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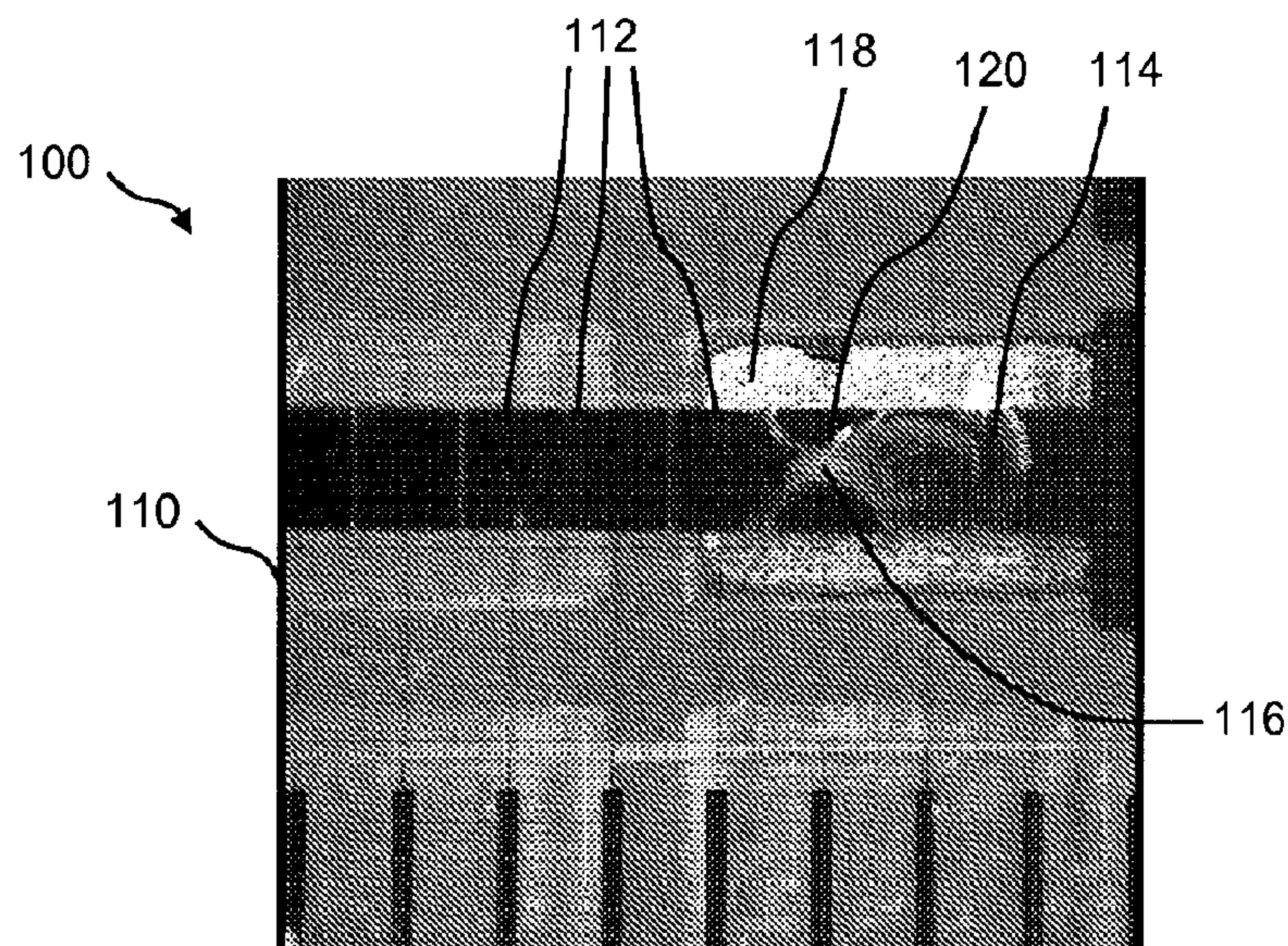
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(54) **Title: DROPLET ACTUATOR DEVICES AND METHODS EMPLOYING MAGNETIC BEADS**



(57) **Abrégé/Abstract:**

A method of providing a droplet in contact with a magnetically responsive bead and having a reduced quantity of a substance. The method generally includes the steps of (a) providing a droplet actuator (200) comprising: (i) a substrate (210) comprising



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electrodes (214) arranged for conducting droplet operations on a surface; (ii) a starting droplet (218/222) comprising: (1) one or more magnetically responsive beads (220); (2) a starting quantity of the substance; and (3) a starting volume; (b) magnetically immobilizing the one or more magnetically responsive beads at a location which is at a distance from a target droplet splitting zone, (224); (c) conducting one or more droplet operations comprising droplet dividing operations selected to divide the combined droplet to yield a set of droplets comprising: (i) a droplet (218) comprising substantially all of the one or more magnetically responsive beads and having a decreased quantity of the substance relative to the starting concentration; and (ii) a droplet (222) substantially lacking the magnetically responsive beads.

