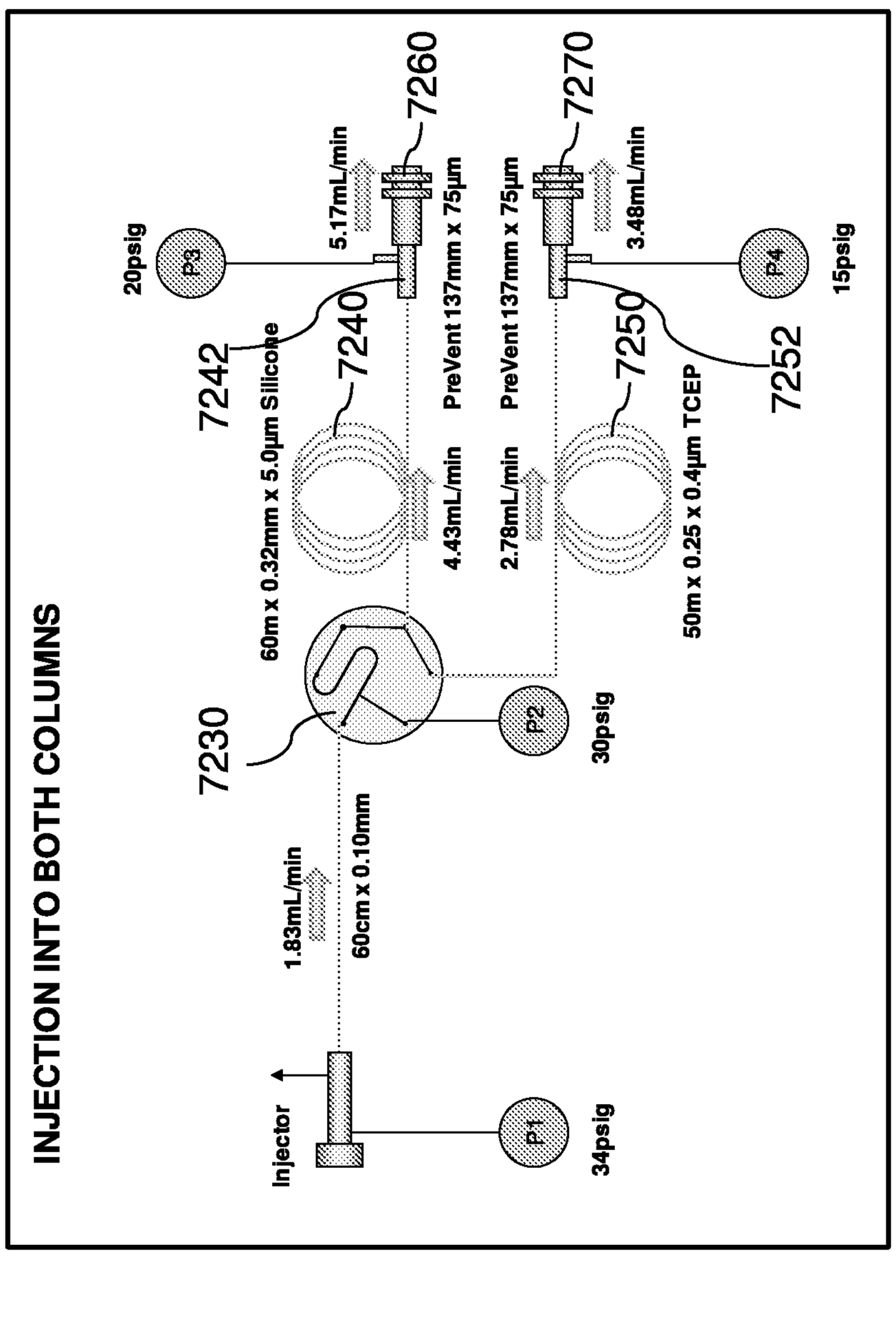

INJECTION INTO BOTH COLUMNS
Injector
1.83mL/min
60cm x 0.10mm
7230
60m x 0.25 x 0.4µm TCEP
50m x 0.25 x 0.4µm TCEP
P1
34psig
P2
30psig
7240
7242
7250
7252
4.43mL/min
2.78mL/min
PreVent 137mm x 75µm
PreVent 137mm x 75µm
7260
7270
5.17mL/min
3.48mL/min
20psig
P3
P4
15psig

