(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 January 2002 (03.01.2002)

PCT

(10) International Publication Number
WO 02/01184 A1

(51) International Patent Classification[7]: G01N 1/28, 35/00

(21) International Application Number: PCT/US01/19954

(22) International Filing Date: 22 June 2001 (22.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/213,865 23 June 2000 (23.06.2000) US

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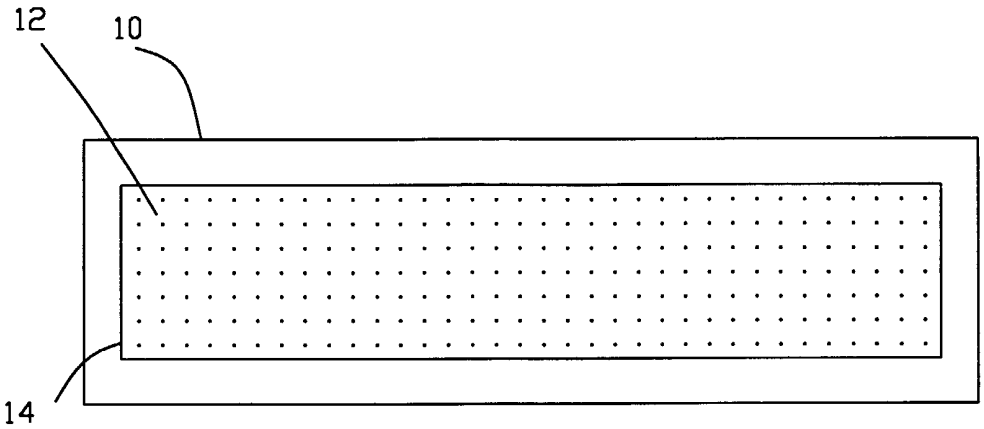
(81) Designated States *(national)*: CA, JP.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FLUID MIXING IN MICROFLUIDIC STRUCTURES



(57) Abstract: A device for assisting in fluid mixing within microfluidic sized structures. Chemicals and other biological specimens are exposed to a small volume of reagent, and said reagent is delivered to said specimens by a novel mixing technique, thus shortening overall process time.

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FLUID MIXING IN MICROFLUIDIC STRUCTURES

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application claims benefit from U.S. Provisional Application Serial No. 60/213,865, filed June 23, 2000, which application is incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to mixing of fluids and, in particular, to the mixing of small volumes of fluids that are dispersed over a relatively large area. Such mixing is required, for example, when a small quantity of reagent is to be distributed uniformly over a microscope slide. This is desired when spotted microarrays are to be exposed to various reagents.

2. Description of the Prior Art

Spotted microarrays are currently in use for various applications. In most cases, a spotted microarray comprises a glass slide of roughly 1" by 3", onto which several hundred to several thousand spots are deposited. These spots typically contain genetic material or other material of biological interest.

Microarrays are currently exposed to reagents by dipping them into a fairly large volume of fluids. Given the sometimes very high costs of the reagents, it would be desirable to reduce the volume required for exposing the microarrays.

Microfluidic devices have recently become popular for performing analytical testing. Using tools developed by the semiconductor industry to miniaturize electronics, it has become possible to fabricate intricate fluid

systems which can be inexpensively mass produced. Systems have been developed to perform a variety of analytical techniques for the acquisition of information for the medical field.

Microfluidic devices may be constructed in a multi-layer laminated structure where each layer has channels and structures fabricated from a laminate material to form microscale voids or channels where fluids flow. A microscale channel is generally defined as a fluid passage which has at least one internal cross-sectional dimension that is less than 500µm and typically between about 0.1µm and about 500µm. The control and pumping of fluids through these channels is affected by either external pressurized fluid forced into the laminate, or by structures located within the laminate.

A microfluidic device can be constructed that mates with a spotted microarray such that the microarray forms the bottom of a channel that is as wide and as long as the microarray, but has a depth of a microfluidic dimension. Microfluidic devices are defined as having at least one dimension in the range of 1-1000 micrometers. Typically, such a device would have a channel depth of 100 micrometers.