**ABSTRACT**

A method of providing a droplet in contact with a magnetically responsive bead and having a reduced quantity of a substance. The method generally includes the steps of (a) providing a droplet actuator (200) comprising: (i) a substrate (210) comprising electrodes (214) arranged for conducting droplet operations on a surface; (ii) a starting droplet (218/222) comprising: (1) one or more magnetically responsive beads (220); (2) a starting quantity of the substance; and (3) a starting volume; (b) magnetically immobilizing the one or more magnetically responsive beads at a location which is at a distance from a target droplet splitting zone, (224); (c) conducting one or more droplet operations comprising droplet dividing operations selected to divide the combined droplet to yield a set of droplets comprising: (i) a droplet (218) comprising substantially all of the one or more magnetically responsive beads and having a decreased quantity of the substance relative to the starting concentration; and (ii) a droplet (222) substantially lacking the magnetically responsive beads.

# DROPLET ACTUATOR DEVICES AND METHODS EMPLOYING MAGNETIC BEADS

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## 3 Field of the Invention

The invention relates generally to the field of droplet actuators, and in particular, to droplet actuators configured for conducting droplet based protocols requiring droplet operations to be conducted using droplets comprising beads, especially magnetically responsive beads. The invention also relates to methods of making and using such droplet actuators.

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## 4 Background of the Invention

Droplet actuators are used to conduct a wide variety of droplet operations. A droplet actuator typically includes a substrate associated with electrodes for conducting droplet operations on a droplet operations surface thereof and may also include a second substrate arranged in a generally parallel fashion in relation to the droplet operations surface to form a gap in which droplet operations are effected. The gap is typically filled with a

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filler fluid that is immiscible with the fluid that is to be subjected to droplet operations on the droplet actuator.

In some applications of a droplet actuator there is a need for using "beads" for various assays. For protocols that make use of beads, the beads are typically used to bind to one or more target substances in a mixture of substances. The target substances may, for example, be analytes or contaminants. There is a need for an efficient approach to bead washing on a droplet actuator in order to reduce the amount of one or more substances in a bead-containing droplet that may be in contact with or exposed to the surface of the bead or beads.

## 5 Brief Description of the Invention

One aspect of the invention provides efficient immobilization of magnetically responsive beads for droplet operations that make use of magnetically responsive beads in droplet-based applications. Examples include assays that require execution of bead washing protocols, such as pyrosequencing and immunoassay applications. In one example, the invention provides techniques employing magnetic forces for immobilizing magnetically responsive beads during droplet splitting operations. The techniques of the invention are particularly useful in protocols for washing magnetically responsive beads within a droplet actuator. Among other advantages, the techniques of the invention avoid undue clumping or aggregation of the magnetically responsive beads. During droplet splitting operations, the techniques can usefully immobilize substantially all of the magnetically responsive beads within a droplet. Techniques of the invention may ensure immobilization and retention of substantially all magnetically responsive beads during a droplet washing operation. Upon completion of a washing process, the techniques of the invention ensure resuspension of substantially all of the magnetically responsive beads within the liquid and with substantially no clumping or aggregation thereof.

Another aspect of the invention provides improved droplet actuators and related methods for effecting improved droplet-based assay operations. Embodiments of the invention provide mechanisms for reducing the crossover of magnetic fields within a droplet actuator. Other embodiments of the invention provide mechanisms for reducing the carryover of beads and other substances within a droplet actuator. Yet other

embodiments of the invention provide mechanisms for improving droplet detection operations within a droplet actuator.

Another aspect of the invention relates to a droplet actuator device comprising magnet sets and electrode sets, wherein:

- 5           (a)     each electrode set comprises a plurality of electrodes associated with a substrate, each electrode set defining a lane of electrodes;
- (b)     two or more electrode sets are:
  - (i)     arranged to form an array of electrodes; and
  - (ii)    configured for conducting one or more droplet operations;
- 10          (c)     each magnet set comprises one or more magnets;
- (d)     each magnet set is arranged in sufficient proximity with an electrode set such that magnetic field(s) exerted by the magnet set restricts mobility of magnetically responsive particles within one or more droplets located on one or more of the electrodes; and
- 15          (e)     the polar orientation of each magnet set is arranged to be opposite to the polar orientation of its adjacent magnet set(s), such that adjacent magnetic fields cancel and thereby reduce crossover of magnetic fields between adjacent magnet sets.

## 6 Definitions

As used herein, the following terms have the meanings indicated.

5 “Activate” with reference to one or more electrodes means effecting a change in the electrical state of the one or more electrodes which results in a droplet operation.

