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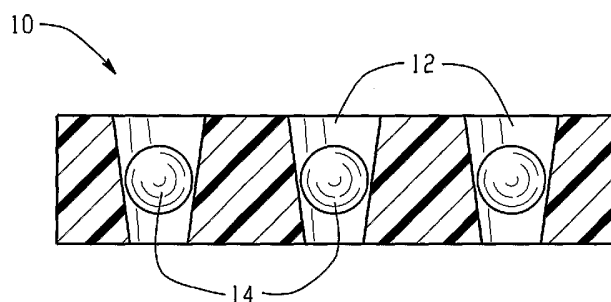
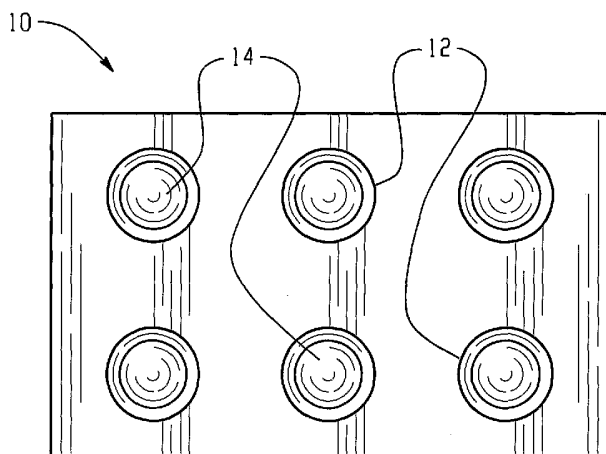
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(54) Title: AN ONLINE FLOW THROUGH MICROCHIP FOR DETECTING HAZARDOUS CHEMICAL AND BIOLOGICAL AGENTS IN DRINKING WATER



(57) Abstract: A flow through microchip is disclosed having physically-registered microetched pores embedded within a wafer. The circular pores have a tapered design having an entrance diameter larger than the exit diameter. Within each pore is placed a small microbead. Each microbead will have monoclonal antibodies bound to its surfaces wherein the monoclonal antibodies are proteins designed and engineered to specifically bind pathogenic microorganisms, biotoxins, pesticides, and other contaminants. The monoclonal antibodies covering the surface of the microbead will capture the contaminants as water flows over the bead.



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An Online Flow-Through Microchip for Detecting Hazardous Chemical and Biological Agents in Drinking Water

5 REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Application No. 60/427,813, filed November 20, 2003.

BACKGROUND OF THE INVENTION

10 The present invention relates generally to advanced detection systems and more specifically to the detection of biological and chemical contaminants in a fluid matrix.

Contamination of drinking water by hazardous chemical or biological agents occurs as a result of the episodic and sporadic influx of these agents into water distribution systems. This fundamental aspect necessitates that the development of advanced detection systems designed to prevent pathogenic or toxic agents from entering the drinking water supplies, provide a twenty-four hour surveillance approach to water quality and security. Existing and emerging detection methods are restricted in their capacity to temporarily assess water quality since they are dependent on water sampling. Water sampling cannot be a part of an effective detection and surveillance system since natural or purposeful contamination of water can occur before or after a water sample is taken. Furthermore, the sheer number of biological and chemical hazards that can contaminate water complicates the development of on-line detection systems. These factors require that an effective early warning surveillance system designed to protect

drinking water supplies meet the following criteria; 1) provide twenty-four hour monitoring of water quality; 2) be highly sensitive and specific for detecting a broad range of biological and chemical contaminants rapidly and simultaneously.

5 BRIEF SUMMARY OF THE INVENTION

In view of the aforementioned needs, the invention contemplates a flow through microchip. The microchip is comprised of physically-registered microetched pores embedded within a wafer. The circular pores have a tapered design having an entrance diameter larger than the exit diameter. Within each pore is placed a small microbead.

10 Each microbead will have monoclonal antibodies bound to its surfaces wherein the monoclonal antibodies are proteins designed and engineered to specifically bind pathogenic microorganisms, biotoxins, pesticides, and other contaminants. The monoclonal antibodies covering the surface of the microbead will capture the contaminants as water flows over the bead.

15 Still other objects of the present invention will become readily apparent to those skilled in this art from the following description wherein there is shown and described a preferred embodiment of this invention, simply by way of illustration of one of the best modes best suited for to carry out the invention. As it will be realized, the invention is capable of other different embodiments and its several details are capable of modifications
20 in various obvious aspects all without from the invention. Accordingly, the drawing and descriptions will be regarded as illustrative in nature and not as restrictive.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

The accompanying drawings incorporated in and forming a part of the specification, illustrates several aspects of the present invention, and together with the description serve to explain the principles of the invention. The drawings are as follows:

Figs. 1A and 1B are respective top and side views of the microchip design of the preferred embodiment contemplated by the present invention.

Figs. 2A, 2B and 2C are respectively a top view of the laser-etched pore on the microchip; a side view of a single pore in the microchip; and a top view of the microchip demonstrating the configurations of the pores in relation to each other.

