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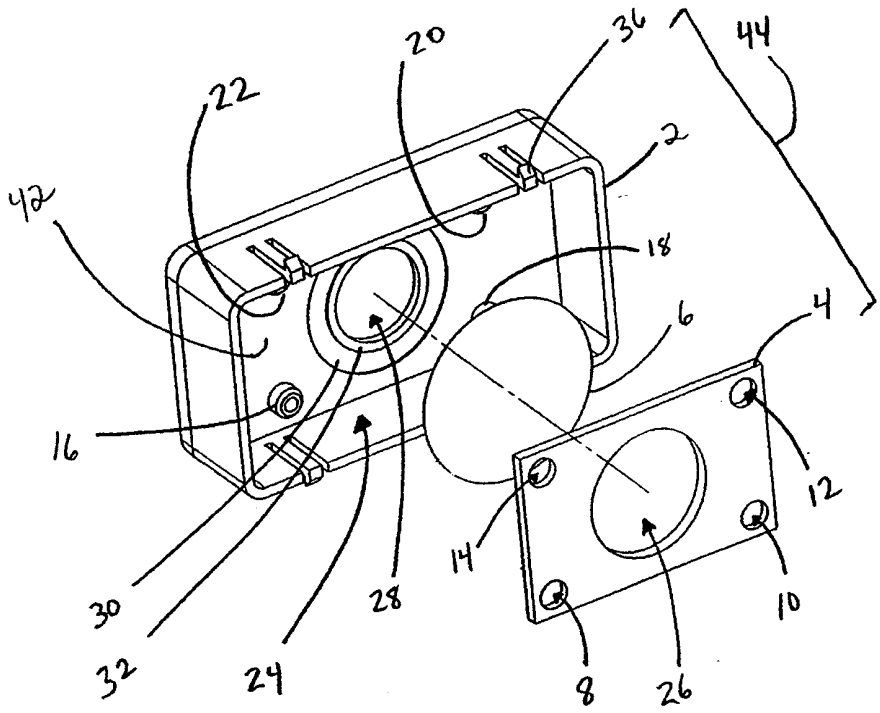
(54) Title: PARTICLE SEPARATION CASSETTE AND RELATED METHODS



(57) Abstract: A device for separating a component from a biological sample includes a platform, a membrane, and a mount. The platform defines a first opening and the mount defines a second opening. The mount and the platform engage in a peripheral interference fit such that the membrane is stretched radially between the first and second openings.

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PARTICLE SEPARATION CASSETTE AND RELATED METHODS

Technical Field

The invention relates generally to an apparatus and methods for separating particles, including whole cells, from a mixture and analyzing the separated particles. More particularly, the invention relates to separating particles from a mixture using a substantially flat membrane and analyzing the separated particles using a laser scanning cytometer.

Background Information

In various applications, cells are separated from a sample mixture and examined to determine characteristics of the separated cells. For example, high throughput diagnostic screening of cells is often used in disease detection. In most screening methods, cells must be separated rapidly based upon one or more distinguishing cellular properties. Accordingly, instruments have been designed to facilitate the separation and/or isolation of a desired group of cells. For example, computer-controlled instruments for measuring multiple optical properties of biological specimens, such as flow cytometers, automated blood cell analyzers, and blood cell differential classifiers, are commonly used.

A flow cytometer is an instrument that hydrodynamically focuses a fluid suspension of cells into a single stream. The stream is then passed through an "examination zone" in which a focused light beam is directed toward the cells. The flow cytometer then measures optical interactions, such as, absorption, scatter, and fluorescence, between the light and the cells. Flow cytometers permit the study of living, unstained, cells in addition to those which have been chemically treated, for example, by staining. Flow cytometry techniques enable certain cellular

constituents, particularly those on the cell surface, to be quantitatively characterized at rates of a thousand or more cells per second.

Blood cell analyzers typically consist of a computerized microscope that classifies various types of blood cells. For example, blood cell analyzers identify and count abnormal cells in a specimen. Most blood cell analyzers are automated. However, most analyzers also allow the operator to view cells manually to stop the analyzer temporarily for making morphological observations, or to review abnormal cells in greater detail. During automatic counting, the X-Y coordinates of every encountered abnormal blood cell are stored. The operator has the option of using a review mode in which abnormal cells are individually accessed, focused, and viewed on a television monitor or through a binocular microscope.

A blood cell differential classifier typically consists of a computer-controlled microscope having a stage that is driven by stepper motors. A light source, such as a xenon arc lamp, illuminates cells and the classifier uses various sensors, such as a silicon photodiode array, to measure the optical interaction between the cells and the light. All of the cells in a given area are illuminated at the same time.

The foregoing devices all are limited by a lack of precision and speed in separating and analyzing cells that is essential in, for example, diagnostics. The present invention addresses this need in the art for a rapid and precise cell separation technique for use in diagnostic and other cell analysis methods.

Summary of the Invention

The present invention solves the problem of rapid, efficient particle separation in a biological sample by providing a separation device having a platform upon which is mounted a radially stretched membrane. The sample is placed on the platform, which preferably has a sink

for containing the sample, and the sample is drawn through the membrane, preferably by an absorbent material contained in a cavity on the underside of the sink.

A device of the invention allows rapid processing of a sample, retaining primarily those particles desired for analysis on the membrane surface. A peripheral interference fit between the device platform and a mount causes the membrane to be stretched in a planar configuration over the opening in the platform. An absorbent material and/or vacuum on the underside of the platform directs sample through the membrane such that particles to be analyzed are captured and distributed uniformly on the membrane. The membrane pore size regulates capture of particles from the sample.

More particularly, the invention provides a cassette comprising a platform, a membrane, and a mount assembled together. A peripheral interference fit between the platform and mount stretches the membrane. As a result of the stretching, the membrane assumes a planar configuration to capture particles on its surface for analysis. The cassette may be engaged with a cartridge for analysis of captured particles.

Accordingly, in one aspect the invention, a device for separating components of a biological sample has a platform defining a first opening, a membrane, and a mount defining a second opening that mates with the first opening. The mount and the platform engage in a peripheral interference fit such that the membrane is stretched radially between the two openings. In certain embodiments, the platform has, on one side, a sink capable of containing a sample, and a cavity for containing an absorbent material on a second side. In some of the embodiments, the first opening is cylindrical, and the platform has a raised ring on the second side that concentrically surrounds the first opening. An annular depression concentrically surrounds the raised ring. A preferred mount has a flange for a radial interference mating with the raised ring on the platform. The flange concentrically surrounds the second opening. Also, in some embodiments, the platform has a plurality of posts on the second surface, and the mount has a

plurality of apertures for receiving the posts when the mount engages the platform. Alternatively, the posts are on the mount and the apertures are on the platform.

A membrane for use in a device of the invention may be any membrane sufficient for isolation of target particles. For example, the membrane may be track-etched or may have a diameter larger than the opening in the platform, the opening in the mount, or the annular depression in the platform. A highly-preferred membrane has at least three detectable coordinate points for determining the position of the membrane. The coordinate points may be optical markers, such as fluorescent markers and other markers detectable by laser scanning cytometry or other means. Finally, the absorbent material for use in a device of the invention may be any material having sufficient wicking properties to allow sample to be drawn across the membrane. A suction or vacuum device may be used as an alternative to, or in addition to, the absorbent material.

In a preferred embodiment, a device for separating components of a sample as described above is contained in a cartridge that has a housing adapted to contain the device. The cartridge is preferably compatible for insertion into a laser scanning cytometer and is preferably adapted for conducting one or more assay(s) to determine a biological property of the particles isolated on the membrane. For example, the cartridge may have reagent wells capable of containing reagents used in a bioassay of particles on the membrane. Such a bioassay may be automated in, for example, a laser scanning cytometer. Cartridges may be reusable or recyclable, or may be adapted for a single use.

In certain preferred embodiments, a device for separating a component from a biological sample has a platform defining a first opening, a membrane, a mount, and a trough. The platform defines a cavity that contains an absorbent material contained within the cavity. The mount defines a second opening that mates with the first opening. The mount and the platform engage in a peripheral interference fit such that the membrane is stretched radially between the

first and second openings. Additionally, the trough is provided for introducing a liquid to a region between the membrane and the absorbent material. Such an introduction of a liquid induces a sample to flow substantially homogeneously through the membrane. In certain embodiments, the trough contains the absorbent material, and/or a secondary absorbent material is in contact with the absorbent material. Also, in some embodiments, a third opening in the platform aligns with the trough such that liquid can be applied to the absorbent material, via the trough, through the third opening.

