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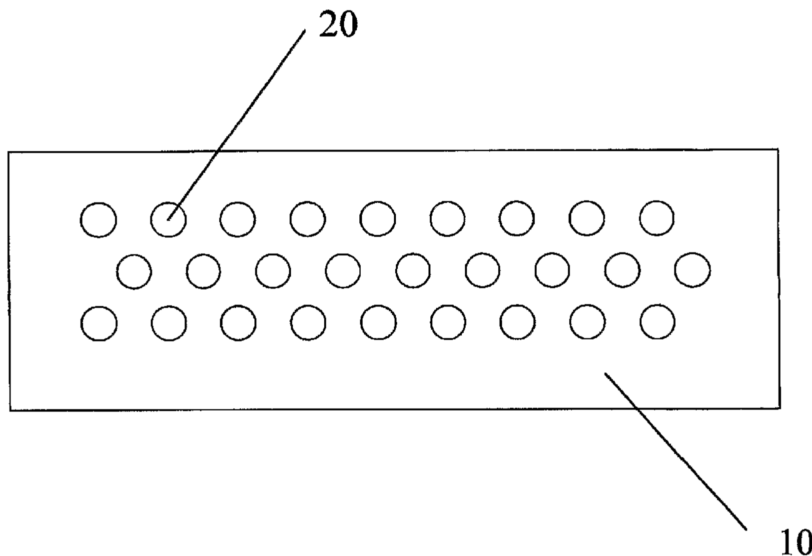
(54) Title: BIOSURFACES FOR RECEIVING, RETAINING & DISPLAYING BIOLOGICAL MOIETIES



(57) Abstract: This invention has application in a variety of biological and biochemical fields. The invention describes multilayer substrates that are configured to receive and retain biological entities or molecules suspended in a fluid that is passed through any of a plurality of separate and distinct permeable locations on the substrate. The permeable features arise from at least one of the layers of the multilayer substrate. The purpose of the retention includes, but is not limited to, further interaction with other biological molecules, further processing, or examination of said biological moieties at the locations on the substrate.

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BIOSURFACES FOR RECEIVING, RETAINING & DISPLAYING BIOLOGICAL MOITIES

RELATED APPLICATION

This application is related to and claims priority from U.S. provisional application entitled "Biosurfaces for Receiving, Retaining & Displaying Biological Moieties," serial number 60/819,619, filed July 10, 2006, which provisional application is incorporated by herein by reference in its entirety.

TECHNICAL FIELD

[0100] The present invention relates to substrates that are configured to receive and retain biological entities or molecules from a fluid passing through any of a plurality of separate and distinct permeable locations of the substrate. The purpose of the retention includes, but is not limited to, further interaction with other biological molecules, further processing, or examination of said biological moieties at the locations on the substrate.

BACKGROUND ART

[0101] The preparation of biological specimens is a well-established science. Techniques for capturing cells, tissues or other larger biological entities or moieties are well known. It is customary to place the specimen onto a glass slide (of standard size i.e. 25mmx75mm) in preparation for any appropriate processing. For cells and tissue, this usually involves some form of staining to highlight structures within the specimen. Staining produces the appropriate level of contrast in the structure so that a skilled observer can determine the condition of the specimen.

[0102] A common technique for separating biological moieties from a suspension involves passage through a permeable membrane, usually referred to as a filter medium. There is considerable art associated with filtration. Usually filtration involves multiple steps. In particular, a filtration step, a substrate preparation step, a transfer step and an analysis step. The present invention permits the reduction in the number of steps of this process.

[0103] In general, the reduction of a three-dimensional specimen objects to a two-dimensional format is required in order to permit viewing by high magnification devices such as microscopes. Clearly this reduction causes some loss of valuable positional information. In many cases (i.e. tissue samples) the three dimensionality is regained by viewing (and comparing) adjacent slices of the specimen in the two dimensional format.

[0104] With smaller biological entities, the methods are more limited. Specimens are simply transferred to a glass slide often by a simple smearing process. In other situations, specimens are placed (often in solution form) onto the glass slide surface. The flat, smooth surface of the slide is a limit to the spacing of the individual components of the specimen.

[0105] There are additional problems associated with the hydrodynamics of fluids close to the surface of the slide. There is usually a stationary layer that is contiguous to the slide surface. The ability to access specimens that lie within this layer is governed by diffusional considerations. Thus specimen processing can take a long time. This also applies to other interactions such as hybridization. This invention permits the passage of other fluids directly through the permeable locations. The intimate contact between these fluids and the retained molecules accelerates the processing steps.

[0106] "Passive" biosurfaces (such as glass microscope slides) neither interact nor amplify. The present invention addresses these issues in terms of an "active" biosurface where the structure and properties of the substrate enhances the preparation, processing and display of the specimen. Such surfaces can also amplify the information derived from the specimen.

[0107] This invention addresses two aspects of this process. The use of permeable surfaces to separate (i.e. filter) one class of biological moieties from a complex mixture is well known. The use of such a single substrate with permeability at selected sites that subsequently become display sites is considered novel. The permeable locations receive specimen material only at the sites thus forming the display locations. The three dimensional nature of the permeable locations allows much more efficient mixing and overcome the laminar layer problem. The properties of the material within the permeable locations alter the binding characteristics and lead to larger signal or better retention.

SUMMARY OF THE INVENTION

[0108] The present invention comprises a substrate that is configured to allow the passage of a fluid containing several kinds of biological moieties (i.e. cellular structures, antibodies, proteins etc) through specific permeable substrate locations. As the fluid passes through these locations, biological entities that are large are not able to pass through the material. In some cases, the substrate material is modified to assist in adhering the biological moieties to the surface. In other cases, the permeable material comprises elements that enhance the ability to view the moieties upon the substrate. Other smaller moieties and the balance of the fluid will pass through the material via the defined locations.

[0109] The present invention comprises a material that provides a barrier to biological entities (in this case cellular organisms) that are greater than a predetermined size. Non-cellular entities that are smaller than this size are able to pass through the material. The surface of the material is configured such that the cellular entities are retained and adhered to specified areas of the material. The non-specified areas of the material have no ability to retain and adhere cellular entities.

[0110] In a preferred version, the adherent areas of the material are also hydrophilic. The non-specified areas are generally hydrophobic. The hydrophobic area of the surface will prevent the flow of fluid through the surface. There will be a preferential flow through the hydrophilic areas. This flow will migrate the cellular entities to the hydrophilic areas of the surface.

[0111] The material may be secured to a mesh or other support for the purpose of providing mechanical strength. Alternatively, the material may have sufficient mechanical strength to act as a structural member.

[0112] The invention can be extended to include a laminate assembly of at least two layers of different material. The first material is permeable at all locations. The pores are small enough to deny passage to biological moieties above a certain size and yet still allow free passage of smaller biological moieties and any fluid medium in which such

moieties are dissolved or suspended. The surface properties of the material are configured to retain and adhere large biological moieties to the surface of the material.

