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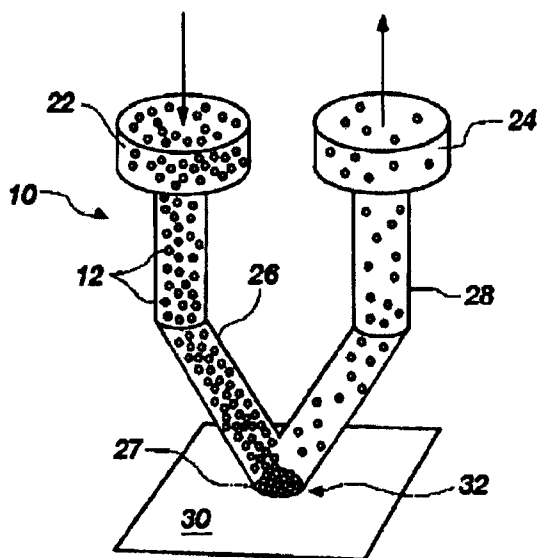
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(54) Title: A MICROFLUIDIC INTERFACE FOR HIGHLY PARALLEL ADDRESSING OF SENSING ARRAYS



(57) Abstract: Disclosed is a spotter device and methods for the formation of microassays, biochips, biosensors, and cell cultures. The spotter may be used to deposit highly concentrated spots of protein, cells, or other substances on a microarray slide, wafer, or other surface. It may also be used to perform various chemistry steps on the same spots. The spotter increases the surface density of substances at each spot by directing a flow the desired substance (or a solution thereof) over the spot area until surface saturation is accomplished. The spotter may be loaded by well plate handling equipment. The spotter uses wells, microconduits, and orifices to deliver the substances. Each orifice may be connected to two wells via microconduits. When the spotter face contacts a surface, a seal is formed between the orifices in the spotter face and the surface. The same or different substances may be flowed to each orifice and over the surface, thereby forming an array of spots on the surface. Any number of wells, microconduits, and orifices may be incorporated into a spotter.

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A MICROFLUIDIC INTERFACE FOR HIGHLY PARALLEL ADDRESSING OF SENSING ARRAYS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with United States government support under grant #DGE 9987616 awarded by the National Science Foundation. The United States Government has certain rights to this invention.

TECHNICAL FIELD

The methods and apparatus disclosed herein relate generally to biotechnology, more specifically to building microassays, biochips, and biosensors. In particular, the methods and apparatus disclosed herein encompass devices with microconduits for the delivery of substances to a surface and methods of use thereof.

BACKGROUND

In recent years, a large number of biological/chemical analysis techniques have been demonstrated using micro-scale systems and have been implemented using micromachining technology. The rationale for using microscale technologies in analytical instrumentation includes reduction in instrument size and cost, reduction in sample and reagent volume, reduction in analysis time, increase in analysis throughput, and the possibility of integration of sample preparation and analysis functions.

High spot density arrays may be produced using robotic spotter systems, such as the GENETIX QARRAY®. One technique uses spotting "pens" which collect the material to be deposited on a needle and then "spots" the material on to a surface. *See, e.g.*, U.S. Patent 6,733,968 to Yamamoto *et al.*, ("968 patent") entitled "Microarray, Method for Producing the Same, and Method for Correcting Inter-Pin Spotting Amount Error of the Same," the contents of which are incorporated herein by reference. The '968 patent notes that when multiple "pens" are used to create an array, not all of the "pens" are microscopically the same size, and therefore each "pen" blots a different amount of material. The patent discloses a method for determining what the errors are for a given set of "pens" so the errors can be mathematically accounted for.

U.S. Patent 6,365,349 to Moynihan *et al.*, entitled "Apparatus and Methods for Arraying Solution onto a Solid Support," the contents of which are incorporated herein by reference, discloses the use of a spring probe to administer material onto a surface.

Similar to the use of "pens" is the use of capillaries. *See e.g.*, U.S. Patent Application 20040014102, Chen *et al.*, entitled "High Density Parallel Printing of Microarrays," the contents of which are incorporated herein by reference. The application discloses the use of capillaries to spot samples onto a microarray. U.S. Patent 6,594,432 to Chen *et al.* ("432 patent"), entitled "Microarray Fabrication Techniques and Apparatus," the contents of which are incorporated herein by reference, also discloses the use of capillaries, such as silica tubes, to spot probes onto a substrate. In the '432 patent, one end of the capillaries may be attached to a reservoir; however there is no return path for the substance that is spotted and therefore no way to flow a substance over a substrate to increase the spot deposition density. The capillary action of the '432 patent is therefore similar to that done with pens. For an additional example see, U.S. Patent 6,110,426 to Shalon *et al.*, entitled "Methods for Fabricating Microarrays of Biological Samples," the contents of which are incorporated herein by reference, which discloses a method for tapping a meniscus at the end of a capillary tube to deliver a specified amount of sample material onto a substrate.

While prior art systems are capable of producing multiple spots of a controlled size, if the desired material for deposition is present in very low concentration, the total amount of desired material that can be deposited on the surface is severely limited for a single spot. The concentration of material in the spots is limited by the concentration of the original material. The Perkin-Elmer BIOCHIP ARRAYER® uses "ink jet printing" technology, but that method has the same concentration limitation as the "pens."

Other systems have been developed which use microfluidic channels on a substrate to pattern genes, proteins, nucleic acids, such as RNA, DNA, oligonucleic acids, or other arrays. For an example of such a system see, U.S. Patent 6,503,715 to Gold *et al.*, entitled "Nucleic Acid Ligand Diagnostic Biochip," the contents of which are incorporated herein by reference. Biochip fabrication methods have been developed that attempt to stir individual microassay spots; however, such systems often require mechanical manipulation of the biochip. *See e.g.*, U.S. Patent 6,623,696 to

Kim *et al.*, entitled “Biochip, Apparatus for Detecting Biomaterials Using the Same, and Method Therefor,” the contents of which are incorporated herein by reference, which discloses spinning a biochip in order to accelerate reaction time. A need exists to simplify the process of developing biochips and biosensors and for providing more control over individual spots on the biochips and biosensors.

Theoretically, a flow deposition system could produce a high surface density if the substrate surface were tailored to bond only to the desired molecules, allowing the unwanted bulk material to be washed away. However, flow deposition systems generally are incapable of producing spot arrays, let alone individually addressed arrays. *See, e.g.*, Japan Patent Application 10084639, Tomoko *et al.*, entitled “Method and Apparatus for Adding Sample,” the contents of which are incorporated herein by reference. That patent application discloses a method wherein a biochip is rotated and centrifugal forces are used to uniformly spread a sample over the entire surface of the biochip. Similarly, U.S. Patent 6,391,625 to Park *et al.*, entitled “Biochip and Method for Patterning and Measuring Biomaterial of the Same,” the contents of which are incorporated herein by reference, discloses a method for making biochips via irradiating portions of the substrate with a laser, and then spin coating probe molecules onto the substrate.

Additionally, current technology is unable to sequentially chemically process individual spots, or to perform layer-by-layer self-assembly (“LBL”) to build up the spot concentration. What is needed is a way to take a material and adhere a high concentration of the material to a surface. This would be particularly advantageous in studying protein function.

Additionally, microarray-type structures are used in forming biosensors and the same problems associated with biochips apply to biosensors. *See e.g.*, U.S. Patent 6,699,719 to Yamazaki *et al.*, entitled “Biosensor Arrays and Methods,” the contents of which are incorporated herein by reference, which discloses using microarray forming techniques in the formation of a biosensor. A need exists to simplify the creation the biosensors.

A need also exists to decrease the cost and time involved in processing microarrays as well. Attempts have been made to address that need, *see e.g.*, U.S. Patent Application 2003/0068253 A1, Bass *et al.*, entitled “Automation-Optimized

Microarray Package,” the contents of which are incorporated herein by reference, which discloses a method for automating microarray processing via a linear strip of microarrays that is processed in an assembly line fashion. Lab-on-a-chip microfluidic devices have included sample wells directly above microfluidic channels; however, a need exists to simplify the cost and time of loading those sample wells.

DISCLOSURE OF THE INVENTION

Disclosed herein are methods and apparatus capable of patterning the surface of microarrays with a high concentration of individually addressed spots. Also disclosed are methods of manipulating the apparatus by well plate handling equipment. The methods and apparatus may be used to increase the surface density at a spot by directing a flow of a substance over the spot area until a desired surface deposition density is accomplished.

One embodiment of the invention is a spotter. In this embodiment, the spotter comprises a support surface. A plurality of wells are formed in the support surface in substantially the same spacing and format as a well plate. Each of the plurality of wells includes a capacity to hold a quantity of fluid. A plurality of orifices are sized and formed in a sidewall of each of the plurality of microconduits. The plurality of microconduits are operably connected to the plurality of wells. The plurality of orifices are arranged along a different surface of the spotter in an ordered array.

Another embodiment of the invention is a method of delivering at least one substance to a surface. The method comprises loading some of the plurality of wells with the at least one substance. The different surface of the spotter is placed in proximity to the substrate surface sufficient to form a seal between the spotter different surface and the substrate surface. The at least one substance is flowed from the loaded plurality of wells to the plurality of orifices. The at least one substance is flowed over the substrate surface. The at least one substance is flowed towards a different some of the plurality of wells.

Another embodiment of the invention is a method of loading a spotter for delivering a substance to a surface. The method comprising positioning a spotter via a positioning means and loading the substance into a plurality of wells of the spotter via a handling means. The spotter comprising a means for holding a plurality of wells, the

wells adapted to hold a quantity of fluid and having a predetermined spacing between adjacent wells. The spotter further comprising a plurality of microconduit means connected to each well, and aperture means within said plurality of microconduits, the aperture means arranged operable to form a seal with the surface.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 idealizes deposition of a substance on a surface.

FIG. 2 is a calibration curve to compare spot deposition concentration yielded by one embodiment of the invention with the spot deposition concentration of a pin spotter.

