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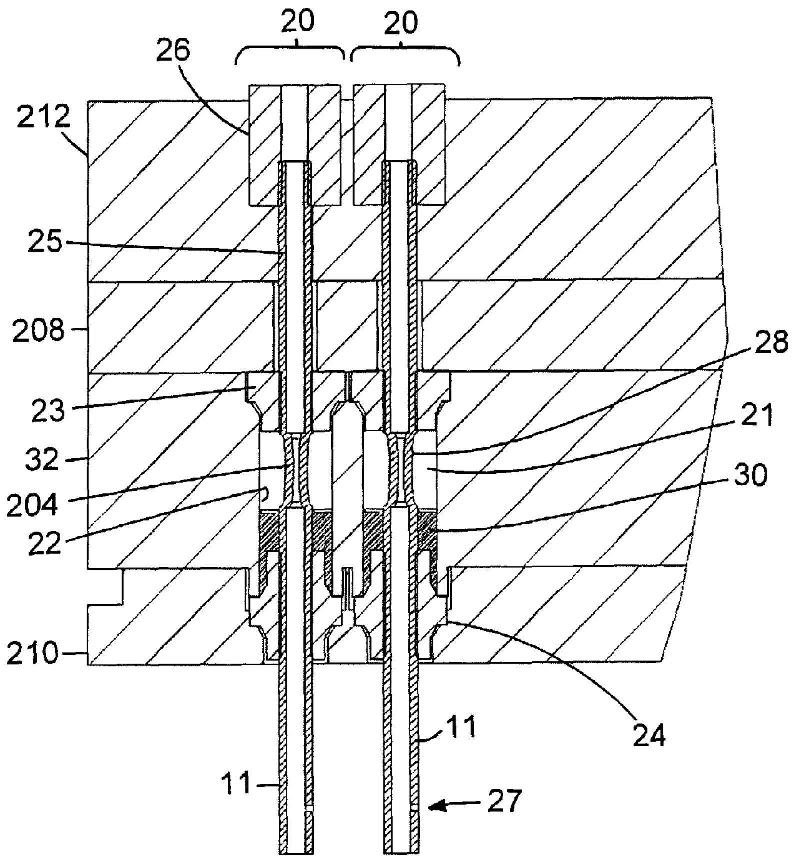
(54) Title: MICROFLUIDIC MIXING AND DISPENSING



(57) Abstract: A micro fluidic dispensing tap (20), such as for use in screening assays, includes a dispensing tube (11) translatable within a sealed reservoir housing (32), The tube has an outer surface and defines an inner cavity (25) open at a lower end of the tube, and a metering aperture (27) extending through a side wall of the tube between the inner cavity and the outer surface to define a known volume. The tube is movable against a seal (24) at a lower end of the reservoir housing between a first position, in which the metering aperture is disposed below the seal, and a second position, in which the metering aperture is disposed above the seal and exposed to the reservoir (21) for entraining a discrete dose of a sample liquid (30) within the aperture. An injector (130) is configured to inject a known quantity ofa diluent (53) into the inner cavity of the tube and into fluidic contact with the dose of sample liquid in the aperture, such that the dose of sample liquid diffuses into the diluent to form a discrete mixture for dispensing from an open end of the tube.

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MICROFLUIDIC MIXING AND DISPENSING

TECHNICAL FIELD

This invention relates to liquid dispensing devices for mixing and dispensing extremely small quantities of liquids, and arrays of such devices for use in microfluidics and laboratory automation.

BACKGROUND

The science and economics of drug discovery has changed with developments in the areas of genomics, combinatorial chemistry and high-throughput screening. The number of targets has increased as a result of genomics while the number of small molecule compounds (samples) has dramatically increased as a result of combinatorial chemistry. This increase in targets and compounds has an exponential effect on the number of tests that need to be performed to increase the likelihood of finding a new chemical entity using high-throughput screening. Microliter amounts of target and sample must suffice for many screening assays, putting pressure on the automation industry to provide new tools to accurately meter, mix and dispense liquids in doses as low as on the order of 10 nanoliters in many instances. Conventional R&D screening efforts use multiple variations of pipetting to move aliquots of the concentrated liquid sample from storage receptacles, to working receptacles, to dilution receptacles where the sample is diluted with a solvent such as pure dimethylsulfoxide (DMSO), and finally to assay receptacles. This "reformatting" process, or "sample preparation" can waste valuable sample or target and increase time and assay cost. Devices and methods are needed for accurately and efficiently handling these valuable liquids in such minute quantities, to increase screening productivity and accuracy.

SUMMARY

The invention features a microfluidic dispensing tap configured to accurately meter and dilute extremely small amounts of liquids, such as sample fluids for screening assays.

According to one aspect of the invention, the tap has a dispensing tube having an outer surface and defining an inner cavity open at a lower end of the tube, the tube also defining a metering aperture extending through a side wall of the tube between the inner cavity and the outer surface, the metering aperture defining a known volume. The tap also includes a reservoir housing defining, together with the tube outer surface, a reservoir cavity for holding a quantity of a first liquid, and a seal between the reservoir housing and the tube outer surface at a lower end of the reservoir, the tube being movable against the seal between a first position, in which the metering aperture is disposed below the seal, and a second position, in which the metering aperture is disposed above the seal and exposed to the reservoir for entraining a discrete dose of the first liquid within the aperture. An injector is hydraulically connected to the inner cavity of the tube and configured to inject a known quantity of a second liquid into the inner cavity of the tube and into fluidic contact with the dose of first liquid in the aperture, such that the dose of first liquid diffuses into the quantity of second liquid to form a discrete mixture for dispensing from the open end of the tube, such as into a well.

Preferably the tap does not contact the well, and the liquid dispensed from each tap breaks contact with the tap before contacting the well aligned with that tap or the contents of the well aligned with that tap. The reservoirs are preferably sealed against air and light. The taps may be configured in an array of reservoir units aligned directly above an array of wells. Each tap may be actuated independently and preferably contains zero dead volume. Examples of suitable multi-well containers are a 96-well microtiter plate, a 384-well microtiter plate and a 1536-well microtiter plate.

In some embodiments, the aperture is a metering capillary and the first liquid is drawn via capillary forces into the metering capillary.

In some cases the tap includes means for cycling the solution up and down within the inner cavity of the tube, such as through a mixing orifice, to thoroughly mix the first and second liquids before dispensing. For example, the injector may be configured to perform such cycling pneumatically.

Some embodiments include means for propelling the mixed solution from the tube utilizing a compressed gas, such as air, nitrogen or argon, that engages an exposed surface of the solution. Alternatively, the fluid can be drawn from the end of the tube by touching the solution to another fluid surface or a solid surface.

The invention also features a device for storing and dispensing liquid into an array of wells in a multi-well container. The device includes: an array of isolated, sealed, tapped reservoir units, each unit containing an integrated metering tap, each tap including a meter capillary. The meter capillary can be sized to draw in, for example, 5 nanoliters to 20 microliters, preferably from 5 to 200 nanoliters of a liquid. The device also includes suitable instrumentation to pump a diluent in through the inner diameter of the tube so that the lower meniscus edge is below the meter capillary, drawing the liquid into the diluent via diffusion or forced vacuum, mixing the liquid and diluent in the tube by hydraulically moving the diluent up and down inside of the tube, and expelling the mixture from the tube by pumping the diluent to the end of the translatable tube. The array of reservoir units can be arranged so that each tap aligns with one well of a multi-well container such as a 96-well microtiter plate, a 384-well microtiter plate, a 1536-well microtiter plate or a flat plate designed to hold small amounts of fluid. However, with suitable equipment, any particular tap can be positioned to dispense into any chosen well. Some embodiments of the invention include a compressed gas inlet port in fluid communication with the fluid output path when the tube is in the dispense position. In addition, some embodiments include a compressed gas path terminating in an annular opening surrounding the fluid output tip. Some embodiments of the invention feature a single channel device that operates independently or operates as an array by placing multiple single-channel units into a frame.

According to another aspect of the invention, a microfluidic dispensing tap includes a dispensing tube having an outer surface and defining an inner cavity open at a lower end of the tube. The tube also defines a metering aperture extending through a side wall of the tube between the inner cavity and the outer surface, the metering aperture defining a known volume. A reservoir housing defines, together with the tube outer surface, a reservoir cavity for holding a quantity of a first liquid. A seal extends between the reservoir housing and the tube outer surface at a lower end of the reservoir cavity. The tube is movable against the seal between a first position, in which the metering aperture is disposed below the seal, and a second position, in which the metering aperture is disposed above the seal and exposed to the reservoir cavity for entraining a discrete dose of the first liquid within the aperture. A tap actuator is hydraulically connected to the inner cavity of the tube and configured to introduce a known quantity of a second liquid into the inner cavity of the tube and into fluidic contact with the dose of first liquid in the aperture, such that the dose of first liquid diffuses into the quantity of second liquid to form a discrete mixture for dispensing from the open end of the tube.

Preferably, the reservoir cavity is sealed against air and light.

In some embodiments, the aperture is a metering capillary and the first liquid is drawn via capillary forces into the metering capillary.

The actuator is preferably adapted to cycle the solution up and down within the inner cavity of the tube to mix the first and second liquids before dispensing, such as by cycling through a mixing orifice. The mixing orifice may be defined at (i.e., aligned with) a detent in the outer surface of the tube, for example. The tap actuator may be configured to perform such cycling pneumatically.

In some cases, the tap also includes means for propelling the mixed solution from the tube utilizing a compressed gas, such as air, nitrogen or argon, that engages an exposed surface of the solution. For example, a compressed gas inlet port may be provided in fluid communication with the inner cavity of the tube when the tube is in a dispense position, or a compressed gas path may terminate in an annular opening surrounding the lower end of the tube.

In some embodiments, the second liquid is introduced into the inner cavity of the tube by injecting the second fluid into the tube at a point where the metering aperture is between the injected second fluid and said open end of the tube. In some other embodiments, the second liquid is introduced into the inner cavity of the tube by being drawn up from the open end of the tube toward the metering aperture.

The metering aperture or capillary preferably has a fixed volume of less than about 20 microliters, more preferably between about 5 and 200 nanoliters.

In some configurations, a multiplicity of the above-described dispensing taps are arranged in an array alignable with an array of wells of a microtiter plate, into each of which the mixed solution is expelled from a corresponding dispensing tap by operating the corresponding tap.

According to another aspect of the invention, a device is provided for storing and dispensing liquid into an array of wells in a multi-well container. The device includes an array of isolated, sealed, tapped reservoir units, each unit containing an integrated metering tap including a dispensing tube having an outer surface and defining an inner cavity open at a lower end of the tube. The tube also defines a metering capillary extending through a side wall of the tube between the inner cavity and the outer surface, the metering capillary sized to draw in a known volume of a liquid. The device includes instrumentation configured to pump a diluent along the inner cavity of the tube so that a lower meniscus edge of the diluent is below the metering capillary; draw the liquid from the metering capillary into the diluent via diffusion or forced vacuum; mix the liquid and diluent in the tube by hydraulically moving the diluent up and down inside the tube, to form a mixture; and then expel the mixture from the tube by pumping the mixture to the end of the tube.