[0113] The second material is an impervious sheet of material containing a number of separate and distinct holes through the sheet surface. Biological moieties are non-adherent to any part of this second sheet.

[0114] A surface of first material is joined with a surface of the second material. A solution or suspension of biological moieties, when presented to this composite assembly will only flow through the assembly at locations defined by the holes in the second sheet of material. The pores defined by the first sheet will prevent passage of biological moieties that exceed a certain size. Small biological moieties will pass through to a substantial extent. In addition to defining locations, the second material provides some mechanical support to the assembly.

[0115] The invention may include a third sheet of impervious material with a substantially similar pattern of holes such that the two impervious sheets provide a first and third layer with the permeable material being the second sheet in a sandwich-like construction. Such an assembly may better mechanical properties but performs essentially the same function. The first and third sheets of materials may be joined along a common edge to form a hinge. The assembly may be closed by applying pressure to the outer faces of the first and third sheets. A method of clamping the non-hinged edges may be included.

[0116] The complete assembly may be used for further processing and examination. Alternatively, the permeable sheet may be removed from the composite assembly and subjected to further processing or examination.

[0117] The permeable sheet may be part of a continuous film or ribbon. The film or ribbon could be moved by a step and repeat process through two perforated impermeable plates that close onto the permeable film of ribbon while the suspension is passed through the composite structure. A second step involves releasing the film or ribbon, moving it along until a new section of the film or ribbon is in place between to two plates. The process is repeated.

[0118] The invention also incorporates multiples of the assembly. Several such sandwich assemblies are formed into a single assembly. The characteristics of the non-woven mat within each sandwich are set to capture a biological moiety of a specific size. Smaller biological moieties that pass through the first sandwich assembly may be captured by the second sandwich assembly. Such a progressive separation may be beneficial in analyzing the distribution of sizes with the population of biological moieties. Alternatively, several sheets with varying permeability may be sandwiched to achieve a similar purpose.

[0119] In particular, this invention comprises the use of laminates of materials that provide a plurality of sites on one surface of the substrate wherein the fluid suspension is able to pass through and leave behind certain biological moieties retained by at least one of the layers within the substrate. The permeability of the locations within the surface also permit easy passage of chemicals designed to prepare the retained biological moieties for further viewing. In other words, the permeable locations not only retain the larger moieties but also provide a support for interaction with other smaller molecules designed to make said moieties easier to view.

[0120] In an alternative use of this assembly, passage of smaller biological moieties through the body (and pores) of the permeable material results in the biological moieties being in contact with the internal walls of the permeable material. It is important that the pore size be appropriate for the passage of these biological moieties through the body of the material. The pore walls of the material may be configured to have received and retained (i.e. anchored) one type of biological moieties in a previous process. In this event, the passage of a second series of biological moieties (i.e. there may be several different kinds of biological molecules) will bring the first and second sets of moieties into close and frequent proximity. If it is possible for certain members of second set of biological moieties to couple together (i.e. hybridize or bind in some way), then the first set of moieties anchored to the walls of the material will couple or bind those members of the second set of biological moieties that match in some way (i.e. are complementary or have surface characteristics that permit binding i.e. antigen to antibody). The rate at which interactions occur will now be dictated by the flow of the fluid through the

material not, as previously, by diffusion processes. Coupling reactions within this kind of medium will proceed to completion at a much faster rate that with an impervious).

[0121] An additional benefit of the invention is that the configuration of the material results in the increase in the number of sites at which interactions can occur. This will increase the number of interactions and will, if some signaling molecules are used to indicate a successful interaction, provide a larger signal.

[0122] Any material described by this invention may be either fully flexible or substantially rigid or combinations thereof

[0123] This invention incorporates a device for capturing the substrate and introducing a series of fluids to one or more of the permeable locations of the substrate for the purpose of further processing the biological moieties received or retained at said locations.

[0124] This device, in its least form, comprises two plates that capture the substrate during the process of flowing at least one of plurality of fluids through said substrate.

[0125] This invention also incorporates devices for the viewing of biological moieties that have been received and retained at any of the permeable locations of said substrate. The viewing of said permeable locations may be directly optical means or by the use of other detection methods including but not limited to fluorescence, radioactivity, or color changes.

[0126] In both cases the use of a movable ribbon through either device is advantageous.

BACKGROUND OF THE INVENTION

[0127] Pathology is the study and diagnosis of the structural and functional changes in cells, tissues and organs that underlie disease. This often involves placing the cells on a surface so that they can be viewed and interpreted. This leads to a need for specific surfaces, called substrates, which are suited for receiving and retaining cells. The

properties of these surfaces, often called biosurfaces, are crucial to the ability of the pathologist to determine the dysfunction associated with the cells arrayed on the surface.

[0128] There are many other biological entities, usually less complex, that can assist in the diagnosis of disease. Examples include antibodies, proteins, antigens, chromosomes and genes. In all cases, it is also highly advantageous to lay these biological entities onto a flat surface so that their interactions can be catalogued. Again a biosurface is a vital part of the procedure. This can permit many useful interactions that result in a diagnostic tool.

[0129] Presently, most biosurfaces comprise either plain, flat glass (in one of several forms but, in particular, microscope slides) or plastic plates of wells arranged in a matrix format (called multi-well or microtiter plates). In general these biosurfaces are substantially smooth. In some cases the physical attributes of the surface assist in the function of the biosurface. Glass has a naturally positive charge and so easily attracts and retains cells. In other cases, various treatments are given to enhance this property. It is known, for instance, that certain plasma treatments will increase the ability of plastic surfaces to retain cells onto their surfaces. With other biological molecules, such as oligonucleotides, some form of linker molecule is used to bridge (i.e. by covalent bonding) between the biosurface and the biological moieties.

[0130] The ability to alter a biosurface so that its performance is improved will enhance the ability of the pathologist to quickly determine dysfunction and to provide a diagnosis.

[0131] This invention describes a class of novel biosurfaces that result in more effective retention of cells and other biological entities upon the surface.

[0132] Two particular applications will benefit from this invention. The first, Liquid Based Cytology, requires the isolation of cells from a suspension with other biological materials and permits the cells to be subject to further examination. These shall be called "Separation Assays". The second application involves tests that require the interaction of two distinct molecules or entities where the successful interaction needs to

be detected. Microarrays (of all types), ELISA assays and many other assays fall into this second category. These shall be called "Coupling Assays".

Separation Assays

[0133] Liquid Based Cytology ("LBC"), as a test for cervical cancer in women, has rapidly displaced the conventional PAP test as a means of detecting cancerous cells. The conventional PAP test relies upon smearing the entire sample onto a simple microscope slide. A battery of processing steps prepares the slide for a visual examination by a skilled cytologist. There are many drawbacks to the procedure. In particular, not all the cells are easily visible due to "clumping" (i.e. the cells are not properly disaggregated and so are obscured). The ability to view the cell is reduced by the presence of blood, mucus and other bodily fluids. Notwithstanding the success of this simple test, LBC addressed these problems. There are several variants of LBC. All involve some form of disaggregation within a liquid medium followed by some form of separation of the cells from the other biological molecules. In the final step, the separated cells are transferred to a flat slide for eventual viewing. In the most popular version of LBC, the "ThinPrep" method, this transfer involves a dabbing process that transfers cells from the outer face of a complex filter assembly to a microscope slide. Usually, the transfer attempts to keep all the cells within a single circular area of 21 mm. This transfer is often incomplete and cells can be left behind. This reduces the probability of a good diagnosis. Additionally, the dabbing process does not assure a single layer of cells. Aggregated clumps can still exist.