FIG. 3 is one embodiment of a four-orifice spotter.

FIG. 3A is a close-up of the orifices

FIG. 4A illustrates a surface with spots deposited with the embodiment of FIG. 3.

FIG. 4B is the same surface shown in FIG. 4A after hydration of the surface.

FIG. 5 is graphical illustration of the data determined from FIGS. 4A and 4B.

FIG. 6 is one embodiment of an eight-orifice spotter.

FIG. 7 is one embodiment of an automated use of the spotter of FIG. 6.

FIG. 8 illustrates a surface with spots deposited with the embodiment of FIG. 6.

FIG. 9 is a graph of data based upon FIG. 8.

FIG. 10 is a graph of data based upon FIG. 8.

FIG. 12 is a graph of data based upon FIG. 8.

FIG. 13 is an embodiment of a 48-orifice spotter.

FIG. 14 is an embodiment of a 48-orifice spotter.

FIG. 15 is a close up of the spotter face of the embodiment shown in FIG. 14.

FIG. 16 illustrates a surface with spots deposited with the embodiment of FIG. 14.

FIG. 17 is a graph of data based upon FIG. 16.

FIG. 18 illustrates deposition of a lipid bilayer.

FIG. 19 illustrates a surface with spots deposited with a twelve-orifice spotter.

FIG. 20 is an illustration of one embodiment of a spotter.

FIG. 21 is an illustration of one embodiment of a 16-orifice spotter.

FIG. 22 is a plan view of FIG. 21.

BEST MODE FOR CARRYING OUT THE INVENTION

Disclosed is a spotter capable of patterning the surface of microarrays with a high concentration of individually addressed spots and methods of using and fabricating the spotter. The spotter is also easily manipulated by well plate handling equipment. The invention includes microconduits of that may be used to increase the surface density at each spot by directing a flow of a substance, such as probe and/or target molecules, over the spot area until a desired surface deposition density is accomplished.

As used herein, the term "substance" includes any material to be sampled, such as probes or target compounds, and any associated carriers and/or processing fluids. Examples of "probes" include: proteins; nucleic acids, including deoxyribonucleic acids ("DNA") and ribonucleic acids ("RNA"); cells; peptides; lectins; modified polysaccharides; synthetic composite macromolecules, functionalized nanostructures; synthetic polymers; modified/blocked nucleotides/nucleosides; synthetic oligonucleotides; modified/blocked amino acids; fluorophores; chromophores; ligands; receptors; chelators; haptens; drug compounds; antibodies; sugars; lipids; liposomes; viruses; and any other nano- or microscale objects. Target compounds include any compounds that are typically flowed over probes or combinations of probes already deposited on a surface. "Carrier" refers to any transport vehicle, such as for transporting probes and target compounds such as a solvent (*e.g.*, any aqueous or non-aqueous fluid and/or gel). "Processing fluids" include any materials necessary for working with a sample material, such as buffers for washing or nutrients for feeding cells.

"Substances" may be entirely or partially fluidic in nature (*i.e.*, liquid or gas). The substances may be in liquid form or in a slurry or paste with particles suspended therein. All that is required is that the substances be flowable for delivery.

"Deposit" as the term is used herein includes adsorbing a substance onto a surface, chemically reacting a substance on a surface or with another substance on the surface already, mechanically interlocking substances with the surface or existing substances on the surface, or any other method of binding a substance to a spot.

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“Delivering” a substance encompasses depositing, as well as, performing various chemistry steps upon the spot, or removing a portion of a substance at the spot, such as an amino acid sequence from a protein.

First, will be described some of the embodiments of the spotter. Next, uses of the spotter will be discussed followed by experimental data generated by some of the embodiments of the spotter.

1.0 Structure

FIG. 1 is an idealized non-proportional illustration of the increasing deposition concentration possible with an embodiment of the invention. Spotter 10 has a well 22 and a well 24. Well 22 is connected to microconduit 26 which in turn is connected to microconduit 28. Well 24 is connected to microconduit 28. At the junction of microconduits 26 and 28 is orifice 27. Orifice 27 forms a seal with the surface 30 upon sufficiently pressing spotter 10 against a surface 30. The area on surface 30 bounded by the orifice is spot 32. Well 22 may be loaded with probes 12. As positive pressure is applied to well 22, probes 12 flow from well 22 to well 24. As probes 12 flow over spot 32, probes deposit on spot 32. This allows probe 12 concentration to increase at spot 32. Microconduits 26 and 28 may be viewed as separate microconduits with a junction at orifice 27. However, microconduits 26 and 28 may also be viewed as a single microconduit extending from well 22 to well 24 with a hole in the sidewall of the microconduit forming orifice 27.

“Microconduits” as used herein encompass channels, microchannels, canals, microcanals, microtubules, tubules and/or tubes. For example, microconduits 26 and 28 of FIG. 1 may be either channels in a block or tubes without a supporting block structure. The cross-section of the microconduits may be circular, rectangular, or any other shape. The width and height of the cross-section may be any distance necessary.

Orifice as used herein refers to the openings in the spotter where a substance may be delivered by the spotter to a surface. “Orifice” encompasses the terms “microconduit holes,” “apertures,” and “spotting holes.” Orifices may be of any shape and have any dimension necessary.

The spotter in FIG. 1 only illustrated two wells; however, a spotter may have any number of wells. It can be beneficial to have the wells in the same number and

placement as a well plate, the function and structure thereof to be described hereinafter. This allows well plate handling devices and technology to be used in loading and unloading the spotter as will be discussed in more detail below. There are a variety of well plate arrays, for example, 8-, 16-, 96-, 192-, 384-, or 1536-well plates. Well plates may also be referred to as micro titer plates. A spotter may include any of the above well plate arrays including larger, non-standard, or different well plate designs that come about in the future. The wells may have any shape such as cylindrical, hemispherical, cuboid, hexagonal prism, or other shapes. The wells may be spaced relative each other in any arrangement. For example, as well plate technology changes, the wells in the spotter may be adapted to mimic new well plates.

The surface of the spotter where the wells are formed is referred to herein as the "top surface" or "support surface" of the spotter. However, that is not to be construed as limiting the orientations in which the spotter may be used. For example, the top surface of the particular 16-well embodiment shown in FIG. 6 may be orientated in any manner needed. Additionally, even though the surface wherein the wells are formed is referred to as the "top" any number of other structures, such as pumping manifolds or glass plates, may be placed over the top of the spotter.

The orifices in the microconduits are preferably arranged along a single surface of the spotter. The surface where the orifices are arranged may also be referred to as the spotter face. FIGS. 14 and 15 illustrate an embodiment of a 96-well spotter where the orifices are placed along a side surface of the spotter. In the embodiment shown in FIGS. 14 and 15, the spotter face 150 is projected away from the side of the spotter 1510 via a projection neck 140. FIG. 15 also illustrates orifices 1527, wells 1522, and microconduits 1526. However, the spotter face 150 may be integrated into the side of the spotter 1510 without a projection neck 140 projecting the spotter face 150 away from the spotter 1510. The spotter face 150 may also be integrated into the bottom surface of the spotter 1510.

The spotter face is adapted to form a seal with a desired surface. Often the surface will be relatively smooth such a microslide or wafer. However, the spotter face and the orifices may be configured to mate with any surface. For example, if a surface has existing wells or canals, the spotter face and orifices can be modified so that the orifices are able to form a seal with the uneven surface.

Generally, the spotter orifices will be arranged in a 2-D array. The array can be in a chess board or honeycomb pattern. However, there may be situations where a different orifice pattern such as a random pattern may be desired. Any number of orifice patterns can be built into the spotter face.

The spotter may include any number of orifices. In one variation, there are two wells for each orifice. Therefore, a spotter with 1536 wells would have 768 orifices. A spotter with 384 wells would have 192 orifices. A spotter with 192 wells would have 96 orifices. A spotter with 96 wells would have 48 orifices. A spotter with 16 wells would have 8 orifices and so on. This variation allows a pumping manifold to be placed over half of the wells and substances placed in the other half of the wells. A pump is connected to the pump manifold, and the pump then delivers alternating positive pressure and vacuum pressure. This structure may cycle the substances back and forth between the wells via the microconduits.

As used herein, the term “pump” includes devices that can deliver positive pressure, alternating positive pressure and vacuum pressure, or just vacuum pressure. Gravity flow may be used as the pump as well. Similarly, “pumping manifold” refers to any device for interfacing between the spotter wells and the pump, regardless of whether positive pressure or vacuum pressure is being delivered. The pumping manifold may be designed to apply the same pressure to each well or to apply different pressures to each well. A single pump and/or valve may be provided for all of the wells. Unique valves and pumps may be provided for each well.

For example, in the 16-well embodiment shown in FIGS. 6 and 7, the front row wells (referring to the “front” as the row closest to the spotter face) serve to interface with the pumping manifold and the back row is loaded with the substances. The wells that interface with the pumping manifold will be referred to as “pump wells.” The wells that are loaded with substances will be referred to as “sample wells.” In this example, there would be eight separate fluid pathways connecting eight pump wells and eight sample wells to eight orifices. Each column of wells in this particular embodiment contains a pump well and a sample well. For example, well 622 is connected to microconduit 626. Microconduit 626 junctions with microconduit 628 at orifice 627. Microconduit 628 connects to well 624. As vacuum pressure is first applied to the front row pump wells, substances are drawn through microconduits

unique to that well, to the orifice where the substance contacts a surface (such as an assay microslide), the substance is then drawn through a second unique microconduit which leads to the pump wells. The process is then reversed by applying positive pressure to the pumping manifold, pushing the substances back through the microconduits and to the sample wells, thus, resulting in oscillating eight potentially different substances over eight spots on the surface to which the spotter orifices are sealed. This process also results in preserving the substances that do not deposit onto the surface spots. Of course, it may be desirable to not oscillate the substance over the spots, and instead to preserve the substance in the pump wells after the substance has come in contact with the surface and any probes on the surface.