In another aspect of the invention, a method for separating cells from a biological sample includes applying a biological sample to a device as described above.

In another aspect of the invention, methods of the invention provide an assay for determining a cellular characteristic of a biological sample. In a preferred embodiment, methods of the invention follow the steps of obtaining a biological sample and exposing the biological sample to labeled reagents capable of reacting with a cellular component in the sample. The sample is applied, either before or after application of reagents, to a device as described above in order to capture at least a portion of the sample on the membrane. The sample components that are captured on the device membrane then are analyzed for at least one cellular characteristic, for example, by detecting the labeled reagents that reacted with the cellular components. If the device has an absorbent material for capturing the sample on the membrane, a liquid can be used to wet the absorbent material and fill a region between the absorbent material and the membrane.

Methods of the invention preferably comprise detecting an optical property (*e.g.*, density, absorbance, or fluorescence) of a cellular component of the sample. Highly preferred methods comprise detecting the optical property by laser scanning cytometry. Methods of the invention also can be used to determine the identity, nature, expression characteristics, or other features of a cell. Specifically, methods of the invention may be used to measure or characterize cell-surface receptors, intracellular receptors, nucleic acids, enzymes, cell morphology, and/or cell type.

Additional aspects and embodiments of the invention are apparent upon consideration of the following drawings and detailed description thereof.

Brief Description of the Drawings

The invention, and exemplary embodiments according to the invention, are more particularly described in the following description, taken in conjunction with the accompanying drawings.

In the drawings, like reference characters generally refer to the same parts throughout the different views. Also, the drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating principles of the invention.

Figure 1 depicts a schematic exploded perspective view of one embodiment of a cassette of the invention;

Figure 2A depicts a schematic top view of the embodiment shown in Figure 1;

Figure 2B depicts a schematic sectional view taken along line A-A of the embodiment shown in Figure 2A;

Figure 3 depicts a schematic top view of one embodiment of a cartridge of the invention in association with the cassette of Figure 1;

Figure 4 depicts a schematic perspective view of the cassette of Figure 1 in association with one embodiment of an absorbent reservoir;

Figure 5 depicts a schematic exploded perspective view of one embodiment of a cassette with dual treatment surfaces in association with a cartridge according to the invention;

Figure 6A depicts a perspective view of the top side of a dual opening housing of the embodiment of Figure 5;

Figure 6B depicts a perspective view of the bottom side of a dual opening housing of the embodiment of Figure 5;

Figure 7 depicts a view of a retainer of the embodiment of Figure 5;

Figure 8A depicts a perspective view of the top of the cartridge of Figure 5 with the cassette of Figure 5 removed;

Figure 8B depicts a perspective view of the bottom of the cartridge of Figure 5;

Figure 9A depicts a schematic exploded perspective view of a cassette that is similar to that shown in Figure 1 and the cartridge that is shown in Figure 5;

Figure 9B depicts a schematic sectional view of the cassette of Figure 9A taken along a line that is generally in the same position as line A-A in Figure 2A; and

Figure 10 depicts a schematic exploded perspective view of a cassette that is similar to that shown in Figure 5 and the cartridge that is shown in Figure 5.

Detailed Description of the Invention

Apparatus and methods of the invention provide for rapid and accurate sample preparation for analysis of cells and cellular components. The invention is especially useful in connection with optically analyzing cells or cellular components, such as with a laser scanning cytometer. A device of the invention has a platform with an opening over which a membrane is mounted by a peripheral interference fit between the platform and a mount. On one side, the platform has a sink for applying the sample to membrane. On the other side, the platform has a well for wicking the sample through the membrane. Based upon the characteristics of the membrane, specified cells or cellular components will remain on the membrane for analysis. In one embodiment, the contents of the membrane are reacted with one or more reagents (*e.g.*, dyes, fluorophores, antibodies, or ligands), and the reacted sample is analyzed using an optical technology, such as laser scanning cytometry.

Laser scanning cytometers typically require a flat surface to efficiently analyze a sample. The invention combines an ability to separate fractions of a sample through a membrane with an ability to present captured sample on the membrane in a configuration suitable for laser scanning cytometry analysis. Moreover, apparatus and methods, according to the invention, provide a flat surface for analysis that is compatible with laser scanning cytometry while, at the same time, allowing for high throughput sample analysis.

More particularly, the invention provides a cassette that holds a membrane flat for application and analysis of a sample. The cassette, in certain embodiments, fits into a cartridge having wells for containing assay reagents and/or the sample itself. In these embodiments, an assay can be conducted in an automated, high through-put configuration. The reagents (for example, a fluorescent marker) and sample are mixed and applied to the membrane, capturing at least a portion of the sample, and the captured sample is analyzed using laser scanning cytometry, for example, by analyzing a fluorescent marker.

Referring to Figure 1, one embodiment of the invention has a housing 2, a retainer 4, and a membrane 6. The underside of the housing 2 defines a cavity 24 and a through-hole 28. In one embodiment, the through-hole 28 has a diameter of 10 mm. Disposed concentrically about the through-hole 24 are a raised ring 32 and a depressed annular region 30. Projecting from a floor 42 of the cavity 24 are mounting posts 16, 18, 20, 22. The mounting posts 16, 18, 20, 22 each have a flat top and a rounded edge that meets a generally columnar structure. The mounting posts 16, 18, 20, 22 are shown arranged in a generally rectangular configuration with the same relative orientation to each respective corner of the cavity floor 42. Other embodiments provide other arrangements of the mounting posts or a different number of mounting posts. The housing 2 can be formed from a polymeric material, such as a plastic. Injection molding can be used to produce the housing 2.

A membrane 4 fits over the through-hole 28, raised ring 32, and depressed annular region 30 and contacts the housing 2. The membrane 6 is slightly larger than the diameter of the depressed annular region 30. In one embodiment, the membrane 6 is about 0.04 inches (about 0.1 cm) larger than the diameter of the depressed annular region 30 (which can be about 0.666 inches (about 1.7 cm) in a particular embodiment). While the membrane 6 is shown as a circle, many suitable shapes exist, such as a square or a rectangle. The membrane 6 can be manufactured from a plastic, such as polycarbonate or polyester. The membrane 6 can be track etched and typically is about 3 μm to about 14 μm thick. The pores through the membrane 6 are of suitable size and density for a particular application, depending upon, for example the size of the cells or particles to be captured on the membrane, the concentration in the sample of the cells or particles to be captured, and/or the deformability of the cells or particles to be captured. Also, the size, concentration, and/or deformability of sample components other than the cells or particles in the sample to be captured can be considered when choosing a pore size and/or density. Depending upon these and other considerations, an appropriate pore size is chosen to maximize the separation of and/or capture of relevant components of the sample. In one embodiment, the pores through the membrane have about a 5 μm diameter, and the membrane has a density of pores of about 4×10^5 pores/cm^2.

A retainer 4 secures and stretches the membrane 6 against the housing 2. The retainer 4 has an aperture 26 that aligns concentrically with the through-hole 28. Mounting apertures 8, 10, 12, 14 through the retainer 4 are provided in the corners of the retainer 4. These mounting apertures 8, 10, 12, 14 align with their respective mounting posts 16, 18, 20, 22. The mounting apertures 8, 10, 12, 14 are slightly smaller in diameter than the mounting posts 16, 18, 20, 22. A raised retainer ring 34 (shown in Figure 2B) that is concentrically disposed about the aperture 26 is included on the retainer 4 on the side of the retainer 4 that faces the membrane 6 and housing

2. In an alternative embodiment, the mounting apertures are located on the housing and the mounting posts are located on the retainer.