FIG. 72

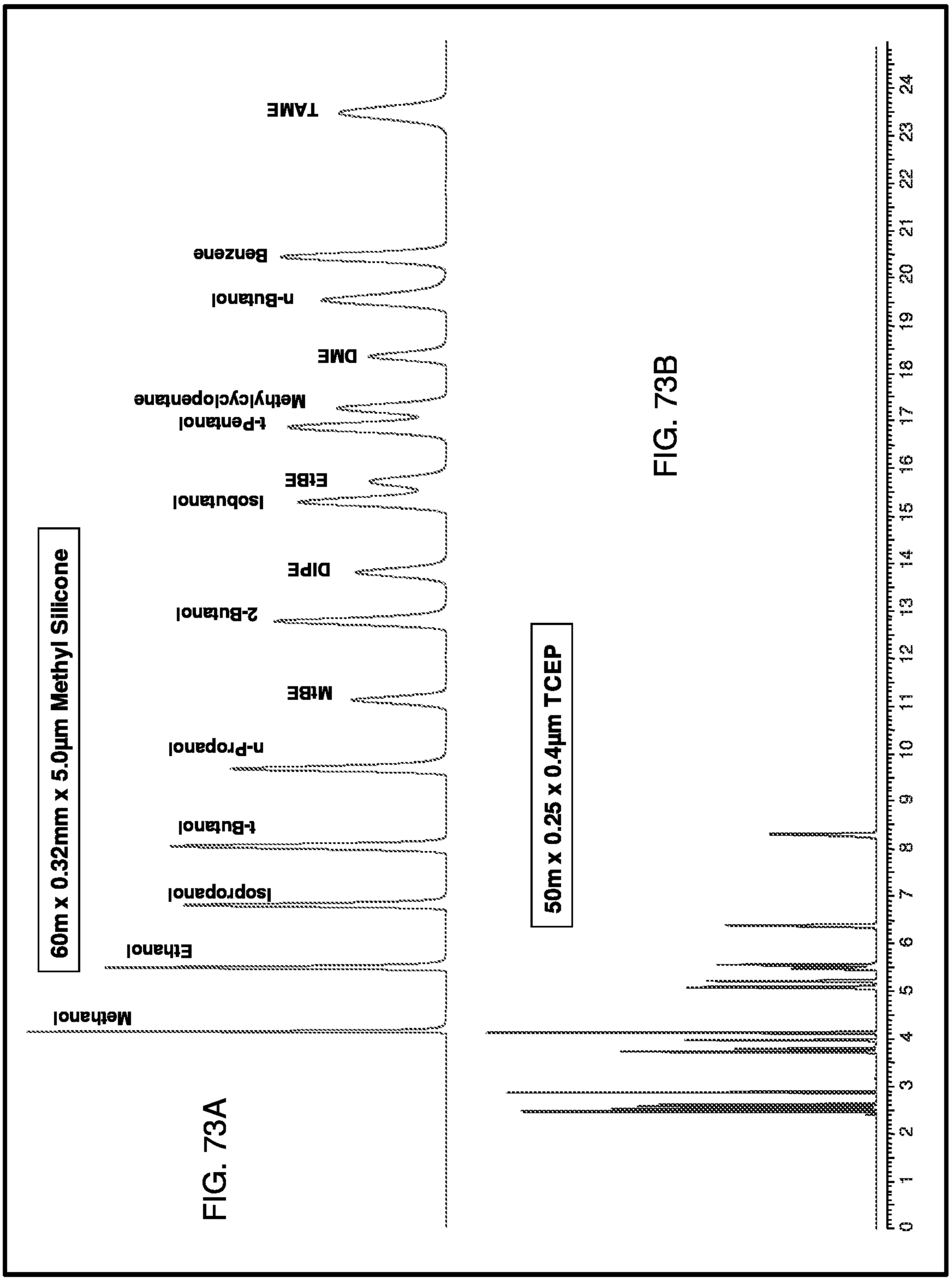


FIG. 73A

FIG. 73B

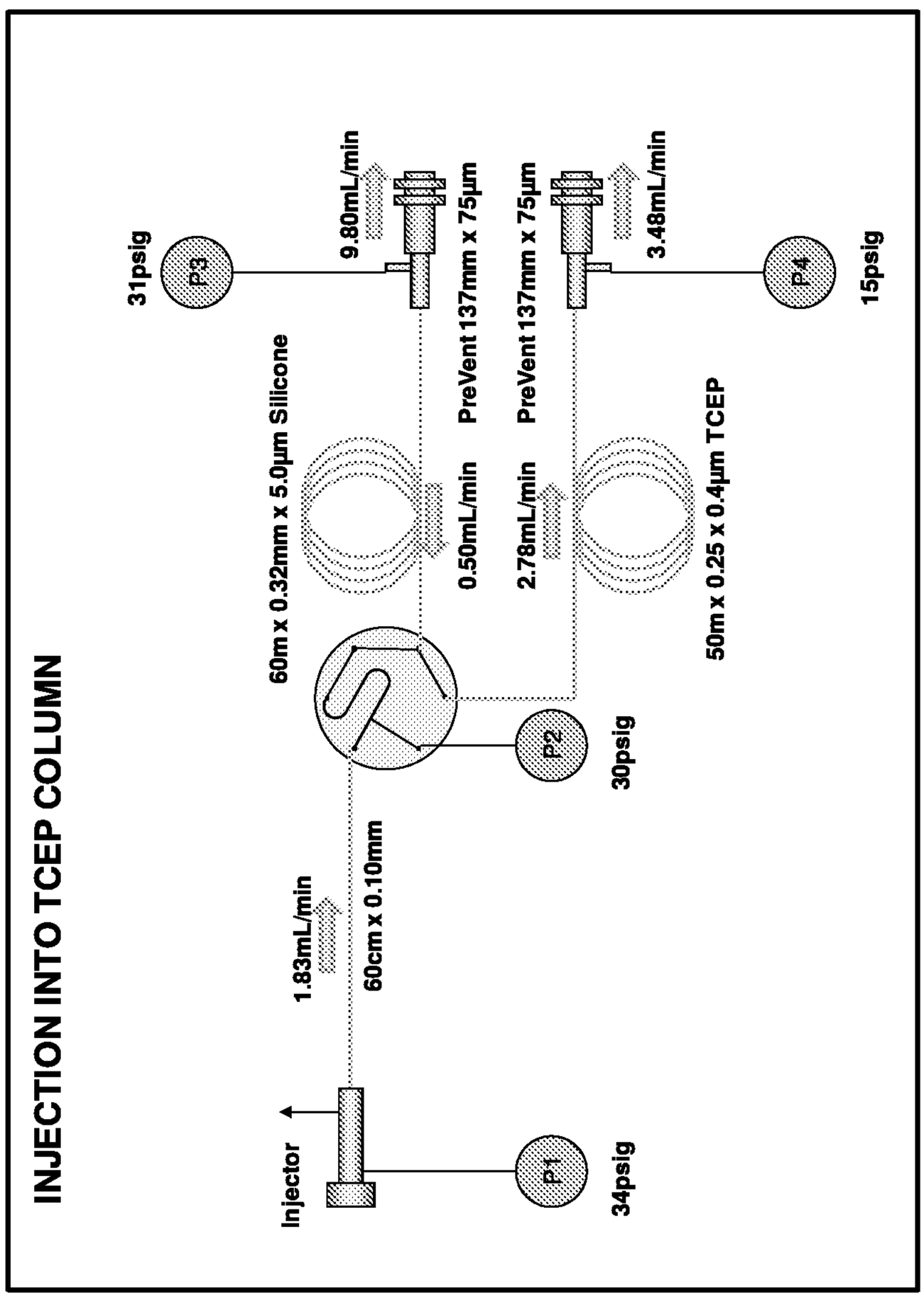

INJECTION INTO TCEP COLUMN
Injector
1.83mL/min
60cm x 0.10mm
P1
34psig
60m x 0.32mm x 5.0µm Silicone
0.50mL/min
PreVent 137mm x 75µm
P2
30psig
2.78mL/min
PreVent 137mm x 75µm
50m x 0.25 x 0.4µm TCEP
P3
31psig
9.80mL/min
3.48mL/min
P4
15psig