Figs. 3A, 3B, 3C, 3D, 3E and 3F are tops views respectively of the uppermost layer of the casing, along with the second, third, fourth, fifth and bottom-most layer of the casing;

Fig. 4 is an exploded view of the layers in Figs. 3A through 3F, showing the assembly of the present casing.

Figs. 5A, 5B and 5C respectively show a side view and a top view of the internal retainer housing layer, and the lower component of the retainer housing;

Fig. 6 is an exploded view showing the arrangement of the components in the retainer housing.

DETAILED DESCRIPTION OF INVENTION

Throughout this description, the preferred embodiment and examples shown should be considered as exemplars, rather than limitations, of the present invention. While the present invention is described for use with drinking water, as those skilled in the art can readily appreciate the present invention can be suitably adapted to any fluid matrix.

As shown in Figs. 1A and 1B, the present invention is a flow through microchip 10 comprising physically-registered microetched pores 12 embedded within a wafer (25 mm x 25 mm x 0.2 mm) made of a suitable material such as the material sold under the name "Kapton" by DuPont. An example of a preferred embodiment is shown in Figs 1A and 1B.

5 The circular pores 12 have a tapered design, having an entrance diameter of 0.1 mm and an exit diameter of 0.05 mm. The pores 12 are spaced 0.150 mm apart (center to center). In the embodiment disclosed herein, the microchip houses 900 pores (30 x 30); however, the number and design of the pores are arbitrary. The design may be modified to include greater or fewer numbers of pores. Furthermore, the pores themselves may be modified,
10 the entrance and/or exit diameters may be increased or decreased as desired.

Within each pore 12 is placed a small microbead 14. For example, for the embodiment shown in Figs 1A and 1B the microbead 14 is preferably 0.070 mm.

Microbeads that meet the design specifications of the chip, for example specific diameters are commercially available from Bangs Laboratories, Inc., 9025 Technology Drive,
15 Fishers, IN 46038-2886; (<http://www.bangslabs.com/company/index.php>). Each individual microbead 14 will have monoclonal antibodies bound to its surface. Monoclonal antibodies are proteins designed and engineered to specifically bind to pathogenic microorganisms, biotoxins, pesticides, etc. The monoclonal antibodies covering the surface of the microbead 14 will bind (or 'capture') the microorganisms or
20 chemicals as water flows over the bead.

The microbeads 14 are dispensed into the individual pores 12 manually or using a commercially available piezoelectric robots, such as those available from the Perkin Elmer Corporation, 549 Albany Street, Boston, Massachusetts, 02118. The piezoelectric

based robotics system can dispense beads to specified sites on the microchip 10, such as the pores 12. This will allow each individual microbead 14 on the microchip to be "self-registered" in terms of both its location on the microchip 10 and by the antibody specificity coating its surface. Fig. 2A is a top view of the laser-etched pore 12 on the microchip 10.

5 The diagram illustrates the physical dimension of the pore entrance. Fig. 2B is a side view of a single pore 12 in the microchip 10, illustrating a tapered pore design where the taper of the hole is at angle of about 11.40 degrees. Fig. 2C is a top view of the microchip 10 demonstrating the configurations of the pores 12 in relation to each other.

Referring to Figs. 3A, 3B, 3C, 3D, 3E, 3F and 4, the laser-etched microchip 10
10 containing the microbeads 14 will be housed in a series of Kapton and borosilicate casings and gaskets. The casings are designed to allow for precise headspace above (approximately 0.050 mm) and below the microchip 10. The nominal headspace above the microchip 10 in conjunction with the tapered hole design will restrict the microbead 14 to the physical confines of the pore 12. An influent (intake) hole 40, and effluent (exit) hole
15 50 in the casing 60 allows the fluid to be dispensed over the microbeads 14. As the fluid flows over the microbeads 14, the monoclonal antibodies on the surface of the microbeads sample the fluid for the presence of hazardous biological and chemical agents or other contaminants. In theory, the preferred embodiment can include 900 microbeads 14, each respectively configured to detect at least 900 individual different pathogens, biologics,
20 chemicals, or other contaminants simultaneously and rapidly.

Figs. 3A, 3B, 3C, 3D, 3E and 3F respectively represent the individual components of the casing 60 that houses the laser-etched microchip 10. The complete casing 60 is composed of a series of laser-etched wafers physically assembled to embed the microchip

10, yet permit water to flow through the entire apparatus. Fig. 3A shows the uppermost layer 20 of the casing 60. The uppermost layer 20 has a single influent hole 40 to allow water into the apparatus. Fig. 3B is a top view of the second layer 22 from the top of the casing 60. The second layer 22 includes an enlarged aperture 42 for receiving and
5 dispersing the water coming through the hole over the embedded microchip 10. The diameter of this layer is critical since it is designed to restrict the microbead 14 to the confines of the pore 12 in the microchip 10. For example, for a bead size of 60 μm , the second layers is 50 μm . Figs. 3C and 3D are top views of the subsequent layers. The laser-etched microchip 10 fits inside the internal square 44 of the third layer 24 and is
10 supported underneath by the internal square 46 of the fourth layer 26. Fig. 3E is a top view diagram of the fifth layer 28 from the top of the casing. Like the second layer 22, it has an enlarged aperture 48 designed to force water out of the effluent hole 50. Fig. 3F is a top view of the bottom-most layer 30 of the casing 60. This layer 30 has a single effluent hole 50 to permit water to flow out of the apparatus. The assembly of these layers with the
15 microchip is shown in Fig. 4.