Of course, the pumping manifold may also be placed over the sample wells. That would merely require first applying positive pressure and then applying vacuum pressure. It is not necessary for there to be a physical distinction between the "pump wells" and the "sample wells." The terms are only useful when one well is initially loaded with a sample, and another well is not. However, in some embodiments all of the wells will have samples and all of the wells may interface with a pumping manifold.

Another variation is where only a few of the wells are pump wells. Well plates are generally organized in 2-D arrays; and the wells of the spotter embodiments shown in the figures are also in 2-D arrays. A single row or column of the well array could be pump wells, and the remaining wells could be for sample wells. That would increase the number of orifices that would be available for spotting.

For example, the particular 16-well spotter shown in FIGS. 6 and 7, spotter 610, could dedicate wells 622 and 624 as pump wells. Therefore, the pumping manifold, instead of interfacing with the front row of wells, would interface only with wells 622 and 624. That would allow for 14 orifices rather eight. Now, instead of each column containing a pump well and a sample well, each row would contain one pump well and seven sample wells. Each of the seven sample wells would each uniquely connect to an orifice. For example, well 621 would connect to a first distinct orifice and well 623 would connect to a second distinct orifice. Separate microconduits would then connect the first and second distinct orifices to well 624. The same would be true of the other five wells in the front row of spotter 610. Therefore, well 624 could serve

as the pump well and the other seven wells including wells 621 and 623 would serve as the sample wells. The same setup would exist for the back row of spotter 610 and well 622. Therefore, there would be 14 sample wells, 14 orifices, and 2 pump wells. It may be desirable in this embodiment to not oscillate the substance back and forth between the wells, particularly when different substances are in the sample wells. Instead, it may be desirable to just draw the substances from the sample wells, across the surfaces sealed by the orifices, and into the common pump wells 622 and 624. Therefore, wells 622 and 624 would also serve as the collective drains for the sample wells.

Similarly, with regard to the 96-well spotter embodiment, if a column of wells were dedicated as pump wells then, the spotter would have 88 orifices. If a row of wells were dedicated as pump wells then, there would be 84 orifices. Similarly, a 384-well spotter could have 368 or 360 orifices. A 1536-well spotter could have 1504 or 1488 orifices. However, any number of wells, in any location on the spotter, may serve as sample wells and pump wells.

Pumping may be accomplished by a variety of devices as will be understood by one of skill in the art. Additionally, any number of pumping mediums may be used for delivering positive and vacuum pressure to the samples. In the examples discussed below air is used; however, any fluid may be used.

As illustrated in FIG. 13, the microconduits may be of varying lengths. When the same pressure is applied to microconduits of the same diameter, containing the same substance, but of different lengths, different substance flow rates result. The microconduits may be modified to create a uniform flow rate between microconduits of different lengths. The pump may be varied as is known in the art to vary the flow rate of a substance. Additionally, the microconduits and orifices may be modified to vary the flow rate of a substance. The wells may also be sized to hold a specific quantity of a substance.

FIG. 3A and the inset in FIG. 13 illustrate one embodiment of an orifice and an accompanying microconduit junction at the orifice. These figures illustrate a rectangular orifice. However, the orifice may be any number of geometries. Also, any number of structures may be incorporated within the microconduit junction near the orifice.

Additionally, more than one sample well may be connected to an orifice. Also, sensing components may be integrated into the spotter for sensing at the spotter face. A few examples of sensing components are wave guides and thermocouples amongst others. However, the sensing components may be built into the surface against which the spotter orifices seal as well.

The spotter in the examples below was fabricated from polydimethylsiloxane ("PDMS"); however, the spotter may be fabricated out of any suitable material that is compatible with the substances to be flowed through the spotter. Examples of compatible materials include, but are not limited to: silicon; silica; PDMS; gallium arsenide; glass; ceramics; quartz; polymers such as neoprene, TEFLON™, polyethylene elastomers, polybutadiene/styrene butadiene rubber ("SBR"), nitriles, nylon; metals, and combinations thereof. It may be desirable to build the spotter out of material for which the substances to be flowed (*e.g.*, a solute) have a low affinity for, thus, reducing binding of the substance within the spotter microconduits. Additionally, the inner diameter of the microconduits may be coated with suitable material to reduce the affinity between the substances being flowed and the microconduits themselves.

The spotter may be loaded and unloaded by well plate handling equipment. Well plate handling equipment may include positioning equipment as well as sample handling equipment. A robot could be constructed that both loaded the spotter, used the spotter to fashion an array, tested the array, and then unloaded any remaining substances in the spotter. Of course, these functions could be carried out by individual devices working in conjunction with the spotter. Additionally, it is possible to integrate the loading and unloading of the spotter as well as its actual use into a computer. A Graphic User Interface ("GUI") program may be developed for manipulating the devices using and working with the spotter. Automated as the term is used herein encompasses manipulating the spotter via robots as well as interfacing the spotter with a pump such as shown in FIG. 7.

FIG. 13 illustrates one embodiment of making a spotter. Spotter layers containing microconduits are produced by molding a silicone polymer onto microfabricated molds. Alternatively, microconduits could be etched into thin layers of an appropriate material. As many layers as necessary may be stacked together. FIG. 13 illustrates the ease with which the spotter may be scaled to include hundreds or

thousands of orifices and microconduits. A top layer containing the wells is added to the stack. Numerous fabrication techniques are known in the art.

FIG. 21 illustrates another embodiment of making a spotter. A first layer may contain holes which will serve as the wells. A second layer may contain grooves that will form microconduits when the first layer is laid on top of the second layer. The second layer may also include flat areas on its top surface that will form the bottom of the wells when the first and second layers are mated. A third layer may contain holes that will form the orifices. The three layers are sandwiched on top of each other by numerous methods known in the art. Thus, in this embodiment, the orifices are located in the bottom surface of the surface of the spotter. FIG. 21 shows the first and second layers as one layer. FIG. 21 also shows a microslide to which the orifices of a completed spotter could be sealed against. FIG. 22 illustrates a top view of FIG. 21. The different layers may be formed and combined by numerous methods known in the art.

FIG. 20 illustrates a potential 3-D fluid flow velocity profile at the junction between two microconduits and an orifice. A substance would essentially dump from one microconduit into an orifice where the substance contacts the spot area and then flow back up into the second microconduit. Each of the microconduits shown in FIG. 20 would also be connected to a separate well. This embodiment may still be viewed as a single microconduit or fluid pathway with a hole in the sidewall where each end of the microconduit is connected to a well.

FIG. 21 illustrates an embodiment with thirty-two wells, thirty-two microconduits, and sixteen orifices. Also, as discussed above, FIG. 21 could be altered to have twenty-eight orifices. Twenty-eight wells would serve as sample wells and four wells as pump wells in a similar manner as discussed above with reference to the 16-well spotter.

The bottom surface of the FIG. 21 spotter would then be pressed against a surface to form a seal with the surface. Additionally, the bottom surface could be patterned by known methods to mate with an uneven surface.

Given the availability of inexpensive materials and the possibility for high volume manufacturing, even disposable spotters may be manufactured.

In addition to all of the embodiment discussed herein, embodiments from PCT Patent Application, Application No. PCT/US05/23895, designating the United States of America, Spotting Device and Method for High Concentration Spot Deposition on Microarrays and Other Microscale Devices, the contents of which are incorporated herein by reference, may be used with the methods and apparatus disclosed herein.

2.0 Uses

The spotter may be used to sequentially flow different substances through the microconduits. Therefore, the spotter may be used to perform surface modifications, deposit biomolecules or cells, carry out wash steps, deliver nutrients, and deliver reagents for chemical reactions. During these different steps, each spot is kept isolated from the other spots and the atmosphere.

Alternatively, each spot of an array may be processed via the spotter, the spotter removed and the entire surface processed at one time, such as by flowing a buffer over the entire surface of an array. Additionally, the spotter could be later realigned with the spots and used again for further processing.

The spotter may be used to deposit highly concentrated spots of chemically- or biologically-sensitive materials on a surface to form an array. It can also be used to perform various chemistry steps on the same spots, or selectively remove biomolecules from microscale sections of a surface for off-surface analysis. The spotter may be used to fabricate protein microarrays. Any molecule that is compatible with the spotter material can be used. The spotter may be used to handle standard Biacore immobilization chemistries on, for example, a FLEXchip on a massively parallel scale. Also, a typical enzyme-linked immunosorbant assay ("ELISA") could be completed using only the spotter. An antibody to be captured could be flowed through the microconduits and deposited on an appropriate surface. The antibody could then be washed and interrogated.

In terms of sensing methods using the spotter, the modes may be hugely varied. Some of the biochemical uses are:

Surface plasmon resonance ("SPR") — spotted recognition molecules such as antibody proteins can spotted in various patterns to create a high-throughput biomolecule assay system using an SPR substrate. The spotter could be used to deposit

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the capture materials followed by the sample. Readout of binding would be made at each point of the array using SPR instrumentation.

Optical — multiple high-concentrate dye spots can be applied in an array format on a surface to act as a multiple-analyte chemical-specific sensor with an increased signal-to-noise ratio, or materials such as cells can be deposited, tested, and labeled using the spotter for fluorescence/absorbance/reflectance detection. The latter application bears significance to the field of drug discovery, by allowing multiple types of cells to be tested simultaneously with varying chemicals. DNA hybridization, is also compatible with the spotter, both for oligonucleotide deposition and labeled-sample interaction.

Quartz crystal microbalance (“QCM”) — antibody-antigen interaction is the most significant application of QCM systems, although any surface-target molecule can be detected by QCM. The spotter could be used to address multiple QCM sites simultaneously.

The spotter can fabricate high-quality arrays, and deliver probes and target compounds to specific spots in a controlled and predictable manner, while keeping spots isolated from each other. The relative low cost of the spotter allows the spotter to be used in both full-scale commercial applications, as well as small research environments for production of custom sensor arrays. These arrays can be used in military applications, laboratories, medical research and diagnostic facilities, or point-of-care settings, for rapid parallel detection of various analytes on a large scale.