FIG. 74

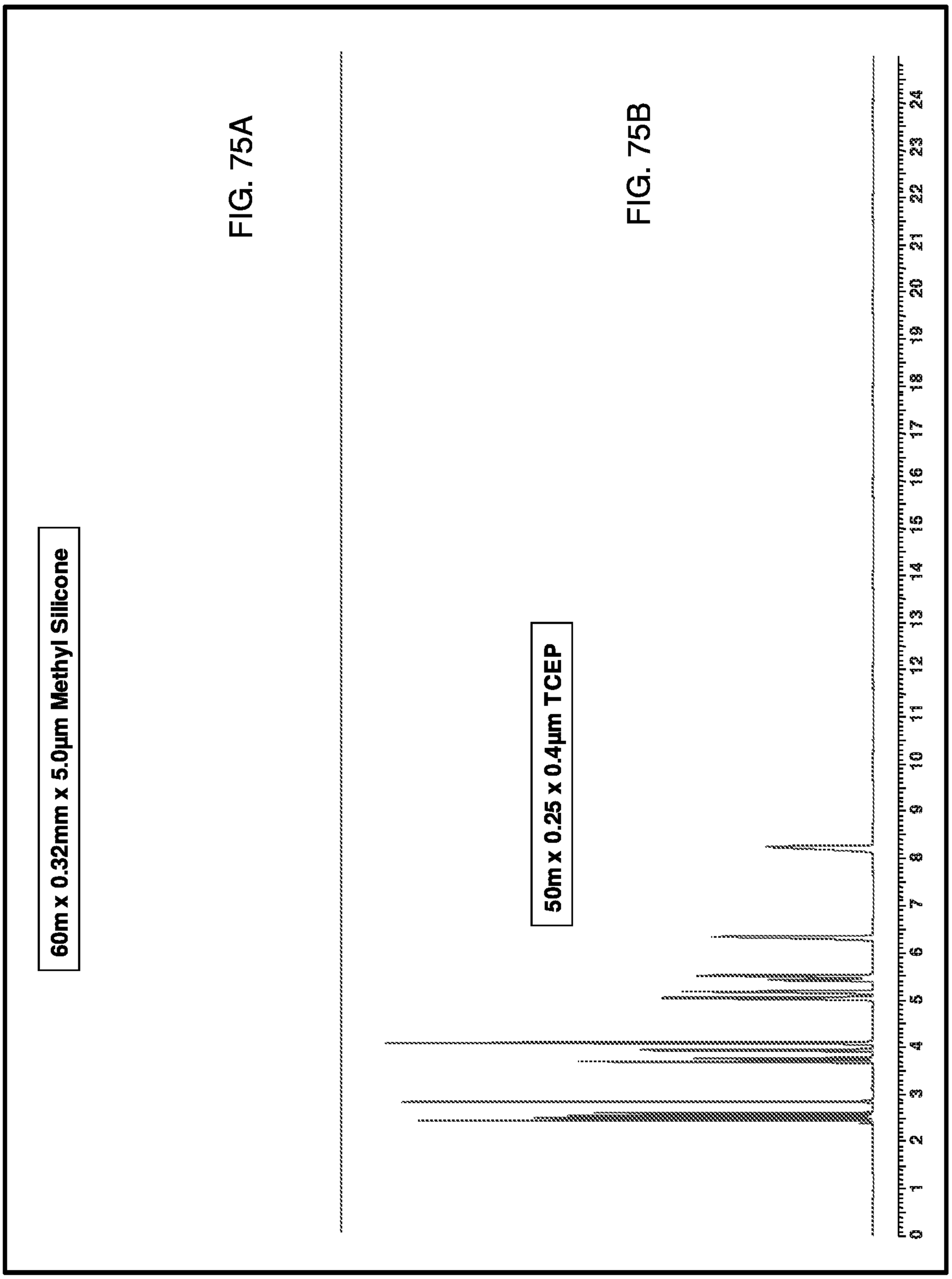


FIG. 75A

FIG. 75B

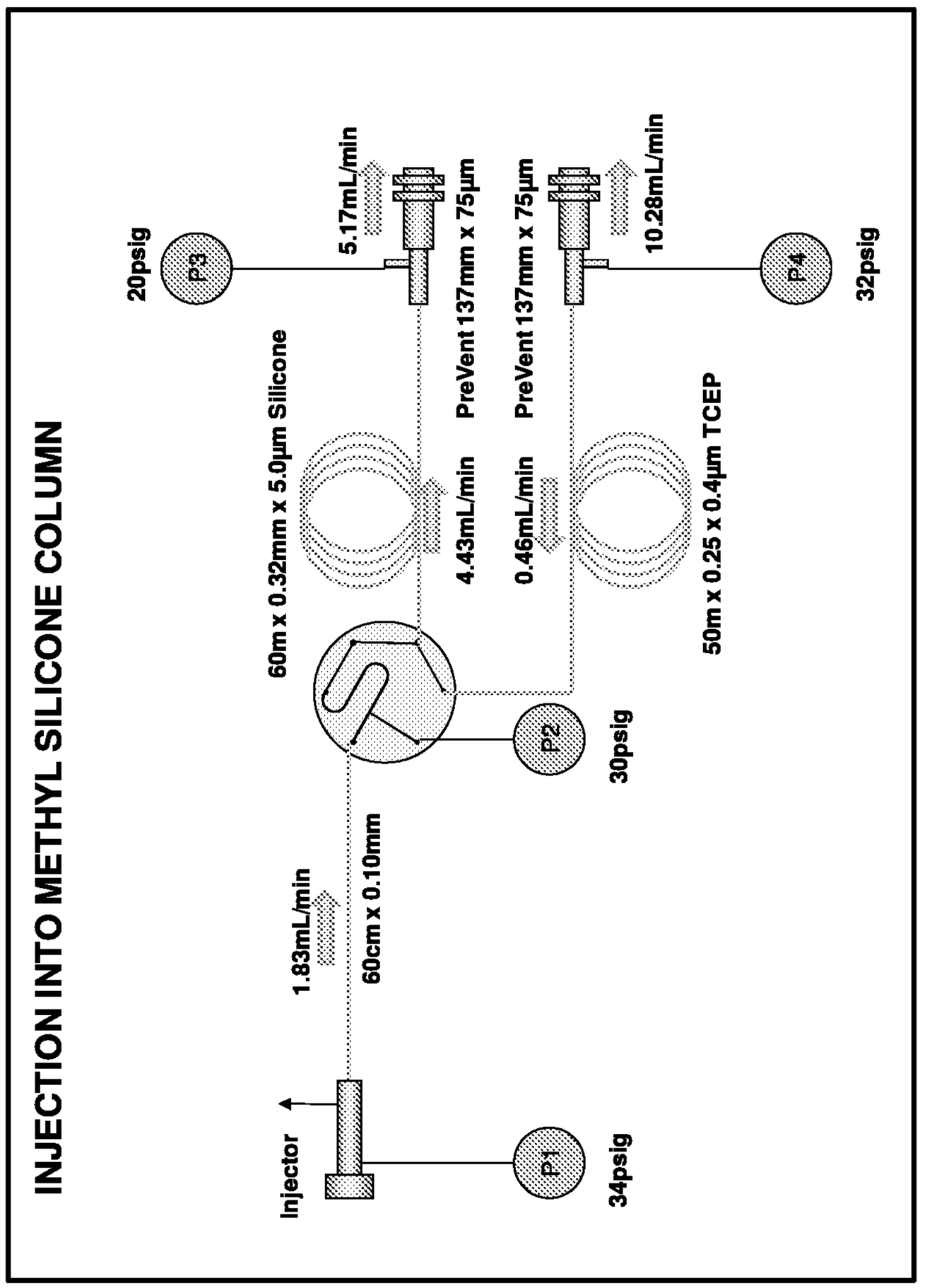

INJECTION INTO METHYL SILICONE COLUMN
Injector
1.83mL/min
60cm x 0.10mm
P1
34psig
60m x 0.32mm x 5.0µm Silicone
P2
30psig
4.43mL/min
PreVent 137mm x 75µm
0.46mL/min
PreVent 137mm x 75µm
50m x 0.25 x 0.4µm TCEP
P3
20psig
5.17mL/min
P4
32psig
10.28mL/min