The housing 2, retainer 4, and membrane 6 are assembled into a unit (Figures 2A and 2B). During assembly of the cassette 44, the membrane 6 is placed over the through-hole 28, raised ring 32, and depressed annular region 30 of the underside of the housing 2. The retainer 4 is then placed on top of the membrane 6 such that the raised retainer ring 34 faces the membrane 6 and that each of the mounting apertures 8, 10, 12, 14 are aligned with their respective mounting posts 16, 18, 20, 22. The retainer 4 is forced down onto the mounting posts 16, 18, 20, 22 with, for example, approximately 80 pounds of force until the retainer 4 contacts the floor 42 of the cavity 24. The mounting posts 16, 18, 20, 22 engage the mounting apertures 8, 10, 12, 14 with a friction fit. When the retainer 4 and the housing 2 mate, the membrane 6 is captured by the housing 2 and the retainer 4. The raised retainer ring 34 engages the depressed annular region 30. The raised ring 32 pushes into the membrane 6. The radial force applied to the membrane 6 by the raised ring 32, raised retainer ring 34, and depressed annular region 30 pulls the membrane 6 taut. This peripheral interference fit system produces a membrane 6 engaged in the cassette 44 with a flatness tolerance suitable for sample capture, sample separation, and sample analysis. The membrane 6 can have a flatness tolerance of less than about 200 μm, preferably less than about 100 μm, and more preferably less than about 20 μm. For example, the surface of the membrane 6 lies between two planes that are separated by less than about 200 μm, preferably less than about 100 μm, and more preferably less than about 20 μm. The flatness tolerances mentioned above can be achieved, for example, over about a 10 mm diameter membrane. The membrane 6 is of a shape and/or size so that the membrane 6 does not interfere with mounting the mounting posts 16, 18, 20, 22.

A sample cavity 38 defined by the housing 2 is located on the side of the housing 2 opposite the cavity 24 and floor 42. The sample cavity 38, in this embodiment, is not as deep as

the cavity 24. When the membrane 6 is in place, the membrane 6 forms the floor of a sample sink 40 and the sides of the through-hole 28 define the sides of the sample sink 40. In some configurations the sample sink 40 can hold approximately 2 ml of a liquid. Depending upon the embodiment, a greater or lesser amount of liquid can be held by a sample sink. Typically, an absorbent reservoir 54 (Figure 4) is included in the cavity 24 that will pull fluid in the sample from the sample sink 40 through the membrane 6. The absorbent reservoir 54 can be constructed from, for example, cellulose acetate. Typically, the absorbent reservoir 54 is adhered to, or in substantially intimate contact with, the membrane 6. At least a portion of a sample in the sample sink 40 is drawn through the membrane 6 (for example, through the pores of the membrane 6) and into the absorbent reservoir 54 due to the absorbent reservoir's 54 absorbing character. The surface of the membrane 6 facing the sample (*i.e.*, the surface of the membrane 6 that forms the floor of the sample sink 40) captures components of the sample that are larger than the pores and/or are not sufficiently deformable to fit through the pores.

Referring now to Figure 3, the assembled cassette 44 can be retained within a cartridge 52. One manner to retain the cassette 44 is through the use of four spring fingers 36, only one being labeled for clarity, that are included in the housing 2. These spring fingers 36 can, for example, engage the cartridge 52 to hold devices of the invention in the cartridge 52 for promoting analysis of the sample. The cartridge 52 can be made from, for example, a plastic. The cartridge 52 can contain sample-holding and/or reagent wells 46, 48 and/or a disposal area 50.

During use of the cassette 44 assembled with the cartridge 52, a sample (for example, blood), that is external to the cassette 44 and cartridge 52, contained in a sample-holding well 46, 48, or contained in the sample sink 40, is combined with reagent(s) (for example, antibodies tagged with fluorochromes). The reagent(s) can be contained in a reagent well 46, 48 or can be contained external to the cartridge 52. Once the reaction(s) between the reagent(s) are at least

sufficiently complete to obtain a reading, if the reaction(s) were accomplished outside of the sample sink 40, the reacted sample is applied to the membrane 6 in the sample sink 40. The applied reacted sample wets the membrane surface and membrane pores. The absorbent reservoir 54 pulls at least a portion of the sample through the membrane as described above. The membrane region is then positioned in the path of an objective lens of an optical reader, such as the objective lens of a laser scanner. The captured sample on the membrane 6 is analyzed.

If a laser scanner is used to analyzed the sample, the laser scanner typically will have a depth of focus requirement. The depth of focus requirement for some laser scanning optics is about ± 10 μm, meaning that the membrane must be positioned within that tolerance over the course of a scan. Thus, the flatness tolerance of the membrane described above is desirable for ensuring that the surface of the membrane where sample is captured is within the laser scanning cytometer's range of focus.

The membrane can include at least three detectable coordinate points in order to facilitate determining the position of the surface of the membrane relative to the laser scanning cytometer lens. These markers, such as fluorescent markers, can be observable with the laser scanning cytometer. The three points define a plane of the surface of the membrane, and the laser scanning cytometer can use this plane for the purposes of analyzing and obtaining location data for a sample captured on the membrane.

In certain embodiments, substantially homogeneous flow through the membrane is facilitated. When applying liquid through a dry membrane, typically, uneven flow through the membrane occurs, for example, due to the fibrous nature of an absorbent reservoir. Liquid will flow preferentially where there is contact between the membrane and absorbent reservoir, leaving areas on the membrane surface where no flow occurs. The result of this preferential flow is an uneven distribution of analyte from the sample on the membrane. In some circumstances uneven

analyte distribution may cause aberrant measurements due to greater than expected densities of analyte on the membrane in areas of high flow through the membrane.

To produce a more homogeneous distribution of analyte across the entire membrane, components are used that allow for wetting the absorbent reservoir prior to applying sample to the membrane. The absorbent reservoir typically is not wet through the membrane, but, rather, is wet through an aperture in the device located away from the membrane mounting region. For example, in Figure 9A, a cassette 144 (in an exploded view and similar to the cassette 44 shown in figures 1, 2A, 2B, and 3), in relation to a cartridge 60 (described more fully below), includes a trough 160 in which the absorbent reservoir 54 sits. The absorbent reservoir 54 rests on a trough floor 168 and is in communication with a slot 166 and a notch 170, both molded into the trough 160. A wetting aperture 164 aligns with the slot 166, and the retainer 4 does not interfere with communication between the wetting aperture 164 and the slot 166. A secondary absorbent reservoir 162 contacts the absorbent reservoir 54 through the notch 170 and is located in a cut-out in the trough 160. The entire assembled cassette 144 and trough 160 fits within the cartridge 60 when assembled together.

A volume of a liquid (for example, but without limitation, a phosphate buffer solution) is provided through the wetting aperture 164 in the device. The liquid drops into the slot 166, bypassing the membrane 6 and retainer 4, and wets the absorbent reservoir 54, which is in proximity to the membrane 6. The liquid flows through the slot 166, which is typically a non-absorbing molded plastic structure, and saturates the absorbent reservoir 54. As the liquid saturates the region of the absorbent reservoir 54 closest to the membrane 6, the secondary absorbent reservoir 162 also is exposed to the liquid, through the notch 170. However, this exposure is not enough to saturate the secondary absorbent reservoir 162.

When sufficient wetting liquid is applied to saturate the absorbent reservoir 54, the absorbent reservoir 54 begins to shed liquid over at least some of its surface area. Because of the

close proximity between the membrane 6 and the absorbent reservoir 54, the liquid shed by the absorbent reservoir 54 fills a region 172 (best shown in Figure 9B) between the membrane 6 and absorbent reservoir 54 (for example, through capillary action), wetting substantially all of the bottom surface of the membrane 6. The region 172 typically is the area where the membrane 6 and the absorbent reservoir 54 are in substantially intimate contact. This contact can be characterized by, for example, a space between the membrane 6 and the absorbent material 54. At certain points, an absorbent material 54 that is in substantially intimate contact with the membrane 6 can touch the membrane 6 such that the space between the membrane 6 and the absorbent reservoir 54 can be irregular. Additionally, capillary forces fill substantially all of the pores in the membrane 6 with the liquid. When other liquids, such as processed sample, are applied to the top surface of the membrane 6, a substantially even flow occurs across the entire membrane 6. The secondary absorbent reservoir 162, which contacts the absorbent reservoir 54 through the notch 170, contains sufficient absorbing character (exercised via the notch 170 and the absorbing reservoir 54) to pull sample through the membrane 6, even when the absorbing reservoir 54 is saturated. This configuration allows for the absorbent reservoir 54 to saturate and remain saturated for a sufficient period of time to allow remaining assay steps to be processed without the liquid in the region 172 evaporating or otherwise escaping. Other absorbent reservoir configurations can be used if they produce a result that involves both filling the region between the membrane and absorbent reservoir while still allowing sample to be wicked through the membrane, particularly in a substantially homogeneous manner. For example, the absorbent reservoir could be a single unit composed of two areas, each with a different absorbing character.