The continuous flow nature of the spotter allows not only high quantity deposition at the spot area to be performed, but also allows multiple layers of biomolecules to be deposited, facilitating sensing on the spots. Thus, one device can do the spotting, assay, and analysis, eliminating multiple pieces of equipment, speeding assays significantly, and improving hybridization conditions. A continuous sensing system is possible where a continuous flow of a substance of interest could be passed through the microconduits and continuously monitored. The continuous flow capable with the spotter is able to speed hybridization, washing, and analyte capture, meaning that a typically ELISA could be reduced from several hours to several minutes. The spotter can be used in a flexible configuration, allowing various sizes of arrays manufactured with the spotter on the same surface.

The spotter may be integrated with numerous microfluidic systems. A few examples are: ink jet printers, mass spectrometry systems, spotting robots, and sample injection systems.

The sample size of substances can be very small. Tests have been performed in some embodiments with as few as 10 microLiters. Additionally, smaller initial substance sample sizes can be used by diluting the substance to take advantage of the concentrating effects of the flow deposition. The dead volume of the microconduit loops from well-to-well for some of the embodiments ranges from 10 to 30 nanoLiters.

A few examples of surfaces that may be used for depositing substances with the spotter include: glass, silicon, streptavidin-gold chips, plain gold FLEXchips, and dextran-coated FLEXchips. Any number of surfaces may be used.

Examples

Deposition of Protein A on a streptavidin gold chip surface was attempted with an embodiment of a single-orifice spotter and on a plain gold FLEXchip surface with embodiments of a four-orifice, an eight-orifice, and a forty-eight orifice spotter. The tests show that Protein A can be immobilized on a streptavidin gold chip and on a plain gold FLEXchip surface via flow deposition through the spotter. Significant variability between the spots occurred in each of the experiments. This was likely due to differences in the flow rates between the microconduits.

Example 1: Single-orifice spotter

Deposition of Protein A was attempted with a single-orifice spotter similar to that shown in FIG. 3, to validate the design concept displayed in FIG. 1. Protein A (ImmunoPure Protein A, cat # 21181, Pierce Inc.) was biotinylated with Biotin (EZ-Link Sulfo-NHS-Biotin, cat # 21217, Pierce Inc.) to provide specific adhesion to a SPR streptavidin gold chip (8500 streptavidin affinity chip, part # 4346388, AB). The protein solution was diluted to a concentration of 0.15 µg/mL in 0.1X Phosphate buffer solution ("PBS") (0.19 mM NaH₂PO₄, 0.81 mM Na₂HPO₄, pH 7.4 and 15 mM NaCl) and supplemented with 100 µg/mL Bovine Serum Albumin ("BSA") to prevent non-specific adhesion. To recirculate the solution over the chip surface, 200µL of protein A solution was loaded into a Phynexus MicroExtractor 100 syringe pump and

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flowed continuously back and forth through the spotter at 75 $\mu\text{L}/\text{minute}$ for 1 hour. A wash step was then performed using 800 μL of 0.1X PBS with 100 $\mu\text{g}/\text{mL}$ BSA. At the end, the sample was removed from the surface by withdrawing air through the assembly, and the chip was then washed using water.

To compare the spotter immobilization to conventional methods, protein A was also immobilized using solid-pin spotting with a Genetix Q array Mini spotter. Increasing protein concentrations were deposited to create a calibration curve of SPR response to deposited concentrations of protein A. This curve was used to calculate an equivalent concentration for use of the spotter, to determine the factor increase in deposition density. The horizontal line extending from the y-axis to the calibration curve shown in FIG. 2 represents the maximal signal from the spotter of 19.256 response change units ("RCU"). Based on the standard curve this signal would correspond to 13 $\mu\text{g}/\text{mL}$ of pin-spotted protein A. Since the concentration of protein A immobilized using the spotter was only 0.15 $\mu\text{g}/\text{mL}$, immobilization via the spotter yielded an 86-fold increase in sensitivity versus solid pin-spotting.

Example 2: Four-orifice spotter

An embodiment of a four-orifice spotter is shown in FIG. 3 & 3A. ImmunoPure Protein A (Pierce, cat #21181) was prepared in PBS buffer (20 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ pH 7.4, 150 mM NaCl) at a concentration of 5 mg/mL. The ports that address each spot were connected together using tubing so that the same sample was flowed across the four spots. The spotter was mounted over a FLEXchip plain gold surface and the sample was flowed over all four spots manually using a syringe for approximately 1 hour. At the end, the spots were washed using PBS buffer and the chip was mounted according to the instructions of the manufacturer.

The protein A-adsorbed chip was inserted in a FLEXchip array instrument. The four areas where protein A was deposited were visible, so the spots could be assigned where the immobilized material is located. An example of the assigned spots is shown in FIG. 4A; the four spots labeled 1-4 correspond to immobilized protein A spots, while the remaining spots are used for monitoring of the binding signal of Immune Globulin G ("IgG") over protein A. For this experiment all spots will be treated the same because we are interested in observing the distribution of binding

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signal on the array based on the spotting pattern. Spots 1-4 correspond to the orifices shown in FIG. 3A.

After the assignment of the spots, the gold chip was blocked using 5 mg/mL BSA. A picture of the chip after hydration is shown in FIG. 4B. The binding assay was performed at 25°C in PBS buffer supplemented with 0.2 mg/mL BSA. The buffer signal was monitored for five minutes at a flow rate of 0.5 mL/minute to establish a stable baseline. Human IgG1, κ (Sigma cat #I5154) was prepared in buffer at 100 nM and injected over the Protein A surface at 0.5 mL/minute for 6 minutes. Buffer was then flowed for an additional 10 minutes to monitor the dissociation phase of the complex.

IgG bound to all the protein A spots, indicating that protein A adsorbed efficiently to the gold surface under the conditions tested. Spot 1, which was the first spot deposited during the spotting protocol, resulted in the highest binding signal, *see* FIG. 5, approximately 195 RCU's. In this region there was leakage to the surrounding spots, as observed by the high binding signals in the binding panel of spot 1. From FIG. 4A, spot 2 appears to cover two spots, which agrees with the observations of the binding experiment shown in FIG. 5. Binding was detected over the two neighboring spots at 120 and 105 RCU. Binding responses were also detected in the spots exactly above the protein A-immobilized spots. Similar to spot 1, binding signals over the spots located down the row from the protein A spot were not detected. The signals over spots 3 and 4 appear much cleaner than the signals of spots 1 and 2 with minimal binding responses over the surrounding spots. This is likely due to a misalignment when the orifice was placed against the surface, leading to poor sealing in spots 1 and 2. Moreover, microscopic grooves were detected on the sealing face due to the method of cutting the spotter face with a razor blade (*see* FIG. 3). Alternative ways of cutting the spotting face to produce a more planar surface will likely produce a more effective seal.

Overall, the results of this experiment were very encouraging, showing that Protein A could be efficiently immobilized over the surface of a gold chip to detect binding of human IgG.

Example 3: Eight-orifice spotter

In Examples 1 and 2, external fluidic connections were made by inserting a 20-gauge needle into the microconduits of the spotters and making connections to the needle (*See* FIG. 3). This technique proved to be robust and sufficient for small scale research devices. However, scaling of the device may have been problematic. Furthermore, a method was necessary that would allow simplified loading of samples. The solution, shown in FIG. 6, was to place sample wells directly above the microconduits. To simplify substance transfer, the wells were placed in the same spacing and format as those of a 16-well plate. This allows researchers to use current sample handling equipment to load and unload the device.

Another significant challenge to parallel fluid delivery was the pumping of the fluids. In Examples 1 and 2, pumping was accomplished by connecting a syringe pump or vacuum line to each individual outlet line. In the embodiment shown in FIG. 6, it is possible to pump all the microconduits at once. Each flow loop is connected to two wells, one for sample loading and one for pumping interface. A simple manifold is placed over the first line of wells and a pump is used to deliver alternating vacuum and positive pressure, cycling the solutions back and forth between the two connected wells.

Example 3 Summary

Binding of IgG to the protein A spots was detected in seven out of the eight spots deposited on the gold chip (*See* FIGS. 9-12). Unlike what we detected with Example 2, binding responses above the baseline were not detectable over the spots in the front of protein A spots suggesting that the spotting is specific over the allocated regions. Signal was detected over the spots following the spotted protein A spots though this is not related to the spotting process but spreading of the samples at high concentrations of protein used for spotting. The IgG binding signals ranged from 100-180 RCU, indicating that there is variability among the amount of protein A deposited over each spot. These results demonstrate that the spotter can be effective in immobilizing ligands to the chip surface yet, this spotter may be further improved to generate more uniform spots.

Example 3 Test Methods and Results

The same sample of protein A that was used in Example 2 was also used for this assay [ImmunoPure Protein A (Pierce, cat #21181) at 5 mg/mL in PBS buffer (20 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ pH 7.4, 150 mM NaCl)]. The spotting was performed over a plain gold FLEXchip and the protein A was flowed over the chip for approximately 1 hour. At the end the spots were washed using PBS buffer and dried. The resulting spots are shown FIG. 8.

Seven of the eight protein A spots were visible after the chip was inserted into the FLEXchip array instrument. The spots were 200 microns square, roughly half the size of the spots deposited over with the four-orifice spotter. A grid of 10x10 spots was assigned to enclose the eight protein A spots and the surrounding area (although the spotting pattern is a 10x10 grid, inclusion of the reference spots results in a grid of 21x11, as shown in the histogram). For reference, the protein A spots are located over the seventh column, spots 2-9. An empty spot was assigned at the top and the bottom of the column to control for contamination of the protein A sample to the surrounding area.