[0134] This invention addresses these difficulties by introducing a special assembly based upon a composite of a porous (e.g., one example is a mat of non-woven) material laminated to an impermeable, cover plate with a series of substantial holes within the cover plate. The plate acts as a mask to the non-woven mat. The liquid suspension is forced to pass through the holes within the cover plate. The (larger) cells will not pass through the non-woven mat. The smaller biological molecules will pass through. The relative dimensions of the interstices in the mat are a critical parameter in achieving the separation. The cells are held in place by the fluid pressure during the

transfer. In order to ensure retention after the fluid flow has stopped, the fibers of the non-woven material can be modified to induce a variety of electrical charge distributions. These assist in the retention.

[0135] In an alternative form, the mat of non-woven, permeable material is sandwiched between two impermeable plates each with an identical pattern of holes such that the holes are aligned when the two plates are compressed onto the mat. It is advantageous to have the two plates joined long one of the edges (preferably the long edge).

[0136] In either case the non-woven mat complete with its complement of cells can be further processed and directly viewed (i.e. no transfer step).

[0137] The holes in the outer plate serve to define the deposition sites for the cells as the suspension is passed through the assembly. As these holes become occluded, the flow of liquid will be reduced and will divert to other nearby holes in the plate. By choosing a small diameter for the holes, it will be possible to collect only a small number of cells at each hole. This will simplify the viewing process and may even make automated screening more feasible.

[0138] Subsequent processing would take place while the cells were adherent to the surface. Staining solutions would be flowed through the substrate and would stain the cells in place. A number of processing cycles would be easy to configure (i.e. washing, stain 1, washing, stain 2 etc)

[0139] With the substrate described by this invention, the cells would be distributed over a number (e.g. 100) of holes. Each hole would accumulate a limited number of cells after the passage of the suspension. Each hole would resemble a matrix of cells. Each hole can be viewed directly (i.e. there is no transfer step). The array of images would be scanned. Viewing such an image will be easier and less interpretive mistakes will be made. It may even be feasible to have image processing software since the image morphology is now considerable less complex.

[0140] A scanning algorithm could them examine the cells at a location and make a series of measurements. (i.e. % light to dark, size of nucleus, any entities outside the

nucleus). A regular lattice would be easiest to process such data but is not essential. The key aspect is the distinct and separate nature of the observation points as well as smallness.

[0141] It should be noted that current microarray scanners are able to quickly scan up to 100,000 points on an oligonucleotide microarray. Thus the technology to scan a small number of locations is well within present industry capability.

Coupling Assays

[0142] Coupling assays are extremely common. The microarray format is perhaps the best-known example of a coupling assay. A first biological moiety is anchored to a surface. A second moiety is introduced in solution or suspension. With the passage of time, the two moieties will meet. Some form of coupling may occur depending upon the nature of the moieties. Once coupled, the second moiety is no longer free to move within the suspension. Some suitable signaling molecule (i.e. a dye) attached to either or both of the moieties detects the coupling.

[0143] In every case the main process dictating the speed of coupling is diffusion. The probability of two molecules or moieties actually meeting and coupling is dependent on many factors (i.e. mass, temperature, hindrance factors). The existence of a liquid boundary layer at the interface between the substrate and the rest of the liquid only serves to slow down the reaction rate. This boundary layer is usually unaffected by macroscopic stirring or agitation. The use of a substrate that prevents the formation of a static boundary layer will increase the interactions between the molecules and lead to faster coupling.

[0144] This invention will describe a substrate that has permeable locations that serve to receive and retain the first biological moiety at many sites within the permeable location. The forced passage of a solution or suspension of the second biological moiety through the permeable location increases the probability of an interaction and so the reaction will proceed at a faster rate. Additionally, of course, there will be more first biological moieties available because of the increased area associated with internal microfeatures of each permeable location.

[0145] One specific example of a coupling assay is the ELISA format assay. Although there are several variants, the common theme is that a complex biological moiety such as an antibody is anchored to a surface. Interactions with unknown samples containing other antibodies (or antigens that key into the surface of the antibody) lead to coupling if particular conditions are met. Further processing allows a color change to indicate the extent to which coupling has occurred. Current ELISA assays are performed within wells of standard multiwell plates. Complex processing sequences of mixing, washing and mixing are required. Always, however, an interaction between two moieties is required and always the anchored moiety is fixed to a massive impermeable substrate with a static fluid boundary layer.

[0146] This invention overcomes this difficulty by using a substrate with permeable locations. The antibody (attached perhaps via an anchoring antigen) is fixed to microfeatures within each permeable location. Liquid containing the second antibody is flushed through (perhaps several times) to maximize contact. Instead of cumbersome pipetting processes, the various liquids can be introduced in flow through mode. Thus a washing step would comprise an injection of a solvent through the mat. The solvent would then be discarded and the next stage would be initiated.

DETAILED DESCRIPTION OF THE DRAWINGS

[0147] Figure 1 shows the invention in its simplest form. The invention comprises an impermeable sheet [10] that is processed so as to create a plurality of permeable locations [20] within its boundary. The sheet can be either flexible or rigid. The sheets can be made from a wide variety of materials including but not limited to metals, plastics or ceramics. In a preferred embodiment, the material comprises a sheet of fibrous material. The fibers may be coated with other materials or treated to enhance various physical parameters (i.e. plasma).

[0148] The impermeability applies to biological moieties of a specified size so that moieties that are greater than a set dimension are prevented from passing through the permeable location but all other biological moieties will pass. It is understood that individuals skilled in the appropriate art are aware of techniques for adjusting the

permeability to meet this condition. Generally such filter media is available in a wide variety of permeabilities where the permeable locations are located uniformly at every location on the surface of the filter.

[0149] The properties of the materials within the permeable locations are also the subject of this invention. This material can be modified to increase its ability to attract and adhere biological moieties passing through the permeable location. As an example, the surface treatment of polymer films with plasmas is known to increase to ability to adsorb cells by making the film positively charged. It is also known that the addition of linker molecules wherein one end is bonded to the surface of a substrate and the other is free to capture and adhere passing biological moieties.

[0150] Figure 2 shows a laminate sheet comprising a permeable sheet [50] sandwiched between two impermeable sheets [30]. Each impermeable sheet has a pattern of holes [40]. The pattern of holes in one sheet is aligned with the pattern of holes in the other impermeable sheet. The combined pattern of holes defines the permeable locations for the laminate sheet.