10 “Bead,” with respect to beads on a droplet actuator, means any bead or particle that is capable of interacting with a droplet on or in proximity with a droplet actuator. Beads may be any of a wide variety of shapes, such as spherical, generally spherical, egg shaped, disc shaped, cubical and other three dimensional shapes. The bead may, for example, be capable of being transported in a droplet on a droplet actuator or otherwise configured with respect to a droplet actuator in a manner which permits a droplet on the droplet actuator to be brought into contact with the bead, on the droplet actuator and/or off the droplet actuator. Beads may be manufactured using a wide variety of materials, including for example, resins, and polymers. The beads may be any suitable size, including for example, microbeads, microparticles, nanobeads and nanoparticles. In some cases, beads are magnetically responsive; in other cases beads are not significantly magnetically responsive. For magnetically responsive beads, the magnetically responsive material may constitute substantially all of a bead or one component only of a bead. The remainder of the bead may include, among other things, polymeric material, coatings, and moieties which permit attachment of an assay reagent. Examples of suitable magnetically responsive beads are described in U.S. Patent Publication No. 2005-0260686, entitled, “Multiplex flow assays preferably with magnetic particles as solid phase,” published on November 24, 2005.

25 The beads may include one or more populations of biological cells adhered thereto. In some cases, the biological cells are a substantially pure population. In other cases, the biological cells include different cell populations, e.g., cell populations which interact with one another.

30 “Droplet” means a volume of liquid on a droplet actuator that is at least partially bounded by filler fluid. For example, a droplet may be completely surrounded by filler fluid or

may be bounded by filler fluid and one or more surfaces of the droplet actuator. Droplets may take a wide variety of shapes; nonlimiting examples include generally disc shaped, slug shaped, truncated sphere, ellipsoid, spherical, partially compressed sphere, hemispherical, ovoid, cylindrical, and various shapes formed during droplet operations, such as merging or splitting or formed as a result of contact of such shapes with one or more surfaces of a droplet actuator.

“Droplet operation” means any manipulation of a droplet on a droplet actuator. A droplet operation may, for example, include: loading a droplet into the droplet actuator; dispensing one or more droplets from a source droplet; splitting, separating or dividing a droplet into two or more droplets; transporting a droplet from one location to another in any direction; merging or combining two or more droplets into a single droplet; diluting a droplet; mixing a droplet; agitating a droplet; deforming a droplet; retaining a droplet in position; incubating a droplet; heating a droplet; vaporizing a droplet; cooling a droplet; disposing of a droplet; transporting a droplet out of a droplet actuator; other droplet operations described herein; and/or any combination of the foregoing. The terms “merge,” “merging,” “combine,” “combining” and the like are used to describe the creation of one droplet from two or more droplets. It should be understood that when such a term is used in reference to two or more droplets, any combination of droplet operations sufficient to result in the combination of the two or more droplets into one droplet may be used. For example, “merging droplet A with droplet B,” can be achieved by transporting droplet A into contact with a stationary droplet B, transporting droplet B into contact with a stationary droplet A, or transporting droplets A and B into contact with each other. The terms “splitting,” “separating” and “dividing” are not intended to imply any particular outcome with respect to size of the resulting droplets (i.e., the size of the resulting droplets can be the same or different) or number of resulting droplets (the number of resulting droplets may be 2, 3, 4, 5 or more). The term “mixing” refers to droplet operations which result in more homogenous distribution of one or more components within a droplet. Examples of “loading” droplet operations include microdialysis loading, pressure assisted loading, robotic loading, passive loading, and pipette loading.

“Immobilize” with respect to magnetically responsive beads, means that the beads are substantially restrained in position in a droplet or in filler fluid on a droplet actuator. For example, in one embodiment, immobilized beads are sufficiently restrained in position to

permit execution of a splitting operation on a droplet, yielding one droplet with substantially all of the beads and one droplet substantially lacking in the beads.

“Magnetically responsive” means responsive to a magnetic field. “Magnetically responsive beads” include or are composed of magnetically responsive materials. Examples of magnetically responsive materials include paramagnetic materials, ferromagnetic materials, ferrimagnetic materials, and metamagnetic materials. Examples of suitable paramagnetic materials include iron, nickel, and cobalt, as well as metal oxides, such as  $\text{Fe}_3\text{O}_4$ ,  $\text{BaFe}_{12}\text{O}_{19}$ ,  $\text{CoO}$ ,  $\text{NiO}$ ,  $\text{Mn}_2\text{O}_3$ ,  $\text{Cr}_2\text{O}_3$ , and  $\text{CoMnP}$ .

“Washing” with respect to washing a magnetically responsive bead means reducing the amount and/or concentration of one or more substances in contact with the magnetically responsive bead or exposed to the magnetically responsive bead from a droplet in contact with the magnetically responsive bead. The reduction in the amount and/or concentration of the substance may be partial, substantially complete, or even complete. The substance may be any of a wide variety of substances; examples include target substances for further analysis, and unwanted substances, such as components of a sample, contaminants, and/or excess reagent. In some embodiments, a washing operation begins with a starting droplet in contact with a magnetically responsive bead, where the droplet includes an initial amount and initial concentration of a substance. The washing operation may proceed using a variety of droplet operations. The washing operation may yield a droplet including the magnetically responsive bead, where the droplet has a total amount and/or concentration of the substance which is less than the initial amount and/or concentration of the substance. Other embodiments are described elsewhere herein, and still others will be immediately apparent in view of the present disclosure.