The binding experiment was performed under conditions similar to Example 2. The gold chip was blocked using 5 mg/mL BSA and the assay was performed at 25°C in PBS/0.2 mg/mL BSA buffer. The buffer signal was monitored for five minutes at a flow rate of 0.5 mL/minute to establish a stable baseline. Human IgG1, κ (Sigma cat #I5154) at 100 nM was flowed over the protein A surface at 0.5 mL/minute for 6 minutes. Buffer was then flowed for an additional 10 minutes to monitor the dissociation phase of the complex.

IgG binding was detected over seven of the eight spots, as shown in FIG. 9. Spot 9 did not show any binding signal suggesting that the microchannel may have been clogged. For the remaining spots, the binding signals ranged from 100 to 180 RCU. Ideally, the responses of these spots should be more uniform with each other since the same material was circulated back and forth at the same rate. The differences are likely due to varying flow rates between the microconduits. As seen in FIG. 6, the microconduits lengths vary due to the sample handling format. Because all the microconduits of this device had equal cross-sectional dimensions, the shorter

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microconduits experienced faster flow rates. Other embodiments may be designed to address this issue.

The leakage problems encountered with the four-orifice spotter have been improved in this embodiment. FIGS. 11 and 12 show the signals detected over the areas in the front and the back of the protein A spots. The left-hand panel shows an overlay of all the signals from the first six columns. Sample leakage was not detected this time, since the signals from all spots overlay at the baseline. Unlike the signal in the front of the protein A spots, spots in the back of the protein A spots (columns 8-11) showed a range of binding responses from 0-50 RCU, suggesting that the back spots are contaminated with protein A. This is not associated with the spotting but rather with the high concentrations of protein (5 mg/mL) used in this assay. Because of such high protein concentrations, it is likely that some of the protein fails to covalently adsorb to the gold surface but instead remains aggregated over the gold chip. As a result when the chip is filled with buffer, the protein flows over the spots following the protein A spots.

The distribution of the binding signals as shown in the histogram (FIG. 12) shows that the contamination is more prominent closer to spots that showed high binding signals, for example spot 2. Spots 5 and 6, which showed low binding responses, showed responses of >5 RCU. Once we have developed an optimized spotting protocol we plan to use proteins of much lower concentrations for spotting which would minimize this effect.

In conclusion, the experiment was successful in terms of showing that the automated eight-orifice spotter can deposit eight spots simultaneously. Although this device shows many improvements over the manual four-orifice spotter, the spot uniformity may need to be further improved. In addition, increasing size of the spots slightly may allow signals to be detected using protein samples of lower concentrations.

Example 4: Forty-Eight Orifice Spotter

In this example, an embodiment of a 48-orifice spotter was used to address a two dimensional 12x4 array, as shown in FIG. 13. The device is designed to integrate with a 96-well plate. Each channel loop system is connected to two wells – one for

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sample substance loading and one for pumping, which is accomplished by applying alternating vacuum and positive pressure. In this embodiment, the lower block of 48 wells is covered by an air manifold and samples are loaded into the upper block of 48 wells.

FIGS. 14 and 15 show an embodiment of an assembled 48 orifice device. Spot sizes are approximately 400 microns by approximately 200 microns. Distance between spots within rows is approximately 400 microns. Distance between spots within columns is approximately 1.5 mm, though some variability exists (FIG. 15). This was due to the use of open molds and irregularities in the levelness of the curing oven. In the future, spotters may be manufactured with closed injection molds, among other techniques, allowing a precise control of layer thicknesses and therefore of spot pitch.

Example 4: Preliminary Tests Results

A dilute solution of Protein A (in PBS at 0.4 mg/mL) was immobilized over a plain gold chip for approximately 30 minutes. An image of the printed chip is shown in FIG. 16. The spots were printed in a 4x12 format. When the chip was inserted in the FLEXchip platform most of the spots in three columns were visible. The device may have been misaligned on the FLEXchip during printing. By comparing FIGS. 15 and 16, it appears that the left most column in FIG. 16 corresponds with the bottom row of spots in FIG. 15, due to the slight spacing irregularities between rows 3 and 4. Therefore, one row of spots was likely deposited outside of the active chip area and the following results examine 36 spots arrayed in a 3x12 configuration. There does not appear to be any substantial spreading around the spots. Also, the concentrations of protein A tested was sufficiently low so when the entire surface of the array was exposed to buffer there was no carryover to the spots further along the direction of the buffer flow.

Human IgG was prepared in running buffer (PBS, 0.2 mg/mL BSA) and tested for binding at 100 nM. The association phase was monitored for 6 minutes at a flow rate of 500 μ L/minute. The binding profile for the spots are shown in FIG. 17. Although most spots produced binding signals, there was some variability among the responses. The average signal was 84.2 RCU with a CV of 9.7%.

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The results show that the levels of protein A immobilized were inconsistent among the different spots. Fast flow rates were observed during the spotting (an uncharacterized pump was used), which may have contributed to the variability. In addition, the microconduit design has not been optimized to provide uniform flow between microconduits. Therefore, the flow rate experienced by each spot varied.

In conclusion, these results show that a multi-layer device can address a two dimensional array of spots without contamination to surrounding spots. Design changes and pumping modifications may be employed to improve deposition uniformity between spots.

Example 5: Lipid Microarrays

Lipid bilayer arrays are normally difficult to deposit because the lipids degrade if the spots dry out. Additionally, the hydrophobic nature of lipid-based solutions make them difficult to deposit using traditional pin-spotter systems, as the lipid molecules do not adhere to the pins. The spotter is suited for lipid arrays because the deposition process can be performed without any exposure to air, and the spotter convective flow does not require hydrophilic solution characteristics.

Small Unilamellar Vesicle (SUV) were prepared from the following Avanti lipids: 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine (DOPC) and Phosphatidyl-ethanolamine (NBD). These small vesicles will self-assemble onto the glass surface to form a bilayer. The sample wells of the spotter were each filled with SUV solution and then flowed to the pump wells. There was an incubation time of 6 minutes and a total flow time of 10 minutes. The wells were then unloaded and rinsed with tris buffer to remove excess vesicles. Nanopure was used to rinse channels until wells were half empty. The slide was slowly removed from the spotter face and placed in a petri dish making sure not to expose the spots to air. The slide was washed once more with nanopure and the image was taken. The uniform spots in FIG. 18 indicate that a lipid bilayer was formed.

Example 6: Sandwich Assay

Anti-mouse IgG with biotin (OEM Concepts, cat # G5-MG10-4 , 10X diluted) was filled in the wells of a 8-well spotter device. Using a pumping manifold and applying vacuum and pressure alternatively, this was flowed through the microconduits over the surface of a streptavidin coated glass slide surface. The anti-mouse IgG was deposited at specific portions on the glass slide through the orifices in the microconduits. The slide was then blocked with 3% BSA (high purity BSA suspended in PBS) for 10 minutes. Once blocked, it was incubated with mouse IgG diluted in 3%BSA for 5 minutes. The mouse IgG bound only to the anti-mouse IgG portions of the substrate. After the incubation, the plate was washed three times with Tris Base Saline with Tween-20 ("TBST"). Then, the substrate was incubated for 5 minutes with Horse Radish Peroxidase ("HRP") labeled goat anti-mouse diluted 1000X in 3%BSA. HRP is a chemiluminescent enzyme used to label antigens and their antibodies to detect their presence. A final wash step was then performed.

The slide was then examined under a scanner. Only the spots deposited by the spotter should be visible since the HRP attaches only to these portions through the mouse IgG.

FIG. 19 compares the effect of using a dilute solution of anti-mouse IgG in the spotter and the same dilute solution in a conventional pin spotter. The spotter performs better in two areas: 1) superior sensitivity with dilute solutions – the pin spotter $\mu\text{g/mL}$ spots are barely visible while the spotter flow deposited spots exhibit significantly more binding; 2) the spotter exhibits higher quality, more uniform, and discrete spots. Embodiments of the spotter may be further refined. The flow time of a solution in the spotter may be increased if the solution is not the desired concentration. Thus, the spotter provides a user with the flexibility to improve spotting results in a short time frame. The results shown in FIG. 19 further indicate that the spotter may be used for a sandwich assay or an ELISA.

While disclosed with particularity, the foregoing techniques and embodiments are more fully explained and the invention described by the following claims. It is clear to one of ordinary skill in the art that numerous and varied alterations can be made to the foregoing techniques and embodiments without departing from the spirit and scope of the invention. Therefore, the invention is only limited by the claims.

CLAIMS

What is claimed is:

1. A spotter comprising:
 - a support surface;
 - a plurality of wells formed in the support surface in substantially the same spacing and format as a well plate and each of the plurality of wells having a capacity to hold a quantity of fluid;
 - a plurality of orifices sized and formed in a sidewall of each of the plurality of microconduits;
 - the plurality of microconduits operably connected to the plurality of wells; and
 - the plurality of orifices arranged along a different surface of the spotter in an ordered array.
2. The spotter of claim 1, wherein the number of the plurality of wells is 8, 16, 32, 96, 192, 384, or 1536 wells.
3. The spotter of claim 1, wherein the plurality of orifices arranged along a surface of the spotter are arranged along either a side or a bottom surface of the spotter.
4. The spotter of claim 1, wherein the different surface is adapted to form a seal against a surface of a substrate.
5. The spotter of claim 1, wherein the ordered array comprises a 2-D array.
6. The spotter of claim 1, further comprising a pumping manifold that interfaces with at least some of the plurality of wells.
7. The spotter of claim 6, wherein the pumping manifold is adapted for connection to a pump/vacuum device.

8. The spotter of claim 1, wherein the number of the plurality of orifices arranged along the surface of the spotter is 4, 7, 8, 14, 16, 28, 48, 84, 88, 96, 176, 180, 192, 360, 368, 768, 1488, or 1504.

9. The spotter of claim 1, wherein the plurality of microconduits are of varying lengths.

10. The spotter of claim 1, wherein the spotter may be manipulated by well plate handling equipment to load and unload the spotter.

