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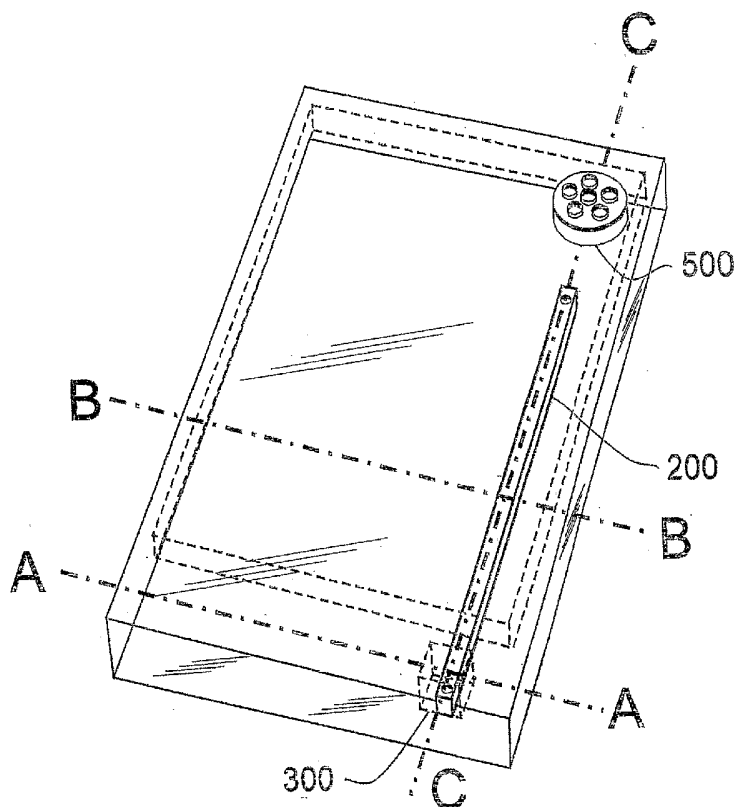
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- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

[Continued on next page]

(54) Title: BIOREACTOR FOR SELECTIVELY CONTROLLING THE MOLECULAR DIFFUSION BETWEEN FLUIDS



(57) Abstract: A diffusion controlling bioreactor (50) that selectively controls the molecular diffusion between fluids through a microchannel (200) in fluid communication with a reaction reservoir (300). A length (230) and a cross-sectional area (250) are selected to obtain a predetermined rate of molecular diffusion between fluids. When the fluids are liquids, flow through the microchannel (200) is laminar and the capillary action of the microchannel (200) and fluid is such that the fluid does not flow into the reaction reservoir (300) unless the pressure of the fluid is increased by an external source, thereby minimizing contamination of the bioreactor (50). The instant invention may also utilize at least one microchannel and reagent reservoir to regulate, rather than prevent, the passage of various molecules into the bioreactor (50). A pressure equalizing vent (400) operating on similar principles to the microchannel (200) may have a structure configured to minimize the chances of fluid leakage from the bioreactor (50), even if the bioreactor (50) is turned in various directions.



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BIOREACTOR FOR SELECTIVELY CONTROLLING THE MOLECULAR
DIFFUSION BETWEEN FLUIDS

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REFERENCE TO RELATED DOCUMENTS

This application claims the benefit of a previous application filed in the United States Patent and Trademark Office by Emilio Barbera-Guillem, M.D., Ph.D.; on February 11, 2005, titled "Bioreactor For Selectively Controlling The Molecular Diffusion Between Fluids;" and given serial number 11/056725.

TECHNICAL FIELD

The instant invention relates to a bioreactor for selectively controlling the molecular diffusion between fluids, particularly, to a bioreactor capable of very slow transfers of molecules into and out of the bioreactor by utilizing the Brownian motion of the molecules in given fluids.

BACKGROUND OF THE INVENTION

Bioreactors are common laboratory and industrial installations used in the areas of cell culture, chemical production, fermentation, testing and analysis, and other biological processes well known to those skilled in the art. A problem inherent in such bioreactors is the need to control the ingress and egress of various compounds to and from the bioreactor. As the size and volume of bioreactors decrease, or as the need to control becomes increasingly exacting, problems occur in the need to precisely control, over a long period of time or in respect of very small quantities of certain compounds, the movement of molecules both into and out of the bioreactor.

Various means have been employed to control small flows of fluids, in a field generally called microfluidics. In general, the means have proposed mechanical devices that are designed to mechanically, but accurately, deliver very small amounts of a fluid

through various microchannels to a delivery point. A typical example is seen in U.S. Pat. No 6,810,713; in which rotors periodically squeeze the microchannels formed in an elastic polymeric substrate to propel small amounts of fluid towards a delivery point by their compressive effect on the microchannel. Another approach is seen in U.S. Pat. No. 6,797,187; in which an electromagnetic field is used to generate a flow of a fluid in a microchannel lined, at least in part, with silicon nitride. Yet another approach is seen in U.S. Pat. No. 6,743,636; wherein pneumatically driven Venturi pumps move fluid through a microchannel system.

These approaches, and others that form the present art, rely on the movement of fluid volumes, albeit small ones, in order to transfer the various components that may be present in such fluids. Therefore, they fall prey to a myriad of problems. As the scale of the device decreases, it becomes increasingly difficult for a mechanical, or even electro-mechanical, device to control the very slow movement of molecules. Even slight variations in the operation of the various pumping mechanisms will result in wide swings, in a relative sense, in the amount of fluids transferred. The instant invention, on the other hand, is novel in its approach to controlling the displacement of various molecules into and out of a bioreactor system by controlling the rate of diffusion at an interface between two fluids, while minimizing any actual mixing of the fluids themselves. Therefore, the delivery of various molecules is inherently stable and predictable, and simple variations in the structure of the bioreactor suffice to control this diffusion.

SUMMARY OF INVENTION

In its most general configuration, the present invention advances the state of the art with a variety of new capabilities and overcomes many of the shortcomings of prior devices

in new and novel ways. In its most general sense, the present invention overcomes the shortcomings and limitations of the prior art in any of a number of generally effective configurations.

In one configuration, the present invention relates to a method of using principles of diffusion to control the transfer of molecules into and out of a bioreactor. Diffusion is a reflection of the fact that molecules, as they vibrate with random motion, called Brownian motion, in a gas or liquid, move toward an equilibrium state where all the molecules in the mixture are uniformly dispersed, and the concentration of any molecular species is the same everywhere.

The diffusion equation (Fick's second law), states that the rate of molecular diffusion is proportional to the second derivative of its concentration. This can be written:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

wherein C is the concentration, t is the time, and x is the distance.

Fig. 1 illustrates a diffusion model for a diffusion gradient along a long axis x, with diffusion progressing in the direction of the arrows. In a laminar flow state, the concentration of the diffusing substance will be equal at all points on a given plane orthogonal to the direction of laminar flow, illustrated as a first plane J1 and a second plane J2. In summary, given a theoretical scheme for diffusion between two compartments separated by a distance (x), as seen in Fig 1, the diffusion equation could be expressed as:

$$\frac{\partial C_E}{\partial t} = D_E \frac{\partial^2 C_E}{\partial x^2}$$

Thus; to reach a certain concentration at plane J₂, given a concentration at plane J₁ of C_B, as seen in Fig. 1, the following variables will determine the diffusion between the planes:

- 1) Distance between planes J₁ and J₂ (x).
- 2) Time (t); and
- 3) Diffusion coefficient of the molecule (D_B), which will be constant for a given molecule for each specific fluid viscosity and temperature.

Therefore, for a given system the combination of distance and time will determine the rate of passage of a molecule from a first location, such as a point on plane J₁ to a second location, such as a point on plane J₂. The magnitude of molecular diffusion will be a function of both distance and time, along with the interface's surface area, that is, the area available for diffusion. Consequently, it is possible to control time of passage from a first location to a second location of a certain amount of a given molecule by setting the other four variables: surface area of the interface, fluid viscosity, temperature, and distance between the locations.

Contemplation will show that since the rate of diffusion is inversely related to the square of the distance between the compartments (x), relatively small increases in distance will have a large effect in slowing the rate of diffusion; and relatively large increases in distance will have an extremely large effect in slowing the rate of diffusion. The calculation of the rate of diffusion in both time and distance is complex; as an application of Fick's second law of diffusion, which can be expressed as follow for long distances:

$$\frac{dC}{dx} = -\frac{C_s}{\sqrt{\pi Dt}} \exp\left\{\frac{-x^2}{4Dt}\right\}$$

where C = concentration of substance in question, t = the time of diffusion, x = distance, C_s = starting concentration, and D = coefficient of diffusion for the molecule in the fluid.

A fast estimate of the rate of diffusion can be realized from the simpler relation that the time, (t), for 37% of the molecules to diffuse over the given distance, x, can be simply calculated as:

$$t = x^{D/2}$$

- 5 The figure of 37% comes from the fact that the process is exponential, e (the base of natural logs) = 2.7183, therefore $1/e = 0.37$, or 37%. This calculation is for the simplest solution for diffusion in one dimension away from a plane. Note that as observed above, the time required for diffusion of a given quantity increases with the square of the distance.

- 10 In order to visualize the important effect of distance (x) in determining the rate of diffusion for a given molecule and fluid, one may compare illustrative applications. In a system where the fluid is water, the diffusing molecules are oxygen, and the system is maintained at 37° C, as seen in Table 1; the time value for 37% oxygen diffusion across three different spaces is calculated, each reflecting a difference in diffusion distance (x). The applications are diffusion: (a) across lung epithelium (having an average thickness of
- 15 5×10^{-7} m); (b) from the atmosphere to the cell layer in a typical laboratory T flask (where the cell layer is separated from the atmosphere by an average distance of 0.003 m); and (c) from the atmosphere to the fluid inside an innovative bioreactor in which the principles of the instant invention are utilized to create a nearly one meter separation between the external environment and the reservoir of fluid within the device.

	x (m)	Oxygen D	Time sec	Days
a	5×10^{-7}	1.8×10^{-9}	0.000035	0.00
b	0.003	1.8×10^{-9}	1,250	0.01
c	0.98	1.8×10^{-9}	133,388,889	1543.85

Table 1. x = distance in meters, D = coefficient of diffusion in water

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Therefore, it is evident that by increasing the distance between compartments, it is possible to greatly slow diffusion, to a point at which diffusion becomes so slow that, for practical purposes, it stops. Therefore, by manipulating the separation between compartments in a bioreactor, as by way of example and not limitation, extending a long tube between otherwise separated compartments, it is possible to delay diffusion.

Manipulation of the length of the tubing will control the rate of diffusion, if other variables are held steady and the movement of fluid is prevented. Nearing the extreme upward limit of delay mentioned above, such a system, as seen in various embodiments of the instant invention, can serve to create a virtual hermetic seal through which, on a practical basis, diffusion times are so long that there is effectively no movement of particular molecules from one end of the seal to the other. In other words, if sufficient distance is created, diffusion becomes so slow that it may be disregarded.

In a preferred embodiment, the instant invention includes a diffusion controlling bioreactor that selectively controls the molecular diffusion between a first fluid and a second fluid. The diffusion controlling bioreactor includes a microchannel in fluid communication with a reaction reservoir. The length and cross-sectional area of the microchannel are selected to obtain a predetermined rate of molecular diffusion between

the first fluid and the second fluid. The flow of fluid through the microchannel is laminar and the capillary action of the microchannel and the fluid is such that the fluid does not flow into the reaction reservoir unless the pressure of the fluid is increased by an external source, which may or may not include the effects of gravity on the fluids.

5 In one of the applications of the instant invention, a microchannel may be configured of such a length and cross-sectional area that, when the microchannel is used to connect the filling means of the bioreactor with the reaction reservoir, microorganisms that may inadvertently contaminate the filling means of the bioreactor are prevented from successfully traversing the microchannel. The laminar flow state of fluid in the
10 microchannel and lack of fluid flow into the reaction reservoir results in non-motile organisms failing to reach the reaction reservoir in generally applicable time frames, and the slow diffusion of nutrients and oxygen from the reaction reservoir toward such organisms results in the death of these organisms before they can diffuse or extend by colony growth to the bioreactor.

15 In addition to the ability to virtually "seal" the bioreactor from contaminants, the instant invention may utilize at least one microchannel and reagent reservoir to effect and regulate, rather than prevent, the passage of various molecules into the reaction reservoir of the bioreactor. For instance, various substances such as drugs in varying concentrations, nutrients, or other agents, may be introduced into the reagent reservoir in the form of a first
20 fluid, which is in fluid communication through the microchannel to the reaction reservoir, which contains a second fluid. The microchannel is sized to have a length and cross-sectional area that may predetermine a rate of diffusion for such substances into the reaction reservoir. The user fills the microchannel and the reagent reservoir only sufficiently to advance the first fluid to the proximal end of the microchannel. At that point,

as long as there is a difference in viscosity between the first and second fluid, and unless the microchannel is pressurized above or below the pressure of the reaction reservoir, there will be no movement of fluid between the microchannel and the reaction reservoir, only diffusion of molecules at the interface between the fluids contained in each.

5 The bioreactor may further incorporate a pressure equalizing vent that operates on similar principles to the microchannel described above. The pressure equalizing vent may have a structure configured to minimize the chances of fluid leakage from the bioreactor, even if the bioreactor is turned in various directions.

10 **BRIEF DESCRIPTION OF THE DRAWINGS**

Without limiting the scope of the present invention as claimed below and referring now to the drawings and figures:

FIG. 1 shows a diffusion model illustrating a diffusion process in a laminar flow state proceeding in the direction of the arrows along the long axis of the model;

15 FIG. 2 shows an embodiment of a bioreactor according to the instant invention in elevated perspective view, not to scale;

FIG. 3 shows detail of the embodiment of the bioreactor of FIG. 2 in elevated perspective view, not to scale, with the microchannel shown separated from the reaction reservoir for clarity;

20 FIG. 4 shows a variation of the detail of the microchannel of the bioreactor of FIG. 2 in elevated perspective view, not to scale;

FIG. 5 shows a cross-section view of a microchannel of the bioreactor of FIG. 2, not to scale;

FIG. 6 shows a cross-section view of a microchannel of the bioreactor of FIG. 2, not to scale;

FIG. 7 shows another embodiment of a bioreactor according to the instant invention in elevated perspective view, not to scale;

5 FIG. 8 shows a portion of the embodiment of the bioreactor of FIG. 7 in elevated perspective view, not to scale;

FIG. 9 shows a portion of another embodiment of the bioreactor of FIG. 7 in elevated perspective view, not to scale;

10 FIG. 10 shows a portion of another embodiment of the bioreactor of FIG. 7 in elevated perspective view, not to scale;

FIG. 11 shows another embodiment of the bioreactor of FIG. 7 in elevated perspective view, not to scale;

FIG. 12 shows another embodiment of the bioreactor of the instant invention in elevated perspective view, not to scale;

15 FIG. 13 shows a portion of the embodiment of the bioreactor of FIG. 12 in elevated perspective view, not to scale;

FIG. 14 shows another embodiment of the bioreactor according to the instant invention in elevated perspective view, not to scale;

20 FIG. 15 shows a portion of the embodiment of the bioreactor of FIG. 14 in elevated perspective view, not to scale;

FIG. 16 shows a portion of another embodiment of the bioreactor of FIG. 14 in elevated perspective view, not to scale;

FIG. 17 shows a portion of another embodiment of the bioreactor of FIG. 14 in elevated perspective view, not to scale;

FIG. 18 shows a portion of the embodiment of the bioreactor of FIG. 17 in elevated perspective view, not to scale;

FIG. 19 shows another embodiment of the bioreactor of FIG. 2 in elevated perspective view, not to scale;

5 FIG. 20 shows another embodiment of the bioreactor of FIG. 19 in elevated perspective view, not to scale;

FIG. 21 shows detail of a surface of the bioreactor of the instant invention in elevated perspective view, not to scale;

10 FIG. 22 shows detail of a surface of the bioreactor of the instant invention in elevated perspective view, not to scale;

FIG. 23 shows detail of two surfaces of the bioreactor of the instant invention in elevated perspective view, not to scale;

FIG. 24 shows detail of two surfaces of the bioreactor of the instant invention in elevated perspective view, not to scale;

15 FIG. 25 shows a portion of the embodiment of Fig 20 of the bioreactor of the instant invention in elevated perspective view, not to scale;

FIG. 26 shows another embodiment of the bioreactor of the instant invention in elevated perspective view, not to scale;

20 FIG. 27 shows a portion of an embodiment of the bioreactor of the instant invention in cross-sectional view, not to scale;

FIG. 28 shows a portion of an embodiment of the bioreactor of the instant invention in cross-sectional view, not to scale;

FIG. 29 shows a portion of an embodiment of the bioreactor of the instant invention in cross-sectional view, not to scale;

FIG. 30 shows a portion of an embodiment of the bioreactor of the instant invention in cross-sectional view, not to scale;

FIG. 31 shows another embodiment of the bioreactor of the instant invention in elevated perspective view, not to scale;

5 FIG. 32 shows another embodiment of the bioreactor of the instant invention in elevated perspective view, not to scale;

FIG. 33 shows a cross-section view of the bioreactor of FIG. 32 viewed across section line A-A in FIG. 32, not to scale;

10 FIG. 34 shows a cross-section view of the bioreactor of FIG. 32 viewed across section line B-B 9 in FIG. 32, not to scale;

FIG. 35 shows a cross-section view of the bioreactor of FIG. 32 viewed across section line C-C in FIG. 32, not to scale;

FIG. 36 shows another embodiment of the bioreactor of FIG. 7, in elevated perspective view, not to scale;

15 FIG. 37 shows a cross-section view of the pressure equalizing vent of the bioreactor of FIG. 35, not to scale;

FIG. 38 shows a cross-section view of another embodiment of the pressure equalizing vent of the bioreactor of FIG. 35, not to scale;

20 FIG. 39 shows a cross-section view of the microchannel of the bioreactor of FIG. 35 with a very thin sidewall; and

FIG. 40 shows a cross-section view of another embodiment of the microchannel of the bioreactor of FIG. 35 with a very thin sidewall.