Because of the wetting step, when a sample is applied to the sample sink, the sample can flow through the membrane in a substantially even fashion. Additionally, because the secondary absorbent reservoir 162 is not fully saturated (*i.e.*, has the capacity to absorb additional liquid), the sample is pulled through the membrane 6. The size of the notch 170 is one way to control

how much liquid saturates the secondary absorbent reservoir 162 as the absorbent reservoir 54 becomes substantially saturated.

Filling the region 172 between the absorbent reservoir 54 and the membrane 6 with a liquid rather than adhering the absorbent reservoir to the membrane avoids uneven flow and/or altering the desired flatness tolerance of the membrane. Additionally, even if the absorbent reservoir is not adhered to the membrane, air trapped in the region 172 can cause uneven flow through the membrane and/or uneven distribution of sample in the sample sink. Allowing liquid flow into the region 172 can reduce these problems associated with trapped air. Moreover, other configurations of components can accomplish the same result of filling the region 172 with a liquid to create substantially homogeneous flow.

In certain embodiments, all or part of steps to analyze the sample occur with the cassette engaged with the cartridge and with the cartridge inserted into an analysis device that includes a laser scanning cytometer. The sample is placed into one of the wells, and the cartridge with cassette is placed into the analysis device. The sample is mixed with reagent(s) such as a label that the laser scanning cytometer can detect. For example, the sample can be stained with a fluorescent dye. Multiple labels can be used at once to detect multiple properties of the sample. As an example, a sample can be analyzed for a nucleic acid present in the sample, an enzyme present in the sample, a receptor present in the sample, and/or a cell type present in the sample. Also, the wells can contain various substances, such as a drug, for application to the sample. The sample may, for example, be stained with a different label if exposed to a drug than if it is unexposed to a drug. Alternatively, competition and/or kinetic studies can be performed. Other tests are possible, and those mentioned above are meant merely to illustrate the wide range of uses for the present invention. Once the sample is mixed with the reagent(s) from the wells, the sample is applied to the sample sink, captured on the membrane, and detected.

The procedure can be fully automated. For example, the analysis device can locate the various wells and sample sink, appropriately mix the materials located in the wells, and apply the sample to the sample sink according to a predetermined procedure. Transfers can occur with a robotic pipetting mechanism and pipette tips can be discarded in the disposal area to avoid cross-contamination of the wells. Once the sample is captured on the membrane, the laser scanning cytometer is used to detect a characteristic of the sample. Because cassettes according to the invention have a particular flatness tolerance, such as the ones described above, the laser scanning cytometer can directly analyze the sample as captured on the membrane.

Now referring to Figures 5-8B, in an alternate embodiment of the invention that is similar to the embodiment shown in Figure 1, two sample locations are provided. The housing 62 of the cassette 79 has two through-holes 68a, 68b disposed within the housing 62. Membranes 66a, 66b are held over the through-holes 68a, 68b with retainers 64a, 64b. Disposed concentrically about the through-holes 68a, 68b are raised rings 112a, 112b and depressed annular regions 110a, 110b. Projecting from a floor of the cavity are mounting posts 102a, 102b, 104a, 104b, 106a, 106b, 108a, 108b. The mounting posts 102a, 102b, 104a, 104b, 106a, 106b, 108a, 108b each have a flat top and a rounded edge that meets a generally columnar structure. The mounting posts 102a, 102b, 104a, 104b, 106a, 106b, 108a, 108b are shown arranged in a generally square configuration surrounding the through-holes 68a, 68b.

The membranes 66a, 66b fit over the through-holes 68a, 68b, raised rings 112a, 112b, and depressed annular regions 110a, 110b and contact the housing 62. The membranes 66a, 66b are slightly larger than the diameters of the depressed annular regions 110a, 110b. The membranes 66a, 66b in this embodiment can have the same properties, can be used in a similar manner, and can be constructed from a similar material as the membrane described above. Retainers 64a, 64b stretch the membranes 66a, 66b and secure them against the housing 62. The retainers 64a, 64b each have an aperture 118a, 118b that aligns concentrically with the through-holes 68a, 68b.

Mounting apertures 92a, 92b, 94a, 94b, 96a, 96b, 98a, 98b through the retainers 64a, 64b are provided in the corners of each of the retainers 64a, 64b. These mounting apertures 92a, 92b, 94a, 94b, 96a, 96b, 98a, 98b align with their respective mounting posts 102a, 102b, 104a, 104b, 106a, 106b, 108a, 108b. The mounting apertures 92a, 92b, 94a, 94b, 96a, 96b, 98a, 98b are slightly smaller in diameter than the mounting posts 102a, 102b, 104a, 104b, 106a, 106b, 108a, 108b. Raised retainer rings 120a, 120b that are concentrically disposed about the apertures 118a, 118b are included on the retainers 64a, 64b on the side of the retainers 64a, 64b that faces the membranes 66a, 66b and housing 62. Two absorbent reservoirs 74a, 74b are located underneath the membranes 66a, 66b and retainers 64a, 64b in indentations 72a, 72b in a cartridge 60. These absorbent reservoirs 74a, 74b can be used to draw a sample through the membrane in a manner similar to that described above. In an alternative embodiment, a retainer is a single piece but has the two apertures as well as the surrounding structures for radially stretching the two membranes.

Because this embodiment has substantially similar parts to those shown in Figure 1, the housing 62, retainers 64a, 64b and membranes 66a, 66b are assembled into a unit in a similar manner to that described for Figure 1. The membranes 66a, 66b are pulled taut as described above and have a flatness tolerance similar to that described above. Because the membrane assembly in this embodiment is similarly constructed and assembled as the one described above, each membrane 66a, 66b forms the floor of a sample sink, and the walls of the apertures 118a, 118b form the sides of the two sample sinks. Thus, two samples are captured on the membranes 66a, 66b in conjunction with the absorbent reservoirs 74a, 74b and can be analyzed simultaneously. Analysis occurs as described above.

Four spring fingers 76 (only one is labeled for clarity) on the housing 62 interact with four respective mating portions 78 (only one is labeled for clarity). Each mating portion 78 has a lip that engages a notch in each spring finger 76. The spring finger 76 is resilient and is moved slightly towards the center of the housing 62 as the spring finger 76 enters the mating portion 78

to slide the leading edge of the spring finger 76 past the mating portion 78 lip. Once the notch is aligned with the lip, the spring finger 76, due to its resiliency, returns to its starting position, away from the center of the housing 62, locking the housing 62 to the cartridge 60.

The cartridge 60 itself (shown, for example, in Figures 8A and 8B) has multiple sample holding and/or reagent wells 86, 88 (only some being labeled for clarity) and a disposal area 80. These areas are used in a similar manner to those described above. On the underside, the cartridge 60 has stabilizing bands 122 (only one being labeled for clarity) that keep the cartridge in a desired shape and keep the wells 86, 88 and/or disposal area 80 from deflecting. The configuration of this cartridge 60, in combination with the dual sample sink cassette 79, allows for two samples to be processed simultaneously. In certain embodiments, multiple sample sinks and corresponding wells can be used to process more than two samples simultaneously. This cartridge and cassette assembly is suitable for use with an automated, preprogrammed analyzer as described above.

Certain dual sample sink embodiments are adapted to create a substantially homogeneous flow of sample through the membranes 66a, 66b. The description above for homogenous sample flow in single sample well embodiments, relating to filling the region between an absorbent reservoir and a membrane with a liquid, such as that shown in and described for Figures 9A and 9B, is applicable to dual sample well and multiple sample well embodiments. Now referring to Figure 10, a slightly modified version 179 of the embodiment 79 shown in Figures 5-8B has a region between each one of the membranes 66a, 66b and each membrane's respective absorbent reservoir 74a, 74b. These regions are filled with a liquid to facilitate a substantially homogeneous flow through the membranes 66a, 66b. Generally, liquid is provided through either one of or both of two wetting apertures 264a, 264b in the housing 62. Each wetting aperture 264a, 264b aligns with a corresponding slot 266a, 266b molded into one of two troughs 260a, 260b, respectively. In this case, the wetting apertures 264a, 264b are located along a midline of

the housing 62. When liquid is dropped through either of the wetting apertures 264a, 264b, the liquid bypasses the membranes 66a, 66b and the retainers 64a, 64b due to the position of the wetting apertures 264a, 264b and the slots 266a, 266b. The absorbent reservoirs 74a, 74b sit in the troughs 260a, 260b on the trough floors 268a, 268b and are aligned with the membranes 66a, 66b. One region (that is ultimately filled with liquid) is formed between one membrane that is aligned with one absorbent reservoir (*i.e.*, there are two regions, one formed between each one of the membranes that is aligned with its respective absorbent reservoir). A single-piece secondary absorbent reservoir 262 contacts both absorbent reservoirs 74a, 74b through the two notches 270a, 270b in the two troughs 260a, 260b. The secondary absorbent reservoir 262 typically does not contact the two absorbent reservoirs 74a, 74b except at the notches 270a, 270b. The two troughs 260a, 260b abut, and the secondary absorbent reservoir 262 mates with the two troughs 260a, 260b in a nested mating configuration.