FIG. 76

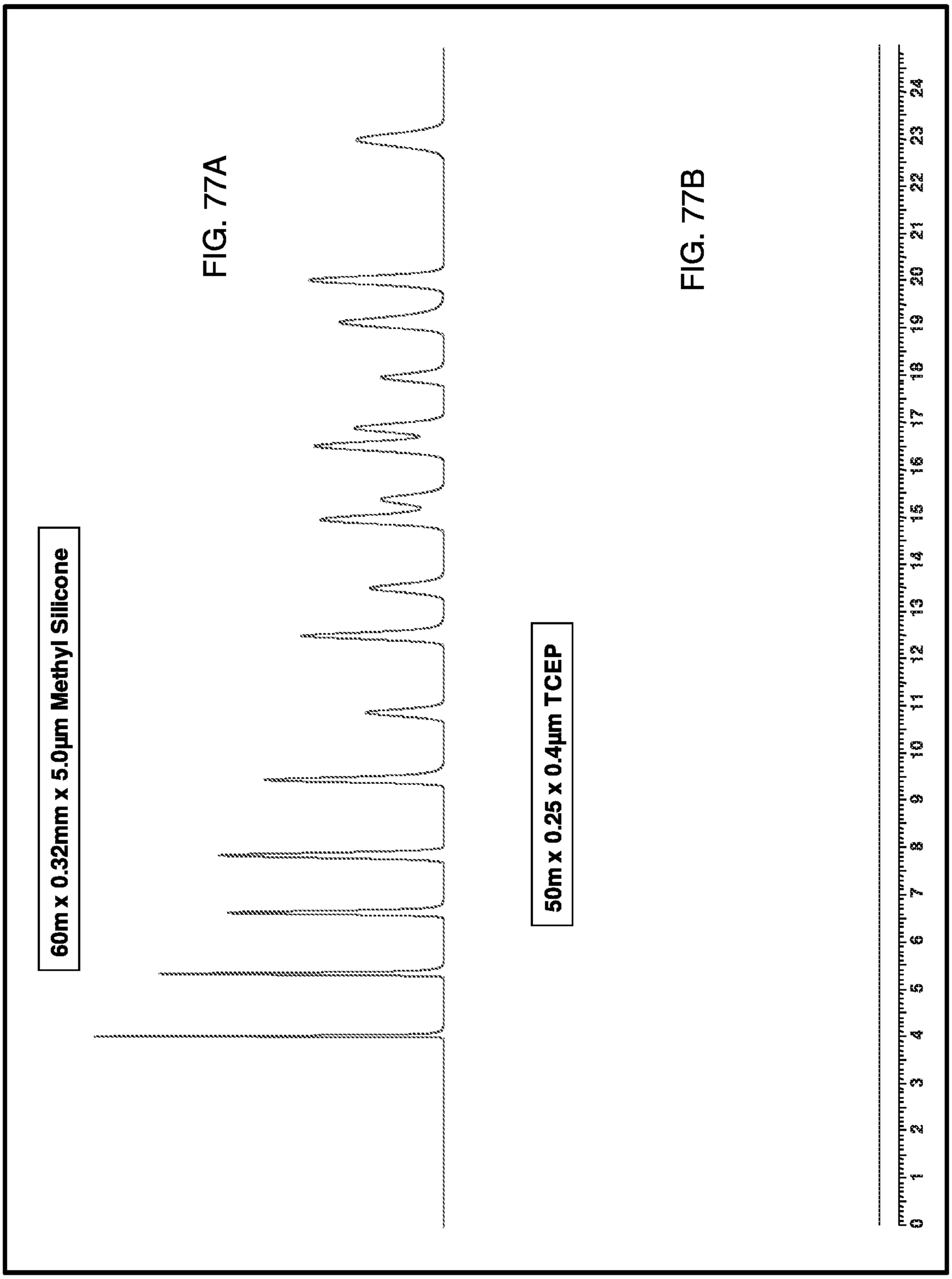


FIG. 77A

FIG. 77B

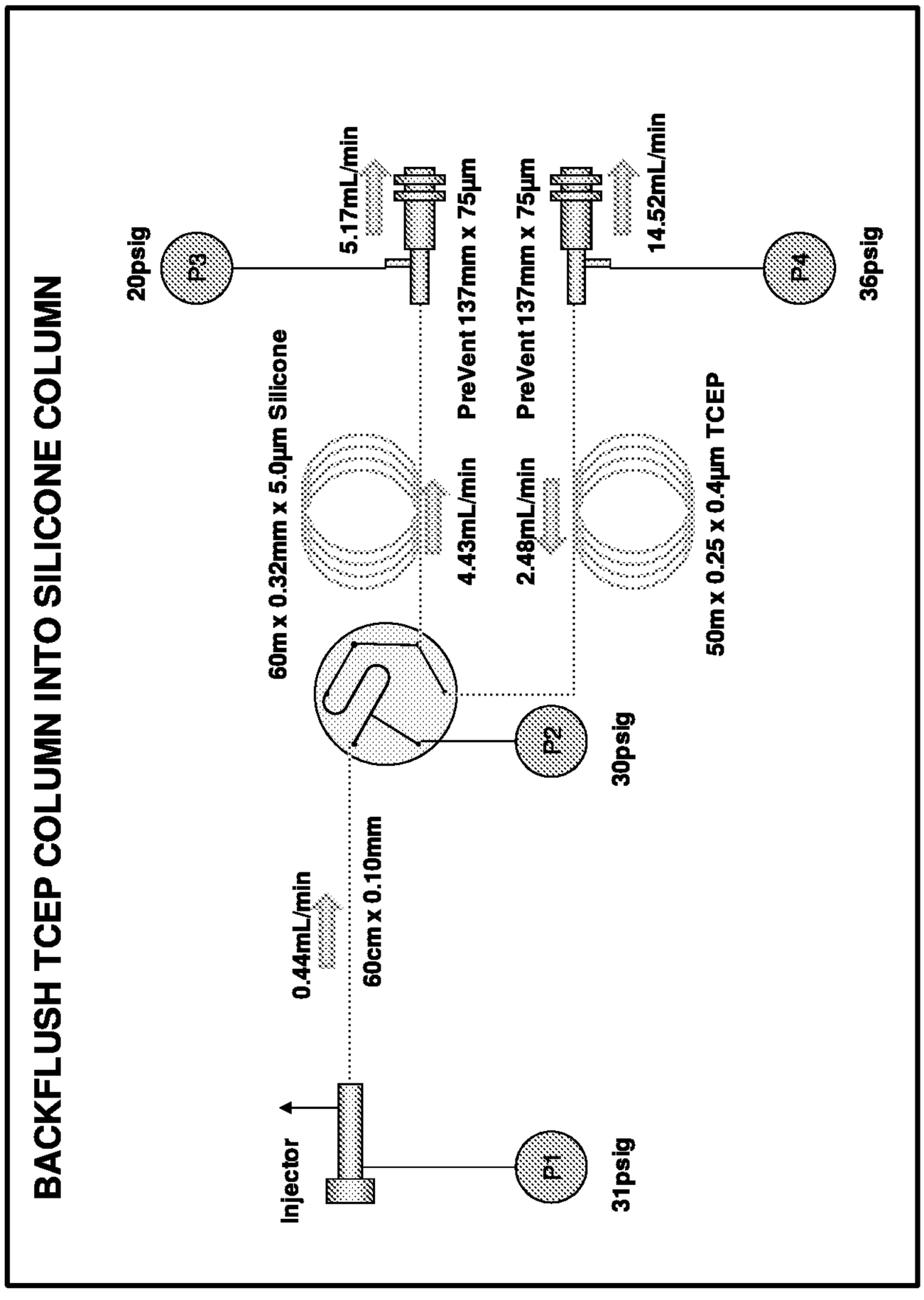

BACKFLUSH TCEP COLUMN INTO SILICONE COLUMN
Injector
0.44mL/min
60cm x 0.10mm
P1
31psig
60m x 0.32mm x 5.0µm Silicone
4.43mL/min
PreVent 137mm x 75µm
5.17mL/min
20psig
P3
P2
30psig
2.48mL/min
PreVent 137mm x 75µm
14.52mL/min
P4
36psig
50m x 0.25 x 0.4µm TCEP