DETAILED DESCRIPTION OF THE INVENTION

The method and materials of the bioreactor for selectively controlling the molecular diffusion between fluids of the instant invention enables a significant advance in the state of the art. The preferred embodiments of the method and materials accomplish this by new and novel arrangements of elements and methods that are configured in unique and novel ways and which demonstrate previously unavailable but preferred and desirable capabilities.

The detailed description set forth below in connection with the drawings is intended merely as a description of the presently preferred embodiments of the invention, and is not intended to represent the only form in which the present invention may be constructed or utilized. The description sets forth the designs, functions, means, and methods of implementing the invention in connection with the illustrated embodiments. It is to be understood, however, that the same or equivalent functions and features may be accomplished by different embodiments that are also intended to be encompassed within the spirit and scope of the invention.

In a preferred embodiment, seen in Figs. 2 through 4, the instant invention includes a diffusion controlling bioreactor (50) that selectively controls the molecular diffusion between a first fluid and a second fluid, and includes a reaction reservoir (100) having at least one reaction reservoir sidewall (110) that defines a reaction reservoir volume (120) and a microchannel (200). The reaction reservoir (100) initially contains the first fluid.

The microchannel (200) is in fluid communication with the reaction reservoir (100). As seen in Fig. 3, the microchannel (200) has a proximal end (210) with a proximal end opening (212), a distal end (220) with a distal end opening (222), a length (230), and at least one microchannel sidewall (240), having a sidewall thickness (245), and defining a

cross-sectional area (250). The length (230) and cross-sectional area (250) are selected to obtain a predetermined rate of molecular diffusion between the first fluid in the reaction reservoir (100) and the second fluid in the microchannel (200). The microchannel (200) is configured in a manner such that when the microchannel (200) is filled with the second
5 fluid, and the second fluid is a liquid, flow of the second fluid through the microchannel (200) is laminar. The capillary action of the microchannel (200) and the second fluid is such that the second fluid does not flow into the reaction reservoir (100) unless the pressure of the second fluid is increased by an external source, such as, by way of example and not limitation, pressurization of the microchannel caused by an increase of temperature.

10 For example, assuming that the reaction reservoir (100) is initially empty, or only contains air, as a user fills the microchannel (200) through the microchannel distal end opening (222) with a second fluid that is a liquid, the capillary action of the microchannel (200) and the second fluid results in the second fluid being drawn to the microchannel proximal end (210), as seen in Fig. 27, until the liquid reaches the proximal end opening
15 (212), as seen in Fig. 28, where it stops. This is also the situation when the reaction reservoir (100) contains a liquid and the liquid is not soluble with the liquid that is introduced and drawn from the microchannel distal end opening (222) to the microchannel proximal end opening (212).

20 One illustrative embodiment of the instant invention, seen in Figs. 5 and 6, utilizes a predetermined rate of diffusion to act as an antiseptic barrier in a diffusion controlling bioreactor (50). Contamination is a consistent problem in the laboratory and commercial uses of most bioreactors, especially in eukaryotic cell cultures. Ports, most often made of penetrable elastomeric septa, are used to introduce and remove various components from bioreactors, most often by means of a pipette-like device that first punctures the septum,

and then adds or removes components from the bioreactor. The septum is designed to resealably close after withdrawal of the pipette. It is well known in the art that such punctures tend to introduce contamination into the system, by either the pipette directly introducing bacteria through the punctured septum at the time of puncture, or by the pipette leaving a very small defect, or defects after repeated use, after withdrawal of the pipette. Organisms, for example, bacteria, yeast, and fungi, eventually migrate through these very small defects.

As applied to the instant invention, the introduced organisms, represented in Fig 5 by small stars, would tend to mix with the fluid present in the distal end (220) of the microchannel (200). Small colonies of bacteria or yeast would tend to begin growth on the interior wall of the distal end (220), or in close proximity to the distal end opening (222), as see in Fig. 5. The microchannel (200) must be sized so that only laminar flow of fluid is possible when the fluid is a liquid. As is well-known in the art, by way of example and not limitation, a microchannel (200) with a cross-sectional area of less than approximately 4 mm² will satisfy this requirement for many liquids.

Once the microchannel (200) is filled with fluid, flow stops and the microchannel remains in a laminar flow state. Should flow resume, or currents develop within the fluid, due to, by way of illustration and not limitation, agitation or heating of the fluid, the sizing of the microchannel ensures that only laminar fluid movement can take place within the microchannel. Non-motile bacteria, due to the exclusively laminar flow characteristics of the microchannel (200), are limited in their movement through the fluid present in the microchannel (200) to diffusion through the fluid. The diffusion constant of non-flagellated bacteria, as is well known in the art, is between around 0.0000001 cm²/sec and 0.0000017 cm²/sec. As a result, for an illustrative embodiment utilizing a microchannel (200) of the

instant invention that is 10 cm long, it will take between 1 and 21 years for most bacteria to diffuse from the distal end (220) of the microchannel (200) to the reaction reservoir (100).

Additionally, living contaminants such as bacteria, yeast and fungi can be transported by direct extension, or colony growth, represented as small stars along the interior walls of the microchannel (200) near the distal end opening (222), as seen in Fig. 6. Most common organisms, certain flagellated or other motile bacteria excepted, will grow confluent in all directions from their initial point of attachment, including along the microchannel (200) of the instant invention towards the reaction reservoir (100). The fluid in the microchannel (200) may contain necessary survival requirements for all microorganisms, such as sugars and amino acids. Furthermore, the fluid may contain oxygen, a necessary requirement for the existence and growth of aerobic life forms. These required substances, if consumed by bacterial growth at the distal end (220) of the microchannel (200) will diffuse through the fluid from the reaction reservoir (100) towards the distal end (220) of the microchannel (200) at a rate predetermined by the cross-sectional area (250) and length (230) of the microchannel (200). Additionally, such growth, along with the normal metabolic needs of the undesirable organisms, will necessarily begin consuming the required substances at the distal end opening (222) of the distal end (220) of the microchannel (200).

The eventual contamination of the reaction reservoir (100) by extending organism colonies becomes a race in time for the organisms to grow to the reaction reservoir (100) before consuming the oxygen and nutrients along the route of growth through the microchannel (200), or oxygen and nutrients that the organisms can obtain due to diffusion of the nutrients and oxygen towards the organisms. The consumption and exhaustion of oxygen and nutrients will lead to stoppage of organism growth, and therefore stoppage of

the migration. It therefore becomes possible for one skilled in the art, knowing the diffusion constants of such common gases as oxygen and carbon dioxide, and such common nutrients as glucose and lipids, to configure a microchannel (200) of such length (230) and cross-sectional area (250) that the resultant diffusion times of various molecules may be easily
5 calculated. In an illustrative embodiment, the aforementioned microchannel (200) with a length (230) of 10 cm will result in a diffusion time, by the equations given in the Background of the Invention above, along the length (230) of the microchannel (200) of 36.2 days for oxygen, 42.2 days for carbon dioxide, and 85.8 days for glucose, in a water based system maintained at 37° C. Even a microchannel (200) that is significantly shorter
10 than the preceding example will have a significant effect on diffusion time. For example, a microchannel that is only 1 cm in length (230), will have diffusion times of 8.7 hours for oxygen, 10.2 hours for carbon dioxide, and 20.6 hours for glucose, in a water based system maintained at 37° C.

Accordingly, it is highly likely that any living contaminants will perish from lack of
15 oxygen, acidosis due to failure of the removal of carbon dioxide from the peri-cellular environment, or deprivation of nutrients, long before they can reach the reaction reservoir (100) of the instant invention, given the usual range of times during which laboratory or commercial biological processes are conducted. Therefore, as seen in Figs. 5 and 6, the microchannel (200) tends to act as an antimicrobial barrier to contamination of the
20 bioreactor (50), even should contaminants pass any physical barriers, such as a cap (332), or a resealable elastomeric septum (333), as seen in Figs 9 and 10, that may be present at the distal end opening (222) of the microchannel (200). Thus, in such an embodiment, the controlled diffusion through the microchannel (200) is used to effect a virtual "seal" between the reaction reservoir (100) and an external environment.

Again as applied to the instant invention, such a virtual “seal” may be used to limit the diffusion of gases into and out of the bioreactor (50) to that which takes place across the walls of the bioreactor. By way of example and not limitation, certain cell cultures, such as stem cells, require a carefully regulated oxygen environment. Unrestricted access by oxygen to these cells can result in cell death or transformation. The bioreactor (50) may be designed with a predetermined surface area to the walls of the bioreactor (50), and be built of a material having a predetermined diffusion constant for oxygen. In this way, the bioreactor (50) may auto-regulate the amount of oxygen available for the biological process within. Therefore, in a cell culture or similar application, the bioreactor (50) may be configured in such a manner and from such a material that a surface area of the reaction reservoir (100) (formed by the surfaces (162, 164, 166, 168, 170, and 172) and the oxygen diffusion constant of the reaction reservoir sidewall (110) material, result in the diffusion of a predetermined amount of oxygen per unit of time, calculated to support the metabolic needs of a predetermined number of cells. By way of example and not limitation, in a cell culture application, the bioreactor (50) may be formulated of a plastic material and with a reaction reservoir surface area such that 4.6 ml of oxygen is diffused, to support the metabolic needs of approximately 2×10^7 cells, at atmospheric pressure and 37° C, per day.

In addition to the ability to virtually “seal” the bioreactor (50) from contaminants and gases, the instant invention may utilize at least one microchannel (200) and reagent reservoir (300) to promote and regulate, rather than prevent, the passage of various molecules into the reaction reservoir (100) of the bioreactor (50).

In another embodiment, as seen in Figs. 7 through 11, there may be a reagent reservoir (300) at the distal end (220) of the microchannel (200) that is in fluid communication with both the microchannel (200) and the reaction reservoir (100). In such

an embodiment, the reagent reservoir (300) has at least one reagent reservoir sidewall (310) defining a reagent reservoir volume (320), seen in Fig. 8, and has a reagent reservoir opening (330) through which the second fluid enters the reagent reservoir (300). The reagent reservoir opening (330) may be closed by a cap (332), or by a penetrable, self-sealing, elastomeric septum (333), among other methods, as seen in Figs. 9 and 10.

Various substances such as drugs in varying concentrations, nutrients, or other agents, may be introduced into the reagent reservoir (300), which is in fluid communication through the microchannel (200) to the reaction reservoir (100). The reagent reservoir (300) is designed to hold a reagent reservoir volume (320), and the microchannel (200) may be configured with a particular length (230) and cross-sectional area (250), as seen in the preceding embodiment illustrated in Fig. 3. Thus, the delivery by diffusion of a plurality of molecules into and out of the bioreactor (50) may be predetermined and easily effected. The length (230) and cross-sectional area (250) of the microchannel (200) is capable of wide variation, as would be known to one skilled in the art, as long as the basic requirement is met that the microchannel (200) be sized so that there is only laminar flow within the microchannel (200). In several embodiments, a cross-sectional area (250) of less than 4 mm² performs well. As would be appreciated by one skilled in the art, the rate of diffusion for a given substance, fluid, and temperature is determined by the length (230) and cross-sectional area (250) of the microchannel (200), and is entirely independent of the shape of the microchannel (200), and in particular is independent of any turns in the microchannel (200). Accordingly, and as seen in Figs. 11 and 36, the microchannel may be configured in a wide array of shapes, including a variety of cross-sectional profiles such as the rectangular and circular profiles seen in the accompanying Figures, in order to

accommodate various lengths (230), reagent reservoir volumes (320), and reaction reservoir volumes (120) and other design criteria.

During use, the user fills the microchannel (200), and the reagent reservoir (300) only sufficiently to advance the first fluid to the proximal end of the microchannel (200). At
5 that point, as long as there is a difference in viscosity between the first and second fluid, and unless either the "interfacial tension" between the first fluid and the second is unbalanced in favor of the second, or the microchannel (200) is pressurized above or below the pressure of the reaction reservoir (100), there will be no movement of fluid between the
microchannel (200) and the reaction reservoir (100), only diffusion at the interface between
10 the fluids contained in each.

As would be obvious to one skilled in the art, the diffusion controlling bioreactor (50) may be configured to selectively control the molecular diffusion between a first fluid, a second fluid, and a third fluid. Such multiple fluid handling capacity is seen in the embodiments illustrated in Figs. 12 and 13, which combine the structures illustrated in Figs.
15 3, 4, and 7. A second microchannel (600) may be in fluid communication with the reaction reservoir (100). As with the first microchannel (200), the second microchannel (600) has a proximal end (610) with a proximal end opening (612), and a distal end (620) with a distal end opening (622) as seen in Fig. 14. This second microchannel may further include a reagent reservoir (300) at the distal end (620) of the second microchannel (600) in fluid
20 communication with both the second microchannel (600) and the reaction reservoir (100). The reagent reservoir (300) has at least one reagent reservoir sidewall (310) which defines a reagent reservoir volume (320) and a reagent reservoir opening (330) through which the third fluid enters the reagent reservoir (300). The second microchannel (600) has a length (630), and at least one second microchannel sidewall (640) defining a cross-sectional area.

The length (630) and cross-sectional area (650) are selected to obtain a predetermined rate of molecular diffusion between the fluid in the second microchannel (600) and the fluid in the reaction reservoir (100). The second microchannel (600) is configured such when the second microchannel (600) is filled with the third fluid, and the third fluid is a liquid, flow of the third fluid through the second microchannel (600) is laminar. The capillary action of the second microchannel (600) and the third fluid is such that the third fluid does not flow into the reaction reservoir (100) unless the pressure of the third fluid is increased by an external source. The instant invention may be configured to have multiple second microchannels and multiple reagent reservoirs, as illustrated in the embodiments seen in Figs. 26 and 31.

In the nature of capillary flow, surface tension produces a non-flat liquid surface, called a meniscus, at the end of the microchannel (200). If a fluid tends to wet the inside surface of a capillary such as a microchannel (200), as seen in Fig. 27, the meniscus is concave until capillary action draws the fluid to the open end of the capillary, at which point it becomes convex, as seen in Fig. 28.

In the illustrative example of Fig. 28, a convex meniscus forms at the end of the microchannel (200). Should any hydrophilic material touch the meniscus, or should the meniscus contact a fluid of higher solubility and the "interfacial tension" between the first fluid and the second becomes unbalanced in favor of the second, the surface tension of the meniscus will be broken, and fluid will begin to wick through the capillary microchannel (200), thus potentially defeating the instant invention goal of the prevention of fluid movement through the microchannel (200) and thereby defeating the controlled diffusion of the instant invention. As is well-known in the art, a capillary having a very short sidewall will support only a very slightly convex or concave meniscus. Accordingly, creating a

section of very thin sidewall (455) at the proximal end opening (212) of the proximal end (210) of the microchannel (200), as seen in Figs. 29, 30, 39, and 40, will result in an area wherein the capillary effect of the very thin sidewall (455) at the proximal end (212) will be insufficient to support a meniscus, resulting in a relatively flat interface between a fluid in the microchannel (200) and a fluid in the reaction reservoir (100). In various embodiments, 5 thicknesses in this section of sidewall (455) of between about 0.1 mm and 0.5 mm work well to create this effect.