The terms “top” and “bottom” are used throughout the description with reference to the top and bottom substrates of the droplet actuator for convenience only, since the droplet actuator is functional regardless of its position in space.

When a given component, such as a layer, region or substrate, is referred to herein as being disposed or formed “on” another component, that given component can be directly on the other component or, alternatively, intervening components (for example, one or more coatings, layers, interlayers, electrodes or contacts) can also be present. It will be further understood that the terms “disposed on” and “formed on” are used

interchangeably to describe how a given component is positioned or situated in relation to another component. Hence, the terms “disposed on” and “formed on” are not intended to introduce any limitations relating to particular methods of material transport, deposition, or fabrication.

5 When a liquid in any form (e.g., a droplet or a continuous body, whether moving or stationary) is described as being “on”, “at”, or “over” an electrode, array, matrix or surface, such liquid could be either in direct contact with the electrode/array/matrix/surface, or could be in contact with one or more layers or films that are interposed between the liquid and the electrode/array/matrix/surface.

10 When a droplet is described as being “on” or “loaded on” a droplet actuator, it should be understood that the droplet is arranged on the droplet actuator in a manner which facilitates using the droplet actuator to conduct one or more droplet operations on the droplet, the droplet is arranged on the droplet actuator in a manner which facilitates sensing of a property of or a signal from the droplet, and/or the droplet has been subjected  
15 to a droplet operation on the droplet actuator.

## 7 Brief Description of the Drawings

Figures 1A and 1B illustrate first and second top views, respectively, of a portion of a droplet actuator in use during a first and second phase, respectively, of a droplet splitting operation;

20 Figures 2A and 2B illustrate a side and top views of a section of a droplet actuator that includes a magnet that is placed at a position which is away from the splitting zone;

Figure 3 illustrates a side view of a section of a droplet actuator that includes two magnets that are arranged above and below droplet;

25 Figures 4A and 4B illustrate side views of a section of a droplet actuator that includes four magnets that are arranged at positions surrounding the droplet;

Figures 5A and 5B illustrate a first and second top view, respectively, of a section of a droplet actuator during a first and second phase, respectively, of a droplet splitting operation;

5 Figure 6 illustrates a side view of a section of a droplet actuator that includes a magnetic shield for reducing the crossover of magnetic fields;

Figure 7 illustrates a top view of a section of a droplet actuator that includes a magnet having poles that are oppositely arranged for reducing the crossover of magnetic fields;

Figure 8 illustrates a map of magnetic fields that are arranged in a relationship such as the one shown in Figure 7;

10 Figure 9 illustrates a top view of a section of a droplet actuator arranged to reduce carryover at a droplet detection region;

Figures 10A and 10B illustrate a top view of a section of a droplet actuator configured to reduce carryover;

15 Figures 11A, 11B, and 11C illustrate side views of section of a droplet actuator configured for improving the sensitivity of droplet detection;

Figure 12 illustrates a top view of a section of a droplet actuator configured for improving the sensitivity of droplet detection;

Figure 13 illustrates a side view of a section of a droplet actuator configured for improving the sensitivity of droplet detection;

20 Figures 14A and 14B illustrate top views of a modular droplet actuator assembly, which provides a universal assembly for orienting a magnet assembly to a droplet actuator; and

Figure 15 illustrates a side view of a modular droplet actuator assembly, which is another nonlimiting example of a universal assembly for orienting a magnet assembly to a droplet actuator.

## 8 Detailed Description of the Invention

The present invention relates, among other things, to droplet actuators configured for immobilizing magnetically-responsive beads and to methods of making and using such droplet actuators. As an example, the droplet actuators are useful for immobilizing beads in droplets on the droplet actuators, thereby facilitating the execution of protocols which require immobilization of such beads, such as bead washing protocols. The invention also provides techniques for reducing or eliminating carryover of substances from droplet to droplet in a droplet actuator and techniques for maximizing signal detection on a droplet actuator.

### 8.1 Bead Loss During Droplet Splitting

Figures 1A and 1B illustrate first and second top views, respectively, of a droplet actuator 100 in use during a first and second phase, respectively, of a droplet splitting operation. Droplet actuator 100 makes use of an arrangement that is *not* suitably arranged for efficiently splitting a droplet using the specific technique shown without loss of magnetically responsive beads. Droplet actuator 100 includes a first substrate 110 and a second substrate (not shown) arranged with a gap therebetween, which serves as a fluid path. First substrate 110 includes a set of droplet operation electrodes 112 configured for conducting droplet operations on slug-shaped droplet 114, which is suspended in a filler fluid and includes magnetically responsive beads 116. A magnet 118 may be arranged in sufficient proximity to droplet operation electrodes 112 to permit some degree of immobilization of the magnetically responsive beads 116.