The retainer housing is shown in Figs. 5A, 5B, 5C and 6. The retainer housing is designed to hold the fully assembled casing 60 with microchip 10 (i.e. the assembled microchip). The assembled microchip 60 is clamped into the retainer housing 70. The retainer housing 70 can be linked to a water source through a spigot and permits the
20 unidirectional flow of water through the assembled microchip 60. Fig. 5A is a side view of the upper layer 72 of the retainer housing. A spigot can be attached to a bore 74 in this upper portion 72 so that water can be pumped through a tube into the assembled microchip 60. Fig. 5B is a top view of the internal retainer housing layer 76. The assembled

microchip 76 is placed inside a suitably shaped cavity 78 in this component 76. This component 76 centers the assembled microchip 60 in the flow stream of the water. Fig. 5C is a lower component 80. A bore hole 82 in the lower component permits the water to flow out of the assembled microchip 60. The upper and lower components include
5 respective surfaces 84, 86 for contacting and receiving the assembled microchip 60. The entire assembly is secured using screws 90 that are received through respective tapped bores 92 in the corners of the respective components. Of course, it is to be appreciated that any suitable-type fastener can be used without departing from the invention.

One application for the present invention is drinking water safety and security. The
10 present invention can be used to monitor drinking water systems for pathogens and other contaminants on a continuous basis (24 hours a day, seven days a week) to ensure the safety and security of drinking water.

Although the invention has been shown and described with respect to a certain preferred embodiment, it is obvious that equivalent alterations and modifications will
15 occur to others skilled in the art upon the reading and understanding of this specification. The present invention includes all such equivalent alterations and modifications.

We Claim:

1. A detecting component for detecting substances in a fluid comprising:
a flow-through microchip comprising at least one microetched pore;
5 at least one microbead received and retained within the at least one
microetched pore;
at least one monoclonal antibody protein layer bound to the surface of the at
least one microbead, engineered to specifically bind to a desired substance in the fluid, to
provide detection thereof.
10
2. The detecting component of claim 1 wherein the at least one microetched
pore has a tapered design.
3. The detecting component of claim 2 wherein the tapered design of the at
15 least one microetched pore has a taper at an angle of about 11.40 degrees.
4. The detecting component of claim 2 wherein the at least one microetched
pore has a tapered design so as to have an entrance diameter of about 0.1 mm and an exit
diameter of about 0.05 mm.
20
5. The detecting component of claim 4 wherein the at least one microbead has
a diameter of about 0.070 mm, so as to be received and retained within the at least one
microetched pore.

6. The detecting component of claim 1 wherein the at least one monoclonal antibody protein layer is engineered to specifically bind to a desired substance in a fluid selected from a group consisting of pathogenic microorganisms, biotoxins and pesticides.

5

7. The detecting component of claim 1 wherein the at least one microetched pore is a plurality of microetched pores, and wherein the at least one microbead comprises a respective plurality of microbeads, received and retained within a respective one of the microetched pores.

10

8. The detecting component of claim 7 wherein at least some of the respective microbeads are coated with respective different monoclonal antibody protein layers, engineered to respectively bind to different desired substances in a fluid selected from a group consisting of pathogenic microorganisms, biotoxins and pesticides.

15

9. The detecting component of claim 7 wherein the flow through microchip is formed on a wafer having dimensions of about 25 mm x 25 mm x 0.2 mm, and wherein the plurality of microetched pores comprises about 900 microetched pores, spaced 0.150 mm apart from center to center.

20

10. The detecting component of claim 1 wherein the flow-through microchip 10 containing the at least one microbead is embedded in a casing having an influent hole and effluent hole to allow fluid flow into and out of the microchip, to allow the fluid to be

dispensed over the microbeads so that the surface of the microbeads sample the fluid for the presence of at least one of hazardous biological and chemical agents and other contaminants..

5 11. The detecting component of claim 10 wherein the casing comprises a predetermined headspace at respective ends of the microchip, so as to restrict the microbead to the respective pore.

 12. The detecting component of claim 10 wherein the casing is formed of a
10 plurality of stacked layers, comprising:
 a first layer having the influent hole to allow water into the casing;
 a second layer including an enlarged aperture for receiving and dispersing the water coming through the influent hole over a surface of the embedded microchip;
 a third layer having an internal square for receiving and retaining the embedded
15 microchip;
 a fourth layer having an internal square for supporting the embedded microchip;
 a fifth layer having an enlarged aperture for forcing water into the effluent hole;
 and
 a sixth layer having the single effluent hole to permit water to flow out of the
20 casing.

 13. The detecting component of claim 10 further comprising a retainer housing for retaining the casing having the microchip.

14. The detecting component of claim 13 wherein the retainer housing is formed of a plurality of stacked layers comprising:

an upper component, having a bore to which a spigot is attached, and linked to a
5 water source to permit a unidirectional flow of water to the casing having the microchip;

an internal retainer housing component having a cavity for receiving the casing having the microchip and centering in the flow of the water;

a lower component having a bore hole that permits the water to flow out of the assembled microchip.