[0151] Figure 3 shows a composite sheet comprising a permeable sheet [50] attached to an impermeable sheet [30] with a pattern of holes [40] . The pattern of holes [40] defines the permeable locations for the composite assembly.

[0152] Figure 4 shows a sheet of hydrophobic material [60] that has been treated to produce locations that are hydrophilic [70] and therefore permeable to solutions or suspensions.

[0153] Figure 5 shows a detail of a permeable location wherein biological moieties [102] that are larger than a set size have been retained on the surface of the sheet. Other smaller biological moieties [103] have been able to pass into the body of the permeable location and have been retained at multiple sites within the permeable location.

[0154] Figure 6 shows detail of a permeable location wherein large biological moieties [200] are able to interact with smaller molecules [201] such as stains. The stain is able to pass through and may be re-circulated. The purpose of the staining is to

highlight important structures within the biological moieties so that visual inspection might reveal the condition of the specimen. This mode is important for separation assays.

[0155] Figure 7 shows detail of a permeable location wherein smaller biological moieties have passed [500] into the body of the permeable location. The further passage of a second biological moiety [502] may lead to a coupling (depending on specific characteristics of the two moieties).

DETAILED DESCRIPTION OF THE INVENTION

[0156] The invention comprises a substrate comprising a plurality of locations wherein the permeability of said locations is materially better than the remainder of the substrate. This configuration will induce fluids introduced to one side of the substrate to flow through only the permeable locations. The nature of the material at the permeable locations is configured to receive and retain biological moieties at the first surface of said substrate. The nature of the material within the permeable locations is also able to receive and retain smaller biological moieties that are able to flow through the body of the permeable locations.

[0157] In both cases (e.g., separation and coupling arrays), the skill has been applied to complete surfaces. The present invention applies these and other methods for the receiving and retention of biological moieties within specified and isolated locations of the substrate. This leads to the final aspect of the invention wherein the moieties, having been received and retained within the permeable locations are subject to further processing. This processing may include, but is not limited to, staining, interactions with other biological molecules and finally preparation for a visual inspection by either a scanning device or an individual skilled in the interpretation of the images. An important aspect of the invention is that the complete assembly becomes the viewing platform. Each permeable location can be viewed or scanned. There is no requirement to transfer isolated biological moieties to other viewing platforms.

[0158] The invention can be incorporated into a device that takes advantages of the properties of the substrate. Once biological moieties have been received and retained by the permeable locations of the substrate, it is customary to further process them in

order to facilitate examination. Present practice requires a series of discrete steps whereby liquids are added, incubated and withdrawn. There may be washing steps. The sequence of steps can be performed either manually or by machine. The manual process is laborious and subject to error. The machines are often quite expensive and involve robotic arms.

[0159] This invention permits the use of a pair of plates that capture the substrate of this invention. The plates are sealed at their periphery so as to form a chamber with the substrate forming a barrier between an upper and lower chamber. Fluids required for the further processing of the biological moieties can be introduced into the first chamber. These fluids will pass through the substrate only at the permeable locations. The fluid can them be removed by collection from the second chamber. This approach is advantageous when a single fluid needs to pass through all locations.

[0160] An alternative approach requires each plate to have a holes located in each plate in a pattern corresponding the pattern of permeable locations on the substrate. When the substrate is captured between the two plates, each permeable location creates a separate chamber such that a fluid introduced through a particular hole in the first plate flows only through the permeable location in closest proximity to that hole. Fluid passing through the said permeable location is evacuated from the corresponding hole in the second plate. In this way, each permeable location can be treated with different fluids in different sequences and for different times.

[0161] In both cases, a series of fluids is passed via the plates and through the permeable locations.

[0162] In both cases, the substrate may be part of a ribbon of material that moves through the assembly comprising the two plates. Plate 1 would be separated from plate 2 while the ribbon is moved. Once the movement is complete, the plates are moved together to fasten to the ribbon.

Example 1:

[0163] A fibrous, non-woven mat made from hydrophobic polypropylene is plasma treated with argon. The surface of the mat is covered with a metallic mask with a

pattern of holes. The plasma is able to reach the surface of the mat only where the holes in the metal mask permit. These sites become hydrophilic and the remainder of the mat retains it hydrophobic nature. The hydrophilic sites also take on a positive charge.

[0164] The mat is mounted on a mesh support and placed into an in-line fluid flow system. A solvent, in this case water, containing a suspension of human squamous cells plus other biological molecules are introduced to one side of the flow system. A differential pressure is created to force the solvent to pass through the mat. At low pressures, the solvent passes easily through the hydrophilic areas of the mat. The mat resists any passage through the hydrophobic areas.

[0165] As the solvent passes through the hydrophilic locations, the larger cells are retained by the surface fibers of the mat. The resident positive charge secures the molecules to the surface of the fibers within the mat. The solvent and the remaining smaller biological molecules are able to pass through the mat and are removed from the flow through system.

[0166] The mat is removed from the flow through assembly and carefully laid out from further processing. This may include staining and various preservation processes. Finally the mat is available for examination by a skilled cytologist using a microscope or other imaging device.

Example 2:

[0167] A non-woven mat made of polyester is treated with an argon plasma. All the fibers in the mat are exposed to the plasma and become hydrophilic and therefore permeable. Additionally the fiber surfaces retain appositive charge and will attract negatively charged species.

[0168] The treated mat is sandwiched between two thin sheets of polycarbonate that have had a pattern of 100 small 1mm holes punched through each surface. Care is taken to align the two patterns so that each ole matches the corresponding hole on the other sheet. The treated mat is exposed wherever there are holes pairs.

[0169] The assembly is clamped so that lateral motion within the treated mat is prevented.

[0170] The assembly is placed within a flow through assembly and a solvent containing squamous cells and other biological molecules is introduced to one side of the assembly. A differential pressure is created to force the suspension to pass through the holes. The larger cells are retained by the surface of the mat within each hole. Material that arrives at the surface of the polycarbonate masks does not stick to the surface. Fluid flow is generally directed to the holes in the assembly and the squamous cells are entrained in the fluid flow and move to the holes where they are captured.

[0171] After the passage of the suspension is complete, further processing is performed to stain the cells that have collected within each hole. The complete assembly is mounted for visual inspection by a trained cytologist. The small number of cells at each hole location reduces the difficulty of detecting a diseased cell.

Example 3:

[0172] A treated polyester non-woven mat is plasma-treated uniformly across its surface. The mat is mounted onto a mesh and placed into a flow through assembly. A solution containing a specific antigen is flowed through the mat. The antigens are attracted to and adhere to the surface of the fibers in the mat. The mat is removed, dried and incubated for 8 hours. The treated mat is again attached to the support mesh and retuned to the flow assembly. A new solution of a specific antibody is flowed through the treated mat. The antibody has been selected so that it will be captured and retained by the antigen anchored to the surface of the fiber. A wash cycle is used to remove excess antibody. The various ELISA protocols can be followed. In all, cases, however, the solutions flow through the permeable locations.