FIG. 78

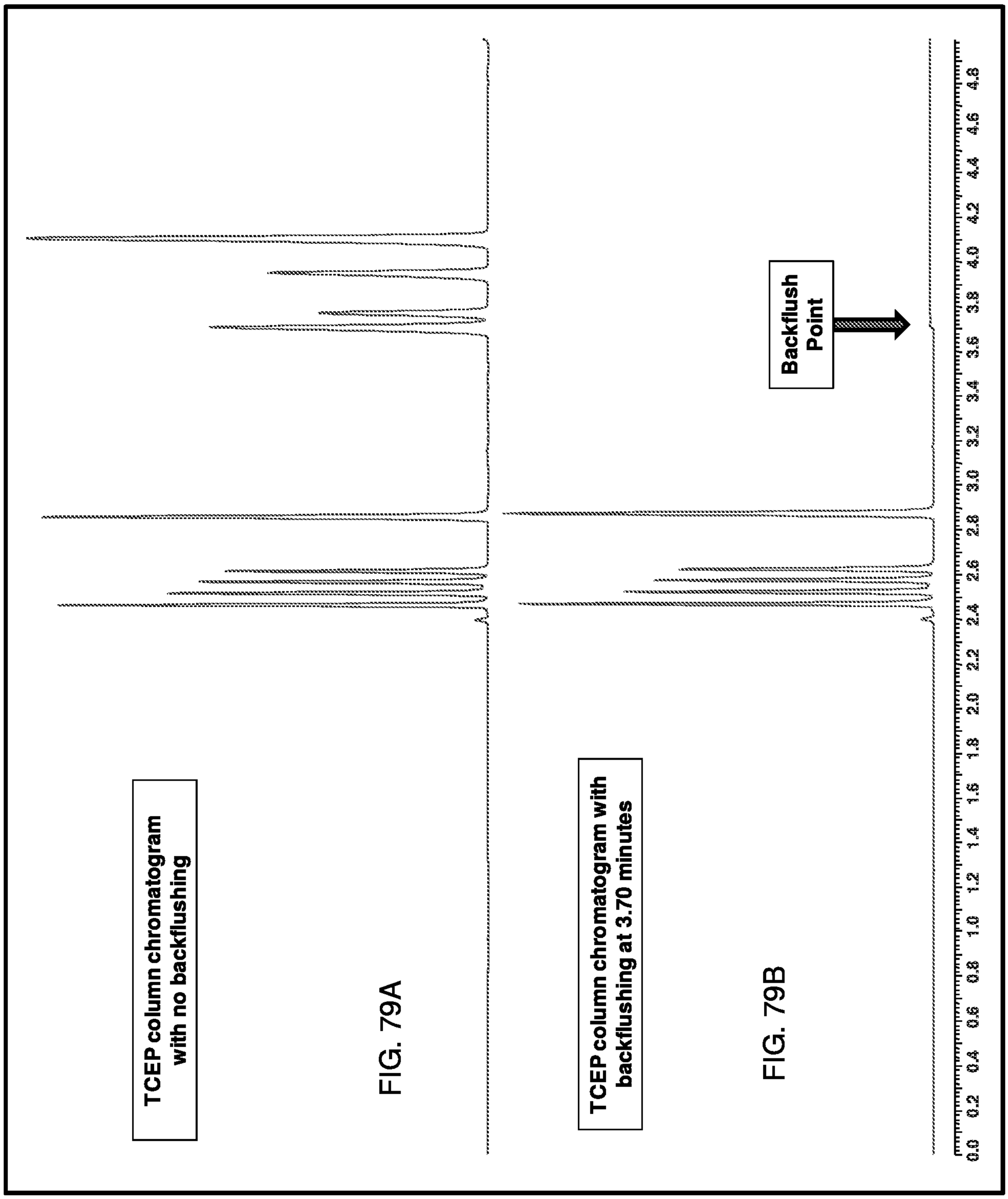


FIG. 79A

FIG. 79B

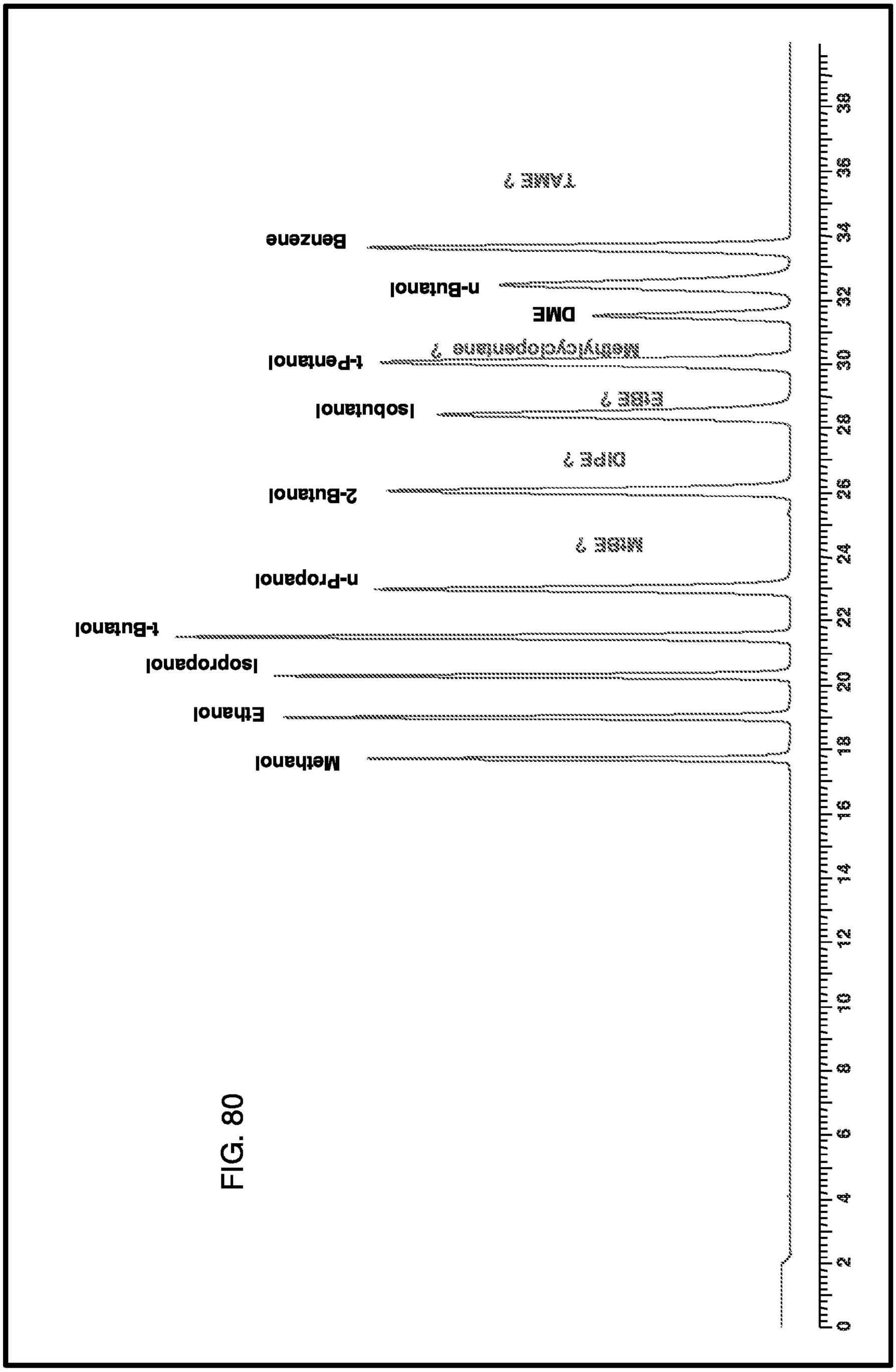

Methanol
Ethanol
Isopropanol
t-Butanol
n-Propanol
MIBE ?
2-Butanol
DIPE ?
Isobutanol
EtBE ?
t-Pentanol
Methylcyclopentane ?
DME
n-Butanol
Benzene
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38