Providing such a device does solve the problem of reducing the reagent volume requirement, but creates another problem: all fluid flow in such a channel is laminar, which implies that, when fluid flows into a such a channel, no mixing other than by diffusion of particles occurs. Particle diffusion is a slow process, depending on the particle size and other fluid parameters, and it can take several hours for fluid particles to diffuse a distance of a few millimeters. Therefore, reactions between the chemicals immobilized in a spot, and those contained in the reagent solution, are rate-limited by the diffusion of reagent particles to the spot. This significantly slows down the reaction, and therefore the required process time for spotted arrays. This invention provides a device and method for moving fluid in such a channel such that each spot is periodically or continuously exposed to a fresh,

unreacted portion of the reagent fluid such that the chemical reaction is no longer diffusion-limited, and the overall process time is reduced.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a method and a device for mixing fluids in wide channels that have a depth of a microfluidic dimension.

It is a further object of the present invention to provide a method and a device for exposing chemicals that are immobilized on a slide to a small volume of reagent while preventing the reaction from becoming diffusion-limited.

These and other objects of the present invention will be more readily apparent in the description and drawings that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top view of a mixing process used in the prior art;

FIG. 2 is a top view of a slide prepared according to the present invention;

FIG. 3 is a top view of a cover slide for use with the slide shown in FIG. 2;

FIG. 4 is a top view of the glass slides of FIGS. 2 and 3 during the mixing process;

FIG. 5 is a top view of an alternative embodiment of the present invention;

FIG. 6 is a front view of an alternative embodiment of the present invention which uses syringe pumps to assist in fluid mixing;

FIG. 7. is a front view of an alternative embodiment of the present invention which uses a bubble pump to assist in fluid mixing;

FIG. 8 is a front view of the device shown in FIG. 7 in rotation;

FIG. 9 is a front view of another alternative embodiment of the present invention which uses rotation of the entire device to assist in mixing; and

FIG. 10 is a front view of the device shown in FIG. 9 showing different locations during rotation of the device.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

FIG. 1 is a representation of the current procedure which is commonly used in laboratories. Referring now to FIG. 1, there is shown a microscope slide 10 containing an array 12 of sample microdots. A reacting liquid is placed on slide 10 covering array 12, and then a cover slip 14 is placed on slide 10, covering array 12. Slide 10 is processed with a heat cycler, and slide 10 is then set aside so that diffusion can take place, as no active mixing occurs during this procedure. Diffusion of the reacting liquid can take as long as 24 hours, and often longer, as the reaction is diffusion-limited. The incubation period for this process can often be very long.

A novel method for performing microfluidic fluid mixing is shown in FIGS. 2-4. Referring now to FIG. 2, a circular slide 20 is shown containing an array 22 of microdots, while the center area 24 of slide 20 contains no microdots, as array 22 comprises a toroidal shape on slide 10. A toroidal cover slide 26, shown in FIG. 3 is also circular in shape, and has a circular aperture 28 located in the central portion of cover slide 26 which aperture corresponds to area 24 of slide 20.

To begin the reaction process, a reacting liquid is placed in aperture 28 of cover slide 26. The liquid will wick under cover slide 26 by capillary action. Cover slide is then rotated in the direction shown by arrow A in FIG. 4. This motion causes the liquid to be completely across the array 22 of microdots, allowing the reaction between the microdots and the reacting liquid. Surface tension at the edges of slide 20 and cover slide 26 will contain the reacting fluid between the slides. The result of this process is a shortened incubation period.

FIG. 5 shows an alternative embodiment of the invention taught in FIGS. 2-4 using different geometries. A rectangular microscope slide 30 is shown having an area 32 in which an array 34 of microdots are located, leaving an area 36 in which no microdots are found. A circular glass slide 38 is initially positioned in area 36.

The mixing process begins as a reacting fluid is added to array 34 and circular slide 38 spins in the direction shown by arrow B while slide 38 moves across array 34 and oscillates back and forth across slide 30. Rotating slide 38 causes local Couette flow as it passes across the microdots in array 34 on slide 30. An external container 40 is used to container slide 30 to inhibit evaporation.