[0173] The experiment yield a much stronger color change and gives a stronger signal since the number of binding s that have occurred is significantly increased by the flow through nature of the reaction.

[0174] In this example the bulk of the mat was permeable. It is also advantageous to use a patterned mat as provided in other examples. In this case the coloration of the mat by the signaling molecule produces a pattern of colored spots that can be easily interpreted by various standard colorimetric devices.

Example 4:

[0175] A porous ceramic sheet (25mmx75mm) of aluminum oxide is permeable substrate. A series of small (i.e. 5mm diameter) metallic (and impermeable) disks are placed on the surface of the alumina sheet. A light tacky adhesive may be used to prevent lateral movement. An epoxy-based adhesive with low viscosity is allowed to flow from the top surface of the sheet (with the disks) and is removed from the lower face. The viscosity of the mixture is selected to allow the adhesive mixture to run through the sheet rapidly. This will minimize lateral flow.

[0176] The alumina sheet is cured in an oven at 300C for 30 minutes to harden the adhesive that has been retained in pores of the alumina.

[0177] The composite sheet is substantially impermeable at those locations where the liquid adhesive ran through the body of the sheet. The material underneath the aluminum disks is substantially permeable (as it was to begin with) because of the shadow effect associated with the disks masking the surface.

CLAIMS

[0178] Claim 1: A substrate comprising at least two layers with at least one permeable location that is configured to receive and retain at least one biological moiety from a fluid passing through any permeable location of the substrate wherein the purpose of retention includes further interaction with other biological molecules and examination of said moieties at the locations on the substrate.

[0179] Claim 2: A substrate of Claim 1 wherein at least one layer of the substrate is comprised of at least two perforations in the layer configured to permit passage of a fluid to other layers in the substrate.

[0180] Claim 3: A substrate of Claim 1 wherein at least one layer of the substrate is comprised of at least two locations that are permeable to the passage of a fluid but do not permit the passage of biological moieties suspended in said fluid.

[0181] Claim 4: A substrate of Claim 1 wherein the perforations of at least one layer are substantially aligned with other perforations in other layers.

[0182] Claim 5: A substrate of Claim 1 wherein the substrate comprises a polymer, a ceramic a metal, or an organic material or a combination thereof.

[0183] Claim 6: A substrate of Claim 1 wherein the sheet is substantially flexible.

[0184] Claim 7: A substrate of Claim 1 wherein at least the permeable locations are comprised of a porous medium with pore sizes in the range of 0.1 micron to 100 microns.

[0185] Claim 8: A substrate of Claim 1 wherein at least the permeable locations are comprised of fibers with diameters in the range 10 nanometers up to 50 microns.

[0186] Claim 9: A substrate of Claim 1 wherein the sheet is substantially rigid.

[0187] Claim 10: A substrate of Claim 1 wherein at least one face of the substrate is attached to a mechanical support that does not substantially impede fluid flow through the permeable sheet

[0188] Claim 11: A substrate of Claim 1 wherein the permeable locations of the substrate are substantially hydrophilic and the remainder of the substrate is substantially hydrophobic.

[0189] Claim 12: A substrate of Claim 1 wherein the permeable locations of the substrate have an affinity for at least one biological moiety.

[0190] Claim 13: A substrate of Claim 1 wherein the permeable locations of the substrate have an excess of positive charges.

[0191] Claim 14: A substrate of Claim 1 wherein the permeable locations of the substrate containing at least one biological moiety are configured to be examined by a variety of physical and chemical means.

[0192] Claim 15: A substrate of Claim 1 wherein substrate locations that are not permeable are resistant to the adherence of biological moieties.

[0193] Claim 16: A substrate of Claim 1 wherein the permeable locations comprise other molecules that are capable of coupling with biological moieties received and retained by said permeable membrane.

[0194] Claim 17: A substrate comprising a permeable sheet of material sandwiched between two sheets of impermeable material each having a at least one perforation such that a perforation on the first impermeable sheet is aligned with a similar perforation in the second impermeable sheet-to produce a plurality of permeable locations that-can receive and retain at least one biological moiety from a fluid passing through any permeable location of the substrate wherein the purpose of retention includes further interaction with other biological molecules and examination of said moieties at the locations on the substrate.

[0195] Claim 18: A substrate of Claim 17 wherein the each sheet of impermeable sheet of material may comprise at least two distinct sheets of material.

[0196] Claim 19: A substrate of Claim 17 wherein each sheet of permeable material may comprise at least two distinct sheets of material.

[0197] Claim 20: A substrate of Claim 17 wherein the sheets of material comprise a polymer, a ceramic a metal, an organic material or a combination thereof.

[0198] Claim 21: A substrate of Claim 17 wherein any of the sheets are substantially flexible.

[0199] Claim 22: A substrate of Claim 17 wherein the permeable sheet is comprised of fibers with diameters in the range 10 nanometers up to 50 microns.

[0200] Claim 23: A substrate of Claim 17 wherein any of the sheets are substantially rigid.

[0201] Claim 24: A substrate of Claim 17 wherein the two sheets of impermeable material are joined along one of the lateral dimensions as a hinge such that the two sheets can be opened to receive the sheet of permeable material.

[0202] Claim 25: A substrate of Claim 17 wherein a mechanism is provided to clamp the two impermeable sheets onto the permeable sheet.

[0203] Claim 26: A substrate of Claim 17 wherein the sheet of permeable material is a section of a ribbon with dimensions larger than those of the sheets of impermeable material.

[0204] Claim 27: A substrate comprising a thin, permeable sheet of material attached to a sheet of impermeable material having at least one perforation to produce at least one permeable location that can receive and retain at least one biological moiety from a fluid passing through any permeable location of the substrate wherein the purpose of retention includes further interaction with other biological molecules and examination of said moieties at the locations on the substrate.

[0205] Claim 28: A substrate of Claim 27 wherein the sheet of impermeable sheet of material may comprise at least two distinct sheets of material.

[0206] Claim 29: A substrate of Claim 27 wherein the sheet of permeable material may comprise at least two distinct sheets of material

[0207] Claim 30: A substrate of Claim 27 wherein the sheets of material comprise a polymer, a ceramic a metal, an organic material or a combination thereof.

[0208] Claim 31: A substrate of Claim 27 wherein the any of the sheets are substantially flexible.

[0209] Claim 32: A substrate of Claim 27 wherein the permeable sheet is comprised of fibers with diameters in the range 10 nanometers up to 50 microns.

[0210] Claim 33: A substrate of Claim 27 wherein any of the sheets are substantially rigid.

[0211] Claim 34: A substrate of Claim 27 wherein the permeable locations of the substrate are substantially hydrophilic and the remainder of the substrate is substantially hydrophobic.

[0212] Claim 35: A substrate of Claim 27 wherein the permeable locations of the substrate have an affinity for at least one biological moiety.

[0213] Claim 36: A substrate of Claim 27 wherein the permeable locations of the substrate have an excess of positive charges.