Figures 1A and 1B show a splitting operation that is taking place in the presence of a magnetic field produced within droplet actuator 100 by magnet 118. The placement of magnet 118 is not suitable for localizing substantially all of magnetically responsive beads 116 in a centralized location (e.g., away from the edges of the droplet) within the portion of droplet 114 that is selected to retain the beads after the splitting operation. Consequently, a certain quantity of magnetically responsive beads 116 bridge splitting zone 120 during the droplet splitting operation, as shown in Figure 1A, and a loss of beads results, as shown in Figure 1B. Figure 1B shows a first droplet 122 that contains a certain quantity of the original quantity of magnetically responsive beads 116 and a second droplet 124 that contains a certain remaining quantity of the original quantity of magnetically responsive beads 116. In other words, the end result of the illustrated

splitting operation is a loss of magnetically responsive beads 116. The inventors have discovered that a contributing factor to the loss of beads is that a full quantity of magnetically responsive beads 116 is not suitably attracted, immobilized, and retained at a centralized location within droplet 114 and/or at a sufficient distance from splitting zone 120.

## 8.2 Magnet Configurations for Preventing/Reducing Bead Loss

Among other things, the invention provides improved droplet actuators that have various magnet configurations in which magnets are arranged for efficiently splitting bead-containing droplets and washing magnetically responsive beads are described with reference to Figures 2, 3, 4, 5A, and 5B. These figures illustrate nonlimiting examples of magnet configurations in combination with a droplet actuator splitting droplets with little or no bead loss, and are, among other things, useful for efficiently washing magnetically responsive beads. One or more magnets may be arranged in proximity to a droplet on a droplet actuator such that magnetically responsive beads are suitably attracted and immobilized within the droplet, preferably at a centralized location away from the neck that forms during splitting operations. In this approach, all or substantially all magnetically responsive beads are retained within a single droplet upon completion of a droplet splitting operation. Similarly, the spitting operation may be performed in a droplet slug which is at a distance from the immobilized beads which is sufficient to reduce or eliminate bead loss. As will be explained with respect to the examples below, one or more magnets may be arranged with respect to the droplet actuator structure above, below, and/or beside, the magnetically-responsive bead-containing droplet and any combinations thereof to achieve this purpose.

### 8.2.1 Position of Magnet Relative to Splitting Zone

Figures 2A and 2B illustrate side and top views of a droplet actuator 200. In this embodiment, the magnet is placed at a position which is sufficiently distant from the portion of the droplet which is breaking during the splitting operation, splitting zone 224 (or vice versa, the splitting zone may be said to be placed at sufficient distance from the magnet position), to reduce or eliminate bead loss during a droplet splitting operation. Further, the magnet is positioned so that the beads are generally centrally located along a lateral diameter L of the droplet (top view). Droplet actuator 200 includes a first substrate

WO 2008/098236

PCT/US2008/053545

210 and a second substrate 212 separated to provide a gap for conducting droplet operations, though only one substrate is required. A set of droplet operation electrodes 214 is associated with one or both of the substrates and arranged for conducting one or more droplet operations. Droplet actuator 200 may include a magnet 216 that is arranged in sufficient proximity to droplet 218/222 to substantially immobilize magnetically responsive beads 220 during a droplet splitting operation. For example, the magnet may be arranged as a component of the droplet actuator and/or in sufficient proximity to the droplet actuator to immobilize the magnetically responsive beads in droplet 218/222 in the gap. Droplet 218/222 may be surrounded by a filler fluid (not shown). Droplet 218/222 contains a quantity of magnetically responsive beads 220 immobilized by magnet 216.

Magnet 216 is positioned relative to one or more droplet operation electrodes 214, in order to localize beads 220 in a region of the portion of the droplet 218/222 that is to form droplet 218 without permitting substantial loss of beads 218 during the droplet splitting operation to droplet 222.

In operation, a splitting operation is achieved without substantial loss of magnetic beads 220 by: as shown in 201, providing a droplet actuator 200 with electrodes activated (ON) to form combined droplet 218/222 and magnet 216 is arranged in a position which causes substantially all of magnetically responsive beads 220 to be attracted to magnet 216 in a zone of droplet 218/222 that prevents substantial loss of magnetically responsive beads 220 to droplet 222. Magnet 216 may be arranged so that magnetically responsive beads 220 attracted thereto are localized at a generally centralized location along lateral diameter L within combined droplet 218/222 and away from the droplet split zone 224. During a droplet splitting operation as shown in 202, an intermediate electrode is deactivated (OFF) to cause splitting at split zone 224. Substantially all magnetically responsive beads 220 are retained in droplet 218, and droplet 222 is formed and is substantially free of magnetically responsive beads 220, as shown in 203.