11. The spotter of claim 1, wherein the spotter material is selected from the group of: silicon, silica, polydimethylsiloxane ("PDMS"), gallium arsenide, glass, ceramics, quartz, neoprene, TEFLON™, polyethylene elastomers, polybutadiene/styrene butadiene rubber ("SBR"), nitriles, and combinations thereof.

12. The spotter of claim 1, wherein each of the plurality of orifices is connected to two each of the plurality of wells.

13. The spotter of claim 1, wherein at least one sensing component is integrated into the different surface of the spotter where the orifices are arranged.

14. The spotter of claim 1, wherein each of the plurality of orifices is connected to at least one of the plurality of wells to which no other of the plurality of orifices is connected.

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15. A method of delivering at least one substance to a substrate surface with the spotter of claim 1, the method comprising:

loading some of the plurality of wells with the at least one substance;

placing the different surface of the spotter in proximity to the substrate surface sufficient to form a seal between the spotter different surface and the substrate surface;

flowing the at least one substance from the loaded plurality of wells to the plurality of orifices;

flowing the at least one substance over the substrate surface; and

flowing the at least one substance towards a different some of the plurality of wells.

16. The method according to claim 15, wherein loading the plurality of wells comprises loading each of the wells with a different substance.

17. The method according to claim 15, further comprising flowing a second substance over the substrate surface without breaking the seal between the spotter different surface and the substrate surface.

18. The method according to claim 15, wherein flowing the at least one substance comprises flowing at least one substance independently selected from the group consisting of: a protein; a nucleic acid, a cell; a lectin; a synthetic composite macromolecule; functionalized nanostructure; a synthetic polymer; a nucleotides, a nucleoside; an amino acids; a ligand; a chelator; a hapten; a chemical compounds; a sugar; a lipid; a liposome; a tissue sample; a virus; and any combination thereof.

19. The method according to claim 15, where the method is used to conduct surface plasmon resonance, enzyme-linked immunosorbent assays, chemiluminescence tests, fluorescence tests, or a quartz crystal microbalance.

20. The method according to claim 15, where the method is used with ink jet printers, mass spectrometry systems, spotting robots, and sample injection systems.

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21. The method according to claim 15, wherein flowing the at least one substance over the substrate surface comprises flowing a protein over a surface of a plasmon resonance substrate or a mass spectrometry plate.

22. The method according to claim 15, wherein flowing the at least one substance over the substrate surface comprises flowing sufficiently to deposit at least a portion of the at least one substance on the substrate surface.

23. A method of loading a spotter for delivering a substance to a surface comprising:

positioning a spotter via a positioning means; and

loading the substance into a plurality of wells of the spotter via a handling means, the spotter comprising a means for holding a plurality of wells, the wells adapted to hold a quantity of fluid and having a predetermined spacing between adjacent wells, a plurality of microconduit means connected to each well, and aperture means within said plurality of microconduits, the aperture means arranged operable to form a seal with the surface.

24. The method of claim 23, wherein loading the substance into the plurality of wells comprises automatedly loading the substance into the plurality of wells.

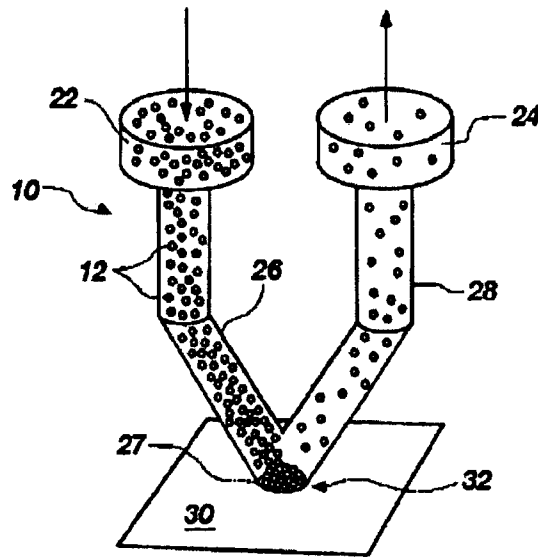


FIG. 1

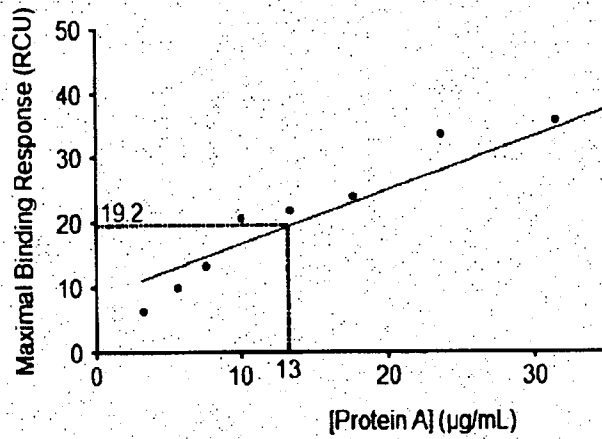


FIG. 2

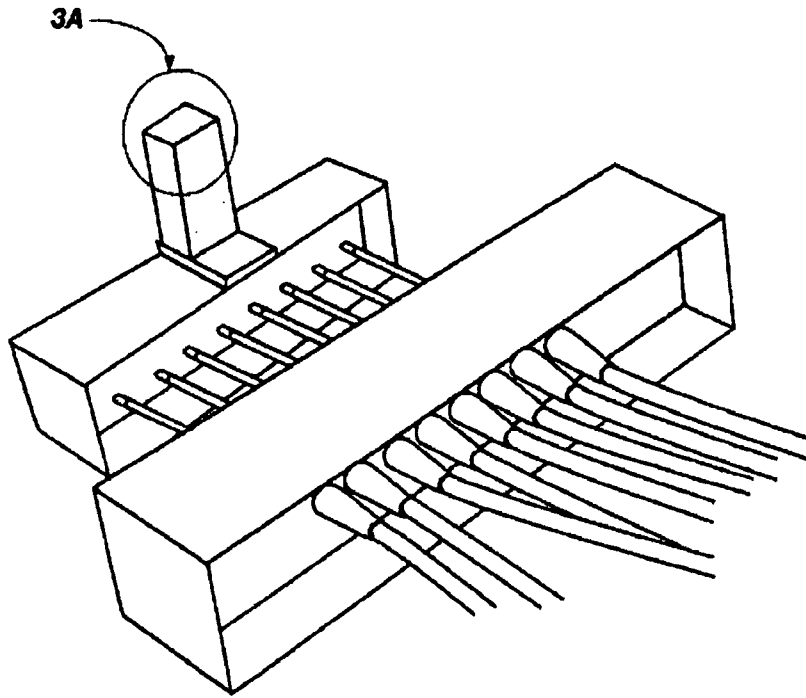


FIG. 3

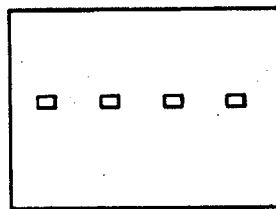


FIG. 3A

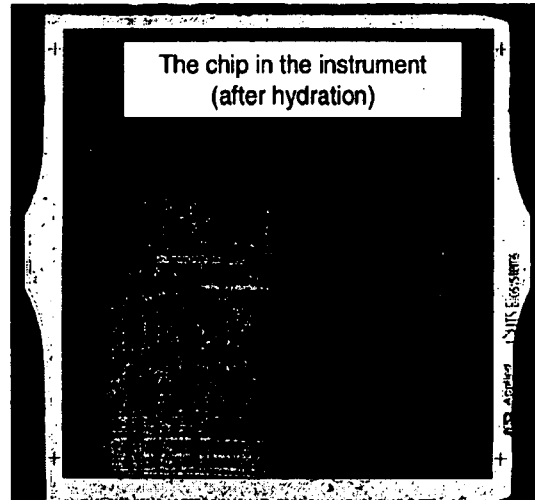
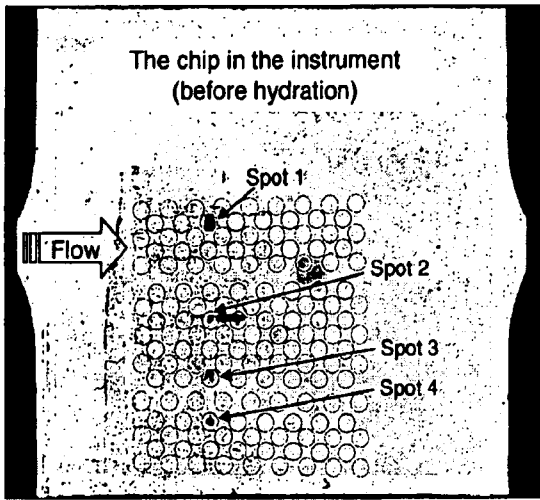