In preferred embodiments, the array of reservoir units is arranged so that each tap aligns with one well of a multi-well container such as a 96-well microtiter plate, a 384-well microtiter plate, a 1536-well microtiter plate or a flat plate designed to hold small amounts of fluid.

According to another aspect of the invention, a method of mixing and dispensing microliter volumes of a sample liquid with a diluent liquid is provided. The method includes:

(a) providing a sealed, tapped reservoir unit containing an integrated metering tap with a dispensing tube having an outer surface and defining an inner cavity open at a lower end of the tube, the tube also defining a metering capillary extending through a side wall of the tube between the inner cavity and the outer surface;

(b) drawing a dose volume of sample liquid into the metering capillary by capillary action;

(c) pumping a volume of liquid diluent along the inner cavity of the tube so that a lower meniscus edge of the diluent is below the metering capillary;

(d) drawing the dose of sample liquid from the metering capillary into the diluent, such as by diffusion or forced vacuum;

(e) mixing the sample liquid and diluent in the tube to form a mixture; and then

(f) expelling the mixture from the tube by pumping the mixture to the end of the tube.

In some cases the volume of liquid diluent is introduced into the inner cavity of the tube by injecting the diluent into the tube at a point where the metering aperture is between the injected diluent and the end of the tube. In some other cases, the liquid diluent is introduced into the inner cavity of the tube by being drawn up from the end of the tube toward the metering aperture.

In some applications, mixing includes hydraulically moving the diluent up and down inside the tube, and may include cycling the sample liquid and diluent through a mixing orifice. The diluent may be moved up and down inside the tube pneumatically, for example.

In some embodiments, expelling the mixture from the tube includes engaging an exposed surface of the mixture with a compressed gas, such as air, nitrogen or argon.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 is a perspective view of a device for integrated storage, integrated dilution and single-channel dispensing of small volumes of liquids, having multiple tap units.

FIG. 2 shows a sectional view of two arrayed tap units, with metering tubes in a down position.

FIG. 3 shows the tap units with the metering tubes in an intermediate up position.

FIG. 4 shows the tap units with metering tubes in an up position.

FIG. 4A is an enlarged view of section 4A of FIG. 4.

FIG. 4B illustrates metering during pressure equalization in a second embodiment.

FIG. 5 shows the embodiment of FIG. 2 with metering tubes in a down position with filled capillaries for dispensing.

FIG. 6 shows the tap units combined with actuation instrumentation and an arrayed assay receptacle.

FIG. 7 shows one of the tap units as shown in FIG. 6, viewed from the right side.

FIGS. 8-10 sequentially illustrate the positioning of a quantity of diluent in contact with the filled capillary, for diffusion.

FIGS. 11-13 sequentially illustrate mixing the diluent and sample, and dispensing the solution into an assay receptacle.

FIG. 14 is an enlarged view of the lower seal detail of FIG. 2.

FIG. 15 schematically illustrates a system for delivering diluent to multiple tap units.

FIGS. 16 and 17 illustrate an alternative embodiment in which diluent is aspirated from a reservoir located below the tap units.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

Referring to **FIG. 1**, a microfluidic dispensing device **10** is useful for storing and dispensing liquid into a conventional 96-well microtiter plate **12**. The depicted device contains a 96-unit array of integrated reservoir/tap units. The 96 units are arranged so that each of the 96 tubes aligns with one well of a conventional 96-well microtiter plate. Protruding from lower surface **13** of device **10** are 96, 384, 1536 or other configuration of flow tubes **11** arranged so that when device **10** is aligned above a 96, 384, 1536 microtiter plate or other receptacle **12**, each flow tube **11** is above a different one of the 96, 384, 1536, or other number of wells or receptacles **14**. On the upper surface **15** of device **10** are 96, 384, 1536, or other number of mechanical interfaces **16** for tap actuation. Operation of each interface **16** actuates a tap whose flow tube **11** is located beneath that interface **16**. Alternatively, a mechanical interface can be provided on the lower end of each tube **11**, with the flow path of tubes **11** reversed.

FIGS. 2 through 5 illustrate sample fluid metering. As shown in **FIG. 2**, each tap unit **20** contains a reservoir **21** around the outer diameter of its tube **11**, defined by a cylinder wall **22** of reservoir plate **32** between upper and lower seals **23** and **24** providing dynamic sealing against the outer surface of the tube **11**. Seals **23** and **24** may be of any suitable material compatible with the fluids to be contained in reservoirs **21**. For use with DMSO as a solvent, molded silicone coated with polytetrafluoroethylene (PTFE) is a suitable seal material for providing a contacting seal surface against the outer surface of each tube **11**. Non-coated silicone may be employed, but DMSO can pull impurities from silicone, resulting in possible sample contamination. The seals may be individual units held in place by upper and lower seal retaining plates **208** and **210** of polypropylene, as shown, or portions of a common sealing member spanning multiple reservoirs. Each reservoir **21** contains a quantity of sample fluid **30**. Each round tube **11** is hollow, defining a central bore **25**, and has a midsection detent **28** of reduced diameter, defining an inner mixing orifice **204**. Tubes **11** have a nominal outer diameter of about 1.5 millimeters, and a nominal inner diameter of bore **25** of about 0.8 millimeter. A mechanical interface **26** is disposed at the upper end of each tube **11** and held within an interface plate **212**, for hydraulically connecting the tube to an actuation instrumentation manifold block (not shown). Each meter/dilution/dispensing tube **11** also defines a meter capillary **27** hydraulically connecting the outside of the tube **11** with the inside bore **25** of the tube, below the mixing orifice **204**. Capillaries **27** define fixed volumes within the thickness of the side wall of each tube. In the illustrated embodiment, tubes **11** are of polypropylene and capillaries **27** are round holes having a diameter of about 0.28 millimeter and a length (equal to the wall thickness of the tube) of about 0.34 millimeter, defining a capillary volume of approximately 15 nanoliters. The metered amount can be from near zero nanoliters to 20 microliters, preferably from 5 to 200 nanoliters. Mixing orifices **204** need not be at the same position along tubes **11** as necked detents **28**.

In operation, the metering tubes **11** are first translated up to an intermediary position (FIG. 3) by moving the interface plate **212** away from upper seal plate **208**. In this position, tube detent **28** is aligned with, and fully spans, upper seal **23** to allow nitrogen or other inert gas to enter the reservoir **21** through open channel **29** defined between upper seal **23** and the outer surface of tube **11**, to balance internal reservoir pressure. Detent **28** may be of any other form suitable for performing this pressure-balancing function, such as one or more longitudinal grooves extending a limited distance along the outer surface of the tube. Tubes **11** continue to be pulled upward to an up position (FIG. 4), where meter capillaries **27** are in direct exposure to stored sample fluid **30** in reservoirs **21**. With reservoir pressure having just been established by the temporary spanning of detent **28** across upper seal **23**, a determinable amount of fluid sample **30** will be drawn into each capillary **27** by capillary action, as a function of capillary volume and surface tension effects, forming a metered fluid dose **31** (FIG. 4A). Typically, the inner meniscus of metered dose **31** will not protrude into the inner tube bore **25**. In the tube position shown in FIG. 4, reservoir **21** is now completely sealed from the outside by cylinder wall **22**, upper seal **23** and lower seal **24**.

Alternately, reservoir pressure equalization may be timed to coincide with metering by repositioning detent **28** with respect to capillary **27**, as shown in FIG. 4B. In the illustrated position of tube **11**, tube detent **28** momentarily spans upper seal **23** to allow nitrogen or other inert gas to enter the reservoir **21** through channel **29** to balance the internal reservoir pressure, while capillary **27** is exposed to sample fluid in reservoir **21**.

As the outside of the tube **11** (at the site of capillary **27**) comes in contact with the fluid, capillary forces and the relationship between the surface tension of the fluid and the surface energy of the tube material will draw fluid into the capillary hole **27** until the volume of that capillary hole is substantially filled. A meniscus forms at the inside surface of the tube **11**. A meniscus is often described in the form of a contact angle between the fluid and the material. The contact angle will vary according to the relationship between the surface tension of the fluid and the surface energy of the tube material. A variation in contact angle will cause the volume of the capillary to vary slightly. The surface tension (fluid) and surface energy (solid) relationship is optimized when the fluid has a low surface tension (e.g. 20-70 dynes per centimeter) and the solid has a relatively high surface energy (e.g. 30-100 dynes per centimeter).

The metering tubes **11** are next moved back to a down position (**FIG. 5**), with the meter capillaries **27** again beneath lower seals **24** and now containing known volumes **31** of sample fluid separated from the remaining fluid in reservoirs **21** by the wiping action of lower seals **24**.

FIGS. 6 through 13 illustrate mixing and dispensing metered sample fluid with a diluent. Referring first to FIG. 6, actuation instrumentation injection block **40** is connected to devices **20** through their mechanical interfaces **26**. Injection block 40 includes means for introducing a known volume of a diluent into each tube 11 and driving the diluent up and down within the tube to engage and mix with the metered sample, as discussed below. Devices **20** have also been connected to an actuation instrumentation manifold block **40** at an opposite end of tubes **11**. A receptacle device **12** is disposed underneath the devices **20** such that each tube **11** is aligned with a corresponding receptacle well **14**, with the capillaries **27** of the tubes each already containing a metered dose **31** of fluid sample. As seen in the side view of FIG. 7, injection block **40** defines both a driving fluid port **50** and a diluent fluid port **51** for each tap unit **20**, hydraulically connected to the bore **25** of tube **11** by an injection sleeve **220** coupled to the upper end of tube **11** at interface **26** to form an air-tight seal. Driving fluid **52** and a discrete plug **53** of diluent fluid of known volume are pumped through injection block **40** to their respective entrances of sleeve **220** (FIG. 8) and then diluent fluid plug **53** is pumped into sleeve **220** (FIG. 9), such as by pneumatic operation. Driving fluid **52** is then forced into sleeve **220** (**FIG. 10**), hydraulically translating diluent fluid plug **53** down through mixing orifice **204** until the bottom edge or meniscus **55** of diluent fluid **53** is below meter capillary **27**. Driving fluid **52** is either air, or is separated from diluent fluid **53** by air gap **54**, such that the driving fluid **52** never comes in contact with the diluent plug **53**. In the condition illustrated in FIG. 10, diluent plug **53** is in direct fluidic contact with the metered dose **31** of fluid sample contained within the meter capillary **27** (FIG. 6). In an amount of time typically less than about 120 seconds, meter capillary fluid sample **31** diffuses into diluent fluid **53**, and may collect at the bottom meniscus **55** of the diluent fluid plug.

Referring next to **FIG. 11**, driving fluid **52** is cycled into and out of injector sleeves **220** to hydraulically translate the diluent fluid plugs **53** (now containing both the diluent and the metered doses of fluid sample drawn from capillaries **27**) up and down a predetermined number of times within the bores **25** of tubes **11**, through mixing orifices **204**, until the solution is sufficiently mixed. The driving fluid **52** is then advanced to hydraulically translate the mixed solution plugs **53** to the bottom of tube bores **25** until proud droplets **56** of mixed solution **53** appear outside of the open ends of tubes **11** (FIG. 12). Compressed dispensing gas is then delivered through port **57** of actuation instrumentation manifold block **41**, between extrusion director sleeves **58** and the outer surfaces of tubes **11** along coaxial flow paths **59**, to engage the exposed surfaces of proud drops **56**. The dispensing gas breaks the liquid-solid attraction between the solution droplets and their metering tubes **11**, overcoming surface tension effects to enable the droplet to fall free of the tube end. Flow paths **59** need not be coaxial with tubes **11** in all cases. The gas flow pushes the proud drop **56** off of the tube **11** and into the bottom of the receptacle well **14** (FIG. 13). The dispensed solution **60** wets the bottom of the receptacle well **14**. The tubes **11** of the device may now be cleaned and dried for further use.