10

15. The detecting component of claim 14 wherein the upper and lower components include respective surfaces for contacting and receiving the casing having the microchip.

16. The detecting component of claim 14 further comprising a plurality of screws that are received through respective tapped bores in the corners of the respective retainer components.

17. A detecting component for detecting substances in a fluid comprising:
20 a flow-through microchip comprising at least one microetched pore;
at least one microbead received and retained within the at least one microetched pore;
at least one monoclonal antibody protein layer bound to the surface of the at

least one microbead, engineered to specifically bind to a desired substance in the fluid, to provide detection thereof;

a casing for embedding the flow-through microchip containing the at least one microbead, wherein the casing comprises an influent hole and effluent hole to allow fluid
5 flow into and out of the microchip, to allow the fluid to be dispensed over the microbeads so that the surface of the microbeads sample the fluid for the presence of the desired substance; and

a retainer housing for retaining the casing having the microchip.

10 18. The detecting component of claim 17 wherein the at least one microetched pore has a tapered design.

19. The detecting component of claim 18 wherein the tapered design of the at least one microetched pore has a taper at an angle of about 11.40 degrees.

15

20. The detecting component of claim 18 wherein the at least one microetched pore has a tapered design so as to have an entrance diameter of about 0.1 mm and an exit diameter of about 0.05 mm.

20 21. The detecting component of claim 20 wherein the at least one microbead has a diameter of about 0.070 mm, so as to be received and retained within the at least one microetched pore.

22. The detecting component of claim 17 wherein the at least one monoclonal antibody protein layer is engineered to specifically bind to a desired substance in a fluid selected from a group consisting of pathogenic microorganisms, biotoxins and pesticides.

5 23. The detecting component of claim 17 wherein the at least one microetched pore is a plurality of microetched pores, and wherein the at least one microbead comprises a respective plurality of microbeads, received and retained within a respective one of the microetched pores.

10 24. The detecting component of claim 23 wherein at least some of the respective microbeads are coated with respective different monoclonal antibody protein layers, engineered to respectively bind to different desired substances in a fluid selected from a group consisting of pathogenic microorganisms, biotoxins and pesticides.

15 25. The detecting component of claim 23 wherein the flow through microchip is formed on a wafer having dimensions of about 25 mm x 25 mm x 0.2 mm, and wherein the plurality of microetched pores comprises about 900 microetched pores, spaced 0.150 mm apart from center to center.

20 26. The detecting component of claim 17 wherein the casing comprises a predetermined headspace at respective ends of the microchip, so as to restrict the microbead to the respective pore.

27. The detecting component of claim 17 wherein the casing is formed of a plurality of stacked layers, comprising:

a first layer having the influent hole to allow water into the casing;

a second layer including an enlarged aperture for receiving and dispersing the water
5 coming through the influent hole over a surface of the embedded microchip;

a third layer having an internal square for receiving and retaining the embedded
microchip;

a fourth layer having an internal square for supporting the embedded microchip;

a fifth layer having an enlarged aperture for forcing water into the effluent hole;

10 and

a sixth layer having the single effluent hole to permit water to flow out of the casing.

28. The detecting component of claim 28 wherein the retainer housing is
15 formed of a plurality of stacked layers comprising:

an upper component, having a bore to which a spigot is attached, and linked to a water source to permit a unidirectional flow of water to the casing having the microchip;

an internal retainer housing component having a cavity for receiving the casing having the microchip and centering in the flow of the water;