FIG. 4A

FIG. 4B

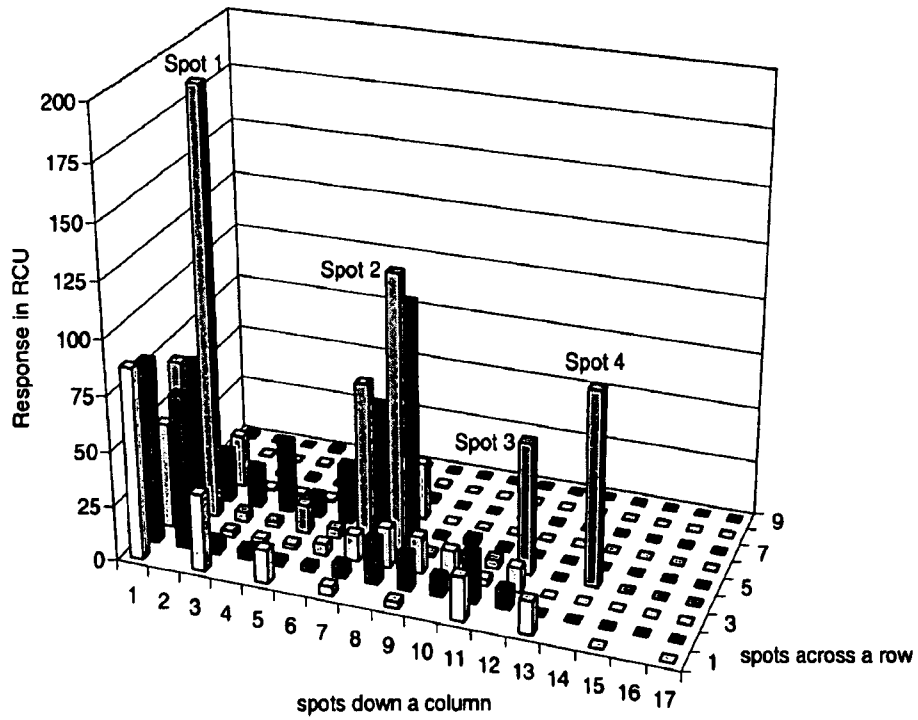


FIG. 5

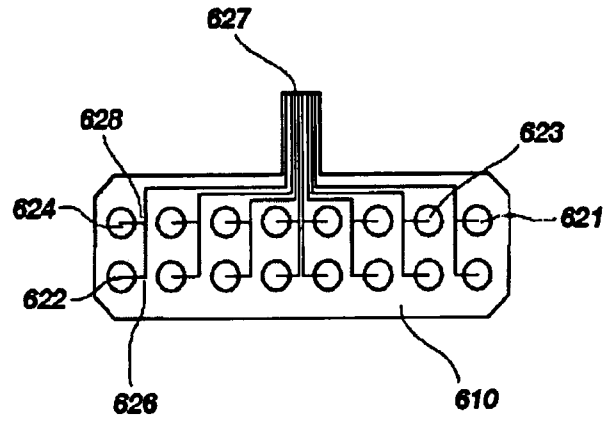


FIG. 6

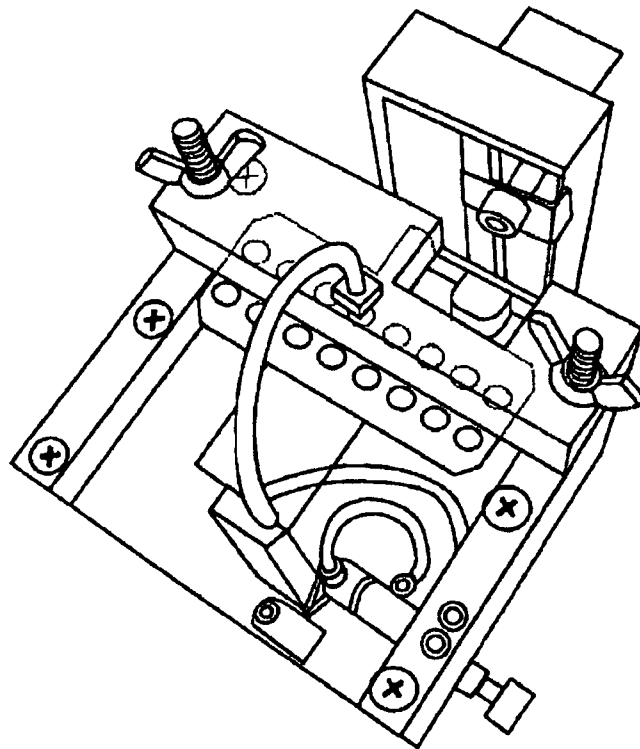


FIG. 7

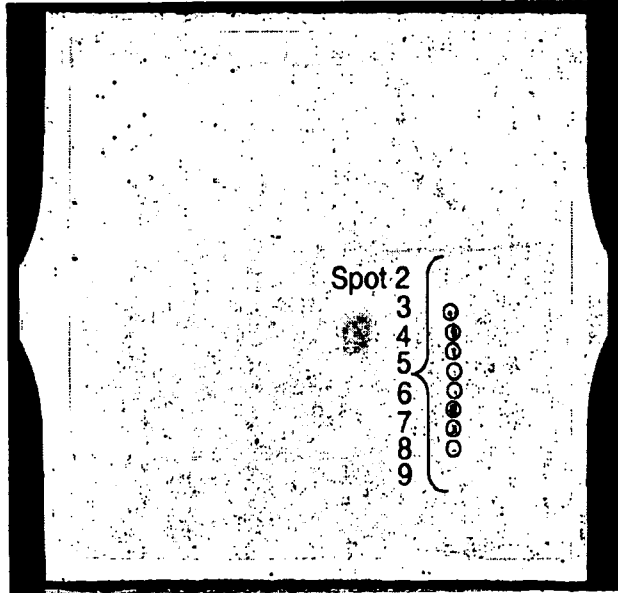


FIG. 8

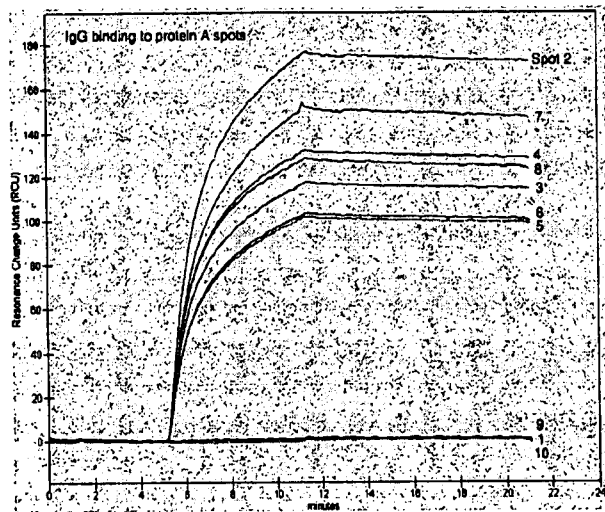


FIG. 9

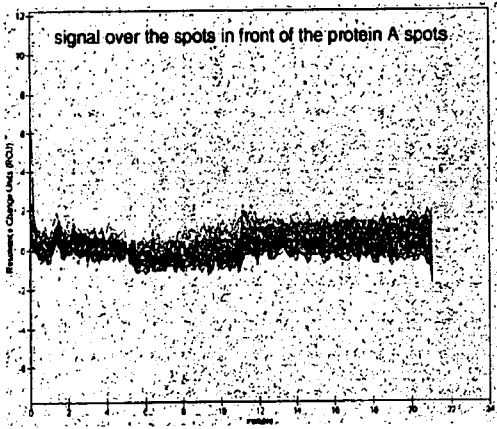


FIG. 10

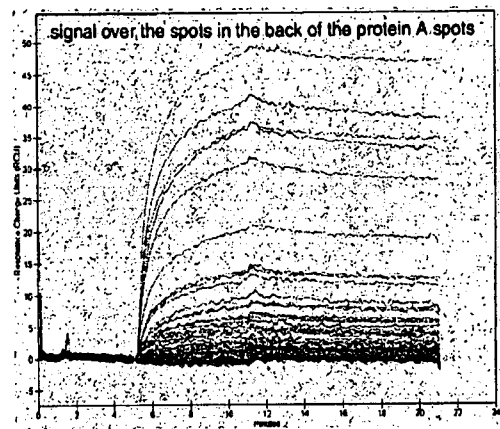


FIG. 11

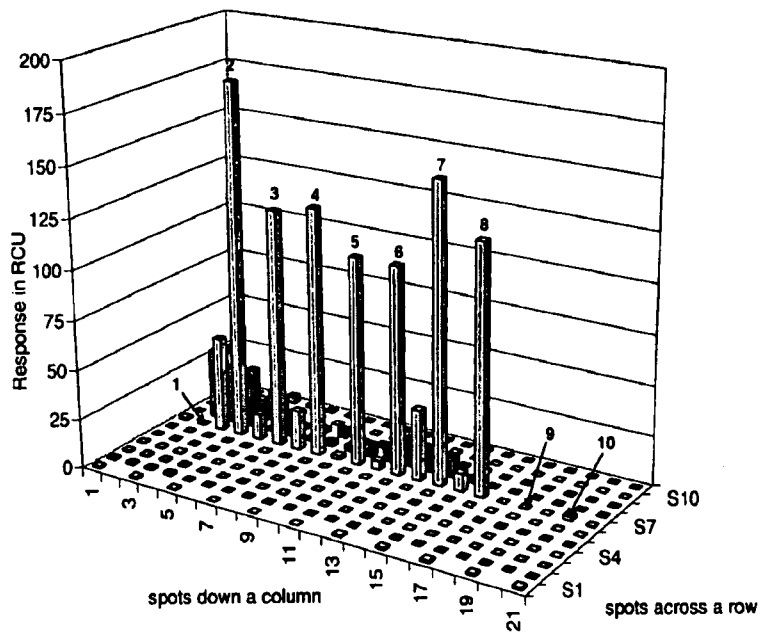


FIG. 12

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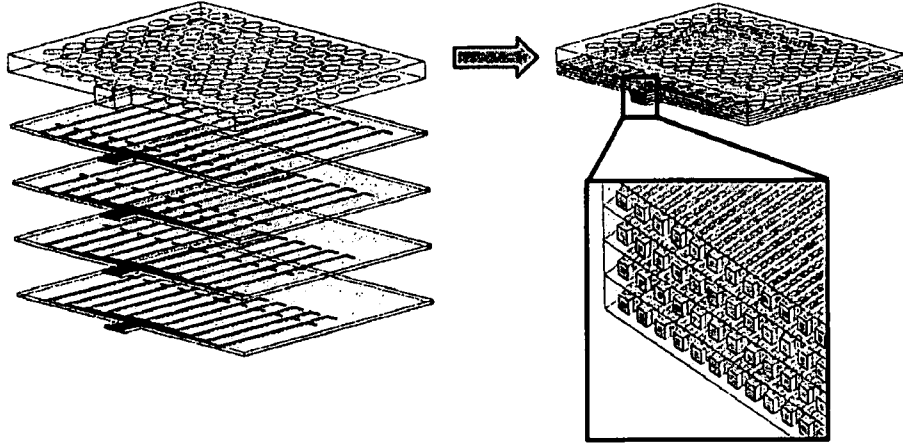