Liquid is dropped through the wetting apertures 264a, 264b and into the slots 266a, 266b of the troughs 260a, 260b. The liquid runs through the slots 266a, 266b and wets the absorbent reservoirs 74a, 74b. Saturation of the absorbent reservoirs 74a, 74b with liquid, filling of the regions between the membranes 66a, 66b and the absorbent reservoirs 74a, 74b with liquid, and filing of the pores of the membranes 66a, 66b with liquid occur in a similar manner to that described above for the single sample well embodiments. Additionally, the absorbent reservoirs 74a, 74b and the secondary absorbent reservoir 262 interact through the two notches 270a, 270b and pull sample through the membrane 66a, 66b homogeneously, as described for single sample well embodiments. The two trough configuration allows the assembled cassette 179 to function as intended. Fingers 272a protruding from the troughs 260a, 260b (only one finger is labeled for clarity) extend from the troughs 260a, 260b to surround the mating portions 78 upon assembly of the cassette 179 with the cartridge 60 and are arranged such that slots are formed between the trough fingers 272a to accommodate the spring fingers 76 as they pass through the troughs 260a,

260b and mate with the mating portions 78. In certain embodiments the troughs can be a single unit rather than individual units, and, also, the secondary absorbent reservoir can be two pieces or a single piece that includes the absorbent reservoir.

Claims

What is claimed is:

1. A device for separating a component from a biological sample, the device comprising:

a platform defining a first opening;

a membrane; and

a mount defining a second opening that mates with said first opening, wherein said mount and said platform engage in a peripheral interference fit such that said membrane is stretched radially between said first and second openings.

2. The device of claim 1, wherein said platform comprises, on a first surface, a sink capable of containing a sample and, on a second surface, a cavity capable of containing an absorbent material.

3. The device of claim 2, wherein said first opening is cylindrical and wherein said platform defines, on said second surface, a raised ring that is concentric with said first opening and an annular depression that is concentric with said first ring.

4. The device of claim 3, wherein said mount comprises a flange for radial interference mating with said ring, said flange being concentric with said second opening.

5. The device of claim 2, wherein said platform comprises a plurality of posts on said second surface and wherein said mount comprises a plurality of apertures for receiving said posts when said mount engages said platform.

6. The device of claim 2, wherein said mount comprises a plurality of posts and wherein said platform comprises a plurality of apertures for receiving said posts when said mount engages said platform.

7. The device of claim 1, wherein said membrane is a track etched membrane.

8. The device of claim 3, wherein said membrane is larger in diameter than said annular depression.

9. The device of claim 1, wherein said membrane further comprises at least three detectable coordinate points for determining a position of a surface of said membrane.

10. The device of claim 9, wherein said three points comprise optical markers.

11. The device of claim 10, wherein said optical markers comprise fluorescent markers.

12. The device of claim 10, wherein said optical markers are detectable by laser cytometry.

13. The device of claim 2, further comprising an absorbent material.

14. A cartridge for analysis of a cellular sample, the cartridge comprising a housing adapted to contain a device according to claim 1.

15. The cartridge of claim 14 comprising a device according to claim 1.

16. The cartridge of claim 15 further comprising at least one well for placement of a reagent.

17. A method for separating cells from a biological sample, comprising applying a biological sample to a device according to claim 1.

18. An assay to determine a cellular characteristic of a biological sample, the method comprising the steps of:

obtaining a biological sample;

exposing said sample to one or more detectably labeled reagents capable of reacting with a cellular component;

applying said sample containing said one or more reagents to a device of claim 2, thereby to form a captured sample on said membrane; and

analyzing said captured sample to determine at least one cellular characteristic of said biological sample.

19. The method of claim 18, wherein said characteristic is selected from the group consisting of a nucleic acid, an enzyme, a receptor, and a cell type.

20. The method of claim 18, wherein said device further comprises an absorbent material.

21. The method of claim 20, further comprising the step of wetting the absorbent material with a liquid, thereby to fill a region between the absorbent material and the membrane with the liquid.

22. The method of claim 21, wherein the sample flows substantially homogeneously through the membrane.

23. A device for separating a component from a biological sample, the device comprising:

a platform defining a first opening, wherein said platform defines a cavity;

an absorbent material contained within said cavity;

a membrane;

a mount defining a second opening that mates with said first opening, wherein said mount and said platform engage in a peripheral interference fit such that said membrane is stretched radially between said first and second openings; and

a trough for introducing a liquid to a region between said membrane and said absorbent material, thereby to induce a sample to flow substantially homogeneously through said membrane.