Another embodiment of the present invention is shown in FIG. 6 using a pair of syringe pumps. A glass microscope slide 50 having an array 52 of microdots positioned thereon has a pair of syringe pumps 54, 56 positioned at each end. A cover slide 58 is located above array 52 over the microdots. Reacting fluid is loaded into syringes 54, 56 and each syringe is operated 180° out of phase such that fluid is expelled from one syringe as it is taken up by the other syringe. This motion causes a Poiseuille flow across array 52 of microdots.

Another embodiment of the present invention is shown in FIGS. 6 and 7, which embodiment operates as a bubble pump. A glass microscope slide 60 having an array 62 of microdots applied to the upper surface is covered with a reacting fluid 64 within an enclosure 66. A slide 68 is located within enclosure 66 covering array 62. Fluid 64 fills enclosure 66 such that an air bubble 70 is trapped within enclosure 66 above cover slide 68.

Another version of this embodiment uses a second fluid which is substantially immiscible and has a different density than said reacting fluid. The second fluid may contain magnetic particles or may have magnetic properties. The second fluid is then oscillated across array 62 by use of a magnetic field, such that the reacting fluid is also moved across array 66. The same result may be accomplished by inserting magnetic particles into the reacting fluid.

Enclosure 66 is then oscillated about a pivot point 72 with a rocking motion indicated by arrow C. The range of rotation is preferably limited to approximately 45° in the counterclockwise direction to 45° in the clockwise direction. As assembly 59 is rotated about point 72, bubble 70 trapped within fluid 64 in enclosure 66 moves from end to end moves to the highest point, as can be clearly seen in FIG. 7, due to the air density being less than the fluid density. This gravity-induced motion will move fluid 64 below cover slide 68 back and forth across array 62.

An additional embodiment showing the present invention is shown in FIGS. 9 and 10. In this embodiment, a glass microscope slide 80 contains an array 82 of microdots positioned on the upper surface. Array 82 is covered with a processing liquid and is then covered by a cover slide 84.

Note that slide 84 only covers the area of slide 80 where array 82 is located. Slide 80 is then moved in a circular pattern without any movement of cover slide 84. Several positions of slide 80 are shown in FIG. 10 as 84a, 84b, 84c, and 84d. This circular translation of slide 84 without rotation of

cover slide 84 creates a form of Couette flow in the liquid covering array 82 between slide 84 and 82. This flow mixes the fluid and brings chemical constituents contained in the liquid closer to the microdots in array 82 so that the diffusion path between the constituents within the liquid and the microdots is reduced, thus speeding up the reaction rate and reducing assembly time.

While the present invention has been shown and described in terms of several preferred embodiments thereof, it will be understood that this invention is not limited to these particular embodiments and that many changes and modifications may be made without departing from the true spirit and scope of the invention as defined in the appended claims.

What is claimed is:

1. A device for incubating a slide containing an array of biological materials, comprising:

a first slide having an array of biological materials comprising microdots deposited thereon;

a fluid containing a substance capable of reacting with at least one of said microdots introduced to said array;

a second slide for covering at least a section of said first slide which contains said array;

and means for moving said fluid over said first slide such that said fluid is moved relative to said first slide in order to incubate said first slide.

2. The device of claim 1, further comprising means for introducing said fluid to said first slide.

3. The device of claim 2, further comprising means for removing said fluid from said first slide.

4. The device of claim 3, wherein said fluid introduction means and said fluid removing means each comprise a syringe pump.

5. The device of claim 1, wherein said first slide has a rectangular shape.

6. The device of claim 1, wherein said second slide has a circular shape.

7. The device of claim 1, wherein said fluid moving means comprises rotating said second slide and moving said second slide along said first slide through the portion of said first slide which contains said array.

8. The device of claim 1, wherein said first slide has a circular shape.

9. The device of claim 8, wherein said array is positioned on said first slide in a toroidal shape.

10. The device of claim 9, wherein said second slide has a toroidal shape corresponding to the arrangement of said array on said first slide.