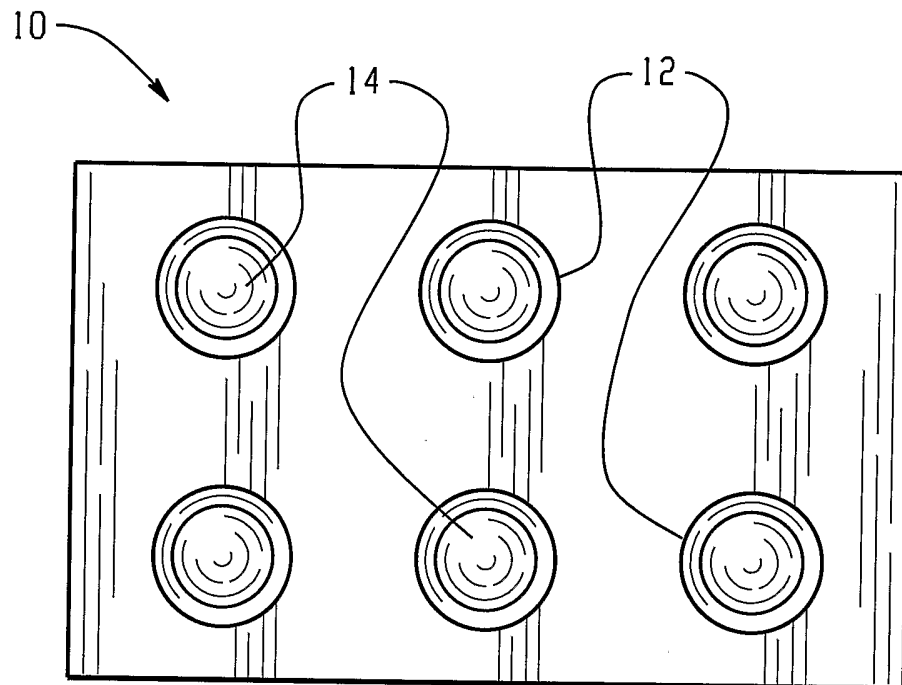
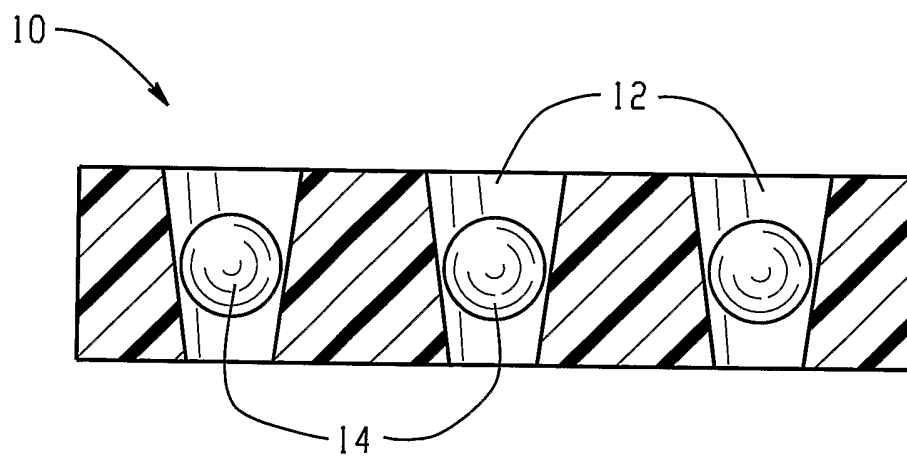
20 a lower component having a bore hole that permits the water to flow out of the assembled microchip.

29. The detecting component of claim 28 wherein the upper and lower

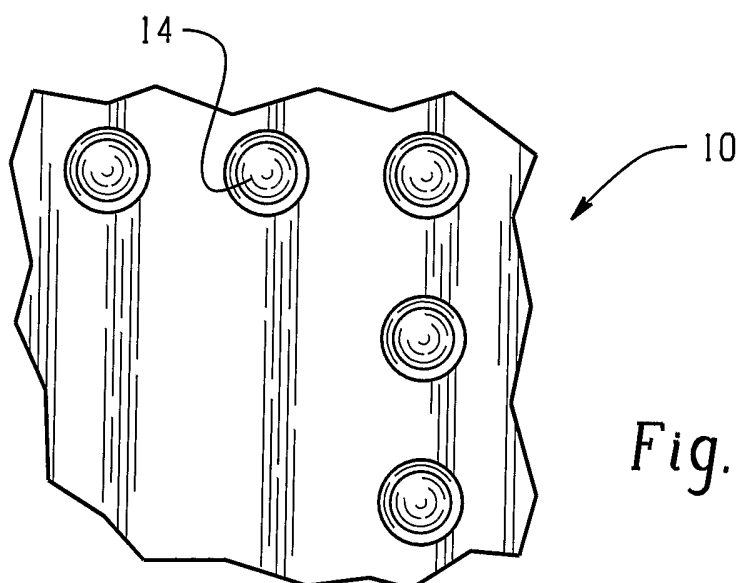
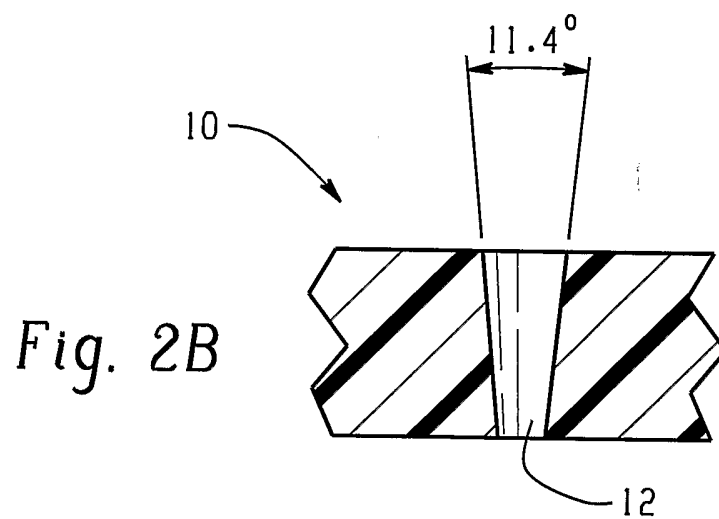
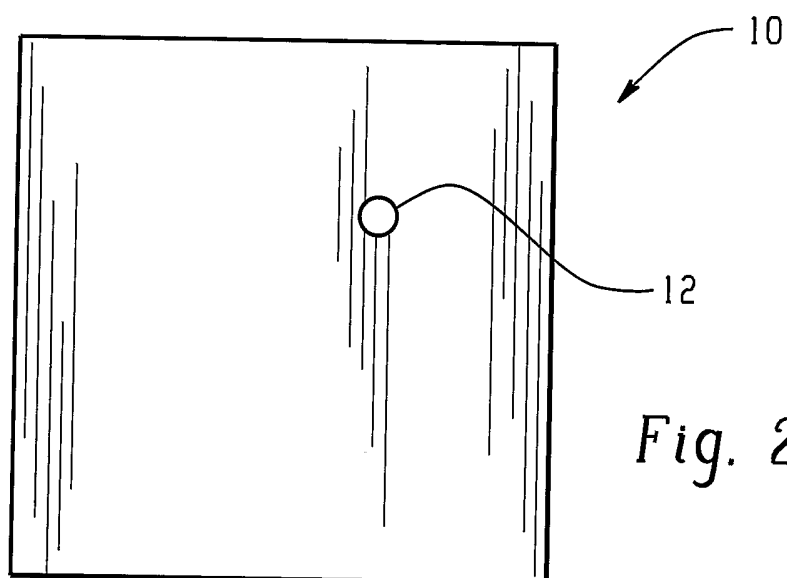
components include respective surfaces for contacting and receiving the casing having the microchip.