[0214] Claim 37: A substrate of Claim 27 wherein the permeable locations of the substrate containing at least one biological moiety are configured to be examined by a variety of physical and chemical means.

[0215] Claim 38: A substrate of Claim 27 wherein substrate locations that are not permeable are resistant to the adherence of biological moieties.

Figure 1: A sheet with a plurality of permeable locations

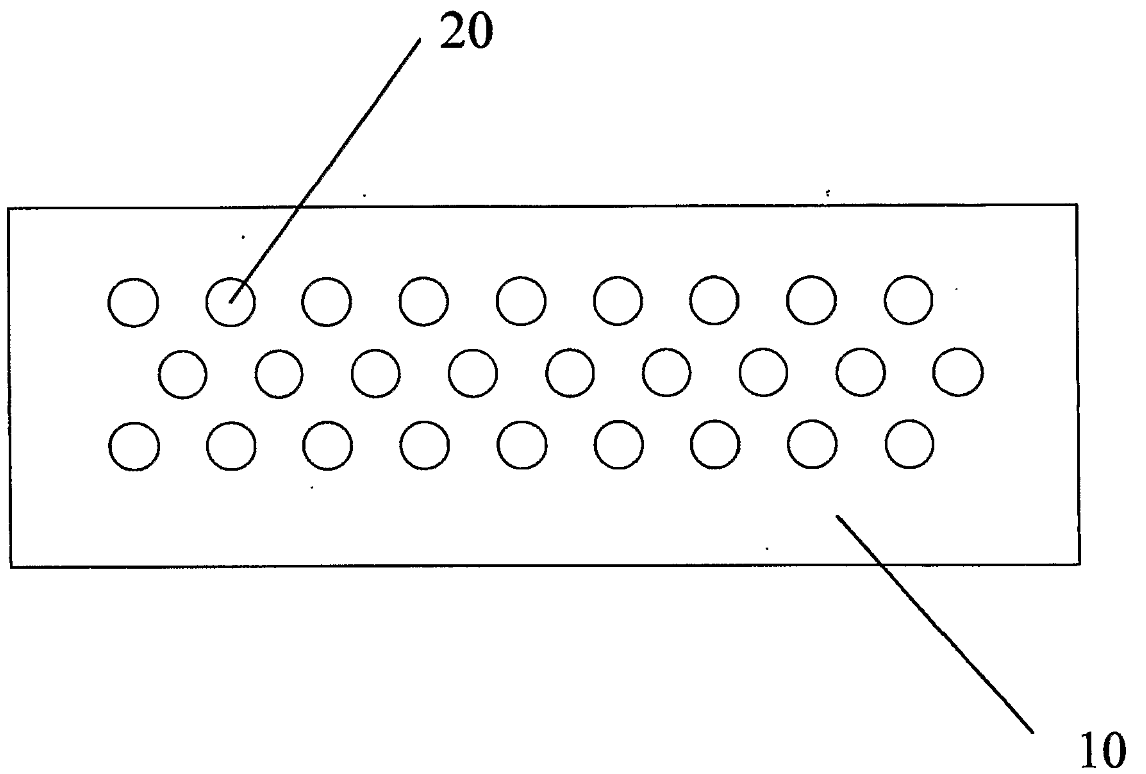

Figure 2: A Laminate Sheet With a Permeable sheet Sandwiched Between Two Impermeable Sheets Each Having A Plurality of Aligned Holes.

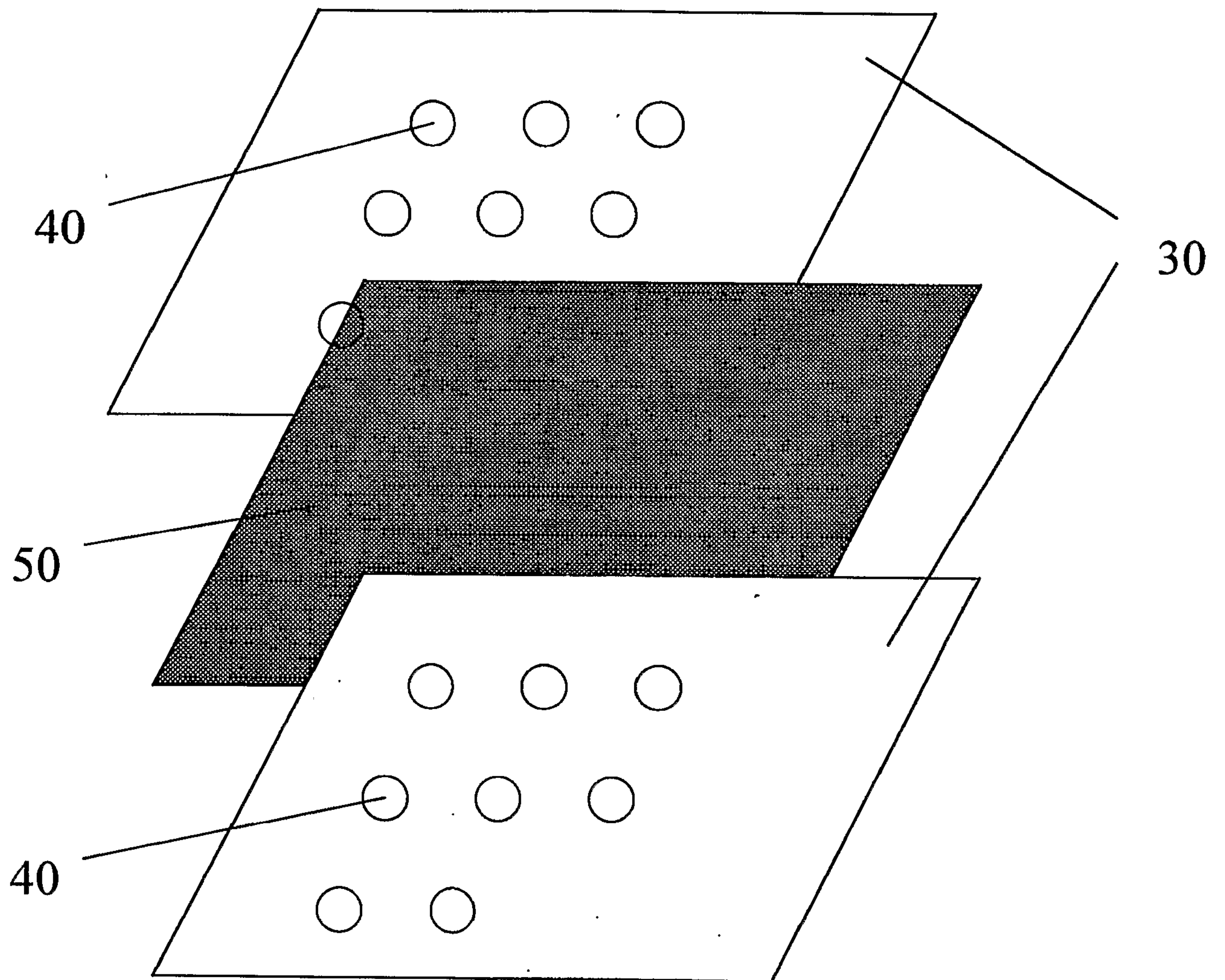

Figure 3: A Composite Sheet With A Permeable First Layer and An Impermeable Sheet With a Plurality of Holes