Referring to **FIG. 14**, lower seals **24** forms a fluid-tight seal with reservoir plate **32** and tubes **11**. Seals **24** each have thin cylindrical protrusions **222** of about 0.6 millimeter thickness that extend up and down the tube surface to help maintain a reliable seal against the tube surface under positive and negative pressure differentials between the reservoirs **21** and the outside environment. Alternatively, seals **24** may be molded without such protrusions **222**, or with the protrusions on either top or bottom in tight engagement with seal plate **210**, rather than spaced apart. Seals **24** are molded of silicone having a hardness of between about 30 and 90 shore A, coated with a layer of PTFE. The sealing bore diameter of each seal should be selected to provide a good seal against the tube surface. Seals with higher durometers can be fashioned to provide less interference against the tube, but softer seals need a tight fit to prevent losing metered fluid as the capillary is forced along the seal.

FIG. 15 shows a schematic of an eight channel injection manifold for controlling the injection of both diluent and cleaning fluid into multiple tap units along a common injection line **139** for each tap unit. Each line **139** is hydraulically connected to the tube bore **25** (FIG. 2) of a respective tap unit. A standard 96 or 384 pipette system with standard or custom tips is indicated by **130**. Prior to use, diluent fluid lines **133** are purged of air for proper channel operation. The injection sequence begins when parallel or serial pinch valves **138** are closed and diluent fluid valve **132** opens and pipette **130** draws diluent fluid from diluent fluid reservoir **131** through diluent fluid valve **132**, through diluent fluid lines **133** into tap unit drive lines **134**. Diluent fluid valve **132** is then closed followed by parallel or serial pinch valves **138** opening. Pipette **130** pushes the diluent fluid along drive lines **134** until the diluent fluid plugs pass through parallel or serial pinch valves **138** and connecting lines **139** to be mixed with fluid sample in each tap unit.

The diluent fluid reservoir **131** is positioned such that the upper surface of the fluid is at nearly the same height as the manifold. This positioning prevents the entry of either too much fluid or not enough fluid air into the diluent fluid lines **133** due to the compressible nature of air. The predictability and accuracy of fluid control is thereby maintained.

The valves **132, 136, 138** preferably include elastomeric elements made of silicone rubber or similar elastomers. During aspiration by the pipettes **130**, i.e. when the pipettes draw fluid from diluent fluid reservoir **131** into diluent fluid line **133**, a control vacuum deflects elastomeric elements in the diluent and cleaning fluid valves **132, 136** away from the individual channel input ports to allow fluid flow into the tap unit drive lines **134**. In the diluent fluid valve **132**, a control pressure forces an elastomeric element onto each seat of the individual channel input port, thereby providing a seal. In the parallel or serial pinch valves **138**, tubes are either pinched so that each channel is closed, or not pinched so that each channel is open.

To clean the tap unit drive lines **134**, the main connection lines **139**, and the individual tap unit lines, cleaning fluid valve **136** is opened. Cleaning fluid (i.e. solvent, air) is pumped from cleaning fluid reservoir **135** through cleaning fluid valve **136**, through cleaning fluid lines **137**, and into tap unit drive lines **134**. The cleaning fluid then passes through connecting lines **139** into each tap unit to be expelled into a receptacle device (not shown).

FIGS. 16 and 17 illustrate an alternative embodiment in which diluent **53** is aspirated from a diluent reservoir **150** defined in a diluent reservoir plate **152** located below the device. Fluid sample metering is identical to that disclosed above with respect to FIGS. 2-5. In this embodiment, a mechanical interface block **154** is first attached to a standard pipettor (e.g., pipettor **130** of Fig. 15) or other aspirating piece of equipment as known in the art. The pipettor mates to the liquid dispensing device **20** through mechanical interface block **154**. A burst of compressed dispensing gas delivered through port **57** blows the metered dose into the center bore **25** of tube **11**.

A diluent reservoir plate **152** is disposed underneath the devices **20** such that each tube **11** is aligned with a corresponding diluent reservoir well **150**, with the metering capillaries **27** of the tubes already emptied of their respective metered doses of fluid sample. In this embodiment, the pipettor driving fluid is air. As the driving fluid is drawn upward by the pipettor, a slug of diluent will be aspirated into the center bore **25** of tubes **11** where it engages and mixes with the sample previously blown into the bore of the tube from the metering capillary. The amount of diluent aspirated is typically between about 0 and 10 microliters.

The driving fluid is cycled up and down a predetermined number of times within the bores **25** of tubes **11**, drawing the mixture of diluent and sample through mixing orifices **204**, until the solution is thoroughly mixed. The driving fluid is then advanced to hydraulically translate the mixed solution plugs to the bottom of the tube bores **25** until proud droplets of mixed solution appear outside of the open ends of tubes **11** (see also FIG. 12).

Devices described herein can be designed for compatibility with various liquids, including aqueous buffers, organic solvents such as DMSO, acids, bases, proteins, oligonucleitides and reagents. Compatibility is achieved by selection of suitable materials for fabrication of components that contact the liquid. Exemplary materials for fabrication of components are stainless steel, nylon, polyethylene, polypropylene, EPD rubber, silicone rubber and PTFE. Suitable materials and fabrication of components is within ordinary skill in the art.

The illustrated embodiments and the features described above build upon our prior work as disclosed in U.S. patent application 09/591,807, filed June 12, 2000, and our corresponding PCT application US01/06174. The entire contents of both of these applications are incorporated herein by reference.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A microfluidic dispensing tap, comprising

a dispensing tube (11) having an outer surface and defining an inner cavity (25) open at a lower end of the tube, the tube also defining a metering aperture (27) extending through a side wall of the tube between the inner cavity and the outer surface, the metering aperture defining a known volume;

a reservoir housing (32) defining, together with the tube outer surface, a reservoir cavity (21) for holding a quantity of a first liquid (30);

a seal (24) between the reservoir housing (32) and the tube outer surface at a lower end of the reservoir cavity (21), the tube (11) being movable against the seal between a first position, in which the metering aperture (27) is disposed below the seal, and a second position, in which the metering aperture is disposed above the seal and exposed to the reservoir cavity for entraining a discrete dose of the first liquid (30) within the aperture;

a tap actuator (130) hydraulically connected to the inner cavity (25) of the tube (11) and configured to introduce a known quantity of a second liquid (53) into the inner cavity of the tube and into fluidic contact with the dose of first liquid (30) in the aperture, such that the dose of first liquid diffuses into the quantity of second liquid to form a discrete mixture for dispensing from the open end of the tube.

2. The dispensing tap of claim 1 wherein the reservoir cavity (21) is sealed against air and light.

3. The dispensing tap of any of the above claims wherein the aperture (27) is a metering capillary and the first liquid (30) is drawn via capillary forces into the metering capillary.

4. The dispensing tap of any of the above claims wherein the actuator (130) is adapted to cycle the solution up and down within the inner cavity (25) of the tube (11) to mix the first and second liquids before dispensing.

5. The dispensing tap of claim 4 wherein the solution is cycled through a mixing orifice (204).

6. The dispensing tap of claim 5 wherein the mixing orifice (204) is defined at a detent (28) in the outer surface of the tube (11).

7. The dispensing tap of any of claims 4 through 6 wherein the tap actuator (130) is configured to perform such cycling pneumatically.

8. The dispensing tap of any of the above claims further comprising means for propelling the mixed solution from the tube (11) utilizing a compressed gas, such as air, nitrogen or argon, that engages an exposed surface of the solution.

9. The dispensing tap of claim 8 wherein the means for propelling the mixed solution from the tube (11) comprises a compressed gas inlet port in fluid communication with inner cavity (25) of the tube when the tube is in a dispense position.

10. The dispensing tap of claim 8 wherein the means for propelling the mixed solution from the tube (11) comprises a compressed gas path (58) terminating in an annular opening surrounding the lower end of the tube.

11. The dispensing tap of any of the above claims wherein the second liquid (53) is introduced into the inner cavity (25) of the tube (11) by injecting the second fluid into the tube at a point where the metering aperture (27) is between the injected second fluid and said open end of the tube. (Fig. 8)

12. The dispensing tap of any of claims 1 through 10 wherein the second liquid (53) is introduced into the inner cavity (25) of the tube (11) by being drawn up from the open end of the tube toward the metering aperture (27). (Fig. 16)

13. The dispensing tap of any of the above claims wherein the metering aperture (27) has a fixed volume of less than about 20 microliters, preferably between about 5 and 200 nanoliters.

14. In combination, a multiplicity of dispensing taps fashioned according to any of the above claims and arranged in an array (10) alignable with an array of wells (14) of a microtiter plate (12), into each of which the mixed solution is expelled from a corresponding dispensing tap (20) by operating the corresponding tap.

15. A device (10) for storing and dispensing liquid into an array of wells (14) in a multi-well container (12), the device comprising

an array of isolated, sealed, tapped reservoir units, each unit containing an integrated metering tap (20) including a dispensing tube (11) having an outer surface and defining an inner cavity (25) open at a lower end of the tube, the tube also defining a metering capillary (27) extending through a side wall of the tube between the inner cavity and the outer surface, the metering capillary sized to draw in a known volume of a liquid (30); and

instrumentation (130) configured to

pump a diluent (53) along the inner cavity of the tube (11) so that a lower meniscus edge (55) of the diluent is below the metering capillary (27);

draw the liquid (30) from the metering capillary (27) into the diluent (53) via diffusion or forced vacuum;

mix the liquid (30) and diluent (53) in the tube (11) by hydraulically moving the diluent up and down inside the tube, to form a mixture; and then to

expel the mixture from the tube (11) by pumping the mixture to the end of the tube (11).

16. The device of claim 15 wherein the metering capillary (27) has a fixed volume of less than about 20 microliters, preferably between about 5 and 200 nanoliters.

17. The device of either claim 15 or 16 wherein the array of reservoir units is arranged so that each tap (20) aligns with one well (14) of a multi-well container such as a

96-well microtiter plate, a 384-well microtiter plate, a 1536-well microtiter plate or a flat plate designed to hold small amounts of fluid.

18. A method of mixing and dispensing microliter volumes of a sample liquid with a diluent liquid, the method including

providing a sealed, tapped reservoir unit containing an integrated metering tap (20) with a dispensing tube (11) having an outer surface and defining an inner cavity (25) open at a lower end of the tube, the tube also defining a metering capillary (27) extending through a side wall of the tube between the inner cavity and the outer surface;

drawing a dose volume of sample liquid (30) into the metering capillary by capillary action;

pumping a volume of liquid diluent (53) along the inner cavity of the tube (11) so that a lower meniscus edge (55) of the diluent is below the metering capillary (27);

drawing the dose of sample liquid (30) from the metering capillary (27) into the diluent (53) via diffusion or forced vacuum;

mixing the sample liquid (30) and diluent (53) in the tube (11), to form a mixture; and then

expelling the mixture from the tube (11) by pumping the mixture to the end of the tube (11).