As may be seen, by way of examples and not limitation only, one skilled in the art will realize that the area of very thin sidewall (455) at the proximal end opening (212) of the proximal end (210) of the microchannel (200) may be formed in a wide variety of 10 manners, not limited to the embodiments illustrated. In particular, as seen in Fig. 40, it may be seen that the area of very thin sidewall (240) at the proximal end opening (212) of the proximal end (210) of the microchannel (200) does not represent any particular requirements as to diameter of the proximal end opening (212).

15 To serve the same purpose, the same may be true for a second microchannel (600), where the area of the proximal end opening (612) of the second microchannel (600) may be configured to interpose a section of very thin sidewall (640) at the proximal end opening (612) of the proximal end (610) of the second microchannel (600), analogous to such as seen in Figs. 29 and 30. As with the first microchannel (200), this will result in an area 20 wherein the capillary effect of the very thin sidewall (640) at the proximal end (612) will be insufficient to support a meniscus, resulting in a relatively flat interface between a fluid in the microchannel (600) and a fluid in the reaction reservoir (100).

In yet another embodiment, seen in Figs. 14 through 18, the diffusion controlling bioreactor (50) may further include a pressure equalizing vent (400) in fluid communication

with the reaction reservoir (100) and a fluidic external environment, wherein the pressure equalizing vent (400) is capable of equalizing the pressure within the reaction reservoir (100) with that of the external environment, as shown in Fig. 15. The pressure equalization vent (400) has a vent microchannel (410) with a distal end (420) with a distal end opening (422) in communication with the external environment. The vent microchannel (410) may further have a length (440), at least one sidewall (450), having a sidewall thickness (455), and defining a cross-sectional area (460), and a proximal end (430) with a proximal end opening (432) in communication with the reaction reservoir (100). The pressure equalizing vent (400) is configured such that when it is filled with a third fluid and the third fluid is a liquid; flow of the third fluid through the pressure equalizing vent (400) is laminar, as with the first microchannel (200) and the second microchannel (600). The capillary action of the pressure equalizing vent (400) and the third fluid is such that the third fluid does not flow out of the distal end opening (422) unless the pressure of the third fluid is increased by an external source.

An illustrative example of such a pressure equalizing vent (400) is seen in Figs. 14 through 18. The filling of the reaction reservoir (100) with a second fluid results in a third fluid flowing from the reaction reservoir (100) into the vent microchannel (410) through the proximal end opening (432) of the vent microchannel (410). Examination will reveal that the operative principles regulating the transport of molecules through such a pressure equalizing vent (400) are essentially identical to those of the embodiment utilizing a reagent reservoir (300) and a reaction reservoir (100), as seen in Fig. 7, with the exception that in those embodiments utilizing a pressure equalizing vent (400), the vent microchannel (410) connects the reaction reservoir (100) with an external environment, rather than connecting the reagent reservoir (300) with the reaction reservoir (100), as with the first

and second microchannels (200, 600). Thus, there may be passage of gas molecules between the external environment and the reaction reservoir (100), subject to the same controlled diffusion principles as discussed above. Again as applied to the instant invention, the formation of a virtual “seal” by the vent microchannel (410), analogous to the virtual
5 “seal” created by the microchannel (200), may be used to limit the diffusion of gases into and out of the bioreactor (50) to that which takes place across the walls of the bioreactor.

The pressure equalizing vent (400) may include, as seen in Figs. 17 through 18, at the distal end opening (422), a filter (500) that is hydrophobic and capable of substantially preventing, at normal operating pressures, flow of liquid through the filter (500). The filter
10 (500) may further include an assembly, seen in Fig. 18, of at least 2 layers with a first layer (510) being adapted to prevent the passage of particles having an average size of at least approximately 80 microns, and a second layer (520) being adapted to prevent the passage of particles having an average size of at least approximately 0.2 microns. Such a filter (500) serves two functions, the prevention of the ingress of contaminants greater than the
15 effective filtration size of the filter, i.e., those particles greater than at least approximately 0.2 microns; and the prevention of particle and fluid movement through the hydrophobic filter (500) and into the external environment should the reaction reservoir become somewhat pressurized above the pressure in the pressure equalization vent (400).

A convex meniscus may form at the end of the vent microchannel (400) similar to
20 the meniscus illustrated in Fig. 28. Should this meniscus touch the material of the filter (500), the surface tension of the meniscus will be broken, and fluid will begin to wick through the vent microchannel (410), thus wetting the filter (500) and potentially decreasing the effectiveness of the filter (500). Just as the creation of a section of very thin sidewall (455) at the proximal end opening (212) of the proximal end (210) of the

microchannel (200), as seen in Figs. 29 30, 39, and 40, will result in an area wherein the capillary effect of the very thin sidewall (455) at the proximal end (212) will be insufficient to support a meniscus at the proximal end (212), an analogous structure, seen in Figs. 37 and 38; an analogous structure at the distal end (420) of the vent microchannel (410) will
5 result in a relatively flat interface between a fluid in the vent microchannel (410) and a fluid, which may be air, between the distal end (420) and the filter (500).

The flatter meniscus is less likely to extend beyond the distal end opening (422) of the vent microchannel (400), and therefore the fluid in the vent microchannel (410) is less likely to contact the filter (500). In various embodiments, thicknesses in this section of
10 sidewall (455) of between about 0.1 mm and 0.5 mm work well to create this effect.

The pressure equalizing vent (400) may have a structure configured to minimize the chances of fluid leakage from the bioreactor (50), seen in Figs. 20 and 25, even if the bioreactor (50) is turned in various directions. In such an embodiment, the reaction reservoir (100) has a maximum first dimension in a primary axis (130), a maximum second
15 dimension in a secondary axis (140), and a maximum third dimension in a tertiary axis (150), seen in Fig. 19. Further, seen in Fig. 20, in such a design, the pressure equalizing vent (400) includes a continuous vent microchannel (410) configured exterior to the reaction reservoir (100). Such a vent microchannel (410) would include at least one component in the primary axis (X) with a magnitude greater than the maximum first
20 dimension (130), at least one component in the secondary axis (Y) with a magnitude greater than the maximum second dimension (140), and at least one component in the tertiary axis (Z) with a magnitude greater than the maximum third dimension (150), as seen in Fig. 19.

In a preferred embodiment, seen in Figs. 21 and 24, the reaction reservoir is a hexahedron having a first longitudinal surface (162), a second longitudinal surface (164), a

first lateral surface (166), a second lateral surface (168), a top surface (170), and a bottom surface (172), thereby defining a reaction reservoir length (180), width (182), and depth (184), as seen in Fig. 20. In such an embodiment, as seen in Fig. 25, the continuous vent microchannel (410) has four sections (471, 472, 473, 474) substantially parallel to the
5 length (180) and greater in magnitude than the length (180); four sections (481, 482, 483, 484) substantially parallel to the width (182) and greater in magnitude than the width (182); and two sections (491, 492) substantially parallel to the depth (184) and greater in magnitude than the depth (184).

Examination of such an embodiment shows that should the bioreactor (50) be
10 rotated though one or more dimensions of space, the configuration is such that the vent microchannel (410) would include at least one component of the sections in the primary axis (X) with a magnitude greater than the maximum first dimension (130), at least one component of the sections in the secondary axis (Y) with a magnitude greater than the maximum second dimension (140), and at least one component of the sections in the
15 tertiary axis (Z) with a magnitude greater than the maximum third dimension (150). This results in at least a portion of at least one section (471, 472, 474, 474, 481, 482, 483, 484, 491, 492) lying above the fluid level in the bioreactor (50) at all times. As such, during rotation, a significant portion of the fluid in the vent microchannel (410) will drain back into the reaction reservoir (100), while a portion will advance into the adjoining section
20 (471, 472, 474, 474, 481, 482, 483, 484, 491, 492) of the vent microchannel (410). This drain and advance process will be repeated with each turn in any dimension of the bioreactor (50), such that only a concerted effort to rotate the bioreactor (50) through sequential turns in more than one dimension, designed to advance the fluid through the vent microchannel (410), will result in a significant amount of fluid reaching the filter (500).

The tendency for the fluid in one section of the vent microchannel (410) to drain back into the reaction reservoir (100) during rotation of the bioreactor (50) also acts to minimize pressure build up at the distal end opening (422) of the vent microchannel (410) due to a static column of fluid, as the weight of the fluid in a draining section tends to counteract, and thereby pull back, against the weight of any fluid advancing in the vent microchannel (410). The minimization of pressure at the distal end opening (422) of the vent microchannel therefore tends to minimize expulsion of fluid at the distal end opening (422) of the vent microchannel (410), should any fluid reach the distal end opening (422), and thereby to minimize the chances of fluid contamination of the filter (500).

In other preferred embodiments, the bioreactor (50) may be configured with a plurality of reagent reservoirs (300), each at the distal end (620) of a second microchannel (600) that is in fluid communication with both the second microchannel (600) and the reaction reservoir (100). In such an embodiment, each reagent reservoir (300) may be in fluid communication with a second microchannel (600) having different second microchannel lengths (630), and/or cross sectional areas (650) from the other second microchannels (600). This, as seen in illustrative embodiments in Figs. 26 and 31, enables multiple simultaneous controlled diffusion processes involving different substances to take place coincidentally, but at different rates. Such multiple arrangements of reagent reservoirs (300) in fluid communication through second microchannels (600) to the reaction reservoir (100) is ideal, as would be appreciated by one skilled in the art, for testing or controlling the effects of diffusion of various drugs, nutrients, or other substances, into the process contained in the bioreactor (50). Additionally, the very slow rates of diffusion that may be achieved by the bioreactor (50) of the instant invention make it ideal for the transport of

very small quantities of substances into the bioreactor (50) in a very stable and accurate manner over long periods of time.

An illustrative embodiment of the instant invention is seen in Figs. 31-35. In this embodiment, the reaction reservoir (100), first microchannel (200) and filter (500) are
5 formed as part of an integral structure, for strength and ease of handling, as seen in Fig. 32. In Fig. 33, a penetrable elastomeric septum (333) closes the reagent reservoir (300) at the distal end (220) of the microchannel. As seen in Fig. 34, the microchannel (200) runs within the integral structure, and inferior to the reaction reservoir (100). In this particular illustrative embodiment, in which the reaction reservoir (100) may be vented as shown,
10 viewed in cross-section in Fig. 35, the relationship between the reaction reservoir (100), a microchannel (200), and a filter (500) may be visualized.

Numerous alterations, modifications, and variations of the preferred embodiments disclosed herein will be apparent to those skilled in the art and they are all anticipated and contemplated to be within the spirit and scope of the instant invention. For instance, it is
15 understood that the specification of a first fluid, a second fluid, and a third fluid are for illustration, and not limitation, only. The fluids may all be of the same composition, or may be different. Additionally, the illustration of particular features in various embodiments is for illustration, and not limitation, only. Any or all of the various features of the instant invention maybe combined in various illustrated and non-illustrated embodiments, as
20 would be known to one skilled in the art. Further, although specific embodiments have been described in detail, those with skill in the art will understand that the preceding embodiments and variations can be modified to incorporate various types of substitute and or additional or alternative materials, relative arrangement of elements, and dimensional configurations. Accordingly, even though only few variations of the present invention are

described herein, it is to be understood that the practice of such additional modifications and variations and the equivalents thereof, are within the spirit and scope of the invention as defined in the following claims. The corresponding structures, materials, acts, and equivalents of all means or step plus function elements in the claims below are intended to

5 include any structure, material, or acts for performing the functions in combination with other claimed elements as specifically claimed.

INDUSTRIAL APPLICABILITY

The diffusion controlling bioreactor answers a long felt need for selectively controlling molecular diffusion between fluids in a bioreactor. The diffusion controlling bioreactor is used for precisely controlling the movement of molecules both into and out of the bioreactor and avoids the inherent problems of fluid delivery with mechanical and electromechanical devices. The bioreactor has utility, among other uses, in experimental and commercial cell culture and other biological processes. The present invention discloses a microchannel in fluid communication with a reaction reservoir. The length and cross-sectional area of the microchannel are selected to obtain a predetermined rate of molecular diffusion between a first fluid and a second fluid.

I CLAIM:

1. A diffusion controlling bioreactor (50) that selectively controls the molecular diffusion between a first fluid and a second fluid, comprising:

5 a reaction reservoir (100) having at least one reaction reservoir sidewall (110) thereby defining a reaction reservoir volume (120), wherein the reaction reservoir (100) initially contains the first fluid;

a microchannel (200) in fluid communication with the reaction reservoir (100), having a proximal end (210) with a proximal end opening (212), a distal end (220) with a
10 distal end opening (222), a length (230), at least one microchannel sidewall (240) defining a cross-sectional area (250), wherein the length (230) and cross-sectional area (250) are selected to obtain a predetermined rate of molecular diffusion between the first fluid and the second fluid, and wherein the microchannel (200) is configured such when the
15 microchannel (200) is filled with the second fluid, and the second fluid is a liquid, that flow of the second fluid through the microchannel (200) is laminar and the capillary action of the microchannel and the second fluid is such that the second fluid does not flow into the reaction reservoir (100) unless the pressure of the second fluid is increased by an external source.

20 2. The bioreactor of claim 1, wherein the distal end opening (222) is closed by a penetrable, elastomeric, self-sealing septum (333) covering the distal end opening (222).

3. The bioreactor (50) of claim 1, further including a reagent reservoir (300) at the distal end (220) of the microchannel (200) and in fluid communication with both the

microchannel (200) and the reaction reservoir (100), wherein the reagent reservoir (300) has at least one reagent reservoir sidewall (310) thereby defining a reagent reservoir volume (320) and a reagent reservoir opening (330) through which the second fluid enters the reagent reservoir (300).

5

4. The bioreactor (50) of claim 3, wherein the reagent reservoir opening (330) is closed by a penetrable, self-sealing, elastomeric septum (333) covering the reagent reservoir opening (330).

10

5. The bioreactor (50) of claim 1, wherein the proximal end opening (212) further includes a section of the microchannel sidewall (240) configured to have a reduced thickness sufficient to minimize formation of a meniscus at the proximal end opening (212) by a fluid within the microchannel (200).

15

6. The bioreactor (50) of claim 3, wherein the proximal end opening (212) blocks the flow of liquid in the microchannel (200) when the pressure of the liquid is not increased by an external source and there is a difference in viscosity between the first fluid and the second fluid, while permitting the diffusion of molecules across the proximal end opening (212).

20

7. The bioreactor (50) of claim 1, wherein the cross-sectional area (250) of the microchannel (200) is less than 4 mm².

8. The bioreactor (50) of claim 1, wherein the length (230) of the microchannel (200) is not less than 1 cm.

9. The bioreactor (50) of claim 1, further including a pressure equalizing vent (400) in
5 fluid communication with the reaction reservoir (100) and a fluidic external environment,
wherein the pressure equalizing vent (400) is capable of equalizing pressure within the
reaction reservoir (100) with that of the external environment, wherein the pressure
equalization vent (400) has a distal end (420) with a distal end opening (422) in
10 communication with the external environment, a length (440), at least one sidewall (450)
defining a cross-sectional area (460), and a proximal end (430) with a proximal end
opening (432) in communication with the reaction reservoir (100), and wherein the pressure
equalizing vent (400) is configured such that when it is filled with a third fluid, and the
third fluid is a liquid, that flow of the third fluid through the pressure equalizing vent (400)
is laminar and the capillary action of the pressure equalizing vent (400) and the third fluid
15 is such that the third fluid does not flow out of the distal end opening (422) unless the
pressure of the third fluid is increased by an external source.

10. The bioreactor (50) of claim 9, wherein the distal end opening (422) of the pressure
equalizing vent (400) further includes a filter (500) that is hydrophobic and capable of
20 substantially preventing flow of liquid through the filter (500).

11. The bioreactor (50) of claim 10, wherein the filter (500) further includes an
assembly of at least 2 layers with a first layer (510) being adapted to prevent the passage of
particles having an average size of at least approximately 80 microns, and a second layer

(520) being adapted to prevent the passage of particles having an average size of at least approximately 0.2 microns.

12. The bioreactor (50) of claim 9, wherein the area of the distal end opening (422) of the pressure equalizing vent (400) further includes a section of the vent sidewall (450) configured to have a reduced thickness sufficient to minimize formation of a meniscus at the proximal end opening (432) by a fluid within the vent (400).

13. The bioreactor (50) of claim 12, wherein the distal end opening (422) of the pressure equalizing vent (400) blocks the flow of liquid in the pressure equalizing vent (400) when the pressure of the liquid is not increased by an external source and there is a difference in viscosity between the third fluid and the external environment, while permitting the diffusion of molecules across the distal end opening (422).