FIG. 80

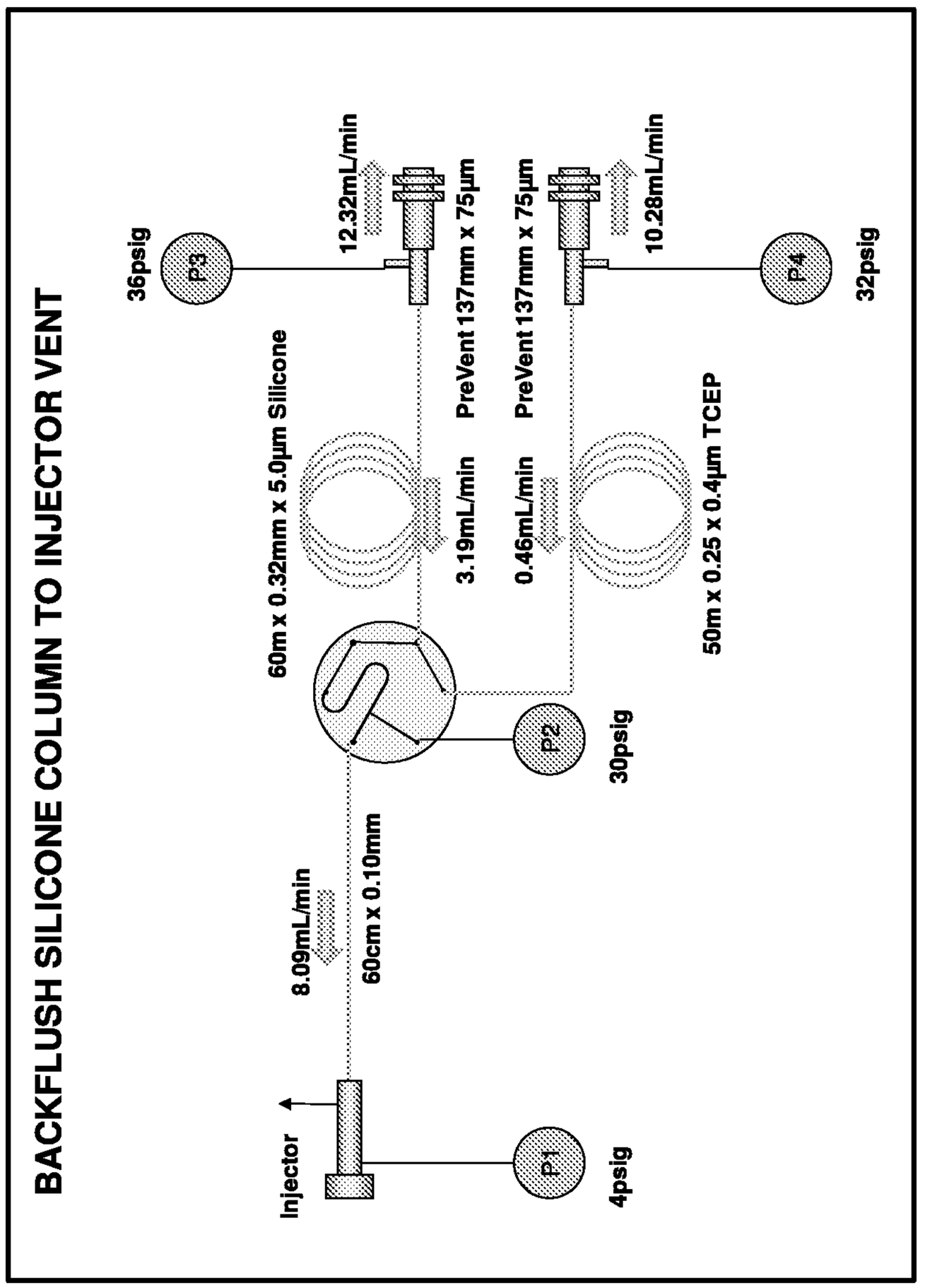

BACKFLUSH SILICONE COLUMN TO INJECTOR VENT
36psig
P3
12.32mL/min
PreVent 137mm x 75µm
3.19mL/min
60m x 0.32mm x 5.0µm Silicone
0.46mL/min
PreVent 137mm x 75µm
10.28mL/min
P4
32psig
50m x 0.25 x 0.4µm TCEP
P2
30psig
8.09mL/min
60cm x 0.10mm
Injector
P1
4psig