A process for washing magnetically responsive beads 220 may, in one embodiment, involve the repetition of droplet merging (with a wash droplet), bead immobilization, splitting, and bead resuspension operations until acceptable levels of washing are achieved.

### 8.2.2 Two-Magnet Arrangement to Produce Bead Column

**Figure 3** illustrates a side view of a droplet actuator 300. Droplet actuator 300 is generally configured as described in Figure 2, except that it includes two magnets, magnet 310a and 310b, arranged below and above droplet 218. The magnets may be integral with droplet actuator 300 and/or arranged in close proximity to the outer side of first substrate 210 and second substrate 212. In general, the magnets 310a and 310b may be arranged such that opposite poles are facing one another. In one example, the north or positive pole of magnet 310a is facing the south or negative pole of magnet 310b, as shown in Figure 3.

Magnets 310a and 310b may be separate magnets or, alternatively, magnets 310a and 310b may be opposite poles of a single U-shaped, C-shaped, or horseshoe-shaped permanent magnet or electromagnet. The arrangement of magnets 310a and 310b may cause magnetically responsive beads 220 to be immobilized and retained in a column-shaped cluster. The magnets are preferably arranged to localize beads within droplet 218 in a position which is away from splitting zone 224 in the portion of the combined droplet (not shown) in which the beads are to be retained. Further, the magnets are preferably aligned to centrally localize the beads along lateral diameter L within the combined droplet (not shown).

### 8.2.3 Multiple Magnet Pairs to Centralize Beads

**Figures 4A and 4B** illustrate side views of a droplet actuator 400. Droplet actuator 400 is generally configured like droplet actuator 200 described in Figure 2, except that it includes four magnets arranged at positions surrounding the droplet. In general, the arrangement illustrates the embodiment in which multiple magnet pairs with positive/negative poles facing each other are arranged to generally centrally locate beads 220 in the combined droplet prior to splitting. As illustrated in Figure 4B, the beads are generally centrally located along a vertical dimension V and a lateral dimension L. In the example illustrated, droplet actuator 400 includes four magnets, such as a magnet 410a, 410b, 410c, and 410d.

Magnets 410a and 410b may be arranged in close proximity to the droplet, equally spaced on either side of the droplet, with opposite poles facing each other. For example, the north pole of magnet 410a may face the south pole of magnet 410b. Magnets 410c and

410d may be arranged in close proximity to the droplet, equally spaced on either side of the droplet, with opposite poles facing each other. For example, the north pole of magnet 410d may face the south pole of magnet 410c. Magnet pair 410a/410b may generally be aligned at right angles around the droplet relative to magnet pair 410c/410d. In the  
5 illustrated embodiment, magnet pair 410a/410b has a vertical orientation around the droplet, and magnet pair 410c/410d has a horizontal orientation around the droplet. Any orientation around the droplet achieving the generally central localization of beads along lateral dimension L and vertical dimension V will suffice to achieve the desired central immobilization.

10 Magnets 410a and 410b may be arranged in close proximity to the outer side of first substrate 210 and second substrate 212, respectively, such that the magnetic field of magnets 410a and 410b may pass through the gap between first substrate 210 and second substrate 212 of droplet actuator 400. Magnets 410a and 410b are arranged such that  
15 opposite poles are facing one another. In one example, the north pole of magnet 410a is facing the south pole of magnet 410b, as shown in Figure 4. Similarly, magnets 410c and 410d are arranged in close proximity to a first side and a second side, respectively, of droplet actuator 400, such that the magnetic field of magnets 410c and 410d may pass through the gap of droplet actuator 400 and perpendicular to the magnetic field of magnets 410a and 410b. Magnets 410c and 410d are arranged such that opposite poles  
20 are facing one another. In one example, the north pole of magnet 410d is facing the south pole of magnet 410c, as shown in Figure 4.

Magnets 410a and 410b may be separate magnets or, alternatively, magnets 410a and 410b may be opposite poles of a single U-shaped, C-shaped, or horseshoe-shaped permanent magnet or electromagnet. Similarly, magnets 410c and 410d may be separate  
25 magnets or, alternatively, magnets 410c and 410d may be opposite poles of a single U-shaped, C-shaped, or horseshoe-shaped permanent magnet or electromagnet. Because the magnetic field of magnets 410a and 410b and magnets 410c and 410d, respectively, intersect at the center of the fluid path within droplet actuator 400, the magnetically responsive beads 220 are magnetically immobilized and retained in a cluster that is  
30 centralized within the combined droplet and retained in droplet 218 following the splitting operation.