11. The device of claim 10, wherein said fluid moving means comprises rotating said second slide on said first slide.

12. The device of claim 1, further comprising an external enclosure surrounding said first slide, said fluid, and said second slide in order to inhibit evaporation.

13. The device of claim 1, further comprising an enclosure, adjacent said first slide, for containing said fluid over said array and partially filled by said fluid such that an air bubble is formed within said enclosure.

14. The device of claim 13, wherein said fluid moving means causes said air bubble within said enclosure to move back and forth within said enclosure.

15. The device of claim 13, further comprising a second immiscible fluid within said enclosure, said second fluid containing magnetic particles.

16. The device of claim 15, wherein said fluid moving means comprises a magnetic field.

17. The device of claim 1, wherein said fluid contains magnetic particles and said fluid moving means comprises a magnetic field.

18. A method for incubating a slide containing an array of biological materials, comprising the steps of:

selecting a first slide having an array of biological materials comprising microdots deposited thereon;

introducing a fluid containing a substance capable of reacting with at least one of said microdots to said array on said first slide;

covering said array on said first slide with a second slide;

and rotating said second slide on said first slide to move said fluid over said array in order to incubate said first slide.

19. The method of claim 17, further comprising the step of:

moving said rotating second slide back and forth along said first slide over said array.

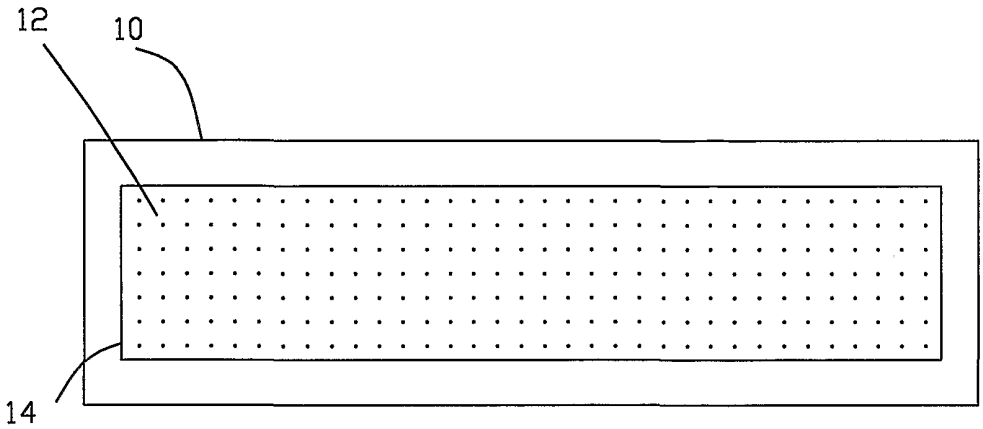

FIG. 1

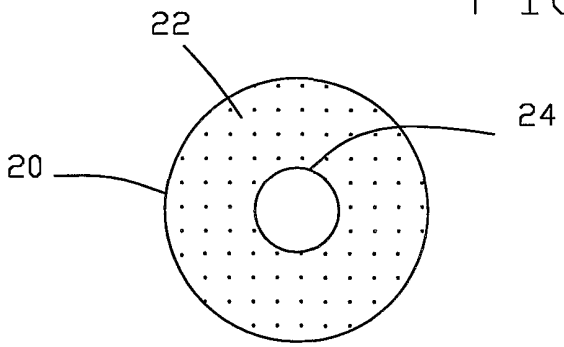

FIG. 2

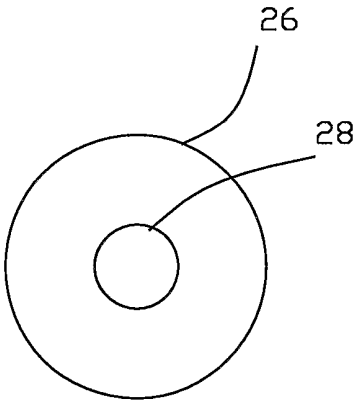

FIG. 3

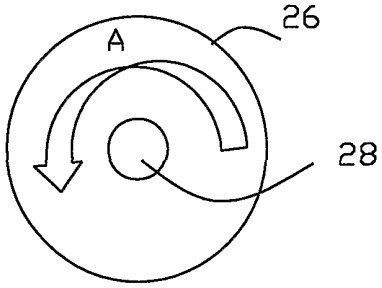

FIG. 4

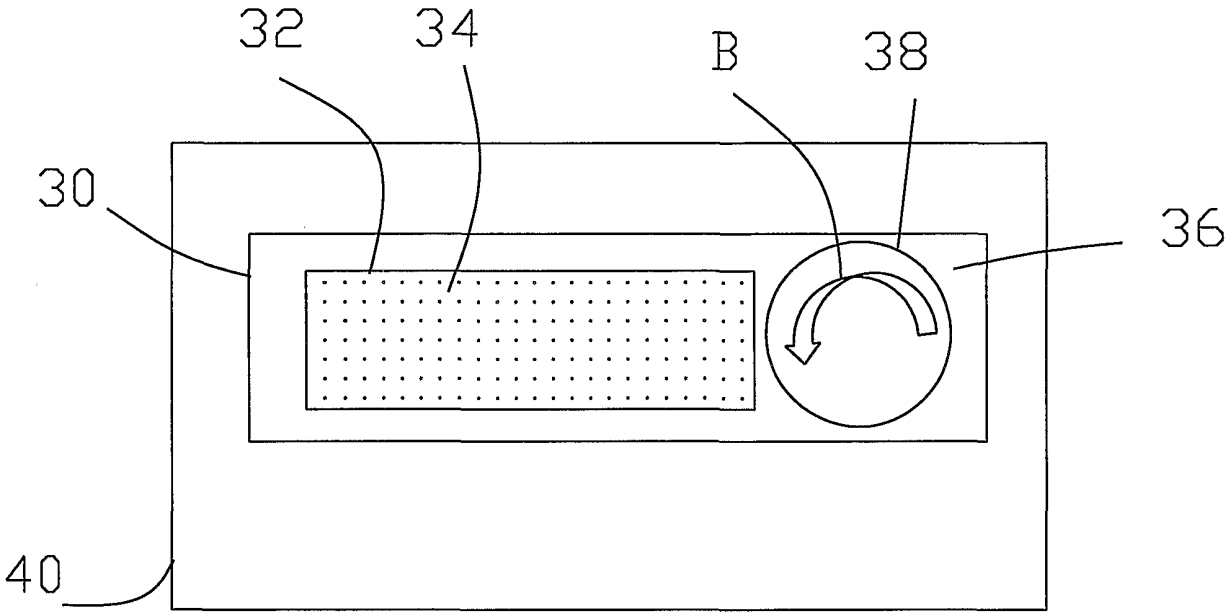

FIG. 5

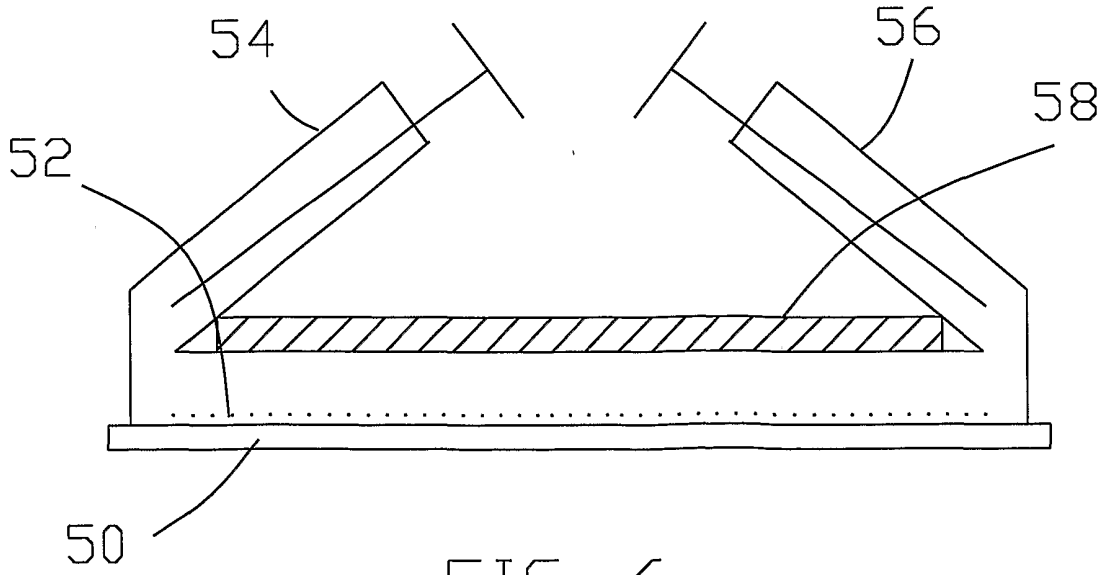

FIG. 6