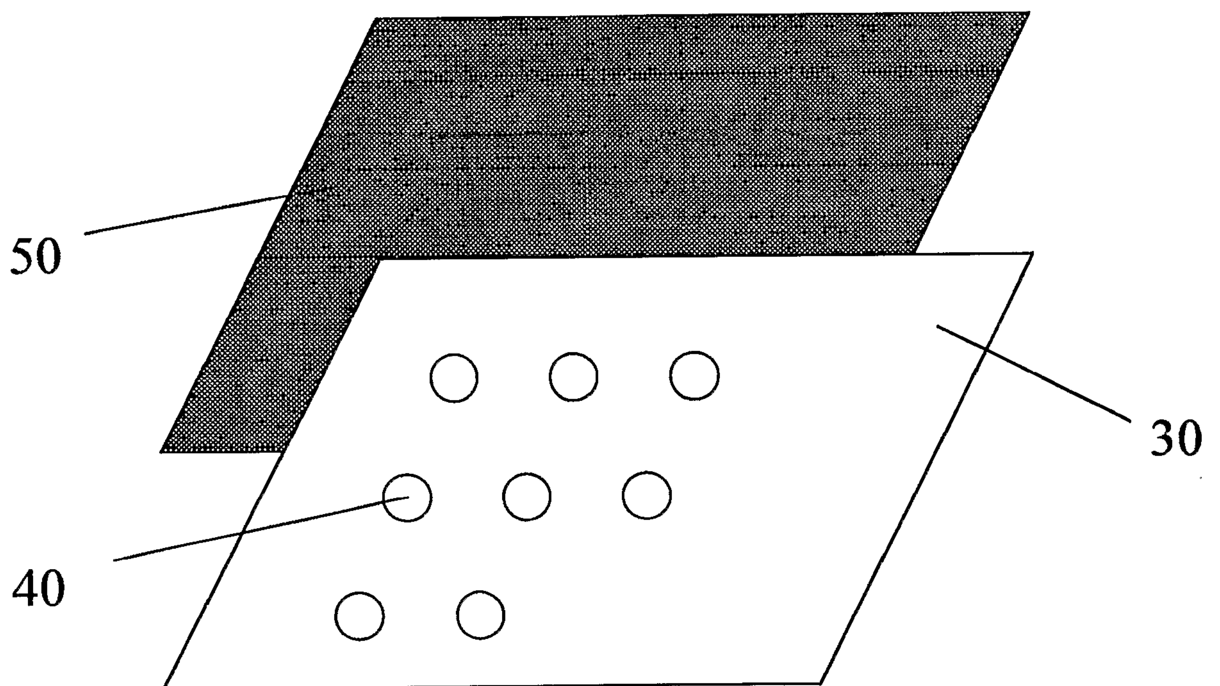

Figure 4: A Permeable sheet with Areas That Are Hydrophilic Surrounded By Areas That Are Hydrophobic

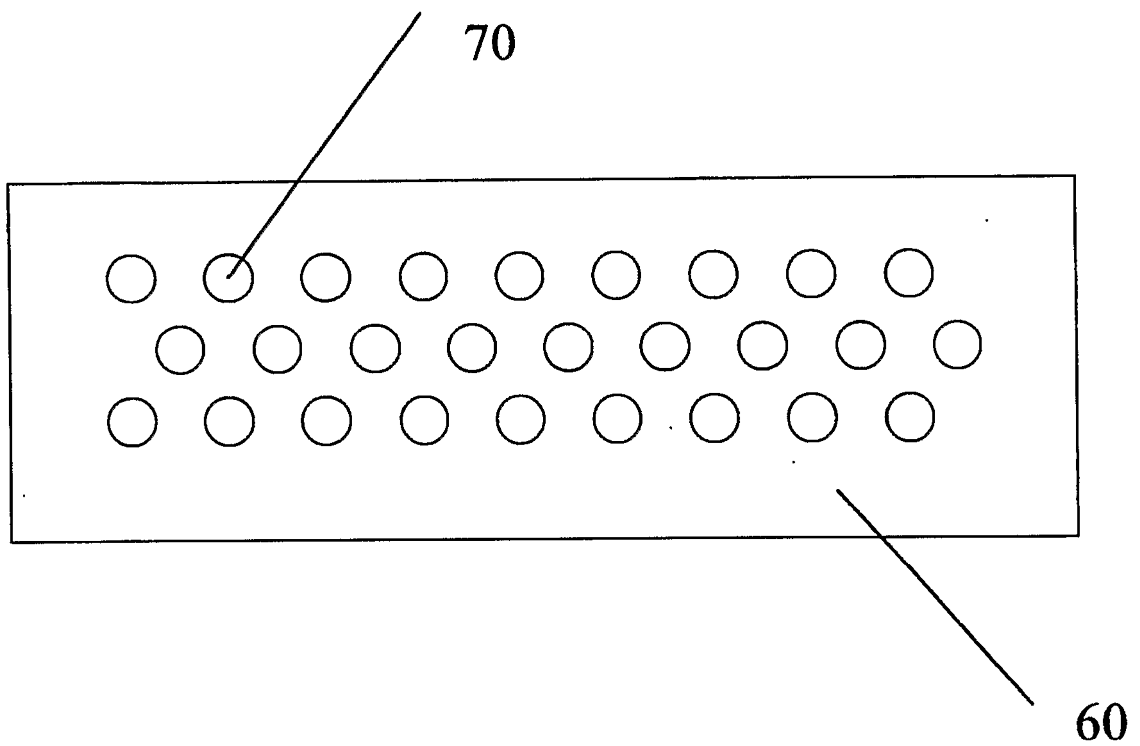

Figure 5 A Permeable Location Showing Biological Moieties Retained a) On the Surface b) Within the Surface