14. The bioreactor (50) of claim 9, wherein the reaction reservoir (100) has a maximum first dimension in a primary axis (130), a maximum second dimension in a secondary axis (140), and a maximum third dimension in a tertiary axis (150), and the pressure equalizing vent (400) is a continuous microchannel configured lying wholly exterior to the reaction reservoir (100) and having at least one component in the primary axis (X) with a magnitude greater than the maximum first dimension (130), at least one component in the secondary axis (Y) with a magnitude greater than the maximum second dimension (140), and at least one component in the tertiary axis (Z) with a magnitude greater than the maximum third dimension (150).

15. The bioreactor (50) of claim 14, wherein the reaction reservoir (100) is a hexahedron having a first longitudinal surface (162), a second longitudinal surface (164), a first lateral surface (166), a second lateral surface (168), a top surface (170), and a bottom surface (172), thereby defining a reaction reservoir length (180), width (182), and depth (184), and wherein the continuous microchannel has four sections substantially parallel to the length (180) and greater in magnitude, four sections substantially parallel to the width (182) and greater in magnitude, and two sections substantially parallel to the depth (184) and greater in magnitude.

16. A diffusion controlling bioreactor (50) that selectively controls the molecular diffusion between a first fluid, a second fluid, and a third fluid, comprising:

a reaction reservoir (100) having at least one reaction reservoir sidewall (110) thereby defining a reaction reservoir volume (120), wherein the reaction reservoir (100) initially contains the first fluid;

a microchannel (200) in fluid communication with the reaction reservoir (100), having a proximal end (210) with a proximal end opening (212), a distal end (220) with a distal end opening (222), a length (230), at least one microchannel sidewall (240), having a sidewall thickness (245), and defining a cross-sectional area (250), wherein the length (230) and cross-sectional area (250) are selected to obtain a predetermined rate of molecular diffusion between the first fluid and the second fluid, and wherein the microchannel (200) is configured such when the microchannel (200) is filled with the second fluid, and the second fluid is a liquid, that flow of the second fluid through the microchannel (200) is laminar and the capillary action of the microchannel (200) and the second fluid is such that

the second fluid does not flow into the reaction reservoir (100) unless the pressure of the second fluid is increased by an external source; and

a pressure equalizing vent (400) in fluid communication with the reaction reservoir (100) and a fluidic external environment, wherein the pressure equalizing vent (400) is
5 capable of equalizing pressure within the reaction reservoir (100) with that of the external environment, wherein the pressure equalization vent (400) has a distal end (420) with a distal end opening (422) in communication with the external environment, a length (440), at least one sidewall (450), having a sidewall thickness (455), and defining a cross-sectional area (460), and a proximal end (430) with a proximal end opening (432) in communication
10 with the reaction reservoir (100), and wherein the pressure equalizing vent (400) is configured such that when it is filled with a third fluid, and the third fluid is a liquid, that flow of the third fluid through the pressure equalizing vent (400) is laminar and the capillary action of the pressure equalizing vent (400) and the third fluid is such that the third fluid does not flow out of the distal end opening (422) unless the pressure of the third
15 fluid is increased by an external source.

17. The bioreactor (50) of claim 16, further including a reagent reservoir (300) at the distal end (220) of the microchannel (200) and in fluid communication with both the microchannel (200) and the reaction reservoir (100), wherein the reagent reservoir (300)
20 has at least one reagent reservoir sidewall (310) thereby defining reagent reservoir volume (320) and a reagent reservoir opening (330) through which the second fluid enters the reagent reservoir (300).

18. The bioreactor (50) of claim 16, wherein the reagent reservoir opening (330) is closed by a penetrable, self-sealing, elastomeric septum (333) covering the reagent reservoir opening (330).

5 19. The bioreactor (50) of claim 16, wherein the proximal end opening (212) further includes a section of the microchannel sidewall (240) configured to have a reduced thickness sufficient to minimize formation of a meniscus at the proximal end opening (212) by a fluid within the microchannel (200).

10 20. The bioreactor (50) of claim 19, wherein the microchannel proximal end opening (212) blocks the flow of liquid in the microchannel (200) when the pressure of the liquid is not increased by an external source and there is a difference in viscosity between the first fluid and the second fluid, while permitting the diffusion of molecules across the microchannel proximal end opening (212).

15

21. The bioreactor (50) of claim 16, wherein the cross-sectional area (250) of the microchannel (200) is less than 4 mm^2 .

22. The bioreactor (50) of claim 16, wherein the length (230) of the microchannel (200)
20 is not less than 1 cm.

23. The bioreactor (50) of claim 16, wherein the vent distal end opening (422) further includes a filter (500) that is hydrophobic and capable of preventing flow of liquid through the filter (500).

24. The bioreactor (50) of claim 23, wherein the filter (500) further includes an assembly of at least 2 layers with a first layer (510) being adapted to prevent the passage of particles having an average size of at least approximately 80 microns, and a second layer
5 (520) being adapted to prevent the passage of particles having an average size of at least approximately 0.1 microns.

25. The bioreactor (50) of claim 16, wherein the area of the distal end opening (422) of the pressure equalizing vent (400) further includes a section of the vent sidewall (450)
10 configured to have a reduced thickness sufficient to minimize formation of a meniscus at the proximal end opening (432) by a fluid within the vent (400).

26. The bioreactor (50) of claim 25, wherein the distal end opening (422) of the pressure equalizing vent (400) blocks the flow of liquid in the pressure equalizing vent
15 (400) when the pressure of the liquid is not increased by an external source and there is a difference in viscosity between the third fluid and the external environment, while permitting the diffusion of molecules across the vent distal end opening (422).

27. The bioreactor (50) of claim 16, wherein the reaction reservoir (100) has a
20 maximum first dimension in a primary axis (130), a maximum second dimension in a secondary axis (140), and a maximum third dimension in a tertiary axis (150), and the pressure equalizing vent (400) is a continuous microchannel configured lying wholly exterior to the reaction reservoir (100) and having at least one component in the primary axis with a magnitude greater than the maximum first dimension (130), at least one

component in the secondary axis with a magnitude greater than the maximum second dimension (140), and at least third component in the tertiary axis with a magnitude greater than the maximum third dimension (150).

- 5 28. The bioreactor (50) of claim 16, wherein the reaction reservoir (100) is a hexahedron having a first longitudinal surface (162), a second longitudinal surface (164), a first lateral surface (166), a second lateral surface (168), a top surface (170), and a bottom surface (172), thereby defining a reaction reservoir length (180), width (182), and depth (184), and wherein the continuous microchannel has four sections substantially parallel to
10 the length (180) and greater in magnitude, four sections substantially parallel to the width (182) and greater in magnitude, and two sections substantially parallel to the depth (184) and greater in magnitude.

29. A diffusion controlling bioreactor (50) that selectively controls the molecular
15 diffusion between a first fluid, a second fluid, and a third fluid, comprising:

a reaction reservoir (100) having at least one reaction reservoir sidewall (110) thereby defining a reaction reservoir volume (120), wherein the reaction reservoir (100) initially contains the first fluid;

- a first microchannel (200) in fluid communication with the reaction reservoir (100),
20 having a proximal end (210) with a proximal end opening (212), a distal end (220) with a distal end opening (222), a length (230), at least one first microchannel sidewall (240), having a sidewall thickness (245), and defining a cross-sectional area (250), wherein the length (230) and cross-sectional area (250) are selected to obtain a predetermined rate of molecular diffusion between the first fluid and the second fluid, and wherein the first

microchannel (200) is configured such when the first microchannel (200) is filled with the second fluid, and the second fluid is a liquid, that flow of the second fluid through the first microchannel (200) is laminar and the capillary action of the first microchannel (200) and the second fluid is such that the second fluid does not flow into the reaction reservoir (100)

5 unless the pressure of the second fluid is increased by an external source; and

a second microchannel (600) in fluid communication with the reaction reservoir (100), having a proximal end (610) with a proximal end opening (612), a distal end (620) with a distal end opening (622), further including a reagent reservoir (300) at the distal end (620) of the second microchannel (600) and in fluid communication with both the second
10 microchannel (600) and the reaction reservoir (100), wherein the reagent reservoir (300) has at least one reagent reservoir sidewall (310) thereby defining reagent reservoir volume (320) and a reagent reservoir opening (330) through which the third fluid enters the reagent reservoir (300), a length (630), at least one second microchannel sidewall (640), having a sidewall thickness, and defining a cross-sectional area (650), wherein the length (630) and
15 cross-sectional area (650) are selected to obtain a predetermined rate of molecular diffusion between the first fluid and the third fluid, and wherein the second microchannel (600) is configured such when the second microchannel (600) is filled with the third fluid, and the third fluid is a liquid, that flow of the third fluid through the second microchannel (600) is laminar and the capillary action of the second microchannel (600) and the third fluid is such
20 that the third fluid does not flow into the reaction reservoir (100) unless the pressure of the third fluid is increased by an external source.

30. The bioreactor (50) of claim 29, wherein the reagent reservoir opening (330) is closed by a penetrable, self-sealing, elastomeric septum (333) covering the reagent reservoir opening (330).

5 31. The bioreactor (50) of claim 29, wherein the proximal end opening (212, 612) further includes a section of the microchannel sidewall (240, 640) configured to have a reduced thickness sufficient to minimize formation of a meniscus at the proximal end opening (212, 612) by a fluid within the microchannel (200, 600).

10 32. The bioreactor (50) of claim 31, wherein the proximal end opening (212) of the first microchannel (200) blocks the flow of liquid in the first microchannel (200) when the pressure of the liquid is not increased by an external source and there is a difference in viscosity between the first fluid and the second fluid, while permitting the diffusion of molecules across the proximal end opening (212) of the first microchannel (200).

15 33. The bioreactor (50) of claim 29, wherein the proximal end opening (612) of the second microchannel (600) further includes a section of the second microchannel sidewall (640) configured to have a reduced thickness sufficient to minimize formation of a meniscus at the proximal end opening (612) by a fluid within the second microchannel
20 (600).

34. The bioreactor (50) of claim 29, wherein the proximal end opening (612) of the second microchannel (600) blocks the flow of liquid in the second microchannel (600) when the pressure of the liquid is not increased by an external source and there is a

difference in viscosity between the first fluid and the second fluid, while permitting the diffusion of molecules across the proximal end opening (612) of the second microchannel (600).

5 35. The bioreactor (50) of claim 29, wherein the length (630) and cross-sectional area (650) of the second microchannel (600) are selected to achieve a predetermined rate of diffusion between the third fluid and the first fluid.

10 36. The bioreactor (50) of claim 29, wherein the reaction reservoir (100) is constructed of a material having a diffusion capacity and with sidewalls (110) having a surface area such that the diffusion of oxygen across the sidewalls (110) is predetermined for a predetermined unit of time.

15 37. The bioreactor (50) of claim 36 wherein the oxygen diffusion across the reaction reservoir sidewalls (110) is approximately 4.6 mg per day at atmospheric pressure and a temperature of 37° C.

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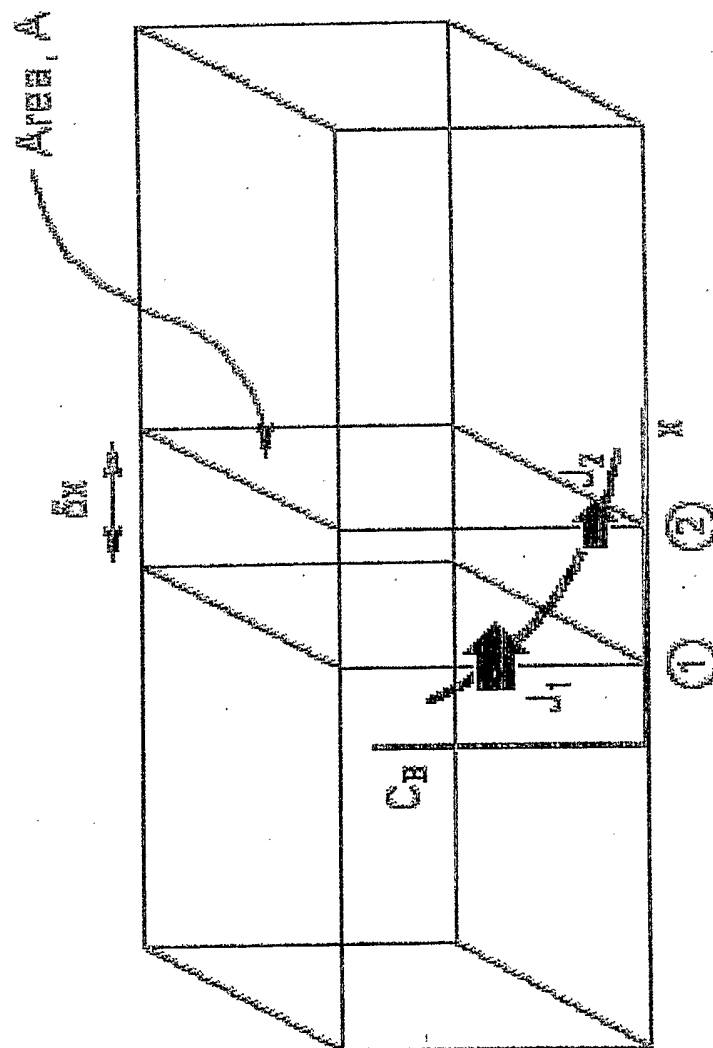


FIG. 1

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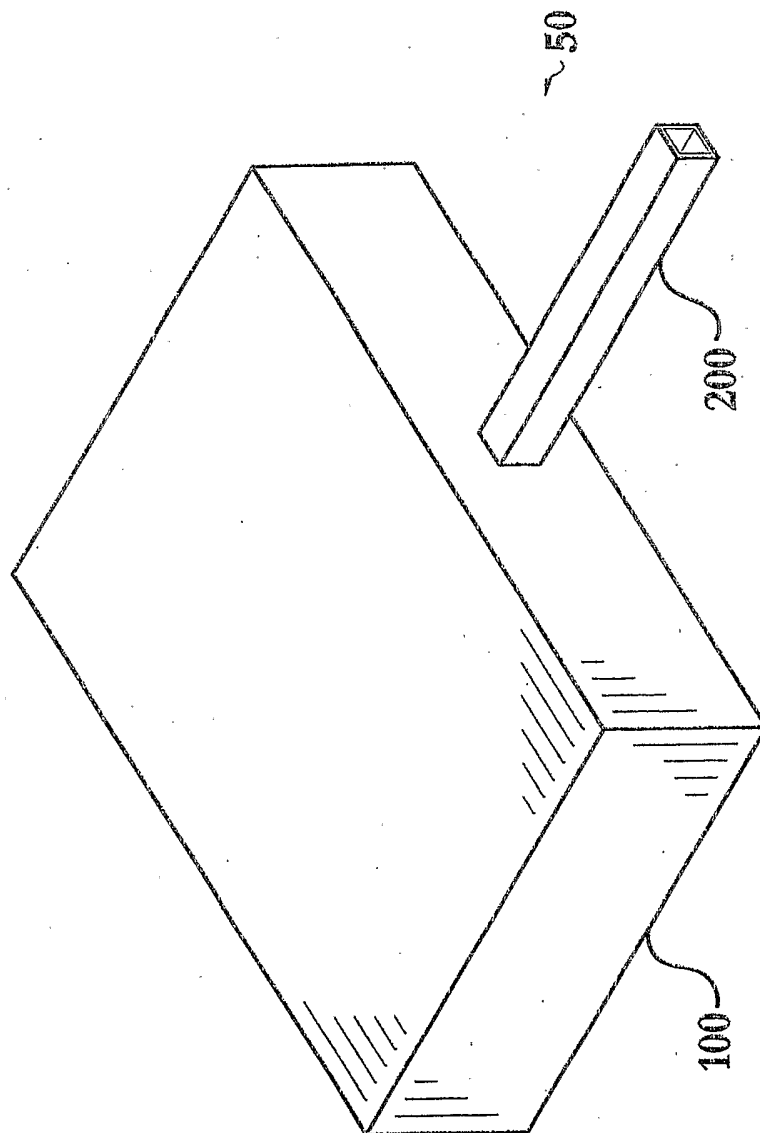