24. The device of claim 23, wherein said trough contains said absorbent material.

25. The device of claim 23, further comprising a secondary absorbent material in contact with said absorbent material.

26. The device of claim 23, wherein said platform defines a third opening, said third opening aligning with said trough.

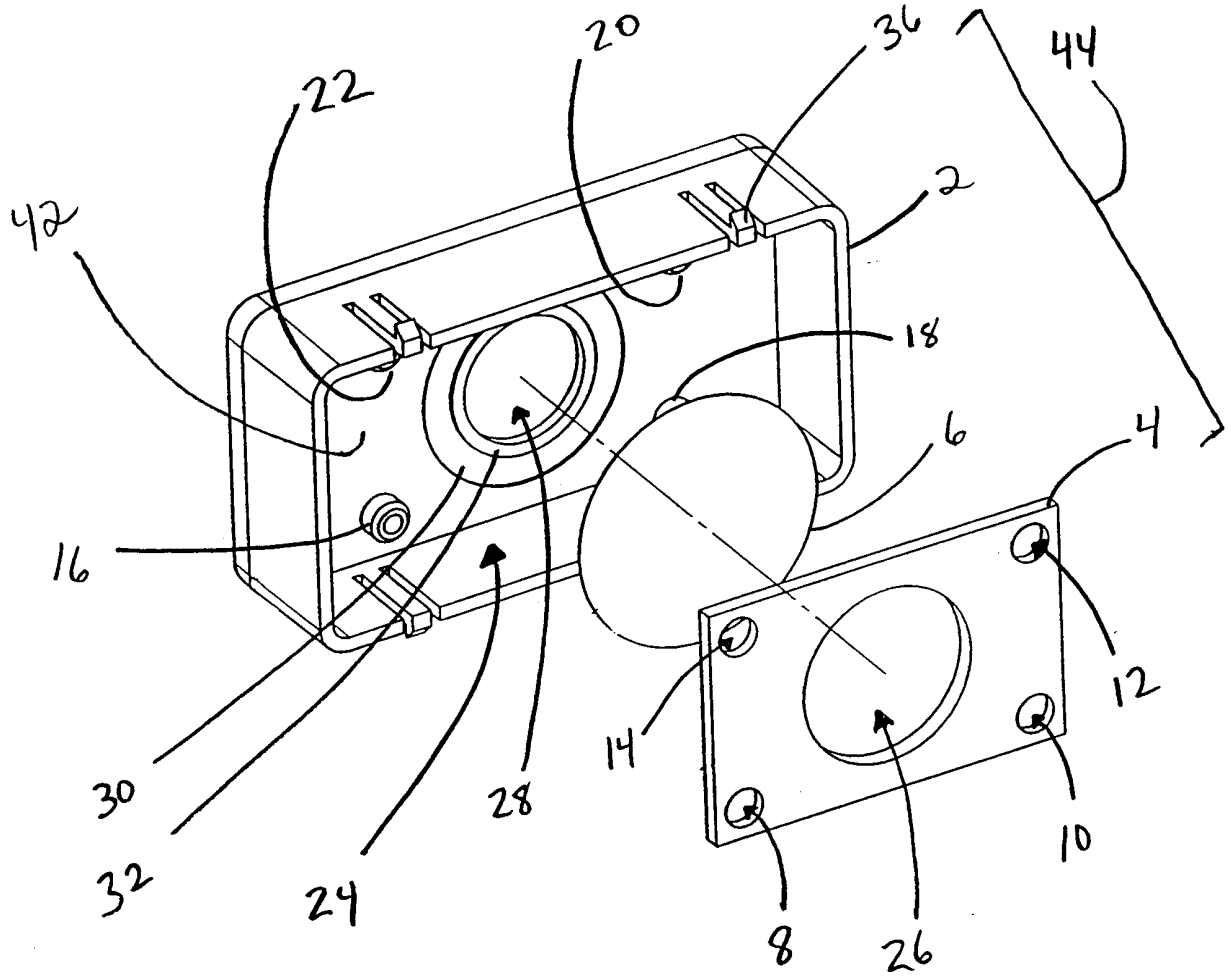

Figure 1

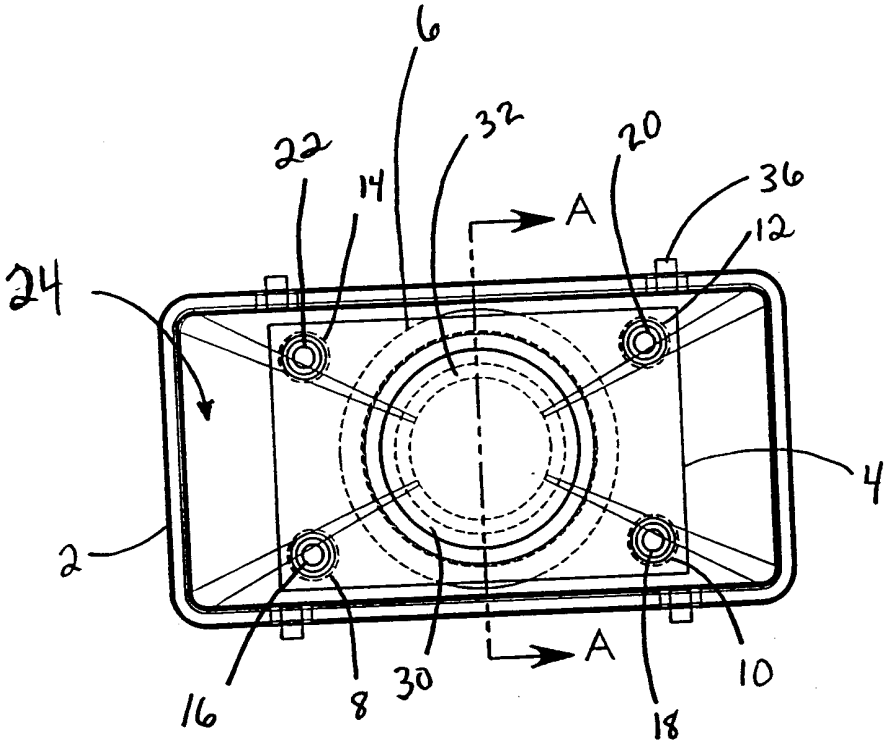

Figure 2A

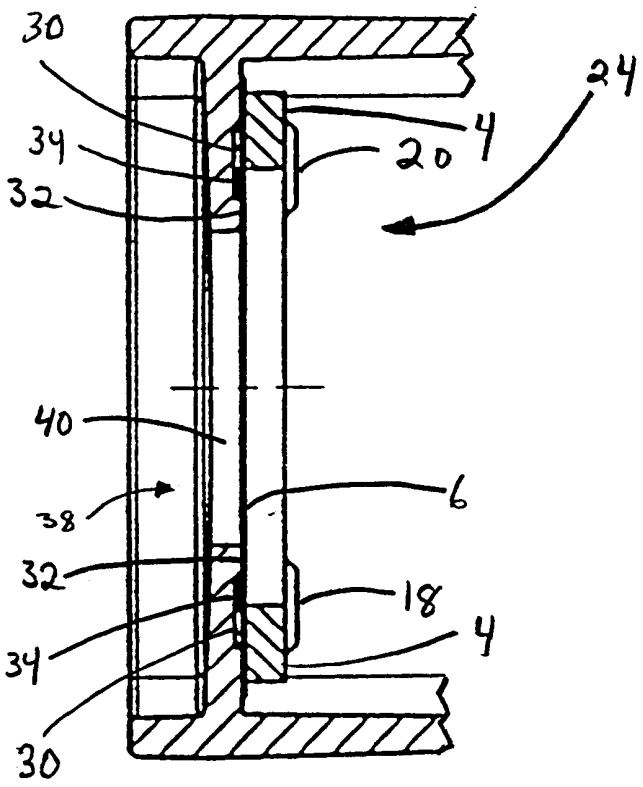

Figure 2B

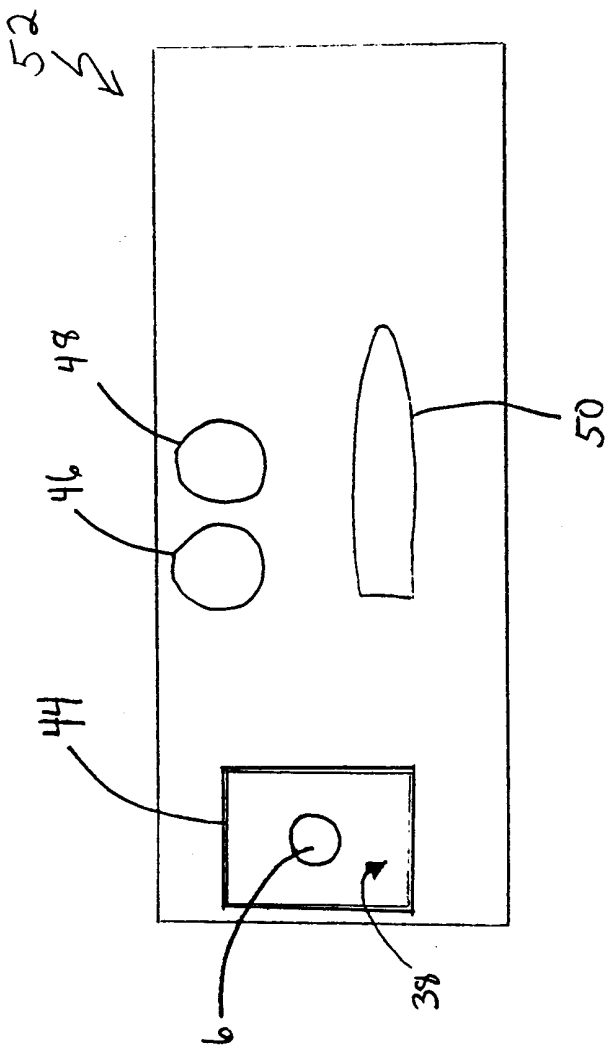

Figure 3

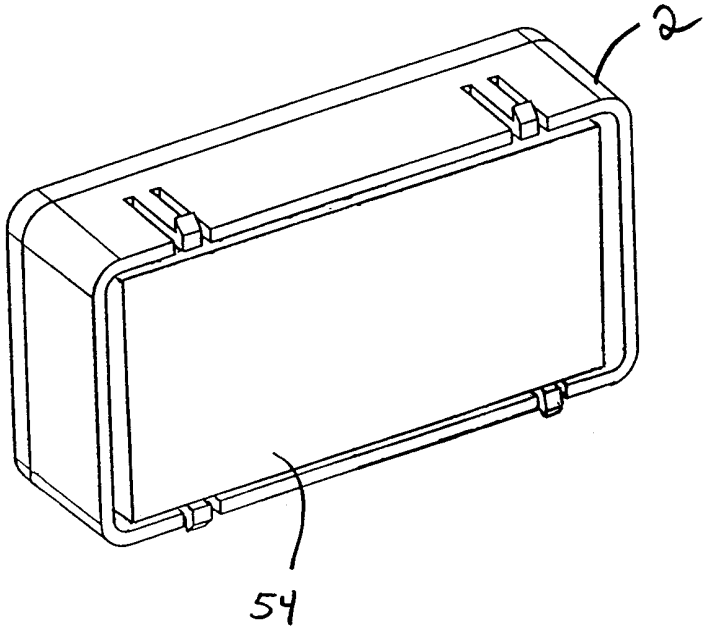

Figure 4

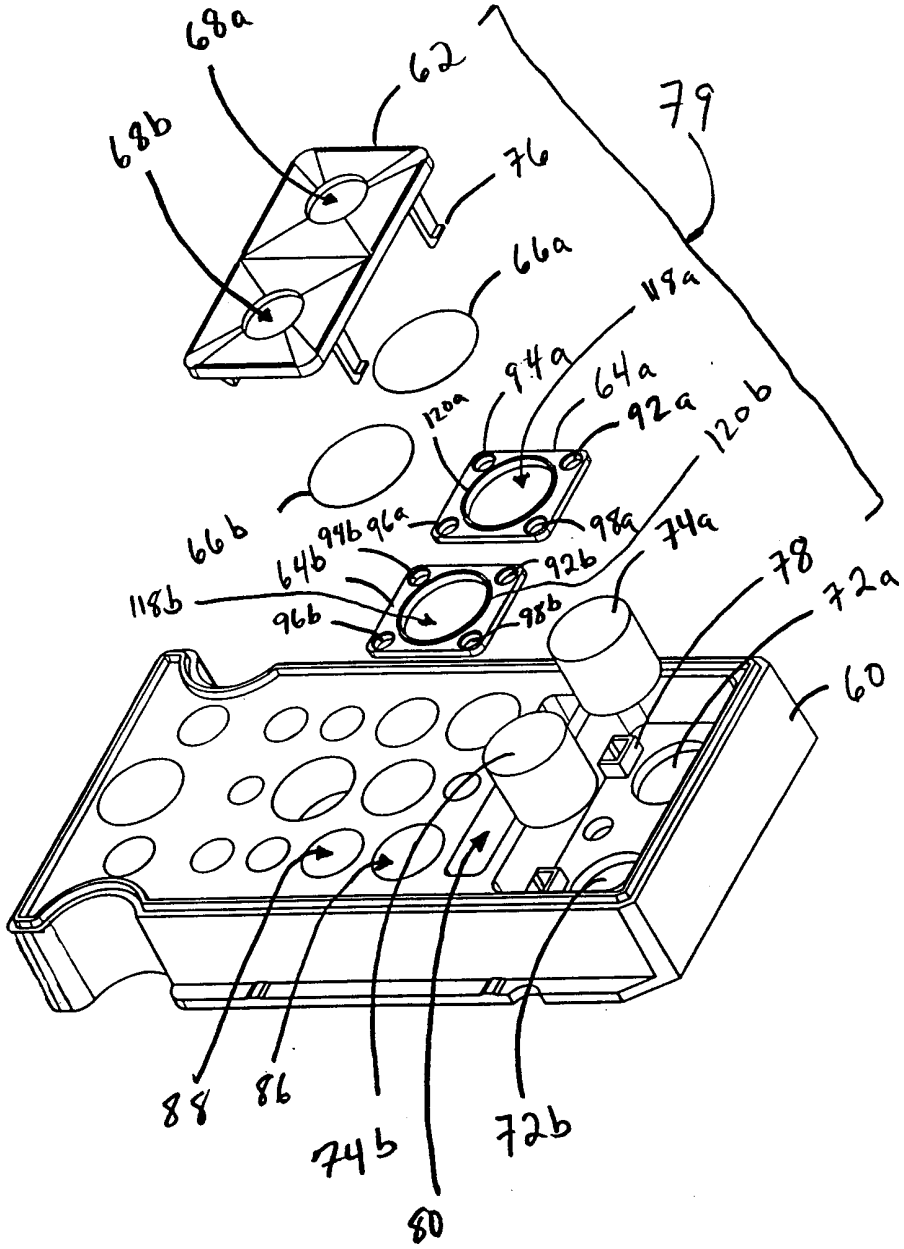
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Figure 5