#### 8.2.4 Illustration of Splitting with Substantially No Bead Loss

Figures 5A and 5B illustrate a first and second top view, respectively, of a droplet actuator 500 during a first and second phase, respectively, of a droplet splitting operation. Droplet actuator 500 may alternatively be configured like any of the example droplet actuators 200, 300, and 400. Droplet actuator 500 of Figures 5A and 5B makes use of magnet forces that are suitably arranged for use in a splitting operation designed to result in substantially complete retention of beads in a single droplet, such as a process for washing magnetically responsive beads.

In particular, Figures 5A and 5B show a splitting operation that is taking place at a sufficient distance from localized beads 220 to permit a splitting operation that results in substantially complete retention of magnetically responsive beads in droplet 218 and a droplet that is substantially free of magnetically responsive beads. The position of magnet face 510 is suitably arranged to magnetically immobilize substantially all of magnetically responsive beads 220 at a centralized location within the droplet and at a distance from the splitting zone that is sufficient to achieve the desired retention of magnetically responsive beads 220 in droplet 218. As a result, substantially no quantity of magnetically responsive beads 220 bridges a splitting zone 512 during the droplet splitting operation, as shown in Figure 5A, and substantially no loss of beads occurs. Figure 5B shows droplet 218 that contains substantially all magnetically responsive beads 220. In other words, the end result of a splitting operation that takes place substantially outside of the magnet forces is that there is substantially no loss of magnetically responsive beads 220 because substantially all of magnetically responsive beads 220 are suitably attracted, immobilized, and retained at a centralized location within the fluid.

### 8.3 Droplet Actuator Configurations with Magnets

#### 8.3.1 Droplet Actuator with Magnetic Shield

Figure 6 illustrates a side view of a droplet actuator 600 that includes a magnetic shield for reducing the crossover of magnetic fields. Droplet actuator 600 includes a top plate 610 and a bottom plate 614 that are arranged having a gap 618 therebetween. An arrangement of electrodes 622, e.g., electrowetting electrodes, may be associated with bottom plate 614 for performing droplet operations and an electrode, such as reservoir electrode 626 that is associated with a fluid reservoir 630 that contains a quantity of fluid

634. One or more droplets (not shown) may be dispensed from the quantity of fluid 634 of reservoir 630 for manipulation along electrodes 622. Additionally, fluid 634 and any droplets dispensed therefrom may optionally contain beads (not shown), which may, in some cases, be magnetically responsive.

5 Droplet actuator 600 further includes a magnet 638 that is arranged in proximity to the one or more electrodes 622. Magnet 638 may be arranged in sufficient proximity to electrodes 622 in order to permit immobilization of magnetically responsive beads (not shown), e.g., in a droplet positioned on an electrode. In one example, the purpose of magnet 638 is to magnetically immobilize and retain magnetically responsive beads  
10 during a droplet splitting operation, e.g., a splitting operation that may be performed in a process for washing magnetically responsive beads.

Additionally, droplet actuator 600 includes a magnetic shield 642 that is arranged in sufficient proximity to fluid reservoir 630 to shield the contents thereof from nearby magnetic fields, such as the magnetic field of magnet 638. Magnetic shield 642 may, for  
15 example, be formed of Mu-metal that has sufficiently high magnetic permeability and that is suitable to reduce, preferably substantially eliminate, unwanted magnetic fields from the magnet within fluid reservoir 630. In one example, magnetic shield 642 may be formed of Mu-metal that is supplied by McMaster-Carr (Elmhurst, IL). Other examples of magnetic shield 642 materials include Permalloy, iron, steel and nickel.

20 Droplet actuator 600 is not limited to one magnetic shield and one magnet only, any number of magnetic shields and magnets may be installed therein. Therefore, by use of one or more magnetic shields, the exposure of magnetic beads (not shown) within droplets to magnetic fields may be limited to desired regions only of droplet actuator 600. Magnetic shields may be included on any surface of the droplet actuator and in any  
25 arrangement which facilitates suitable shielding.

In one example application, a droplet actuator may be employed to perform multiple assays in parallel and, consequently, there may be a need for generally simultaneous washing of the various magnetic beads that may be manipulated within multiple lanes of electrodes. Without magnetic shields, a wash operation or assay that is performed at a  
30 certain location using an associated magnet may be affected by the magnetic field of a distant magnet (i.e., crossover of magnetic fields). By contrast, crossover of magnetic

fields between any two magnets may be reduced, preferably substantially eliminated, via the strategic placement of one or more magnetic shields, such as magnetic shield 642, within the droplet actuator.

### 8.3.2 Droplet Actuator with Alternating Magnet Configuration

5 Figure 7 illustrates a top view of a droplet actuator 700 that includes a magnet whose poles are oppositely arranged for reducing the crossover of magnetic fields. Droplet actuator 700 includes an arrangement of electrodes 710, e.g., electrowetting electrodes, for performing droplet operations on one or more droplets (not shown). Additionally, a magnet 714 is arranged in close proximity to a first lane of electrodes 710, a magnet 718 is arranged in close proximity to a second lane of electrodes 710, a magnet 722 is arranged in close proximity to a third lane of electrodes 710, and a magnet 726 is arranged in close proximity to a fourth lane of electrodes 710. Magnets 714, 718, 722, and 726 may be arranged in sufficient proximity to electrodes 710 in order to permit immobilization of magnetically responsive beads (not shown) within one or more droplets (not shown) located on one or more of the electrodes.