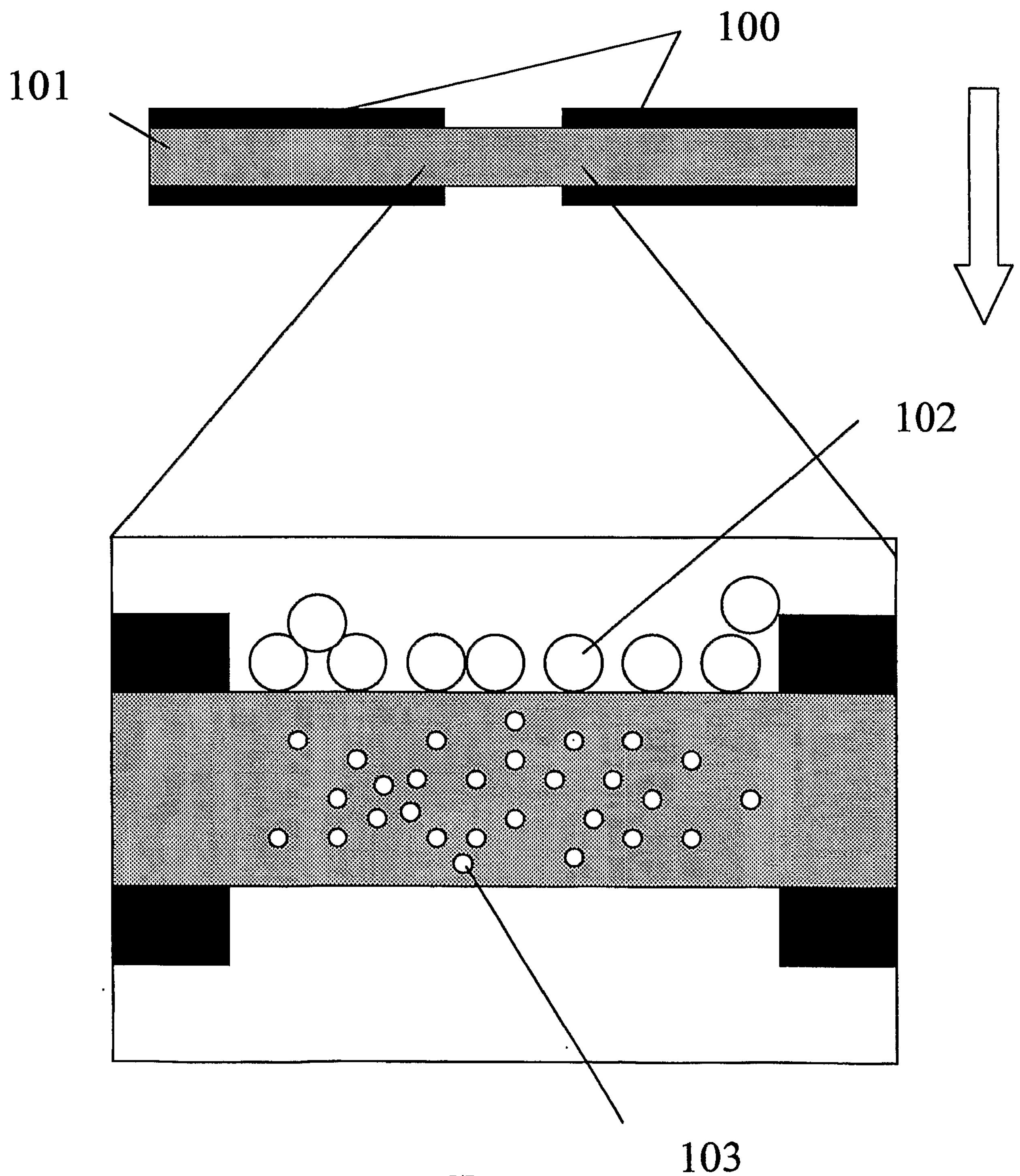

Figure 6: A Permeable Location Showing Biological Moieties on the Surface interacting with Staining Molecules

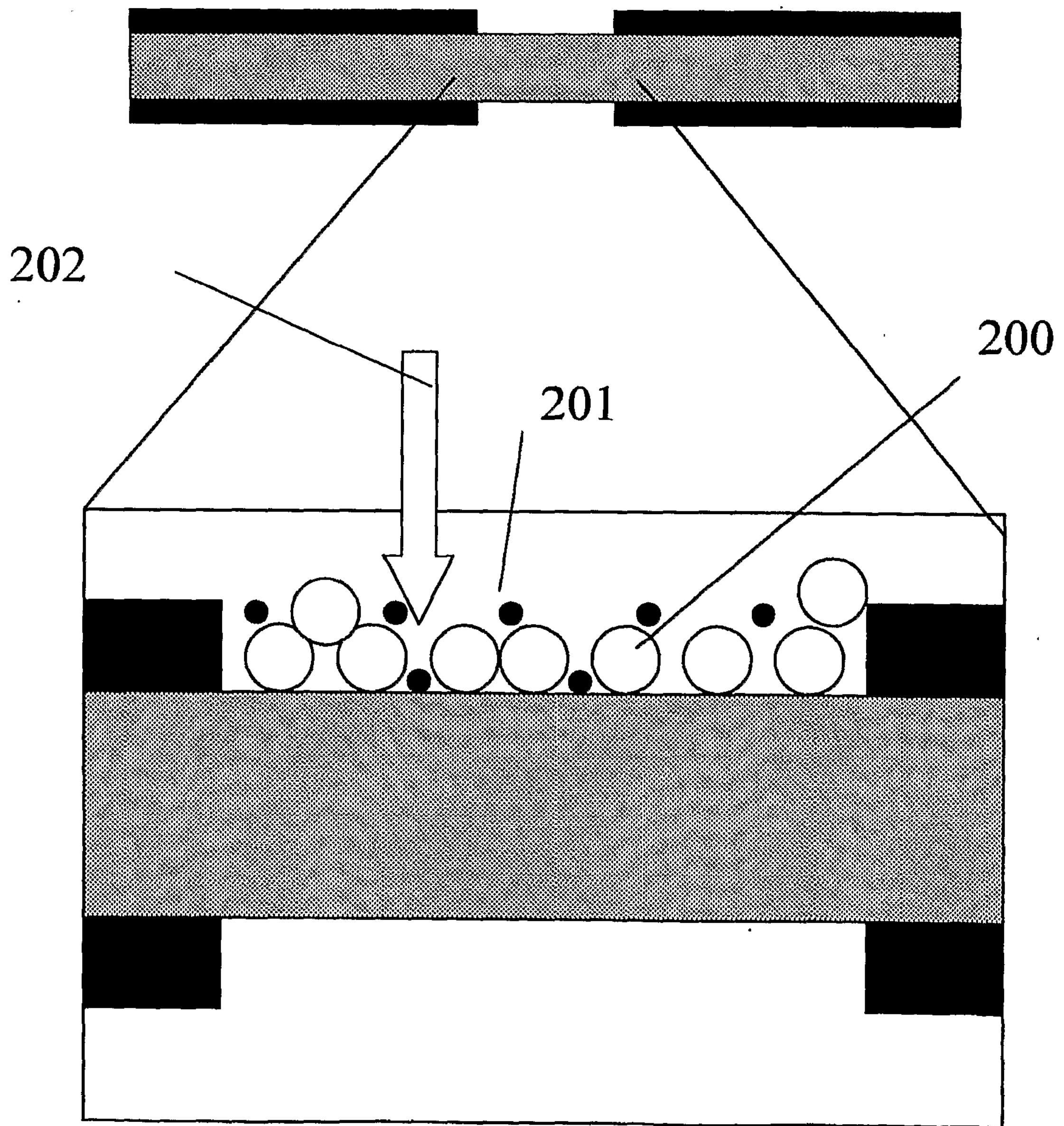

Figure 7:A Permeable Location Showing the Flow Through of Other Biological Moieties and Increased Interaction Between Retaining and Free Moieties