Figure 6A

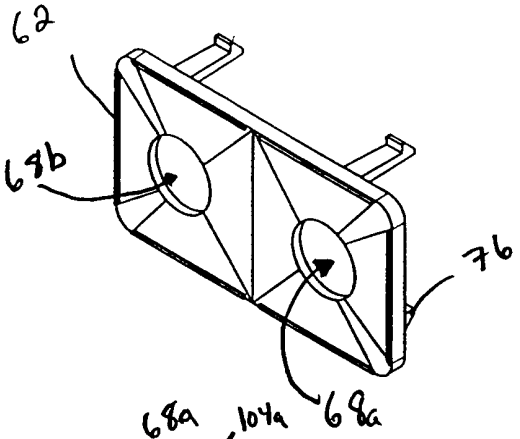


Figure 6B

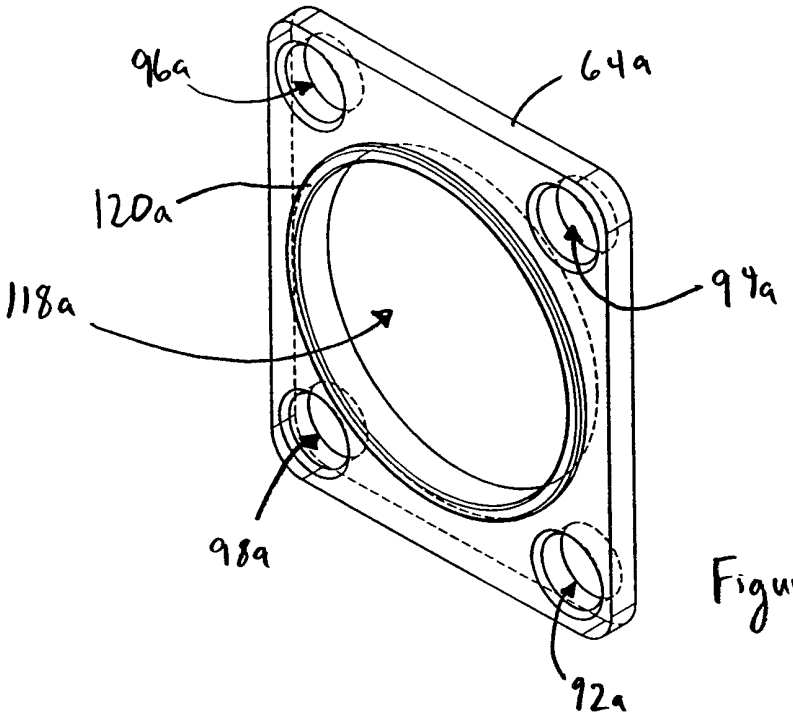


Figure 7

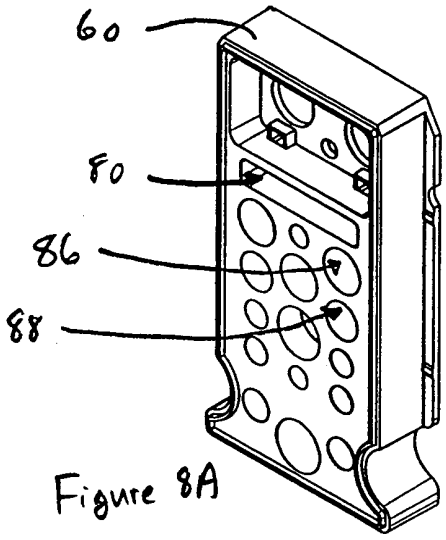


Figure 8A

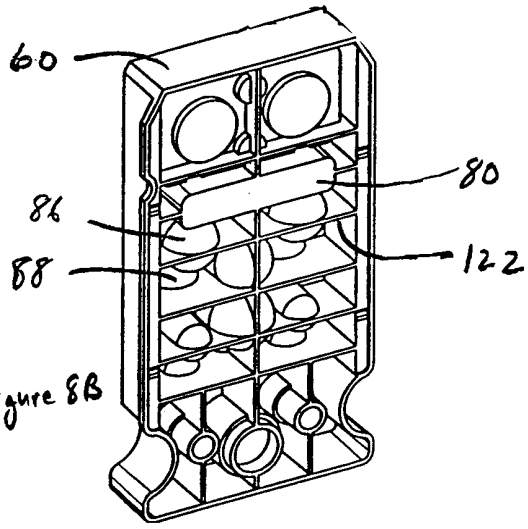


Figure 8B

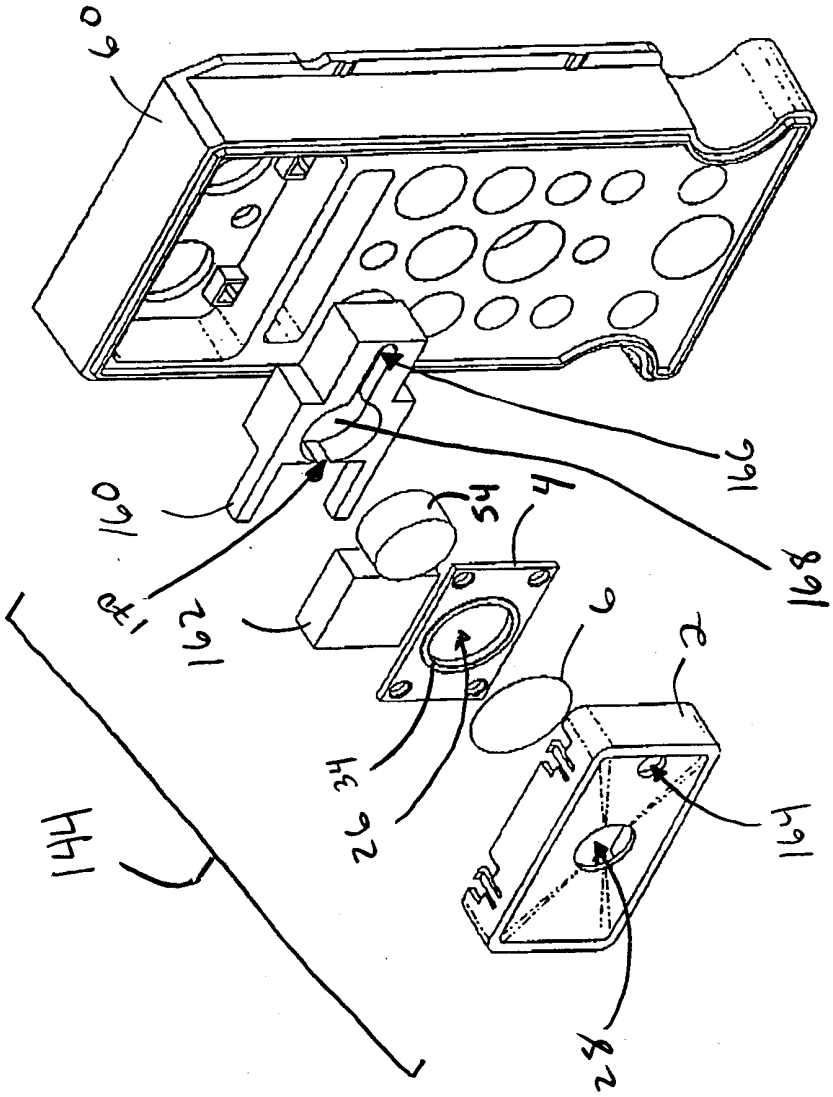


Figure 9A

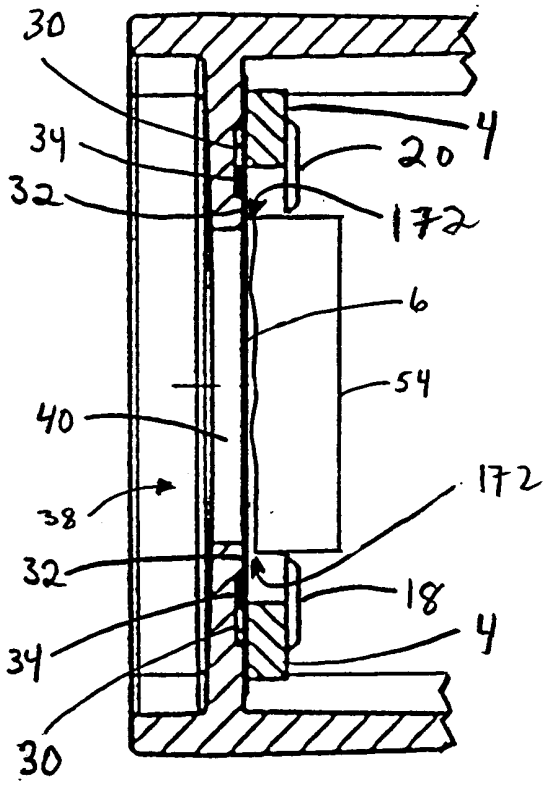
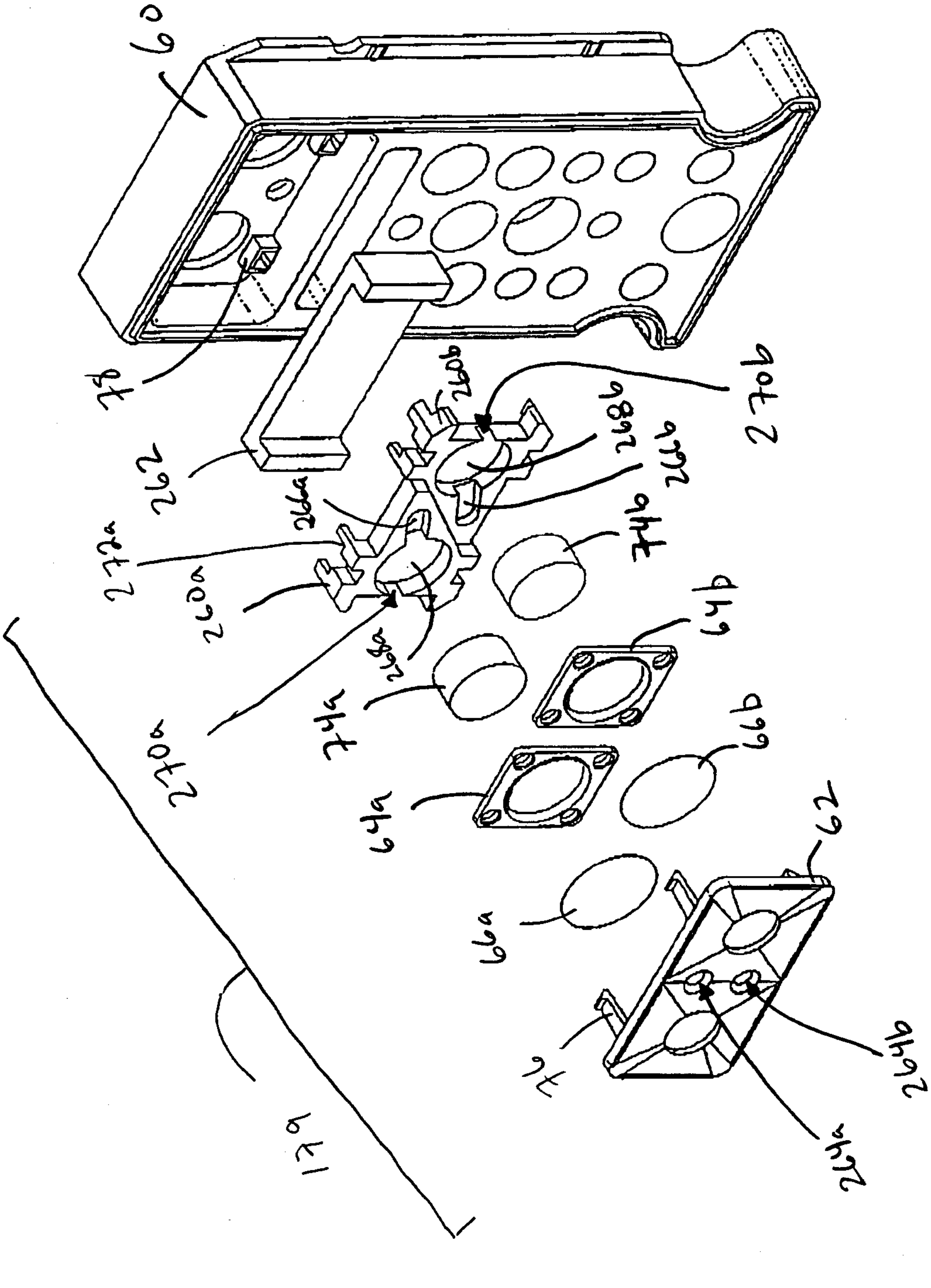


Figure 9B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264b
179

Figure 10