19. The method of claim 18 wherein the volume of liquid diluent (53) is introduced into the inner cavity (25) of the tube (11) by injecting the diluent into the tube at a point where the metering aperture (27) is between the injected diluent and said end of the tube. (Fig. 8)

20. The method of claim 18 wherein the liquid diluent (53) is introduced into the inner cavity (25) of the tube (11) by being drawn up from the end of the tube toward the metering aperture (27). (Fig. 16)

21. The method of any of claims 18 through 20 wherein mixing includes hydraulically moving the diluent up and down inside the tube (11).

22. The method of claim 21 wherein mixing includes cycling the sample liquid (30) and diluent (53) through a mixing orifice (204).

23. The method of claim 21 wherein the diluent is moved up and down inside the tube (11) pneumatically.

24. The method of any of claims 18 through 23 wherein expelling the mixture from the tube (11) further comprises engaging an exposed surface of the mixture with a compressed gas, such as air, nitrogen or argon.

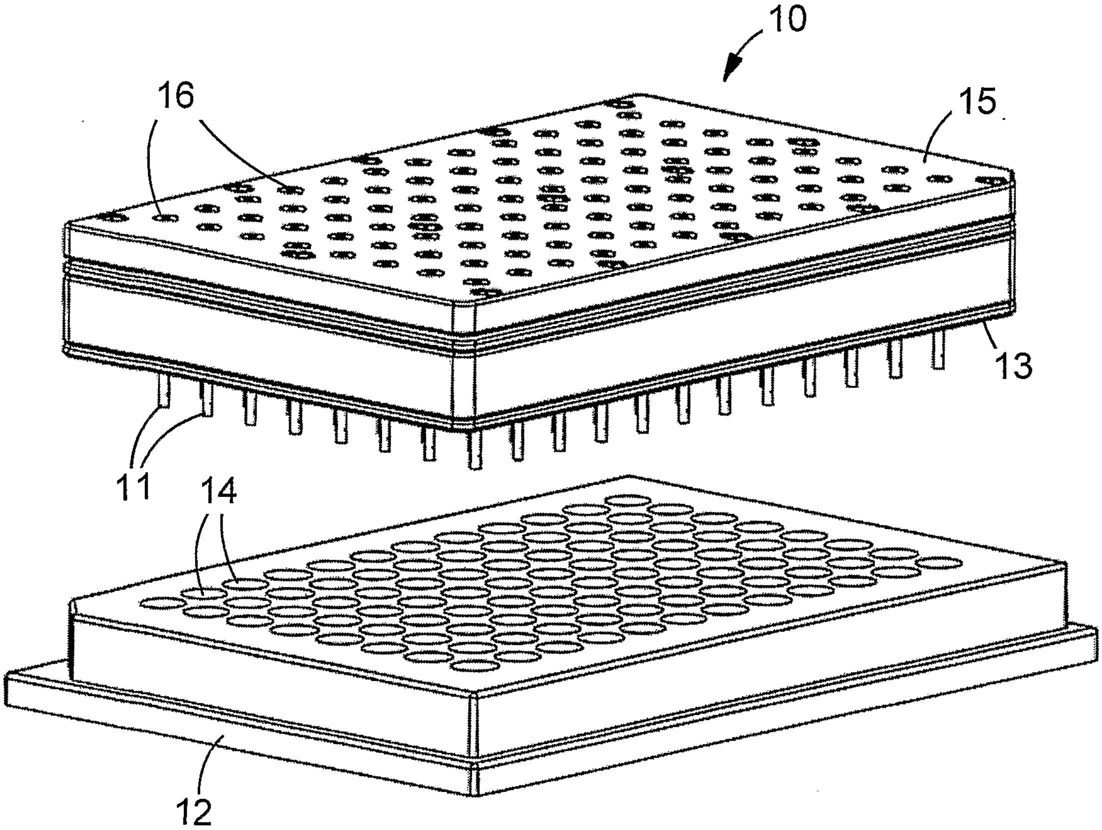

FIG. 1

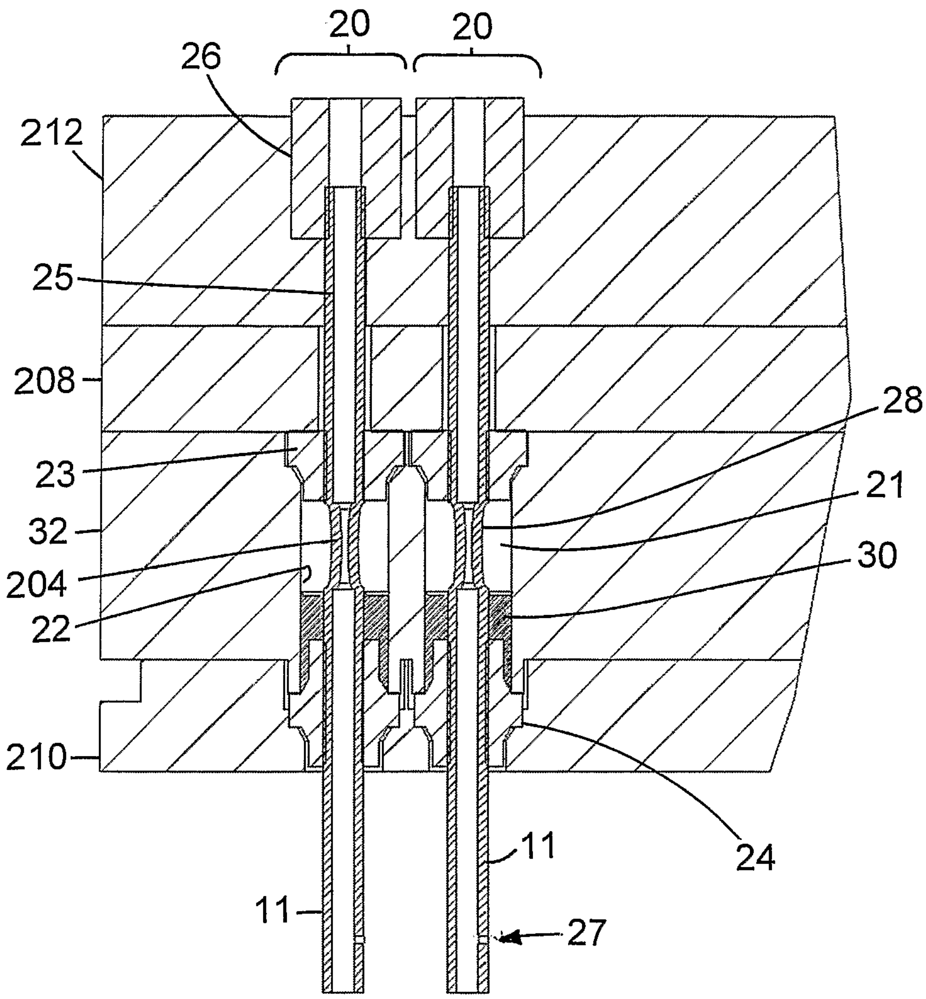

FIG. 2

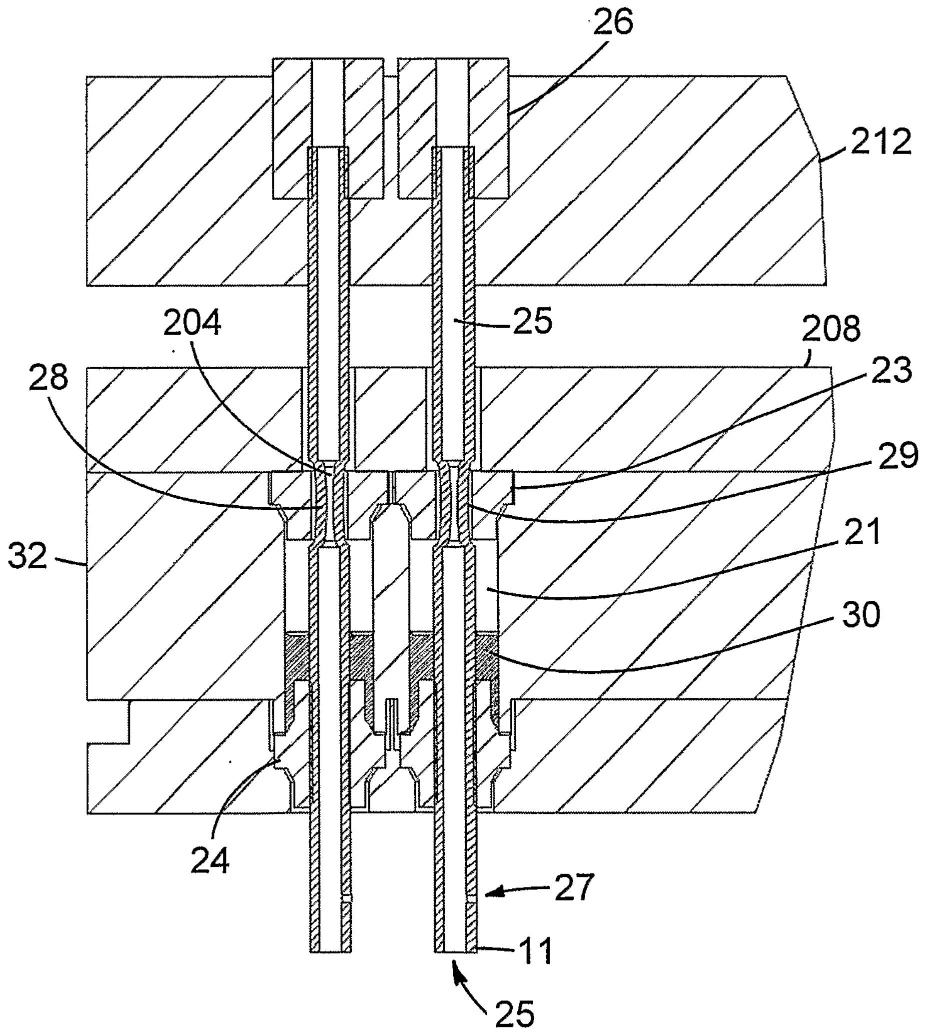

FIG. 3

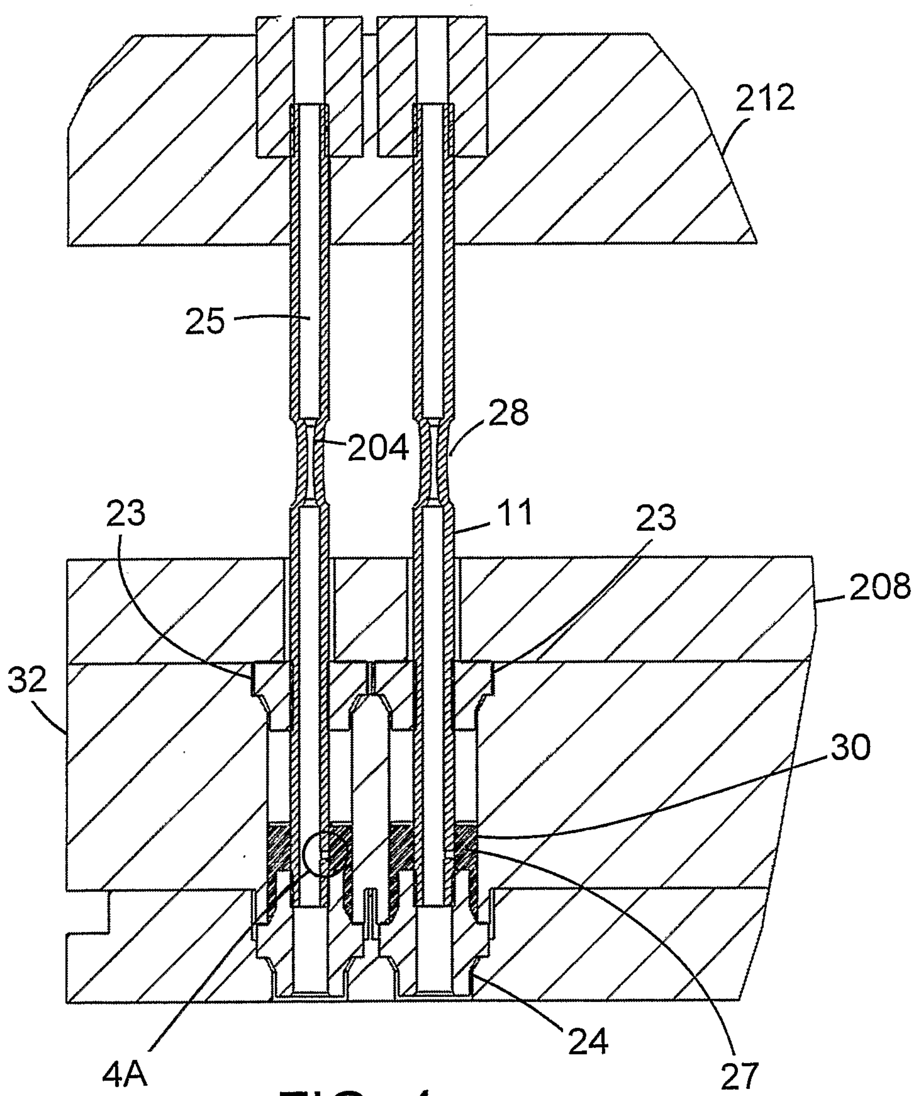