30. The detecting component of claim 28 further comprising a plurality of
- 5 screws that are received through respective tapped bores in the corners of the respective retainer components.

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*Fig. 1A**Fig. 1B*

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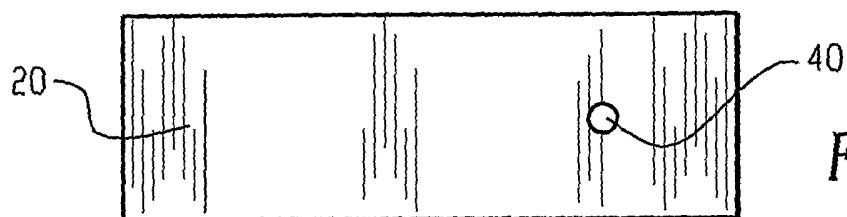


Fig. 3A

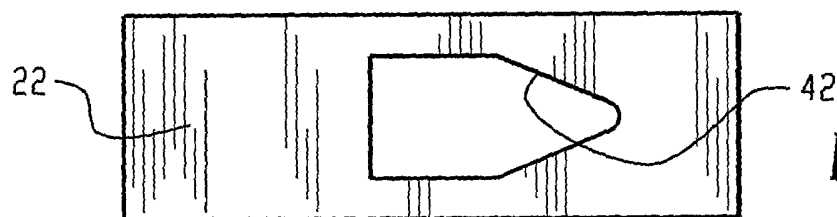


Fig. 3B

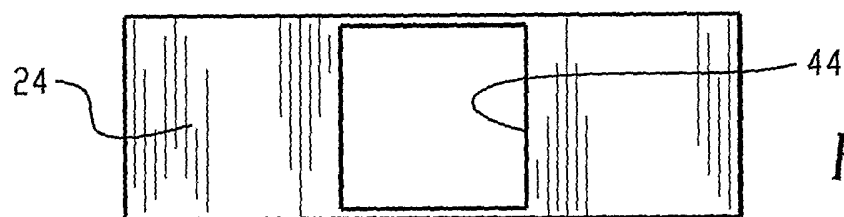


Fig. 3C

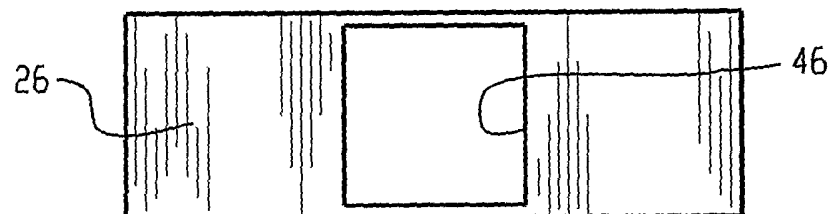


Fig. 3D

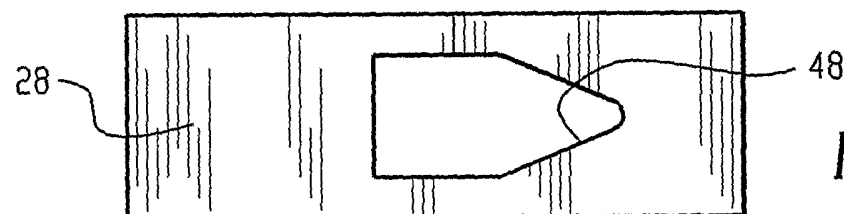
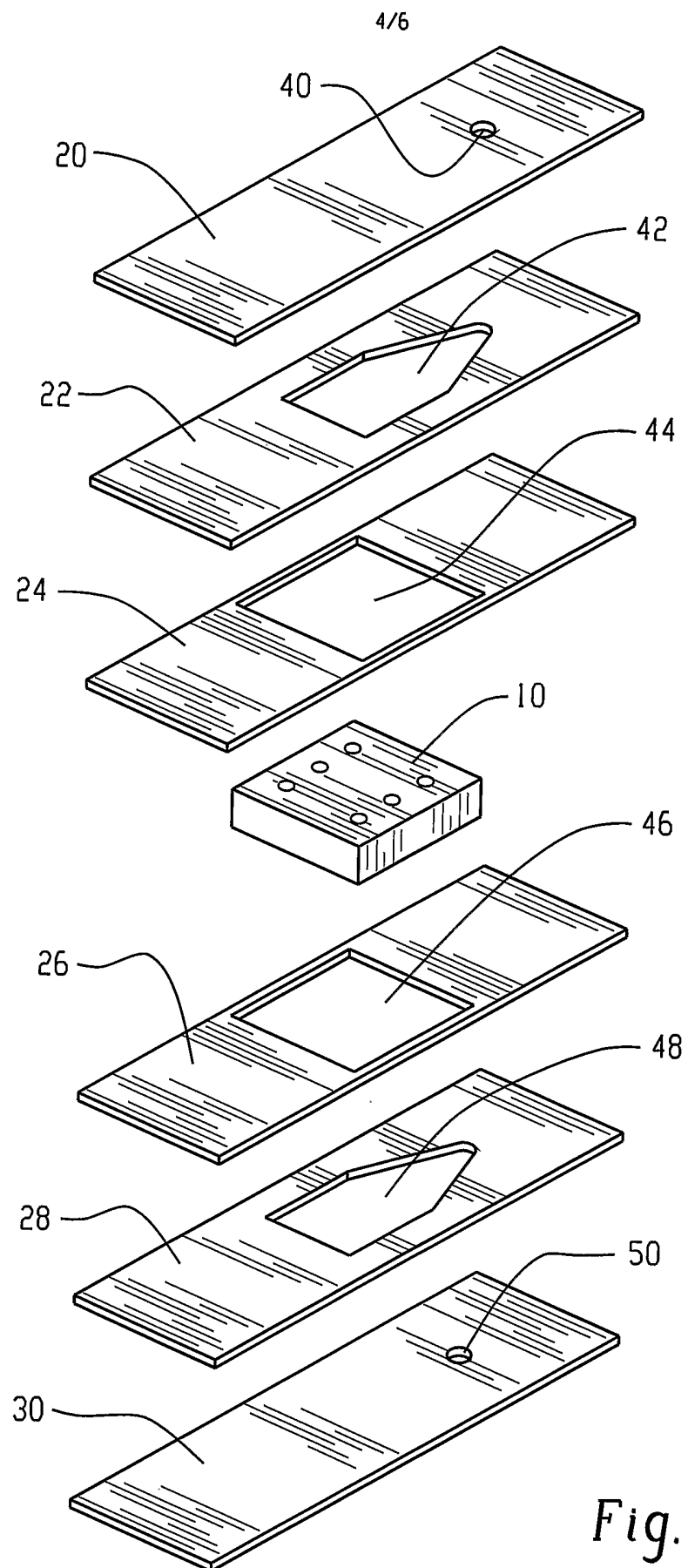


Fig. 3E



Fig. 3F



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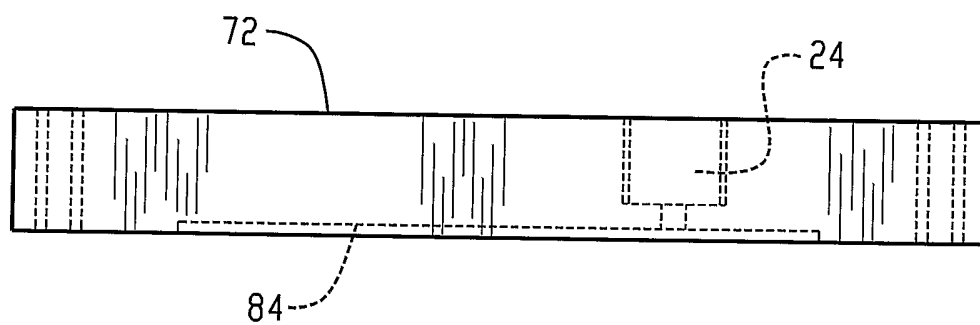