FIG. 81

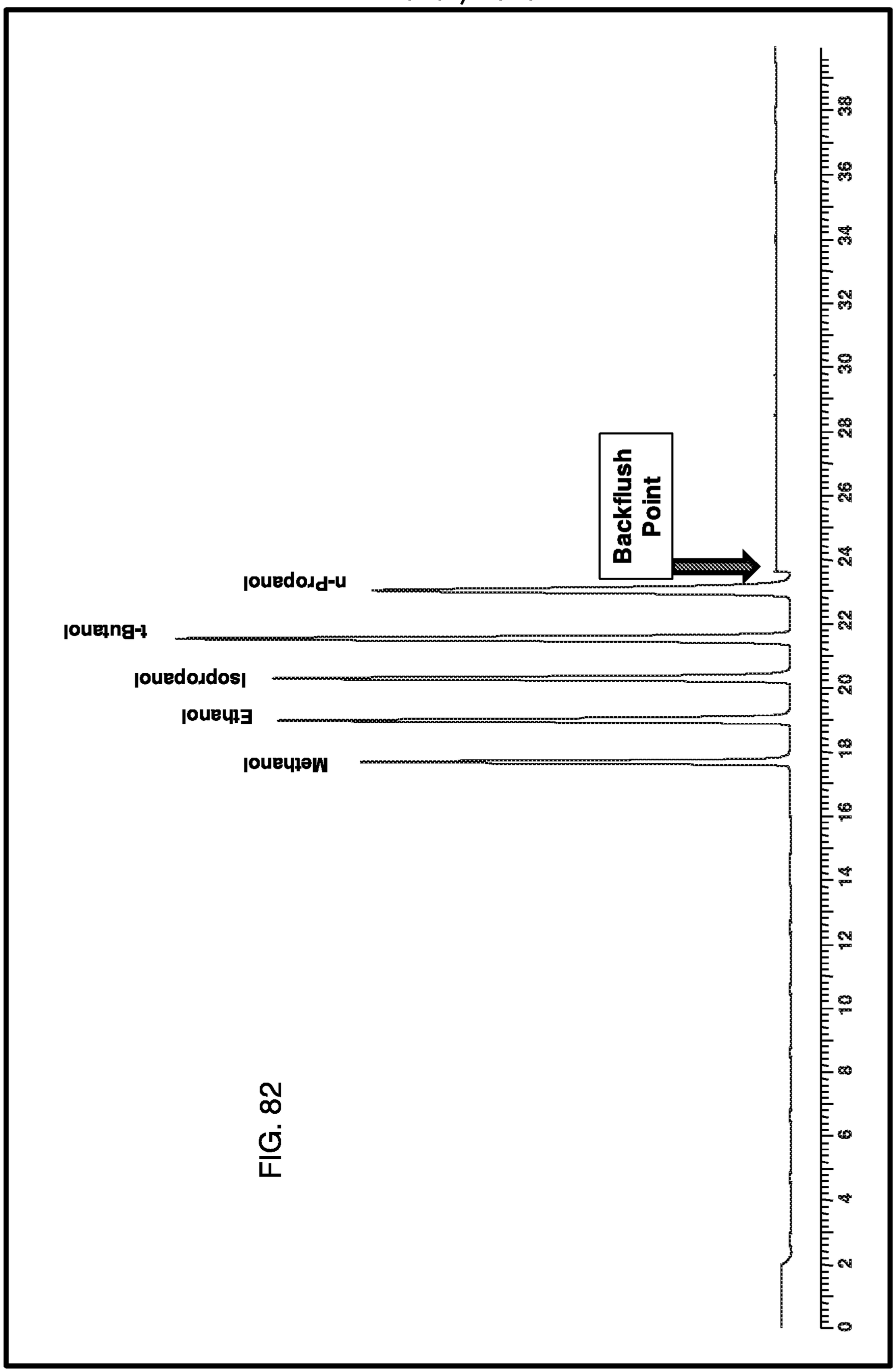
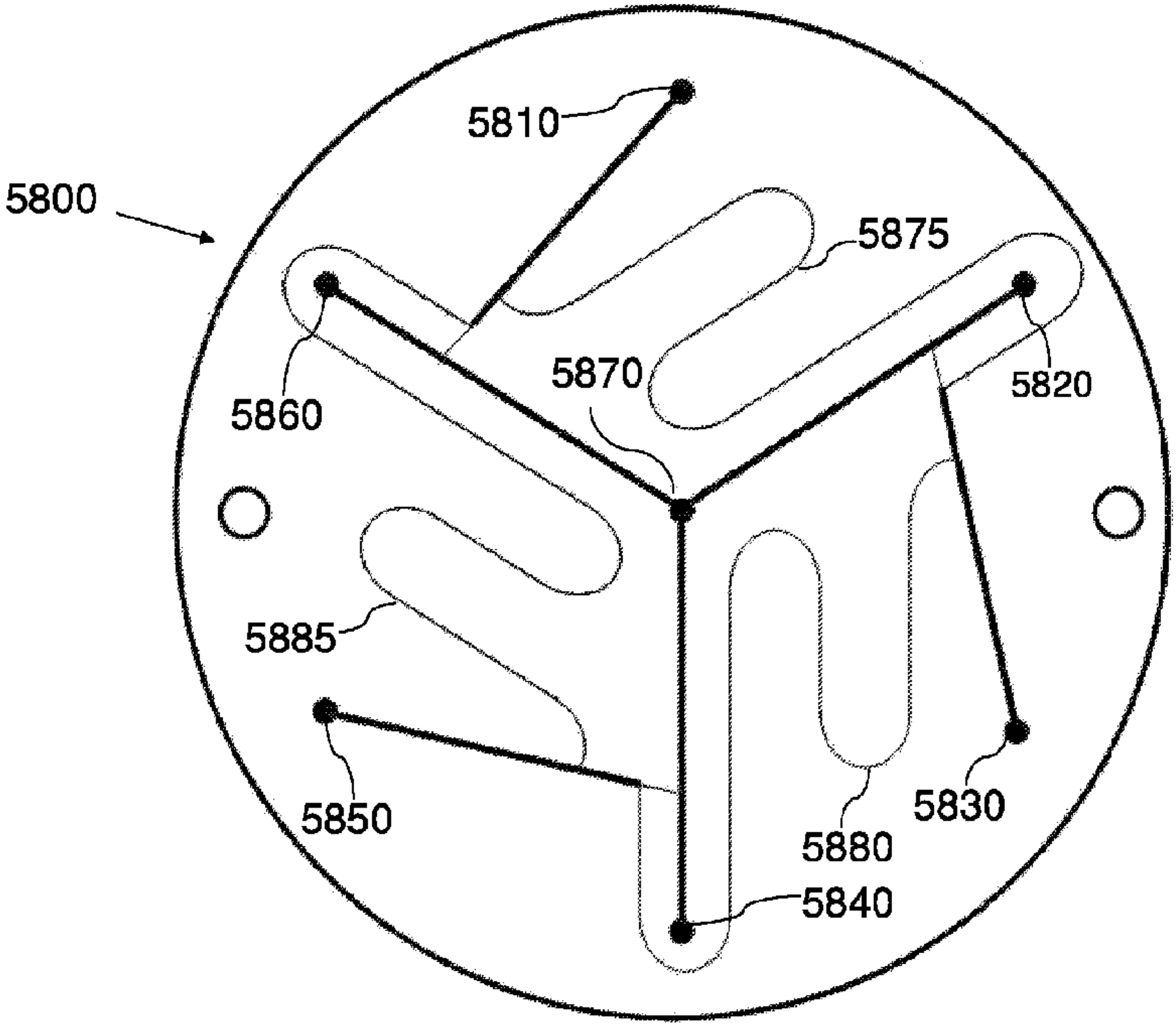

Backflush Point
n-Propanol
t-Butanol
Isopropanol
Ethanol
Methanol
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38

FIG. 82


5800
5810
5875
5860
5870
5820
5885
5850
5880
5830
5840