FIG. 4

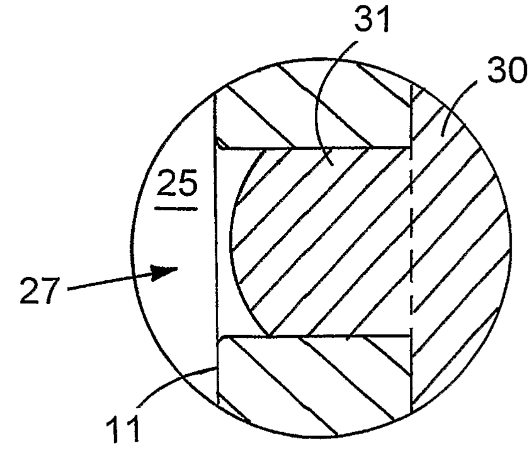

FIG. 4A

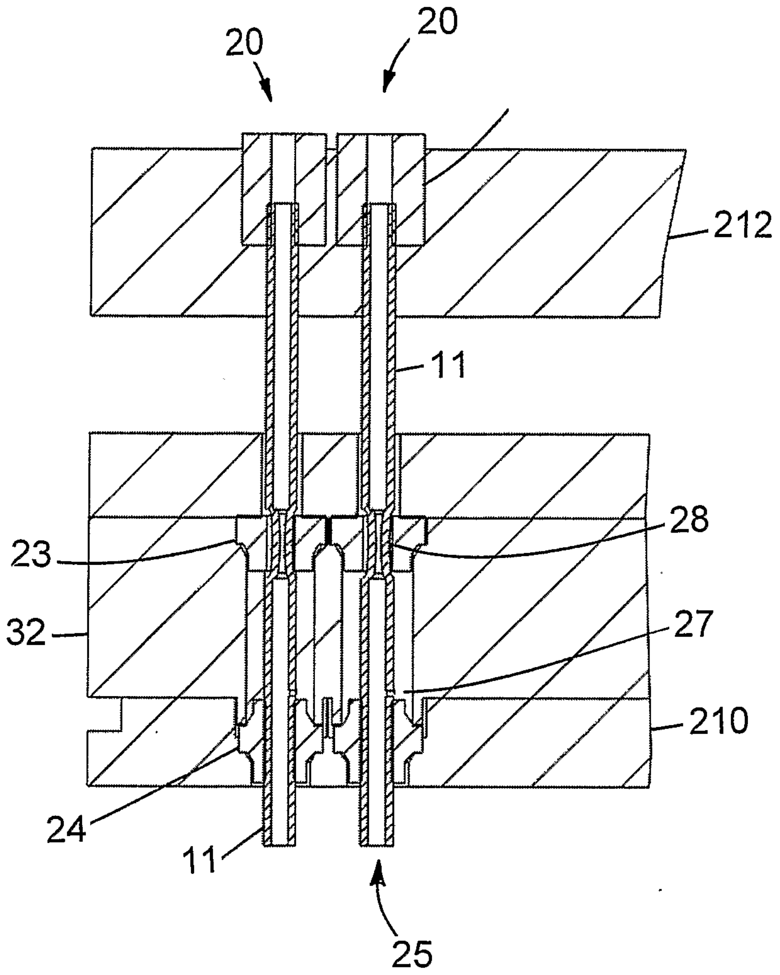

FIG. 4B

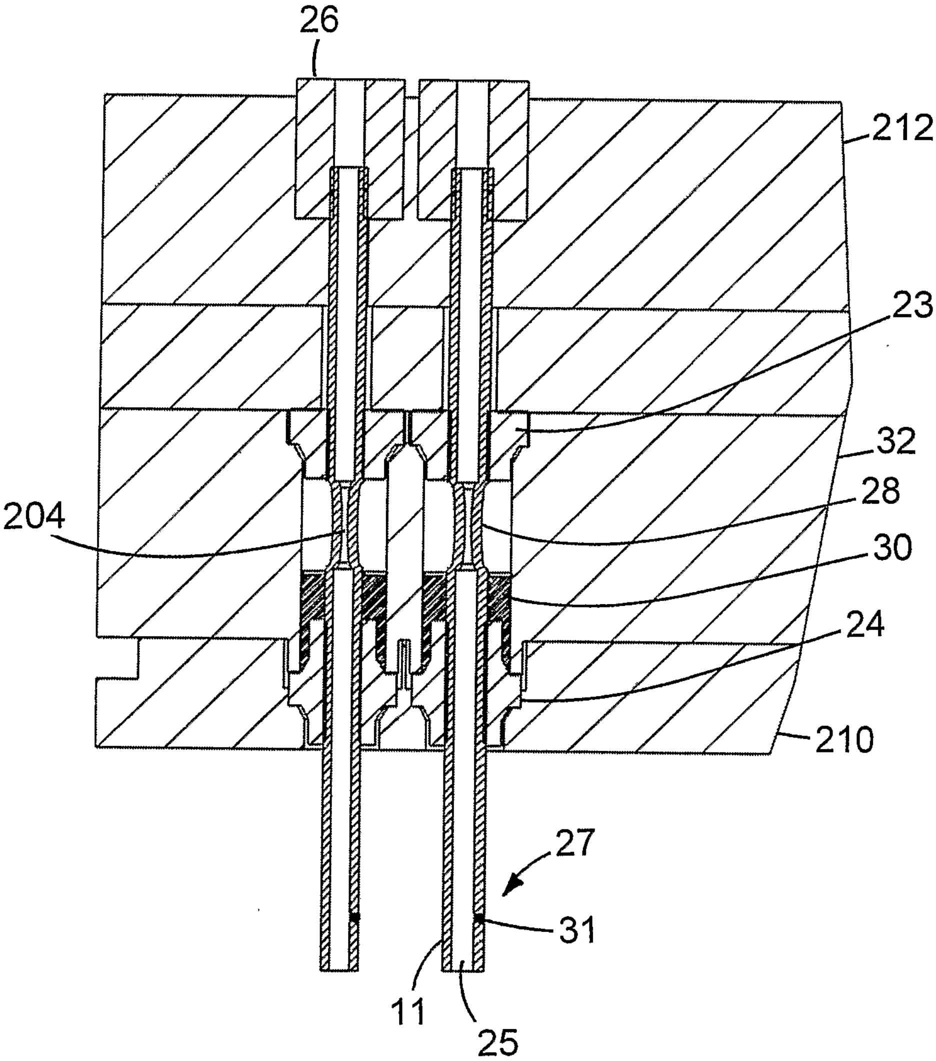

FIG. 5

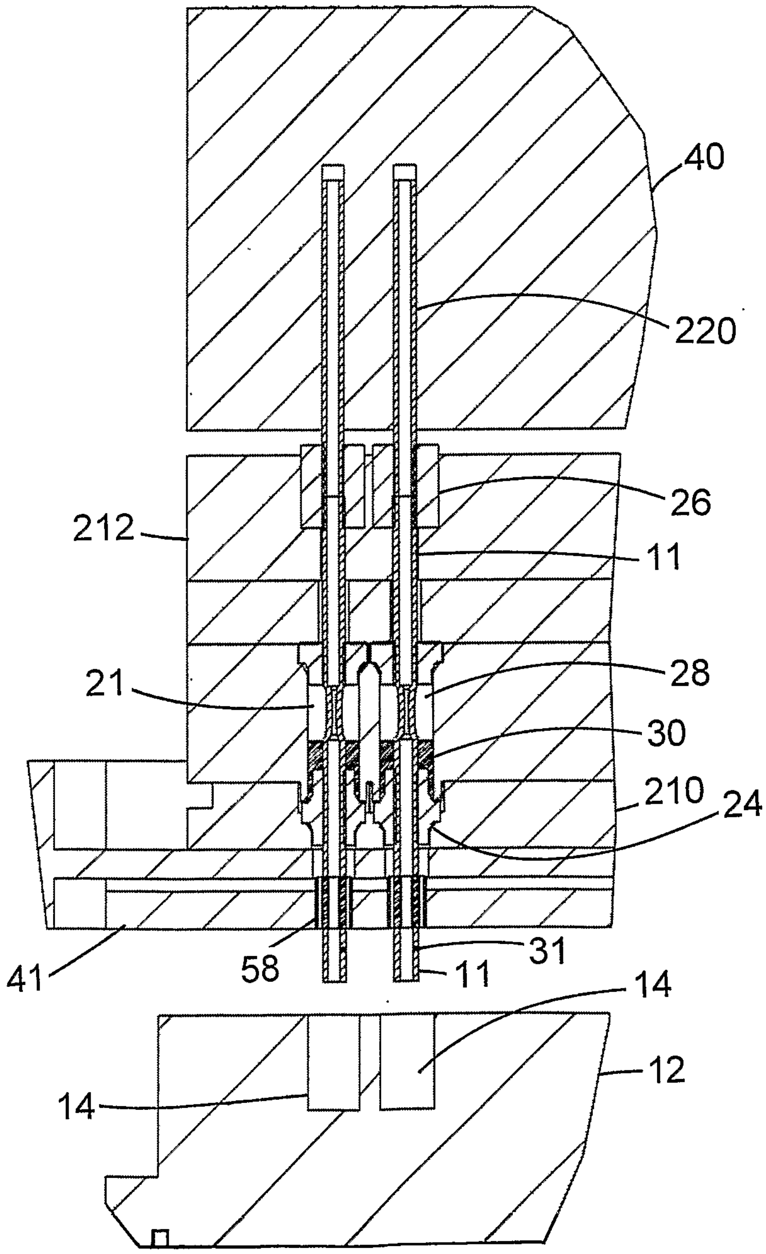

FIG. 6

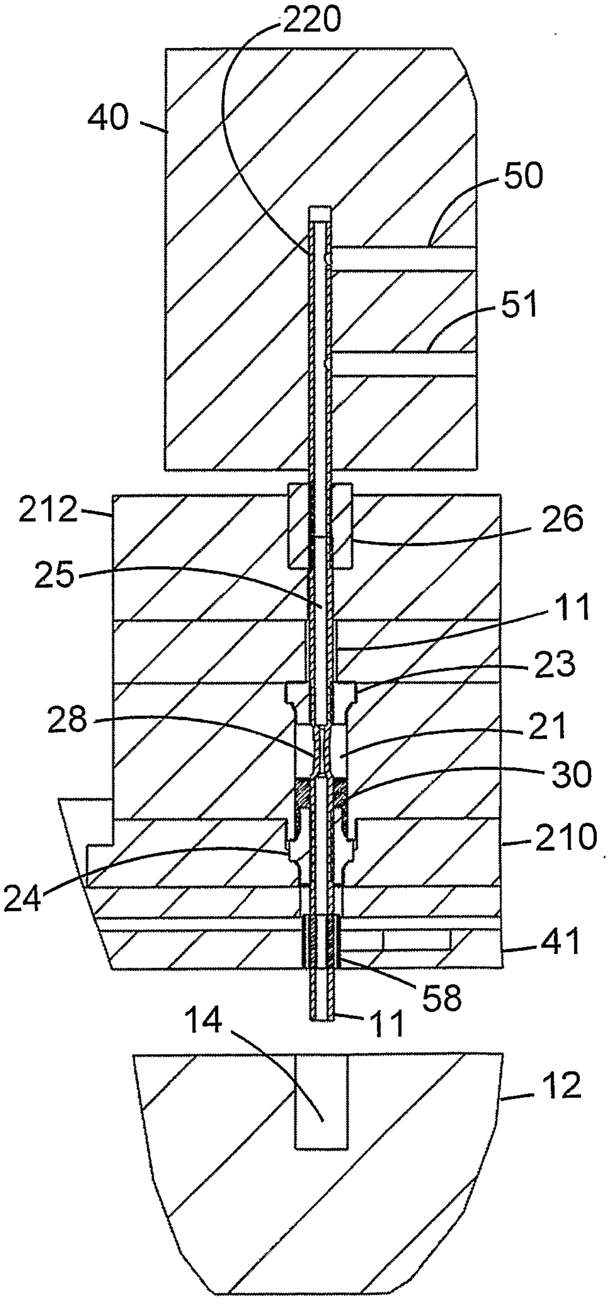

FIG. 7

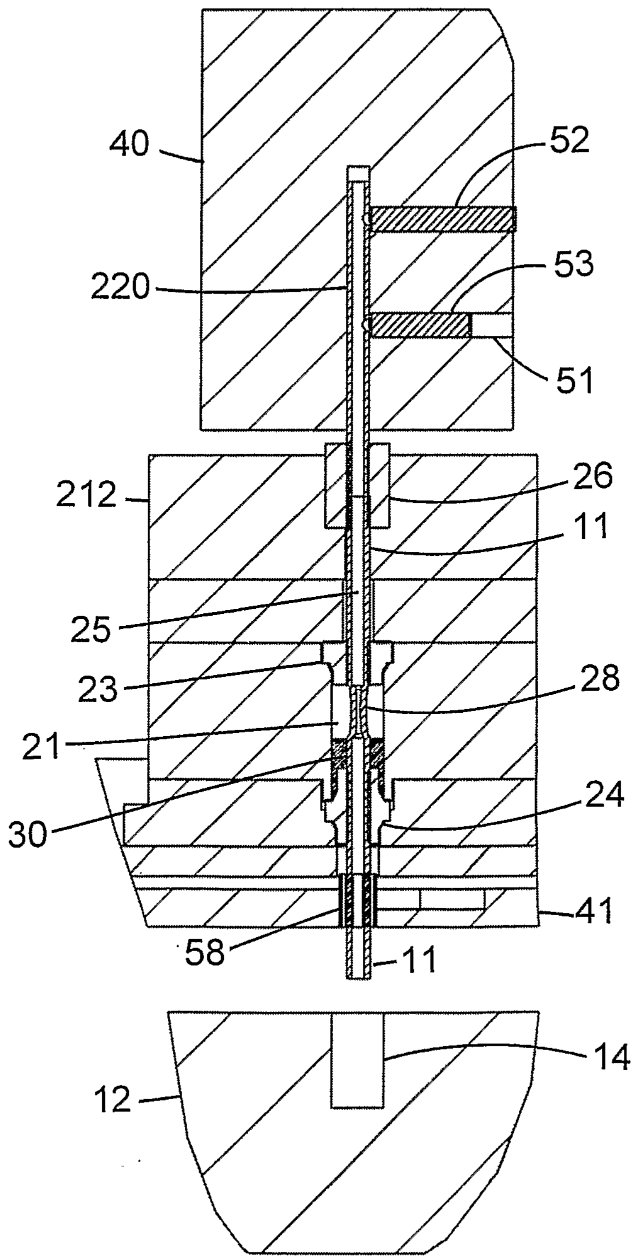

FIG. 8

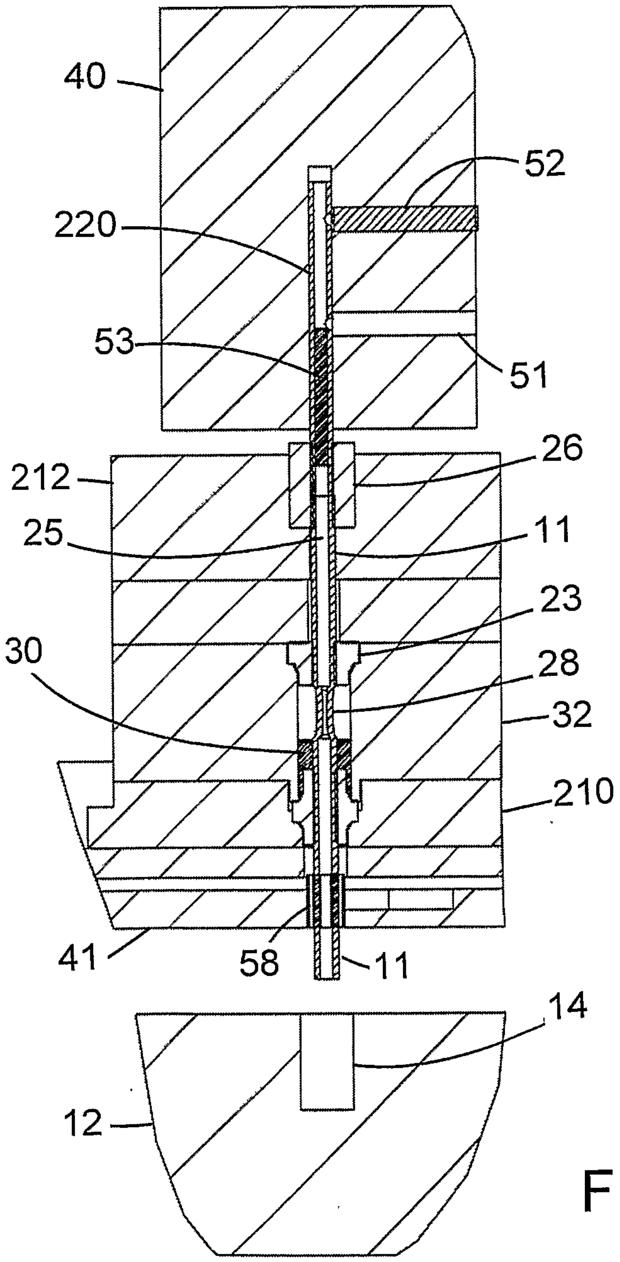

FIG. 9

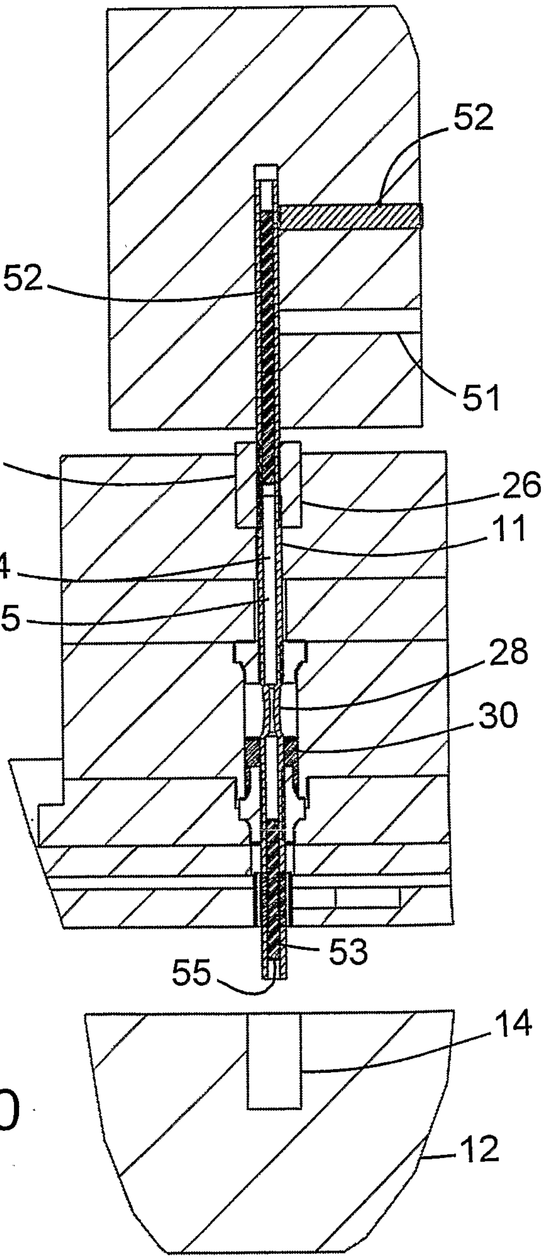