FIG. 2

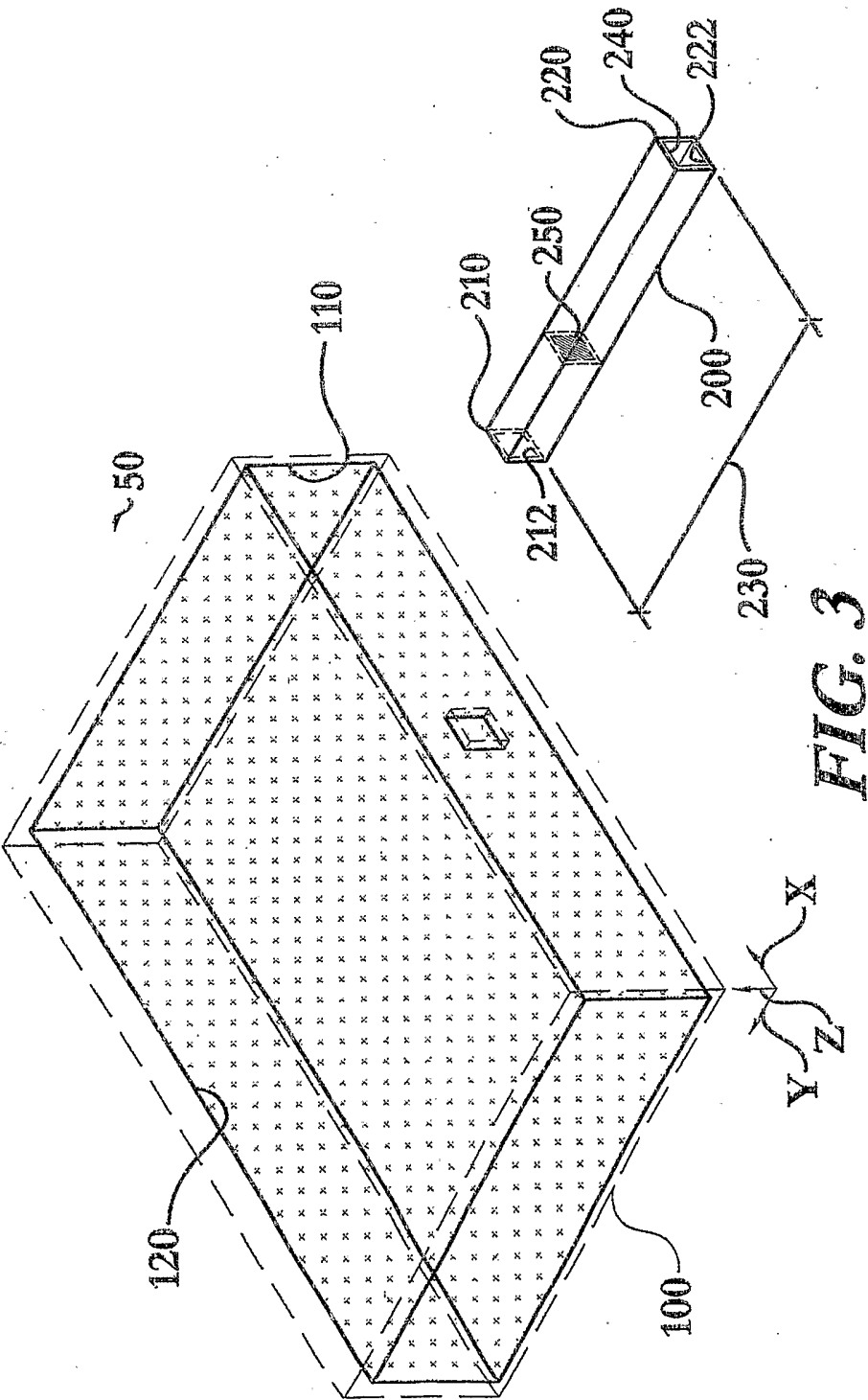


FIG. 3

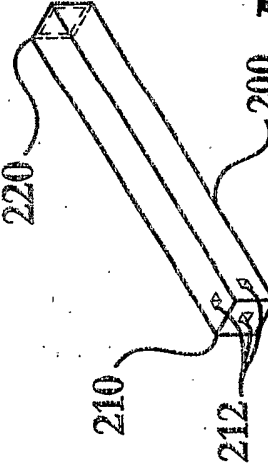


FIG. 4

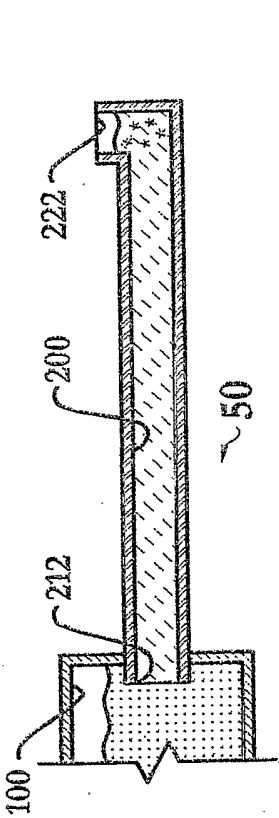


FIG. 5

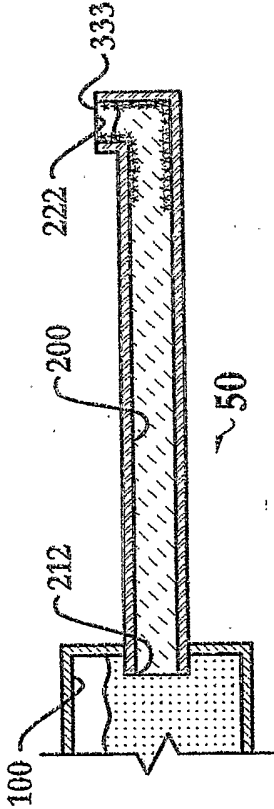


FIG. 6

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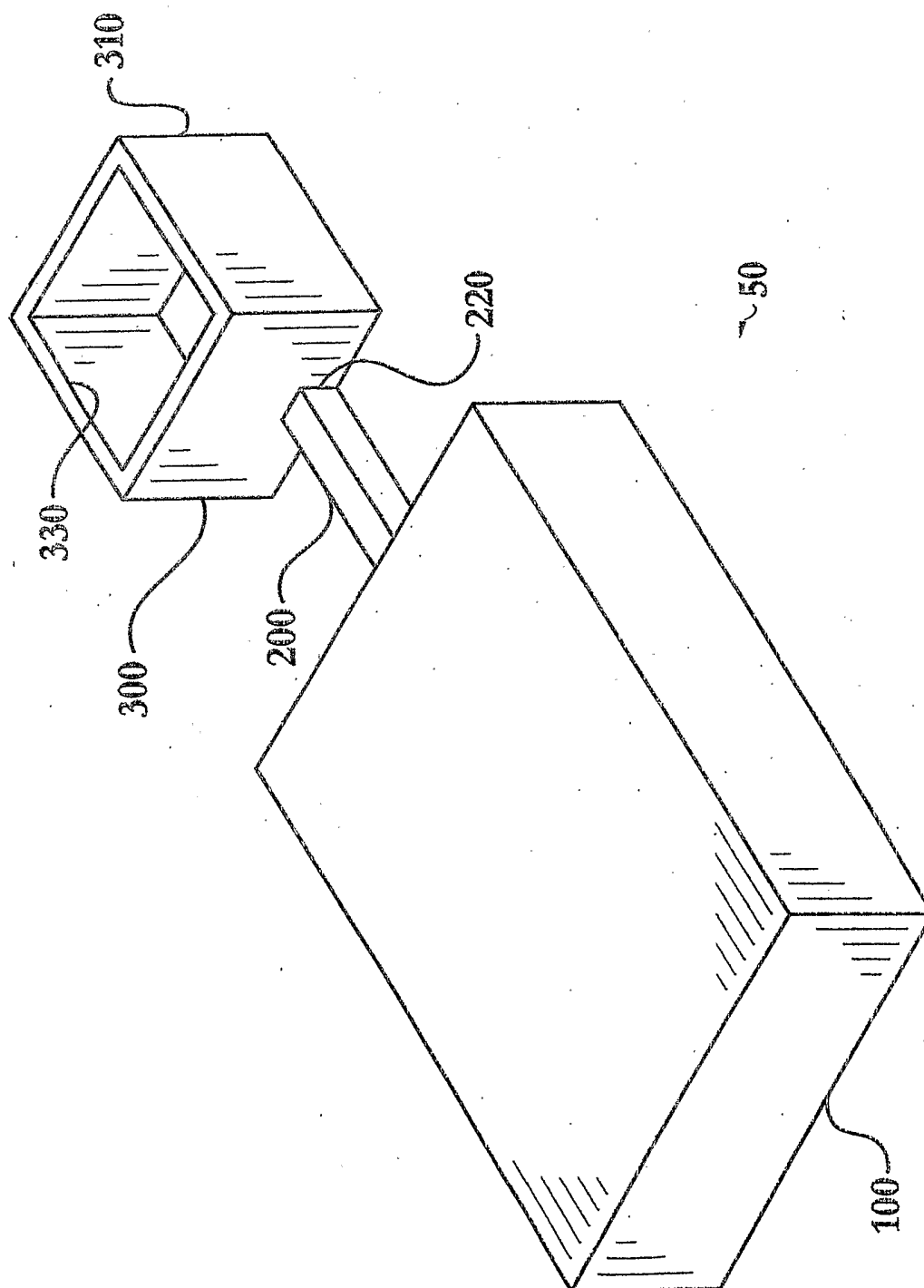
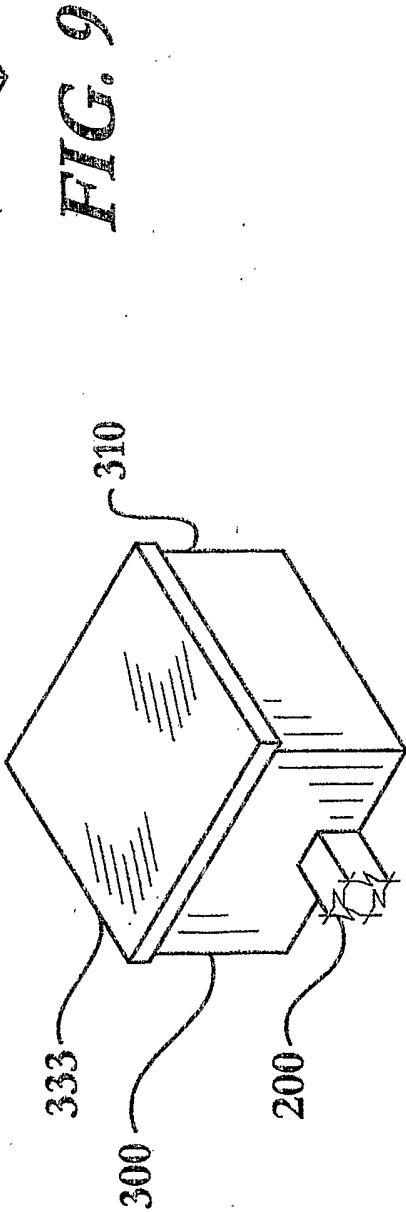
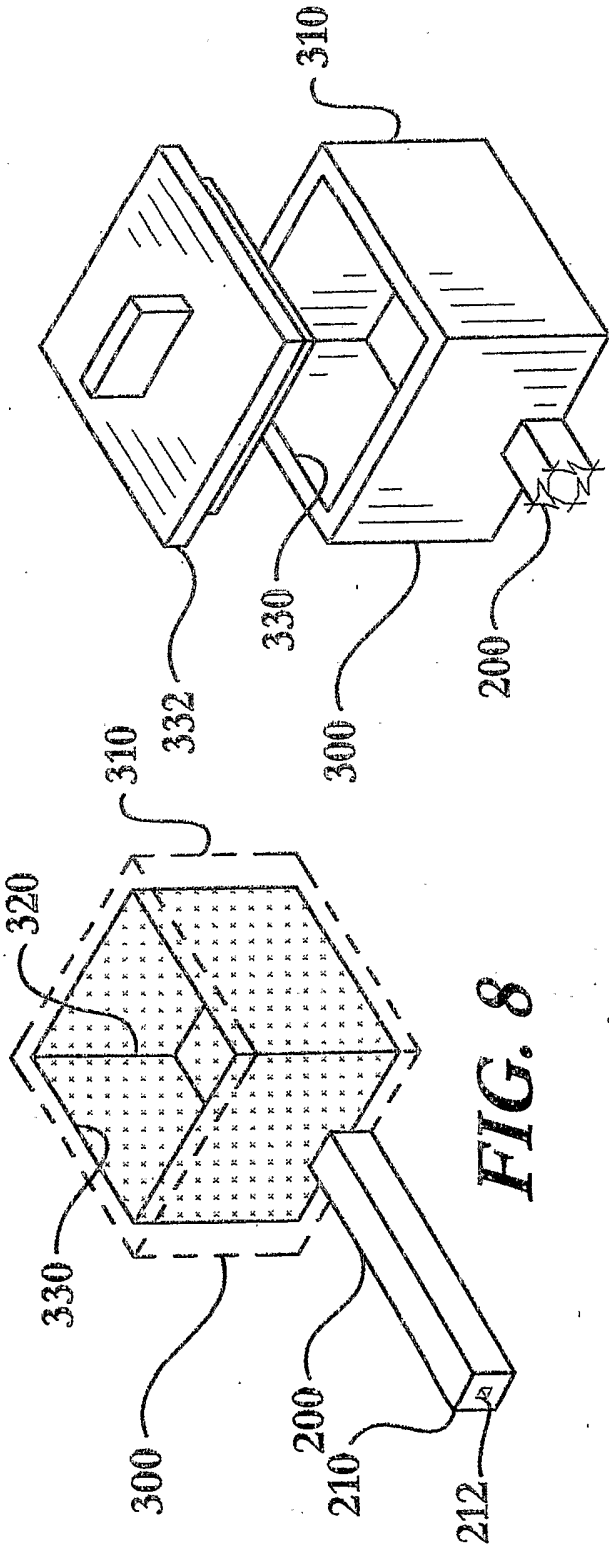