FIG. 13

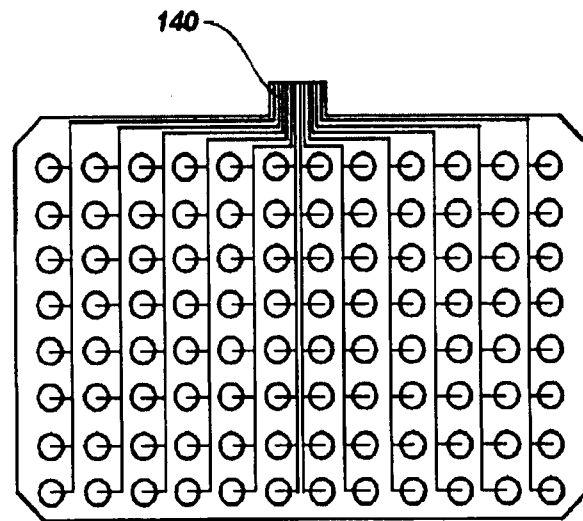


FIG. 14

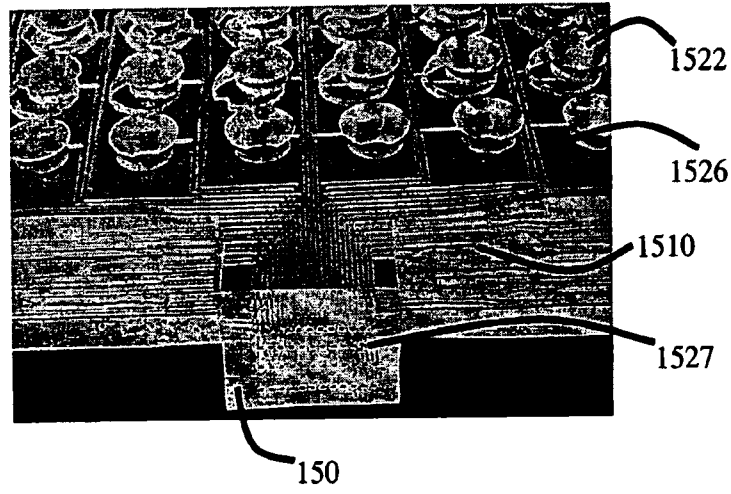


FIG. 15

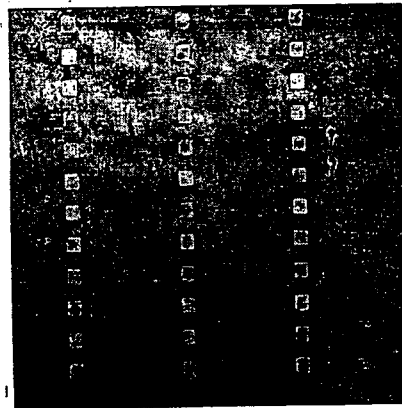


FIG. 16

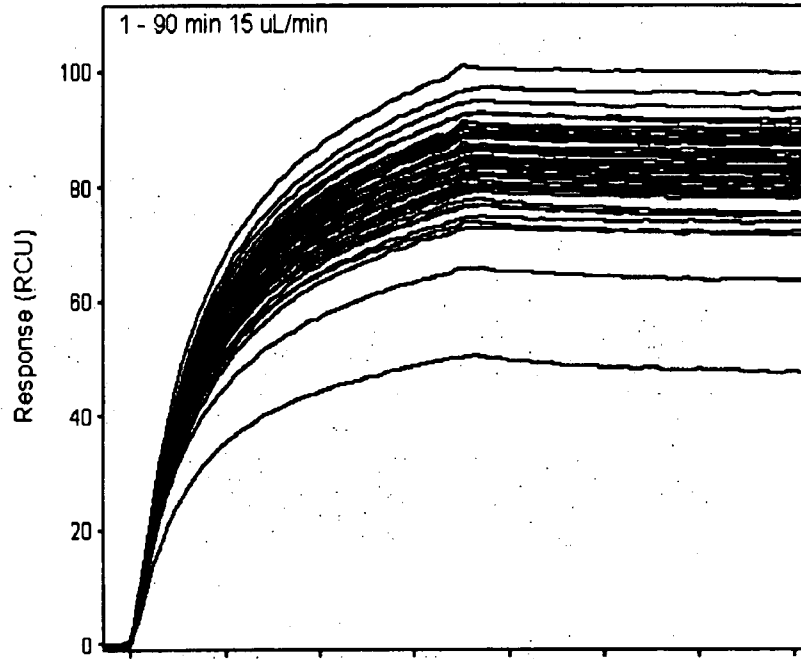


FIG. 17

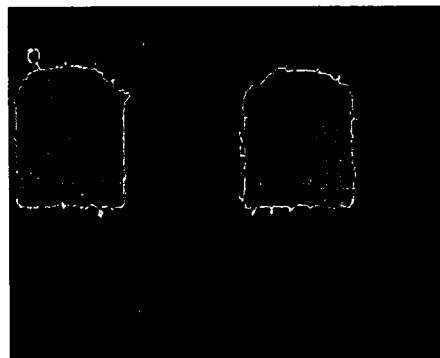


FIG. 18

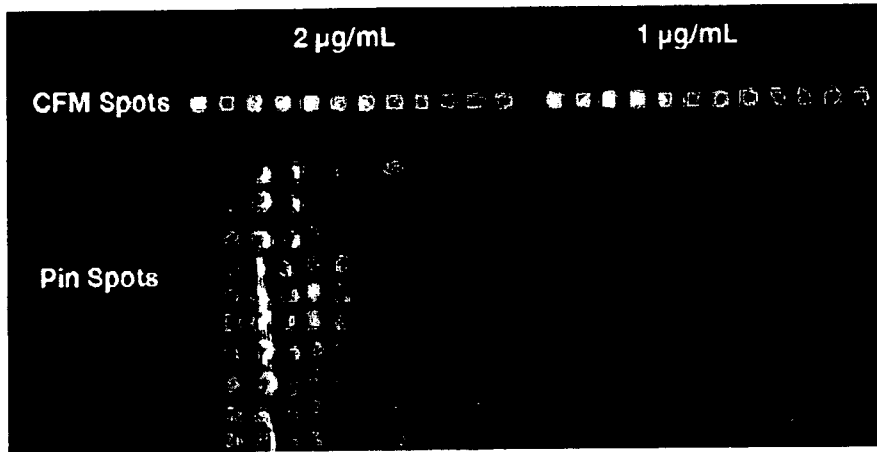


FIG. 19

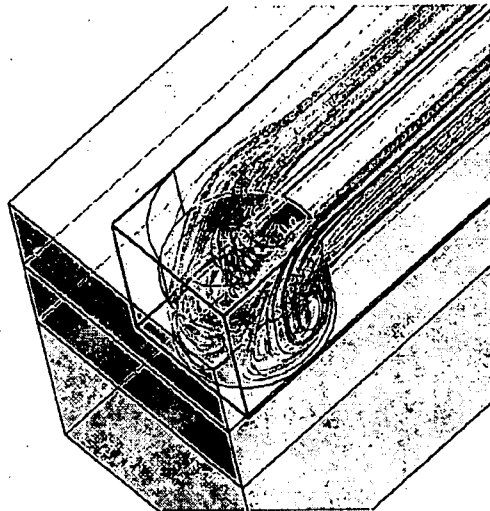


FIG. 20

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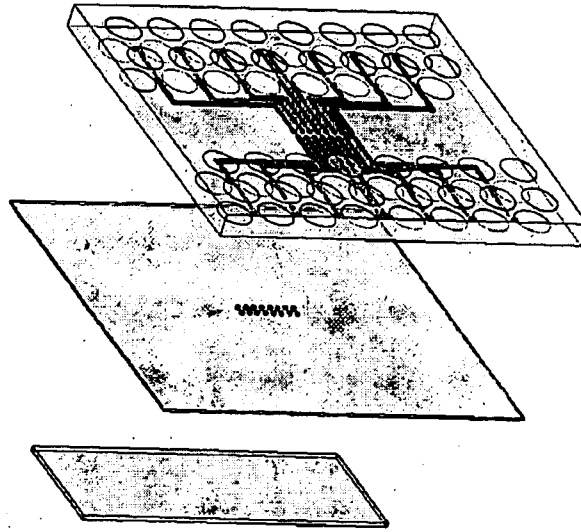


FIG. 21

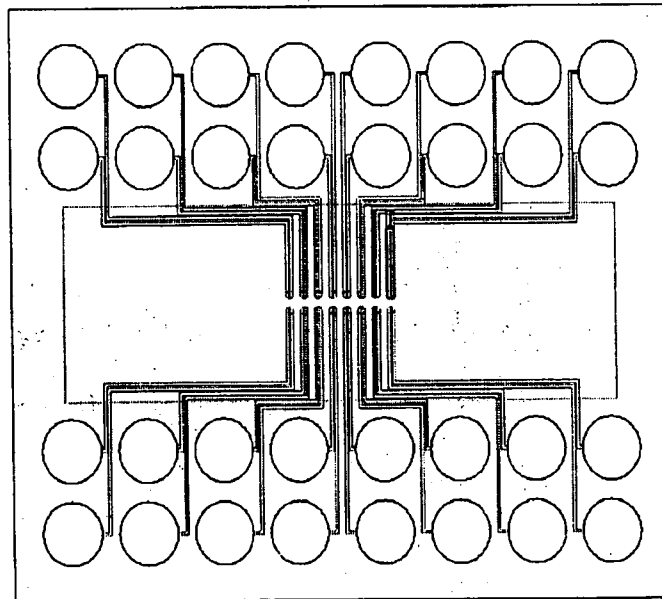


FIG. 22