FIG. 10

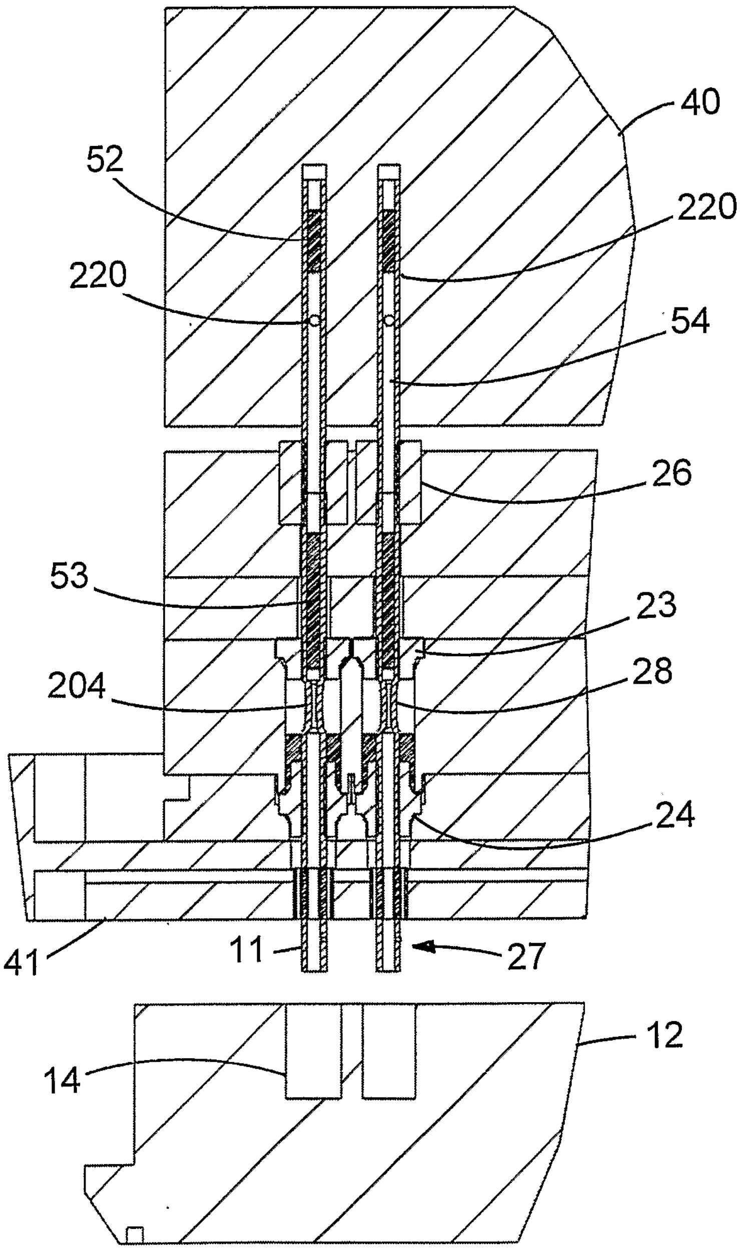

FIG. 11

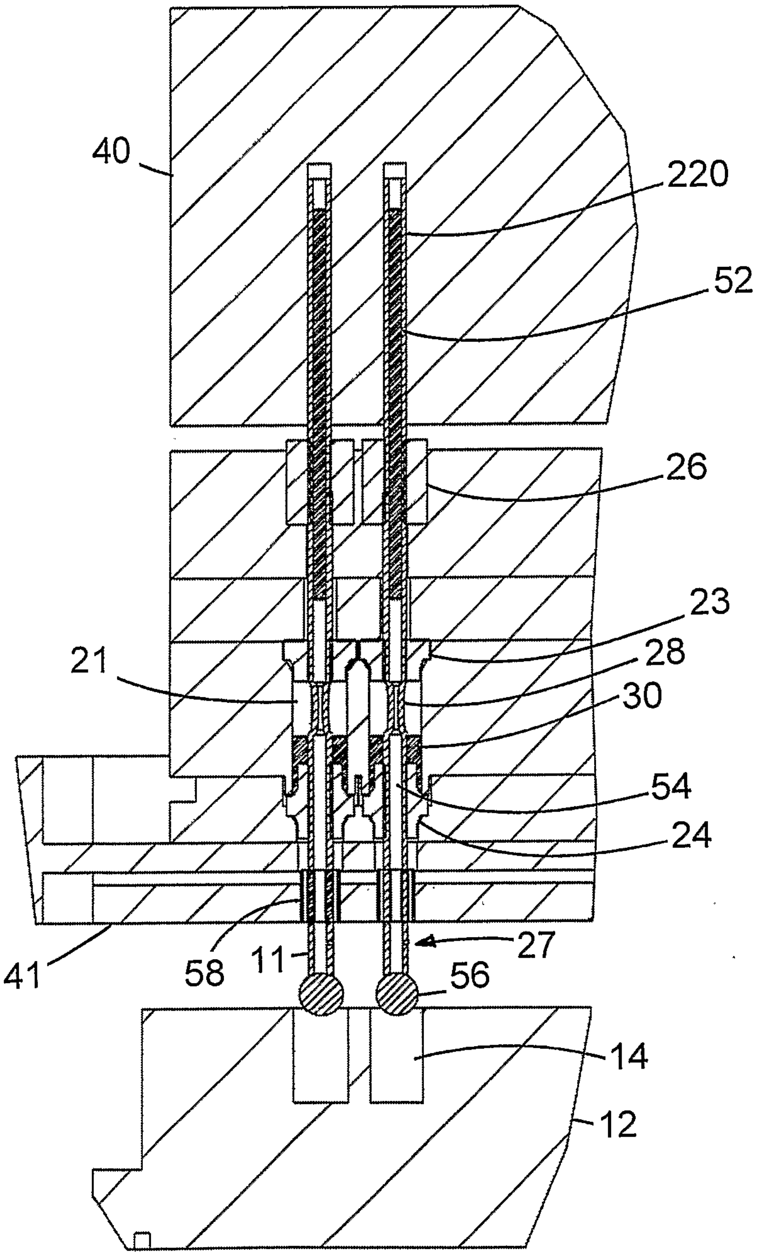

FIG. 12

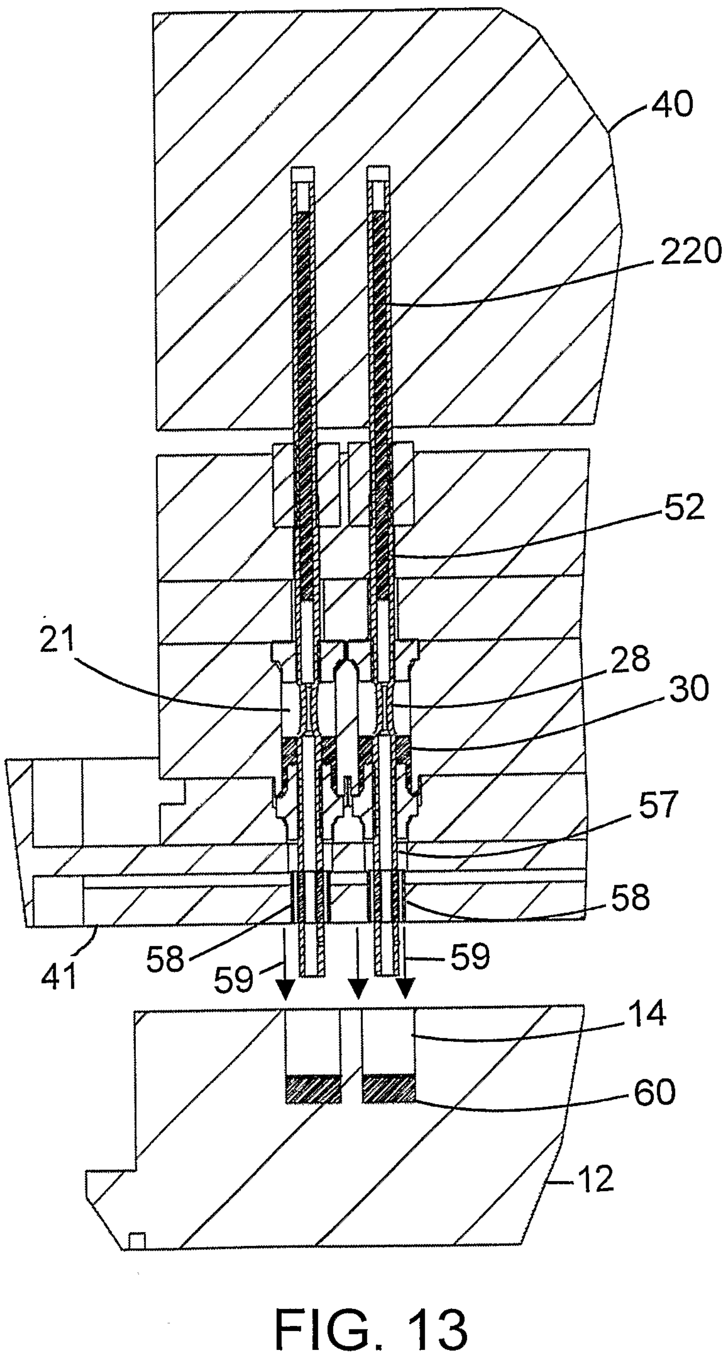

FIG. 13

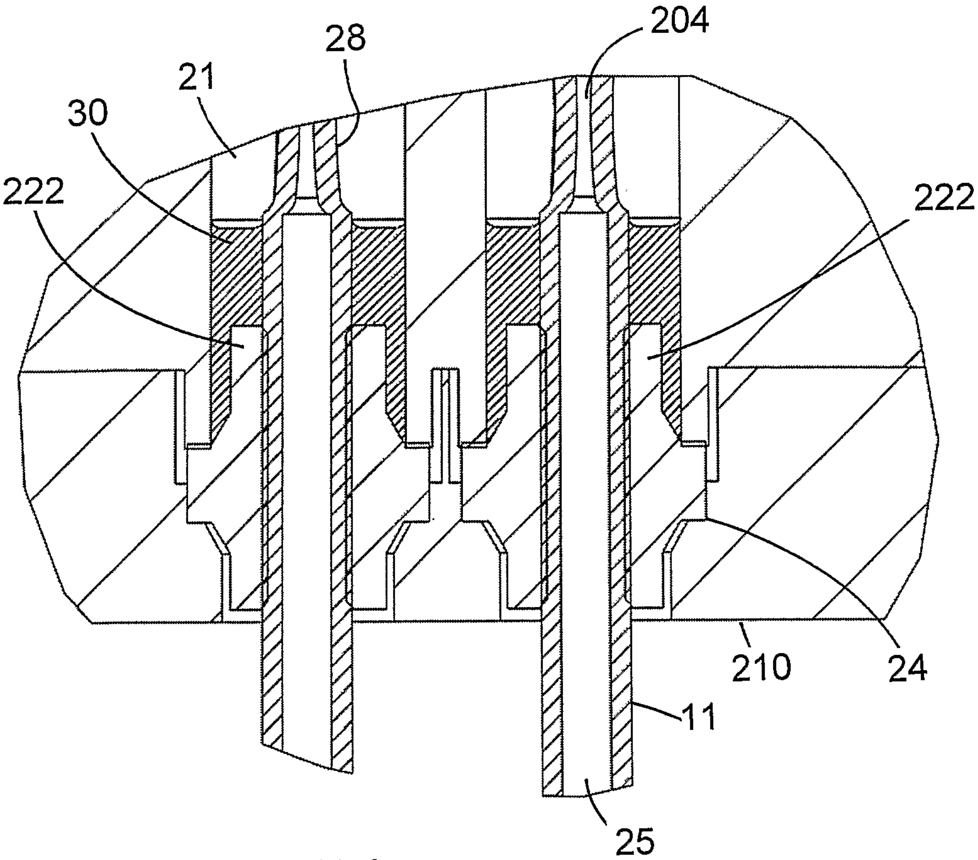

FIG. 14

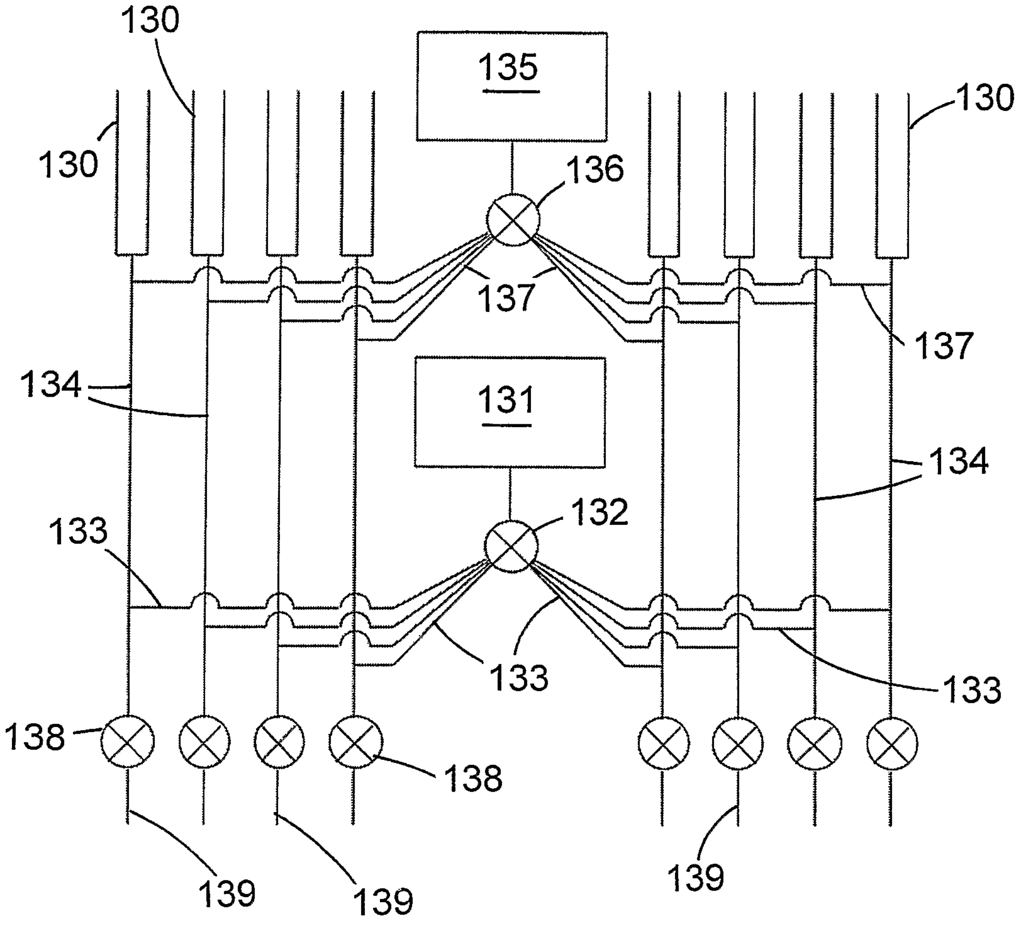
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FIG. 15

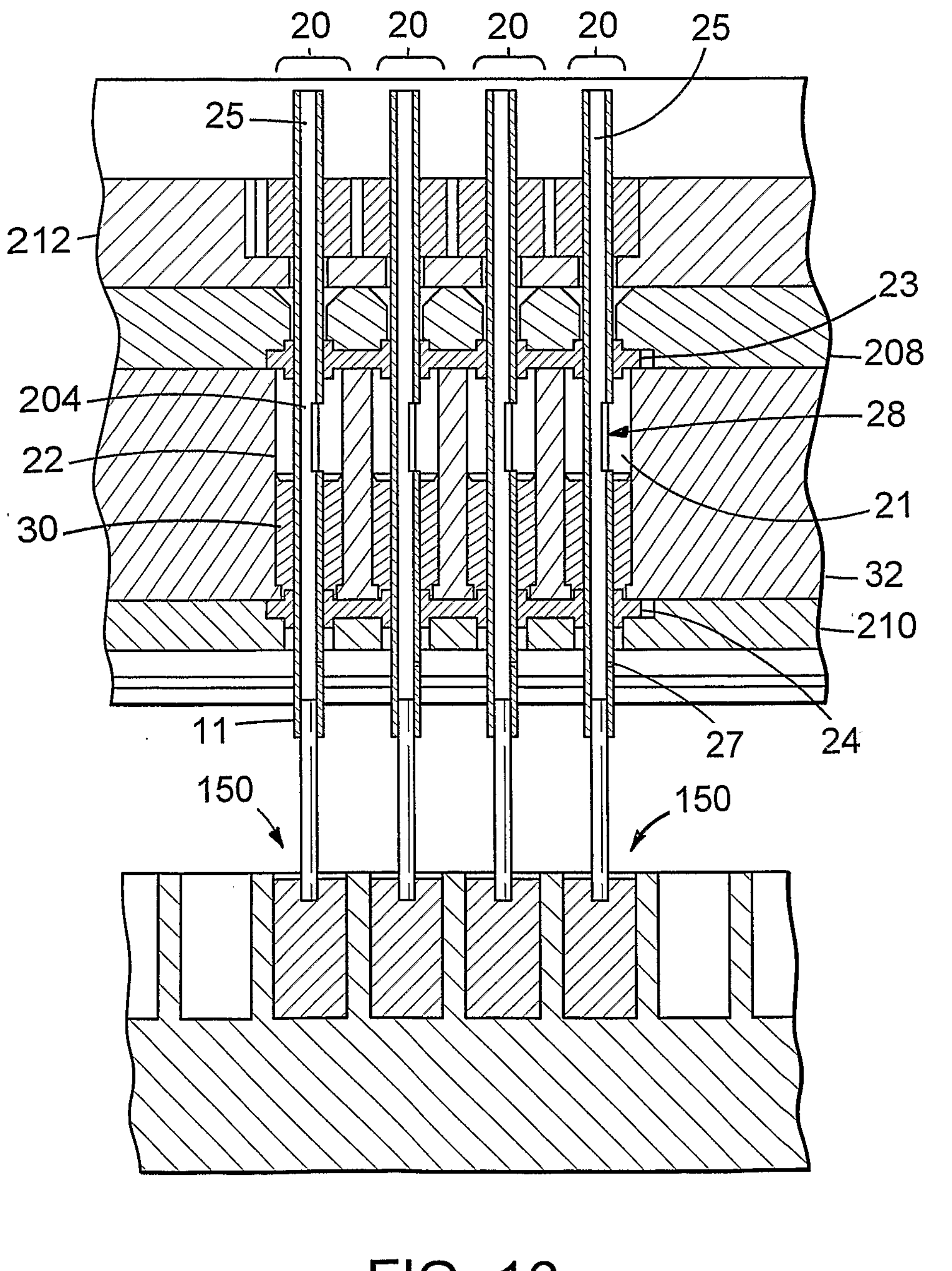

FIG. 16

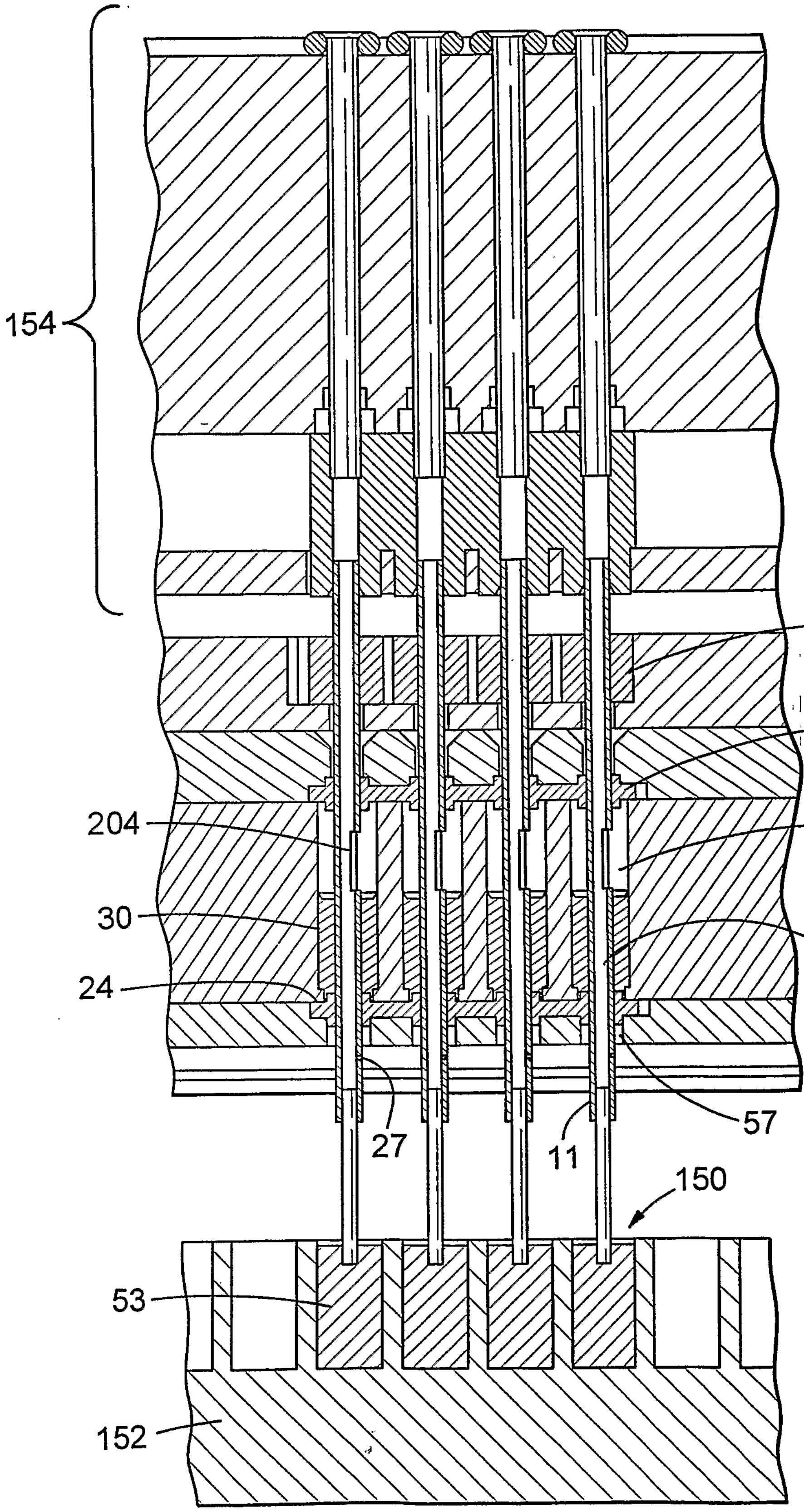

FIG. 17