FIG. 7



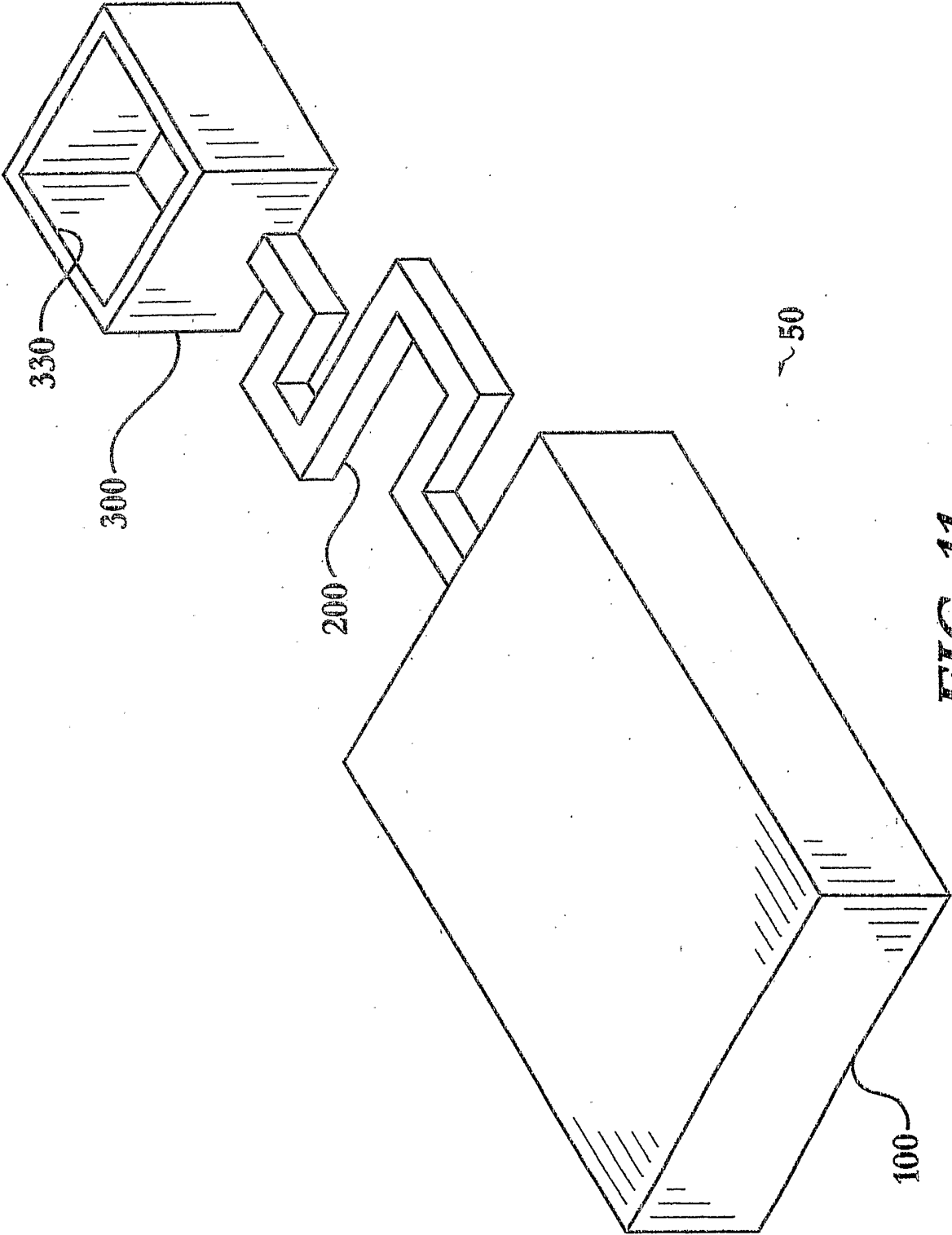


FIG. 11

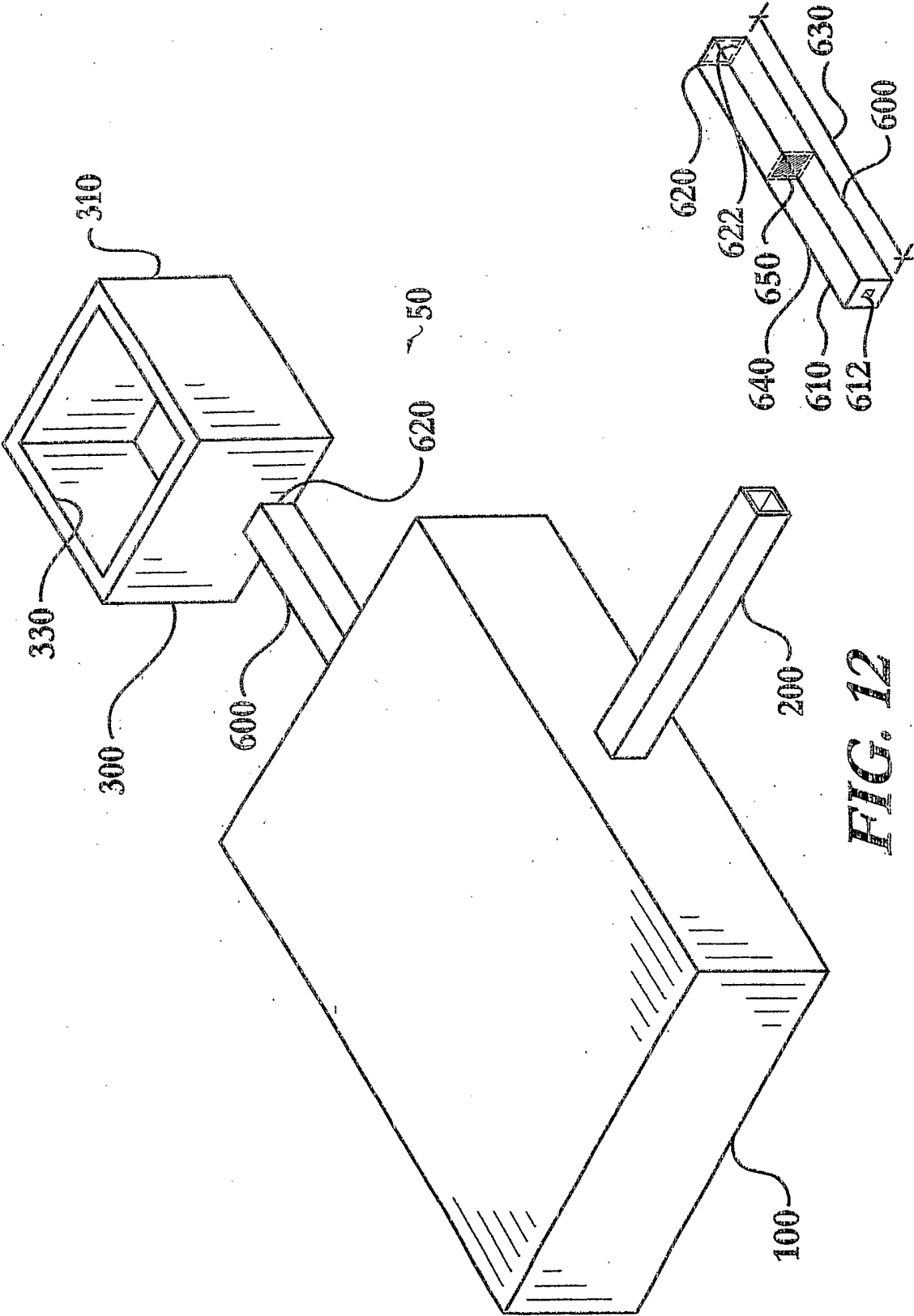


FIG. 13

FIG. 12

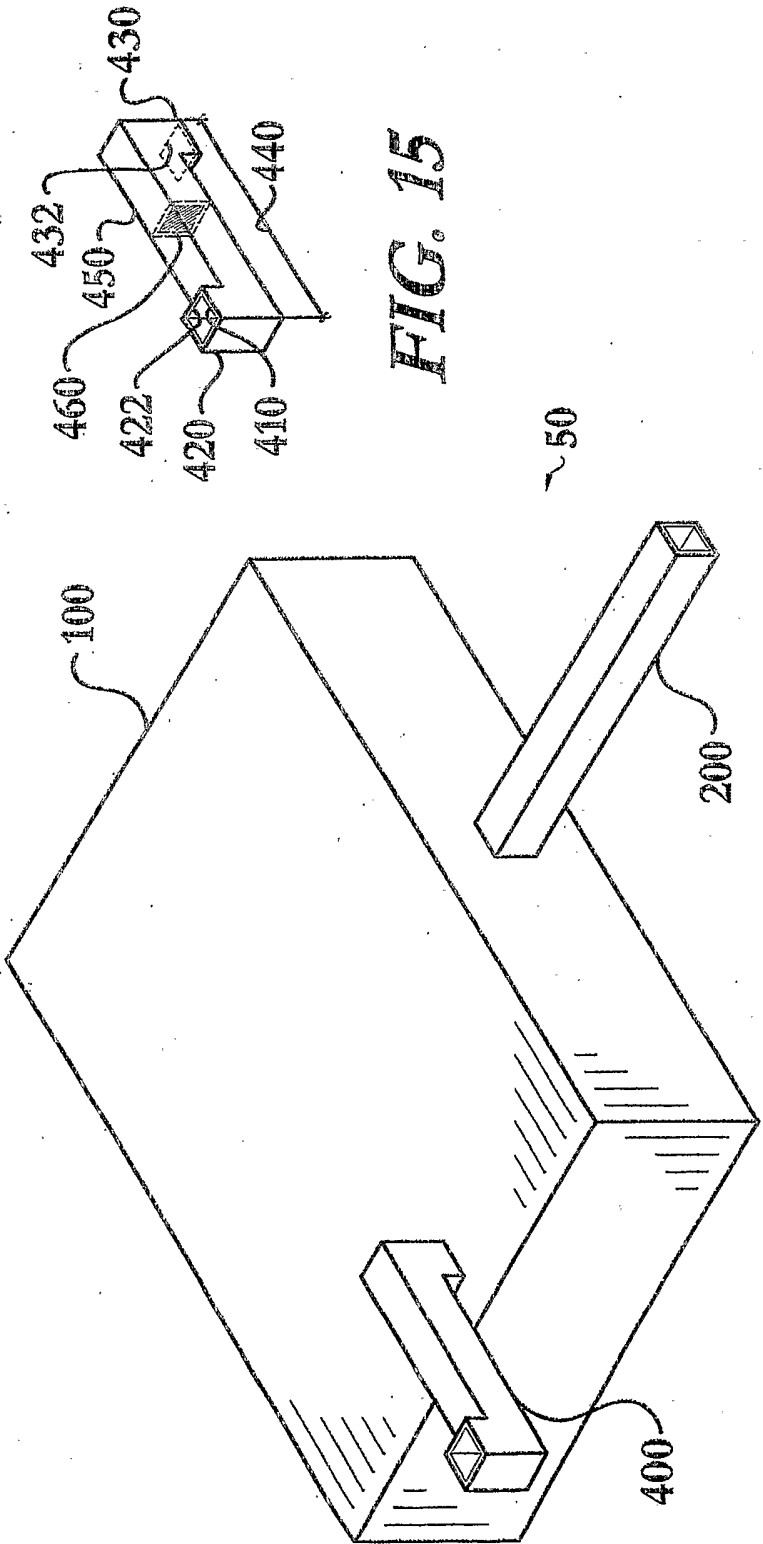


FIG. 15

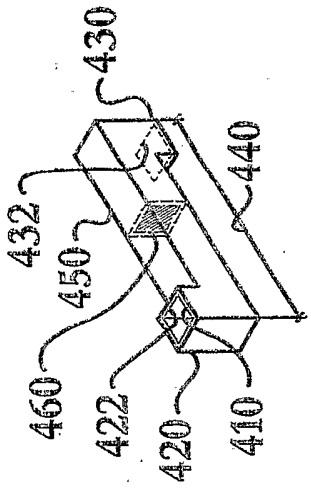


FIG. 16

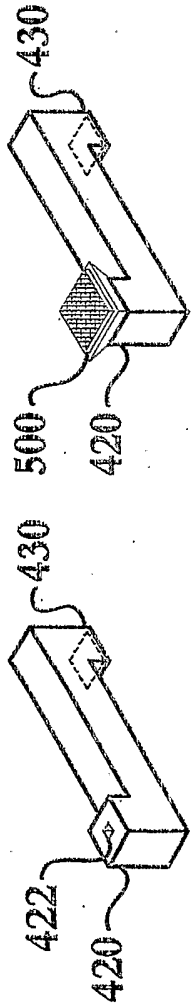


FIG. 17

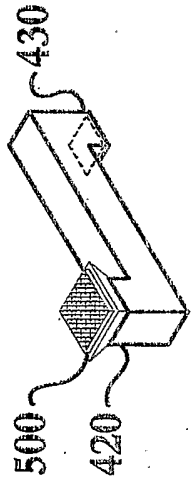
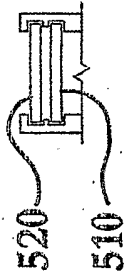


FIG. 18



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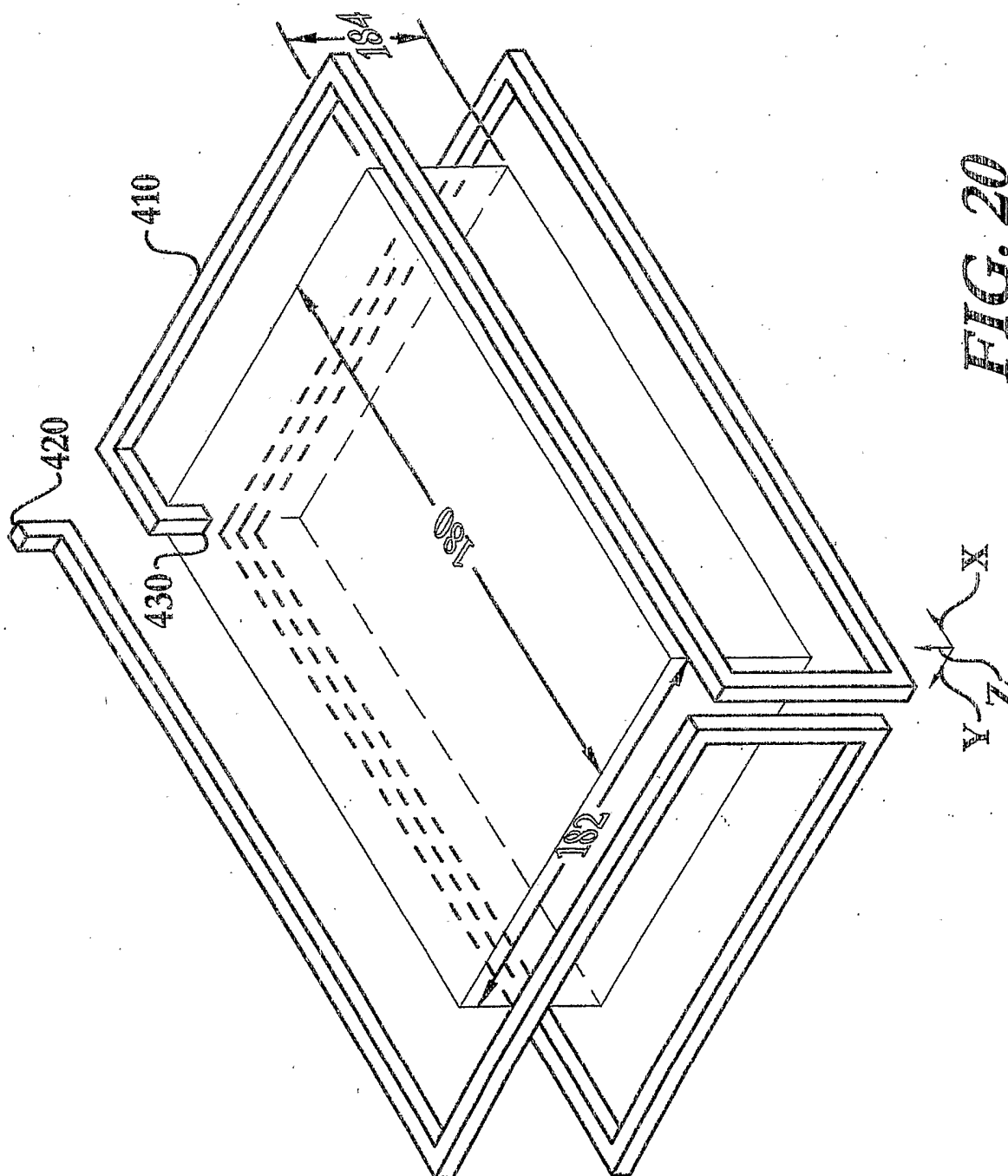