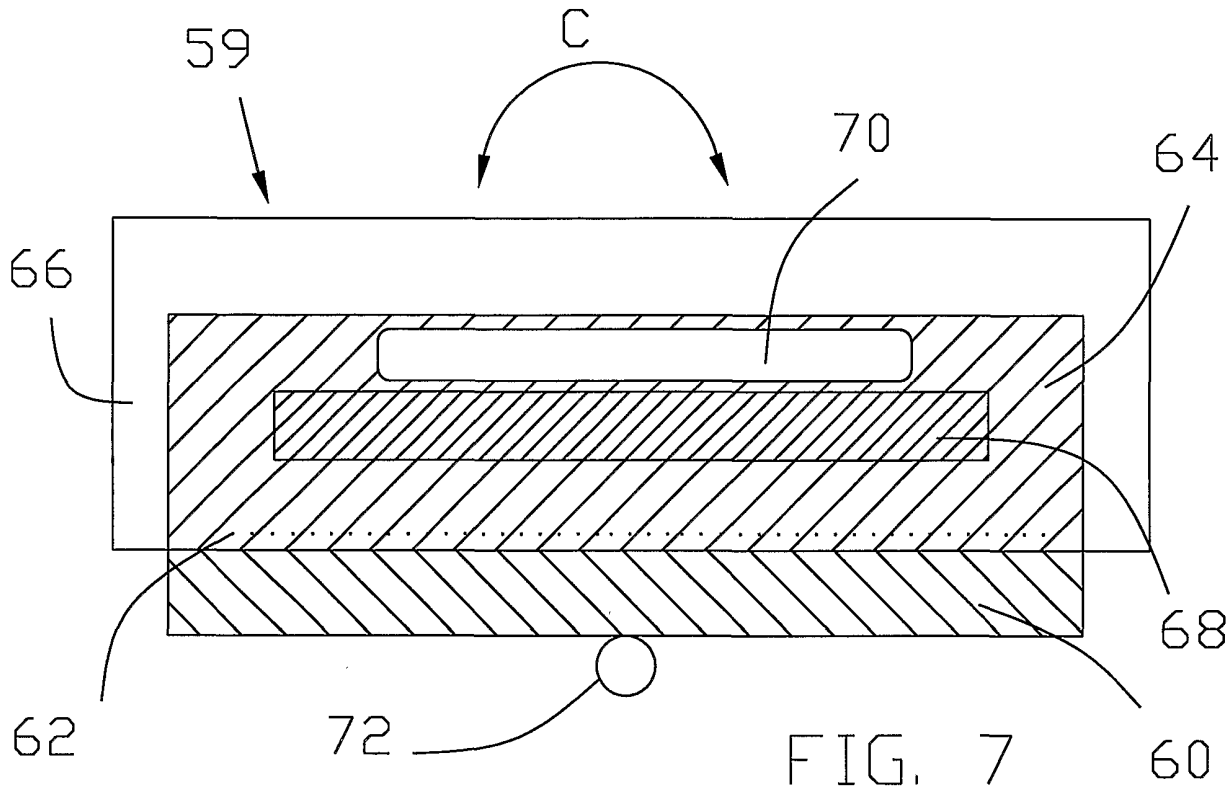

FIG. 7

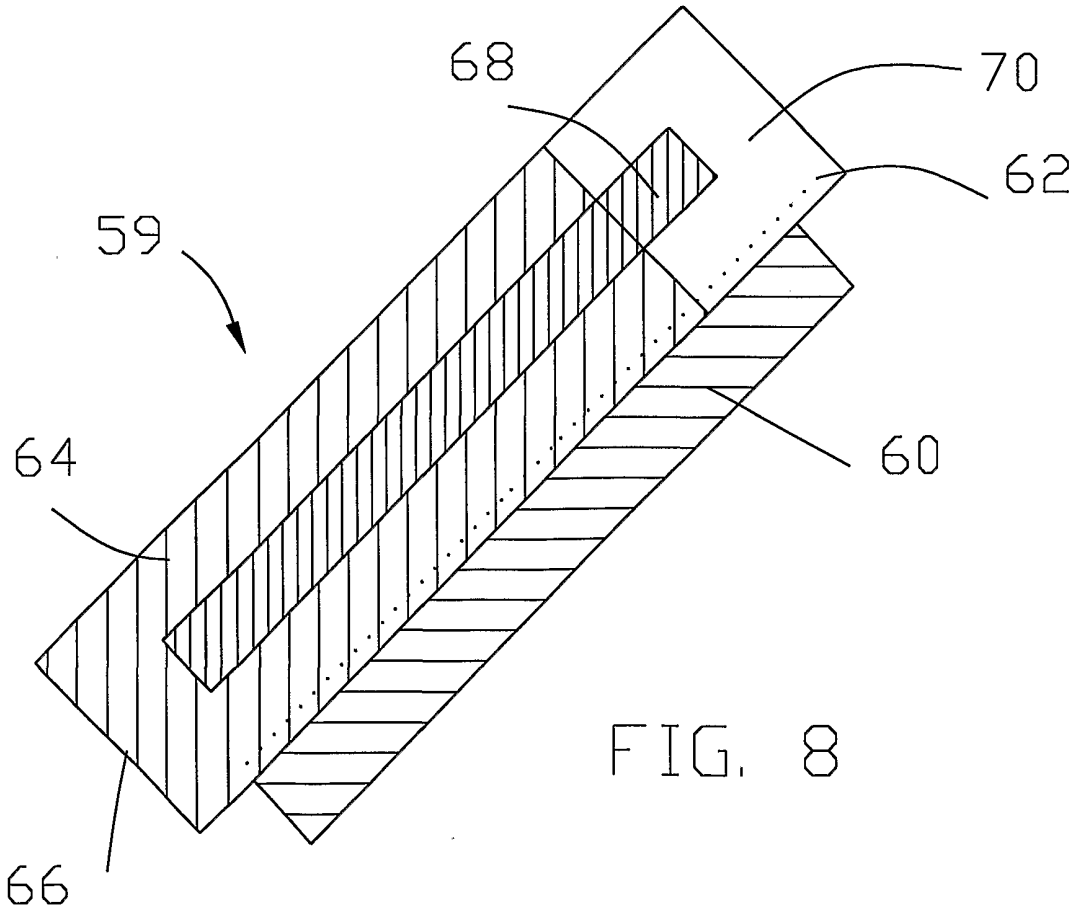

FIG. 8

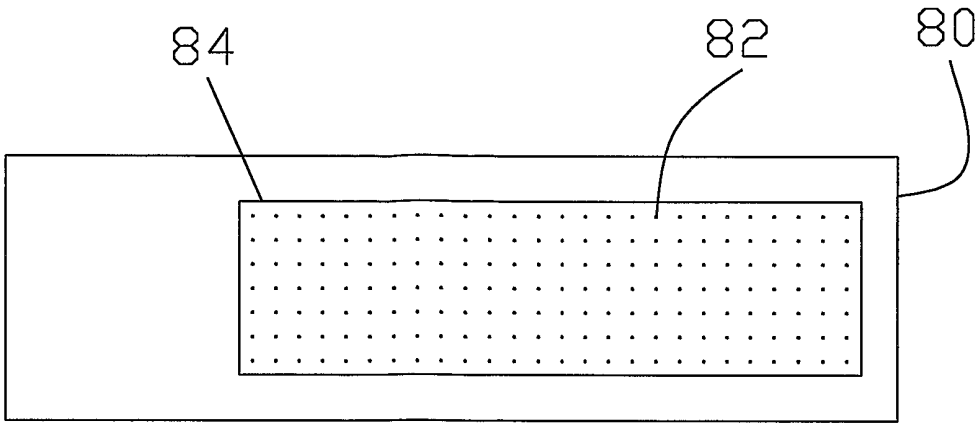

FIG. 9

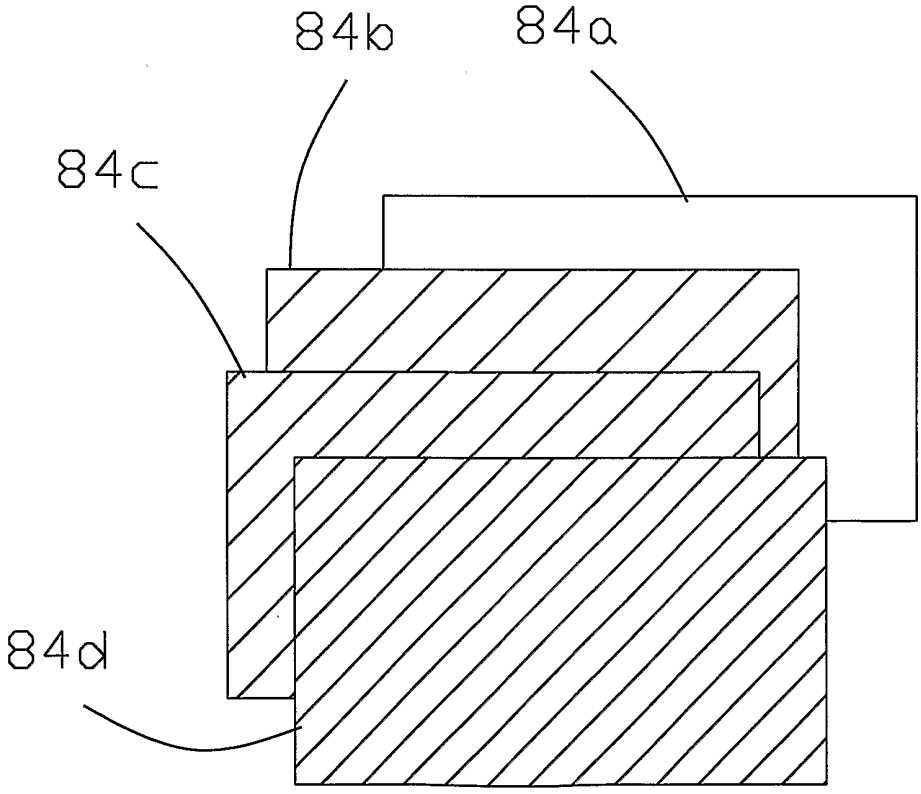

FIG. 10

INTERNATIONAL SEARCH REPORT

I ational Application No
PCT/US 01/19954

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N1/28 G01N35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01L B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 985 669 A (PALANDER JARI) 16 November 1999 (1999-11-16) column 1, line 7 -column 1, line 20 column 3, line 27 -column 4, line 53 column 4, line 60 -column 5, line 14 figures 1-3 ---	1-5,8, 12-19
Y	US 5 670 329 A (OBERHARDT BRUCE J) 23 September 1997 (1997-09-23)	1-5,8, 12-19
X	column 4, line 19 -column 4, line 58 column 7, line 10 -column 9, line 39 figures 2,3,5 --- -/--	1,2, 12-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 December 2001	14/12/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Koch, A

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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Y	US 5 330 625 A (MUSZAK MARTIN F ET AL) 19 July 1994 (1994-07-19) column 1, line 12 -column 1, line 44 column 3, line 24 -column 4, line 2 column 5, line 50 -column 6, line 15 figure 1 -----	8

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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