Fig. 5A

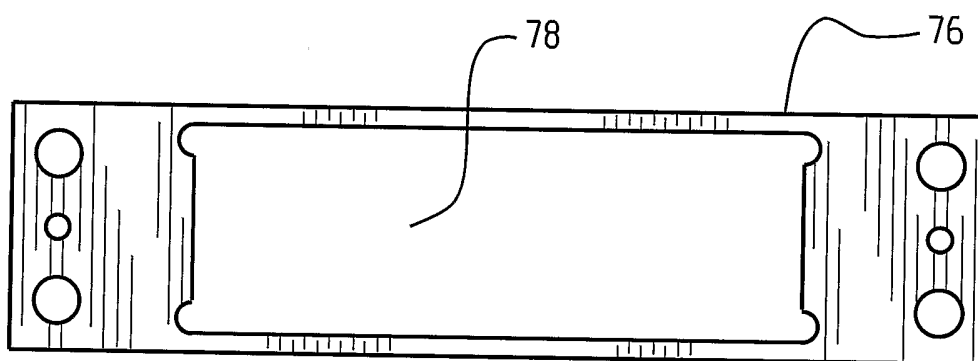


Fig. 5B

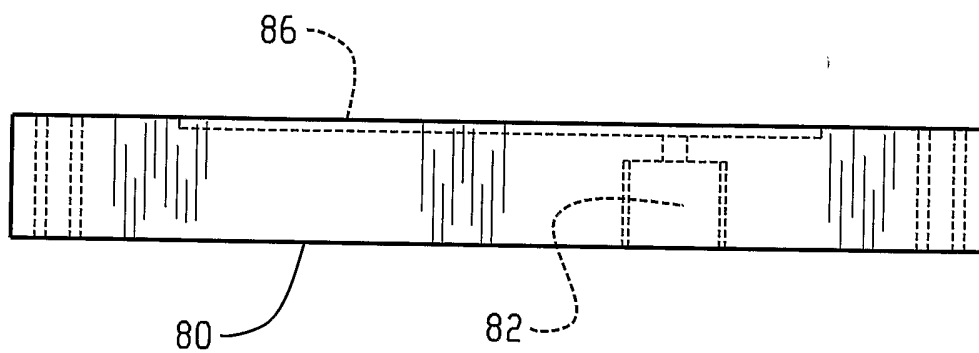
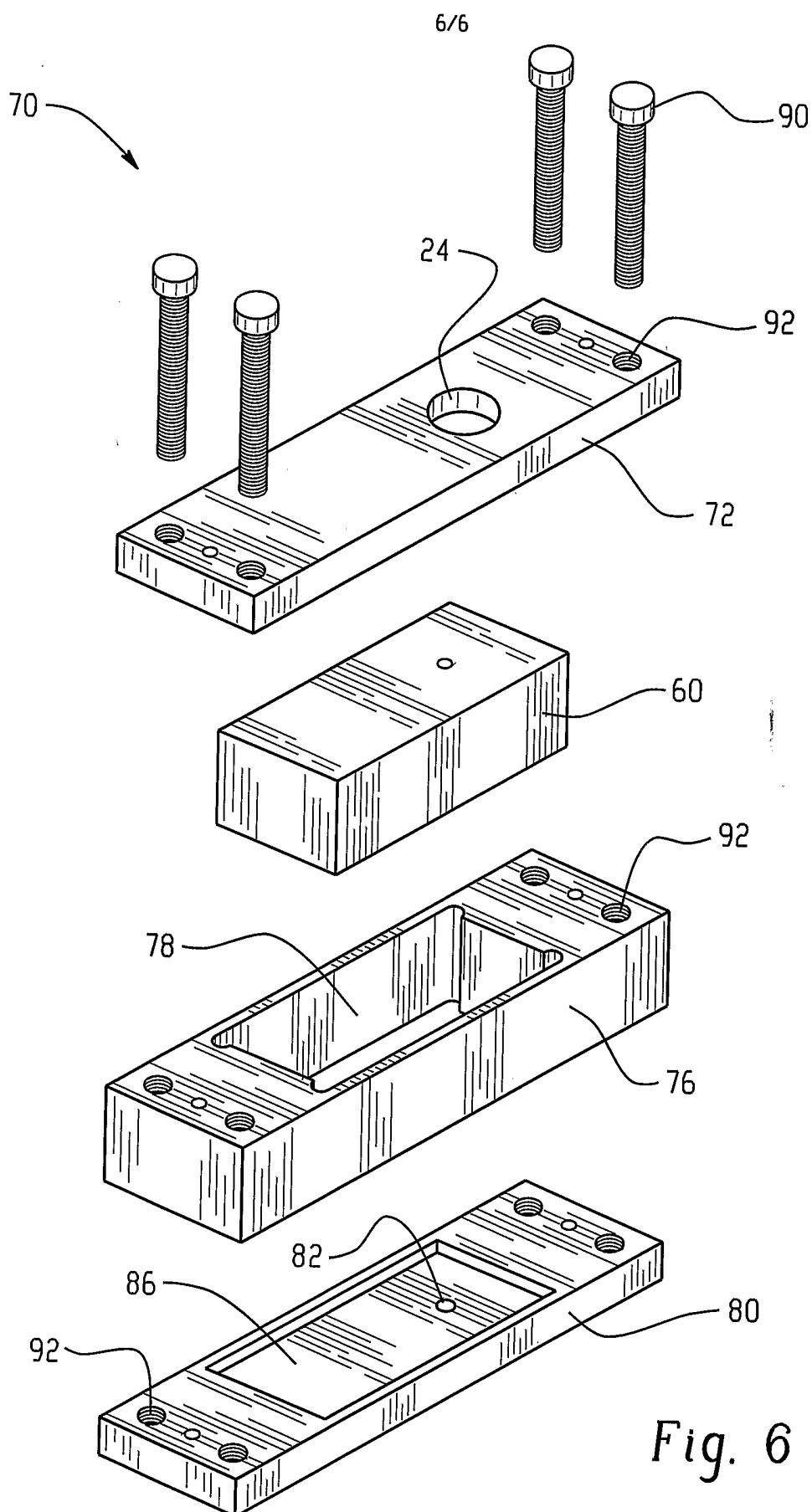


Fig. 5C

*Fig. 6*