FIG. 20

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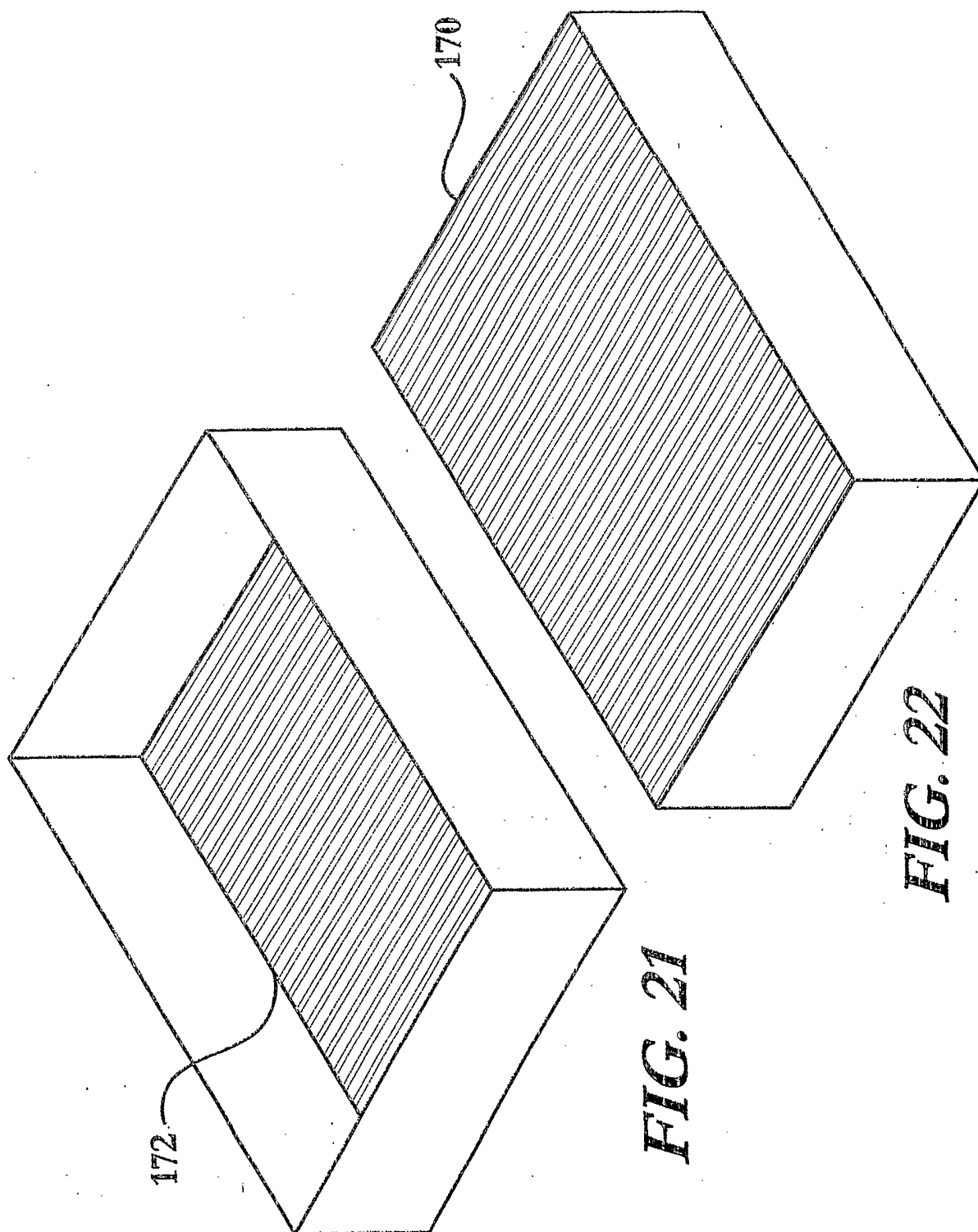
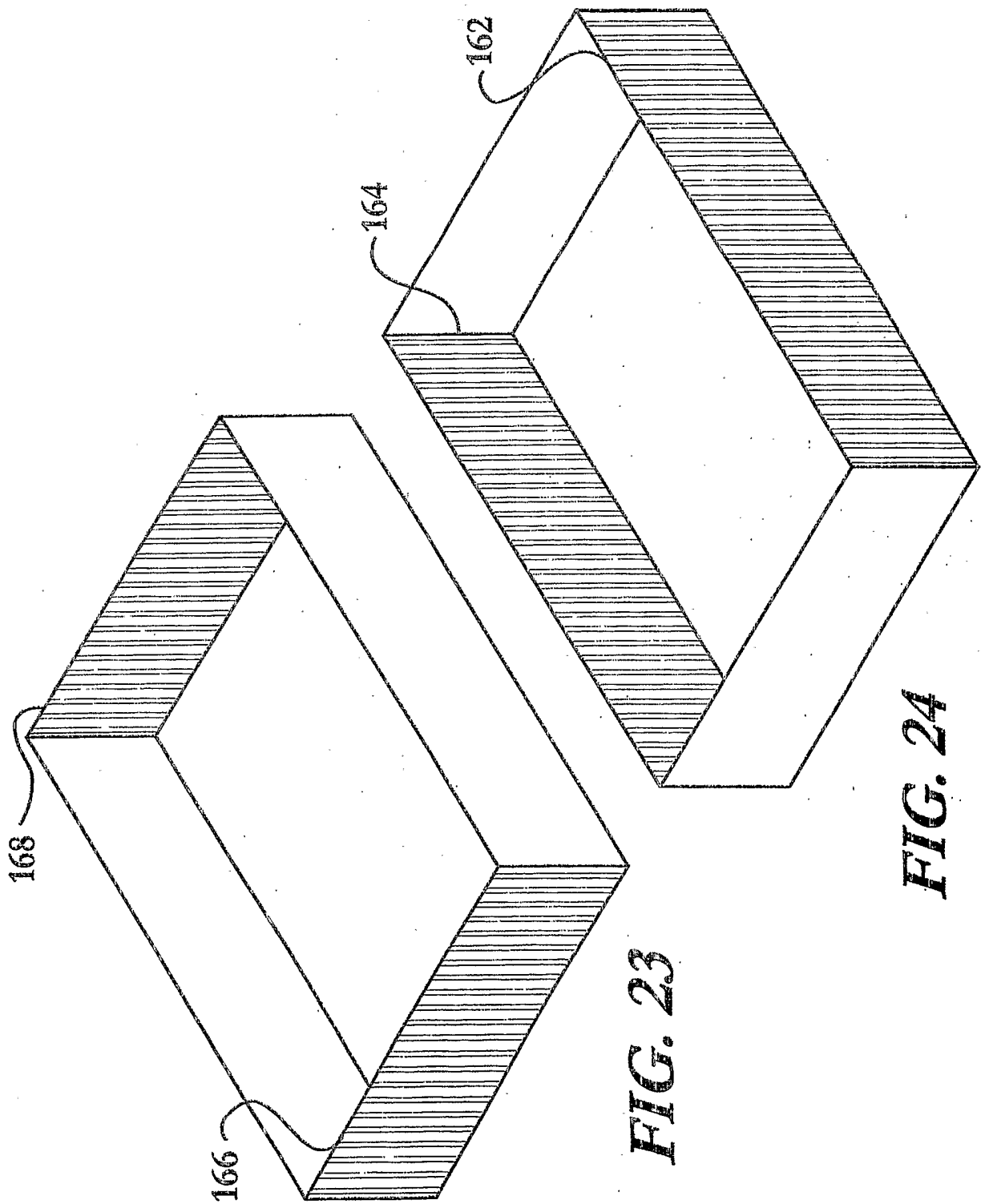


FIG. 21

FIG. 22



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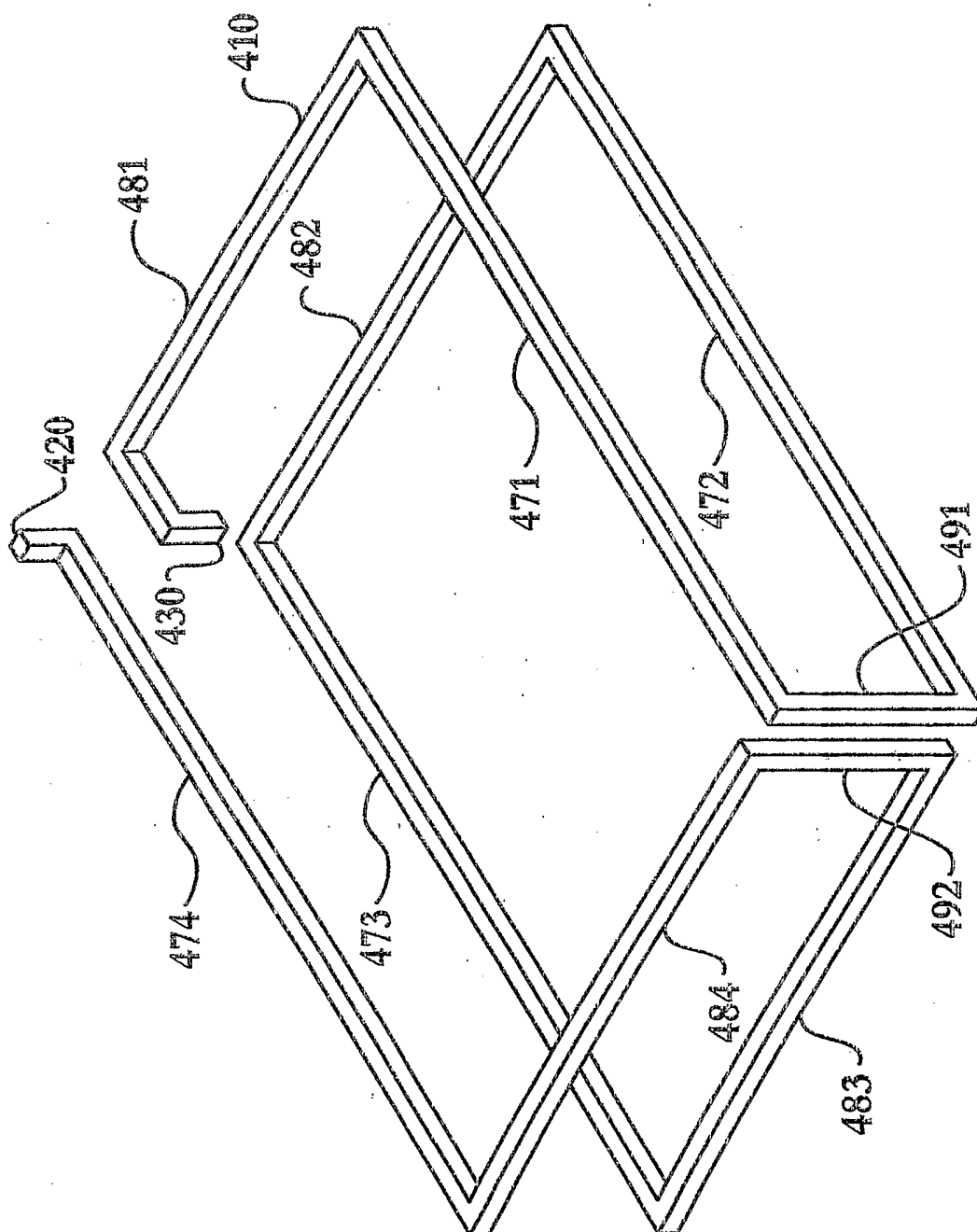


FIG. 25

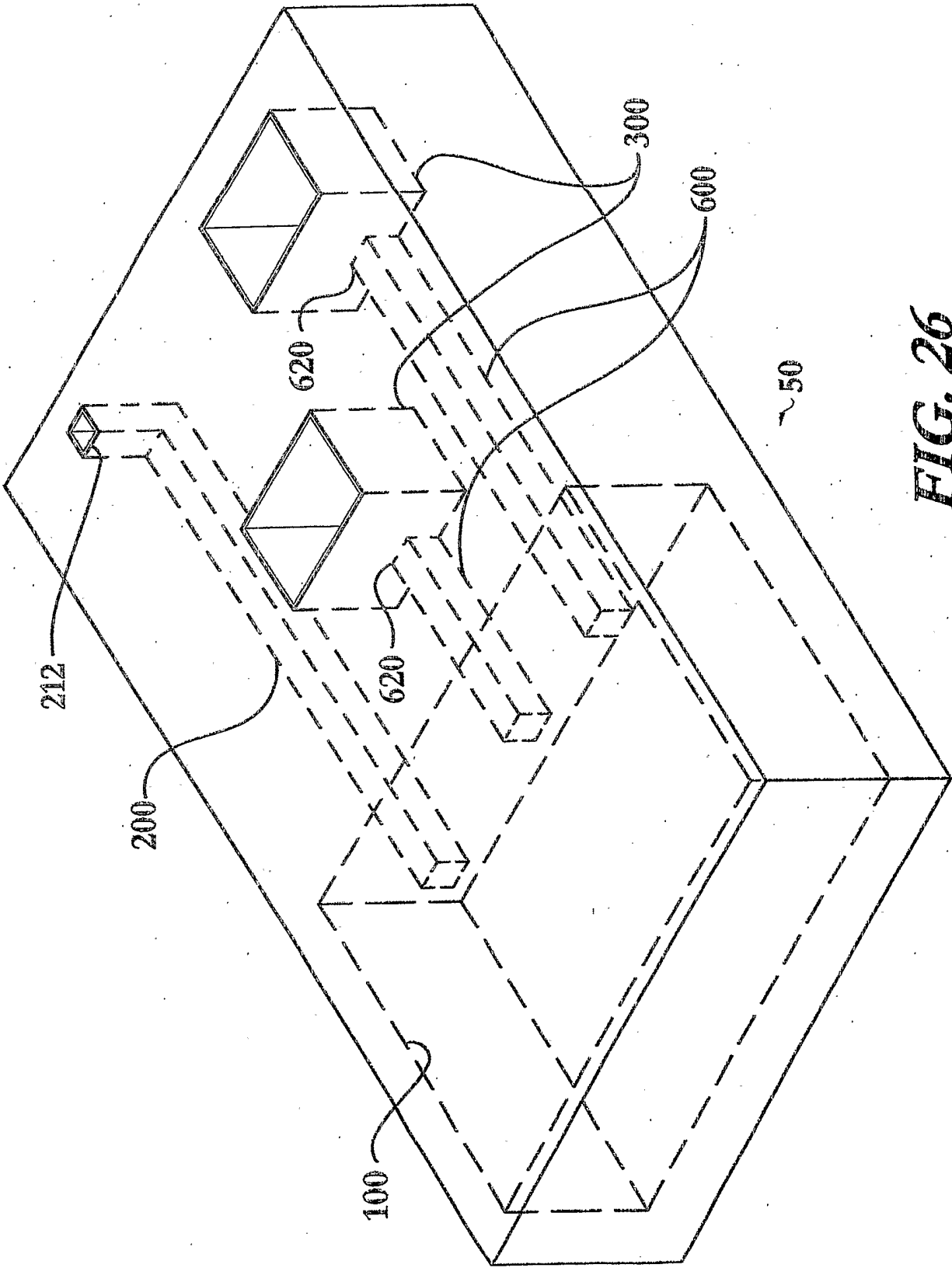


FIG. 26

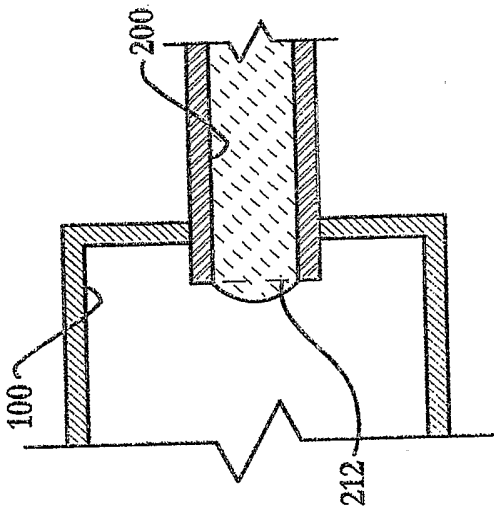


FIG. 27

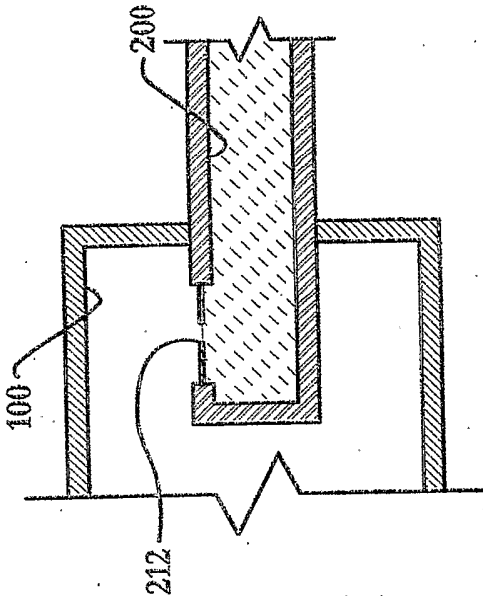


FIG. 28

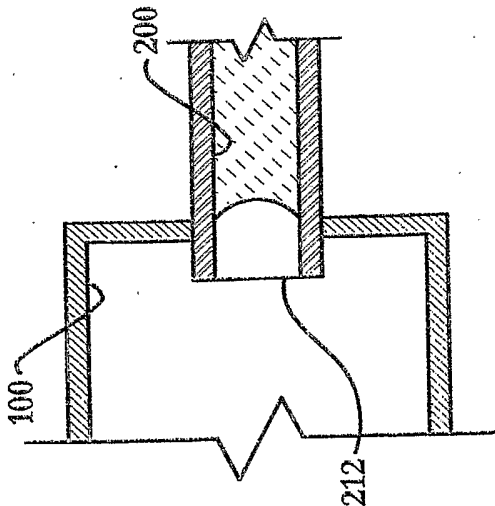


FIG. 29

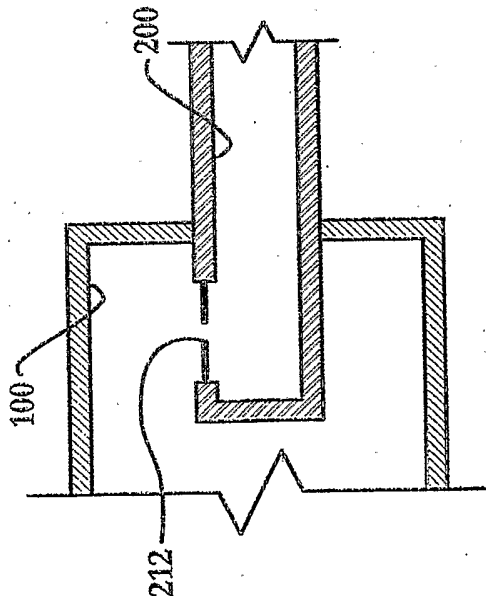


FIG. 30

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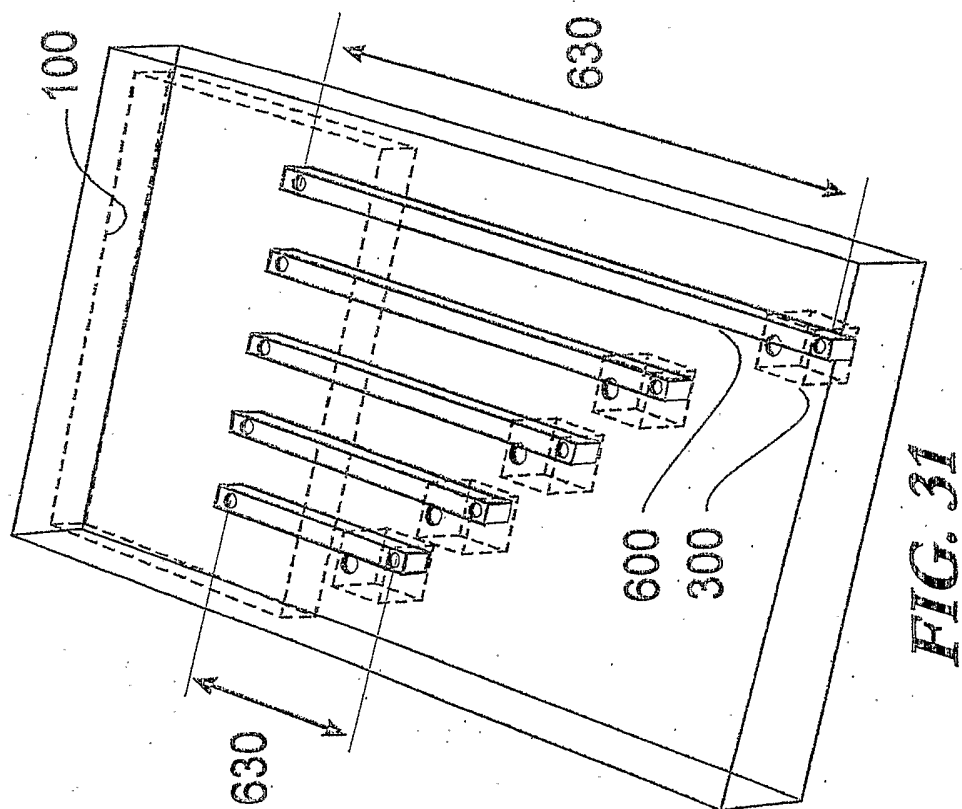
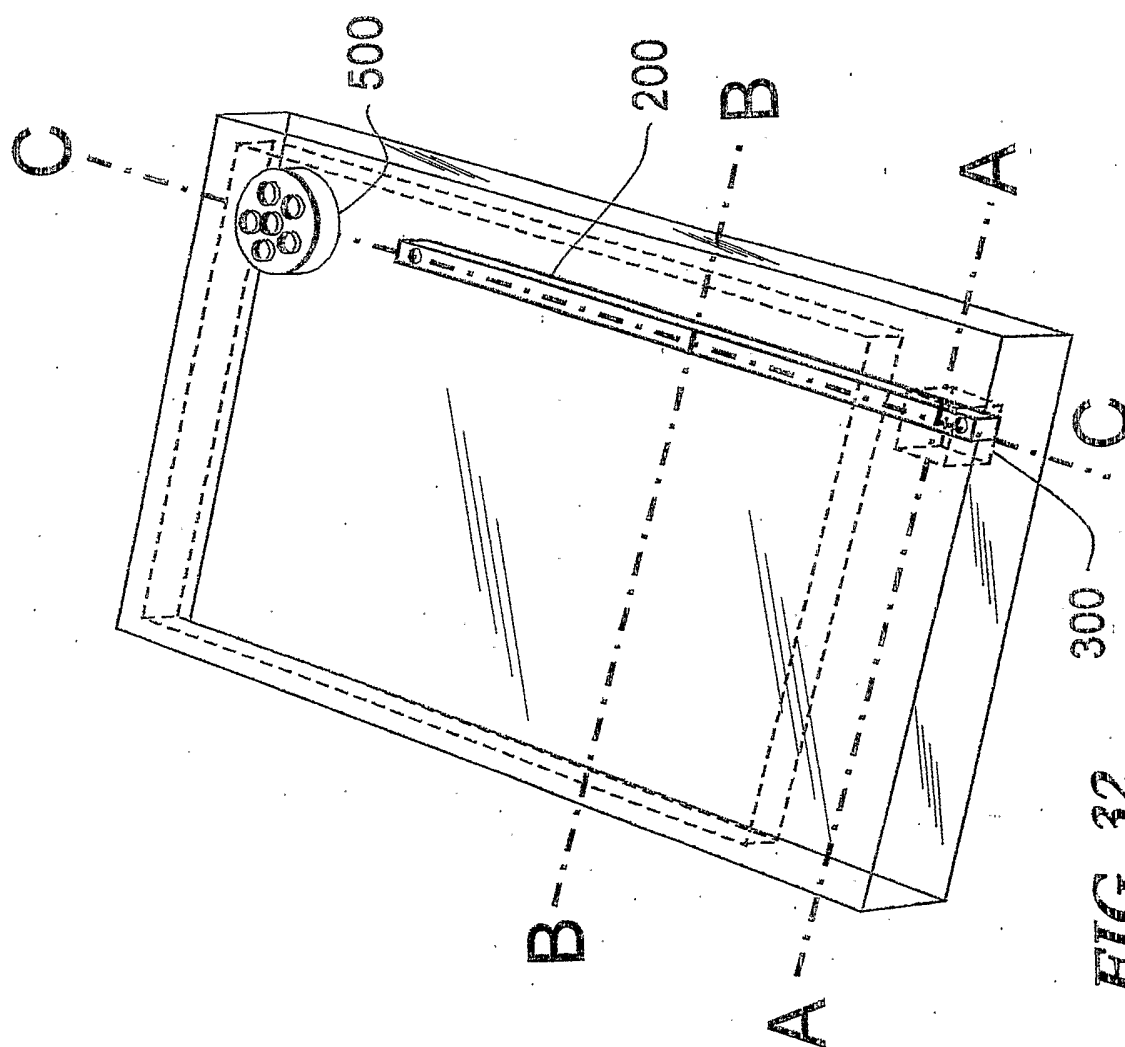
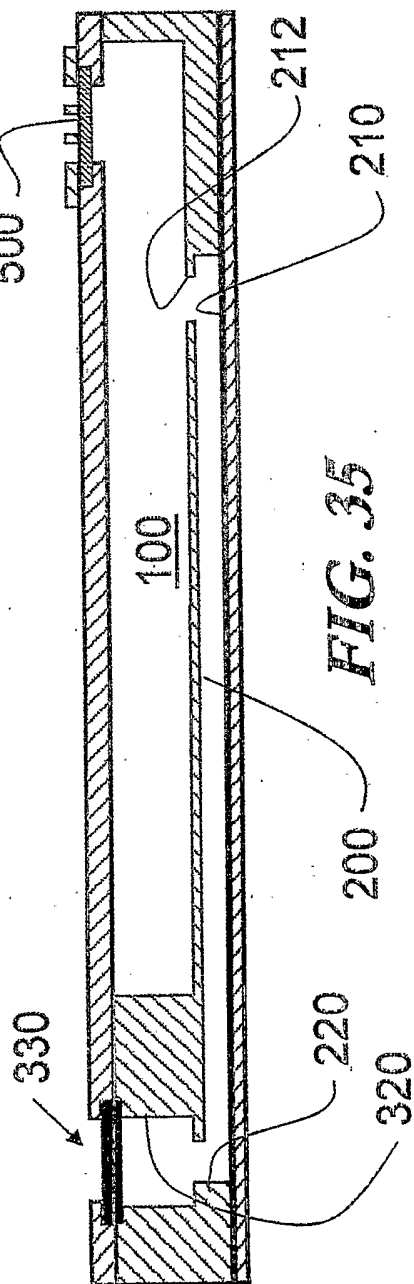
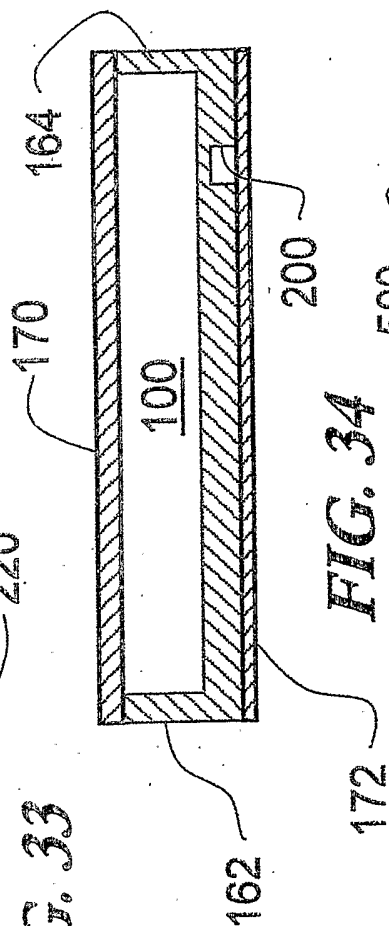
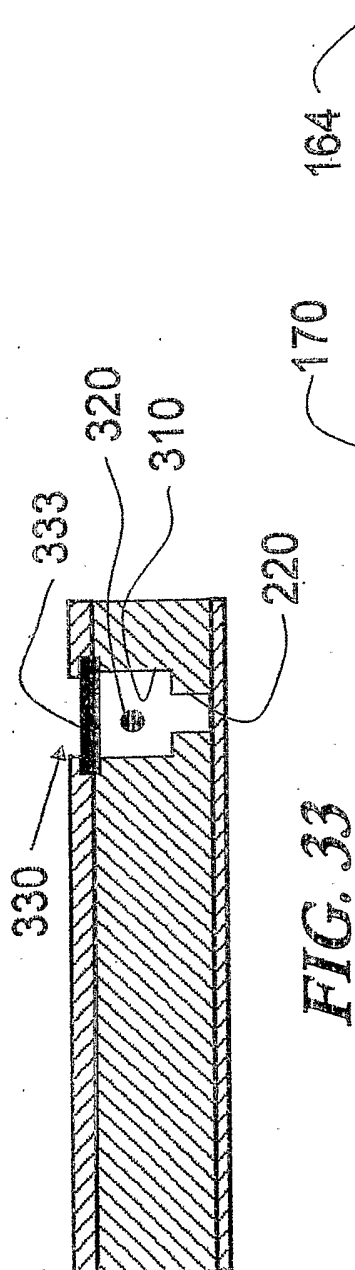


FIG. 31

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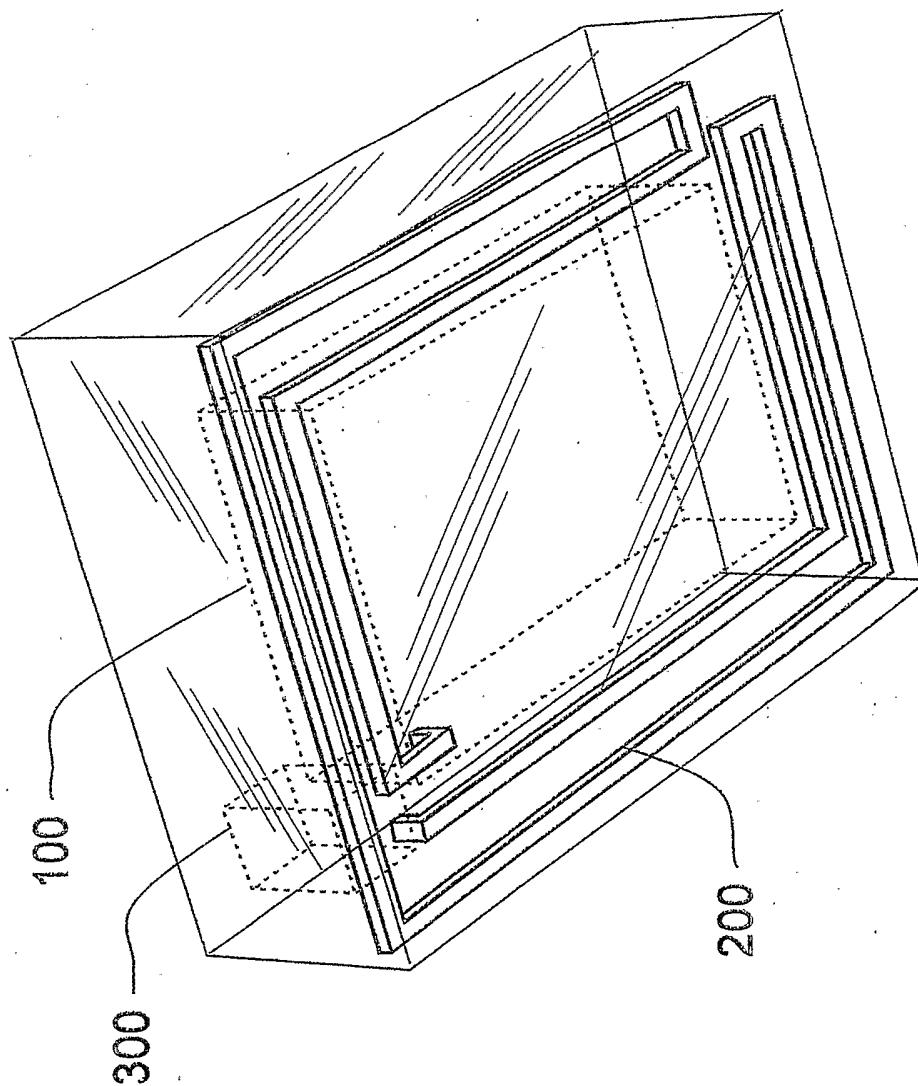


FIG. 36

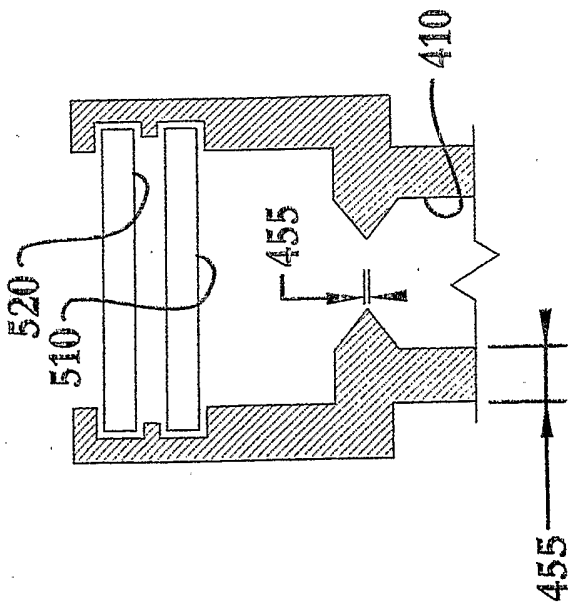


FIG. 37

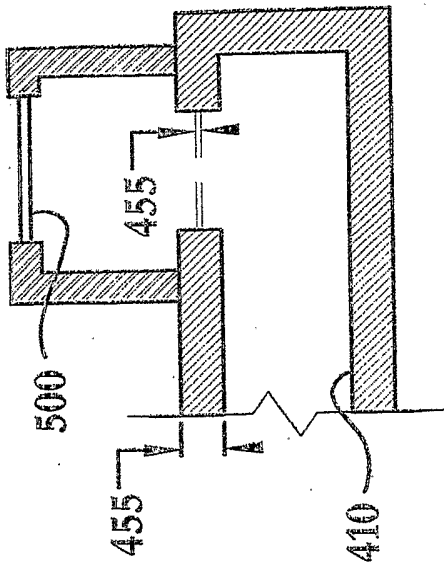


FIG. 38

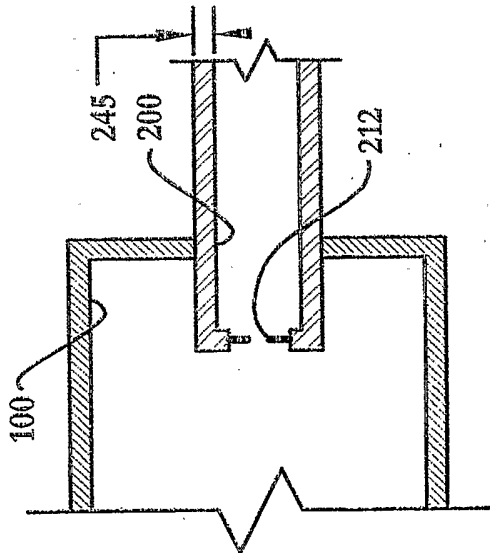


FIG. 39

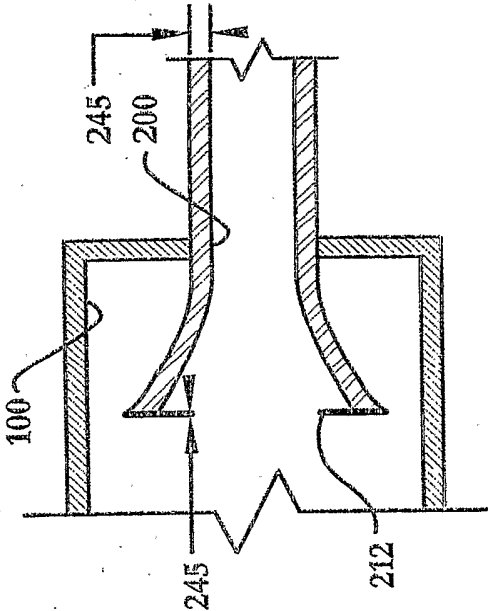


FIG. 40