In order to reduce, preferably substantially eliminate, crossover of magnetic fields between adjacent magnets, the poles of adjacent magnets are oppositely arranged, which causes the adjacent magnetic fields to cancel. For example and referring again to Figure 7, the north pole of magnet 722 is oriented upward, and the south pole of magnet 726 is oriented upward. In this way, the magnetic fields are cancelled and the crossover of magnetic fields between magnets 714, 718, 722, and 726 may be reduced, preferably substantially eliminated.

Figure 8 illustrates a map 800 of magnetic fields arranged in a relationship such as the one shown in Figure 7.

### 25 8.4 Other Techniques

The invention also provides techniques for reducing carryover in a droplet actuator, as well as techniques for improving detection operations.

#### 8.4.1 Technique for Reducing Carryover in a Droplet Actuator

**Figure 9** illustrates a top view of a droplet actuator 900 by which an operation to reduce carryover at a droplet detection region may be performed. Droplet actuator 900 includes an arrangement of electrodes 910, e.g., electrowetting electrodes, for subjecting droplets to droplet operations. Additionally, droplet actuator 900 includes a designated detection region 914 at, for example, a certain electrode 910. Detection region 914 is used to detect droplets positioned thereon or passing therethrough during droplet operations. In one example, droplet detection is performed using a photomultiplier tube (PMT) or photon-counting PMT that is associated with detection region 914. A PMT (not shown) is used to measure light (i.e., detect photons) emitted from a droplet (e.g., due to fluorescence and/or chemiluminescence) when at the electrode that is associated with detection region 914.

In some cases, a build up of substances at a detection region, such as detection region 914, may occur due to carryover, which involves beads or other substances being left behind on surfaces and/or in filler fluid during droplet operations. Carryover may interfere with accurate detection of signals from subsequent droplets and/or interfere with droplet operations by affected electrodes.

Referring again to Figure 9, a droplet sequencing operation of the invention reduces, preferably substantially eliminates, carryover at detection region 914 by providing a series of alternating assay droplets 918 and wash droplets 922. In one example, an assay droplet 918a passes through detection region 914, followed by a wash droplet 922a, followed by an assay droplet 918b, followed by a wash droplet 922b, followed by an assay droplet 918c, followed by a wash droplet 922c, followed by an assay droplet 918d, which is followed by a wash droplet 922d. As assay droplets 918a, 918b, 918c, and 918d have the potential to degrade the function of detection region 914 due to crossover, wash droplets 922a, 922b, 922c, and 922d perform a cleaning operation of the surfaces that are associated with detection region 914. The cleaning process of the invention is not limited to the sequence that is shown in Figure 9. Any sequence is possible as long as the sequence includes a suitable number of wash droplets in order to suitably clean the detection region. For example, depending on the requirements of the specific assay, multiple wash droplets may be provided between assay droplets and/or multiple assay droplets may be provided between wash droplets, e.g., AAWAAWAAW, AAAWAAWAAW, AWWAWWW, AWWAWWWAWWW,

AAWWAAWWAAWW, AAWWWAAAWWWAAWW, etc., where A = assay droplet and W = wash droplet. It should be noted that it is not necessary for assay and wash droplets to be the same size. Assay droplets may be larger or wash droplets may be larger. The larger droplet may be subjected to droplet operations as a slug (e.g., a slug occupying 4 electrodes) or as a single large droplet (e.g., a 4X droplet occupying as many electrodes as it naturally covers without being formed into a slug). Each arrangement may result in a different cleaning result. It is also not necessary for the assay droplets and wash droplets to follow the same path. For example, their paths may intersect at the location needing to be cleaned.

**Figures 10A and 10B** illustrate a top view of a droplet actuator 1000 by which another operation for reducing carryover may be performed. Droplet actuator 1000 includes an arrangement of electrodes 1010, e.g., electrowetting electrodes, for performing droplet operations on one or more droplets, such as assay droplets 1014 and 1018 (Figure 10A) and wash droplet 1022 (Figure 10B). Additionally, a magnet 1026 is arranged in close proximity to a certain electrode 1010. Magnet 1026 may be arranged in sufficient proximity to the certain electrode 1010 in order to permit immobilization of magnetically responsive beads within one or more droplets, such as magnetic beads 1030 within assay droplet 1014.

During, for example, a droplet split operation, a quantity of "satellite" droplets may be left behind at the point at which the split occurs. For example and referring to Figure 10A, a droplet split operation by which assay droplet 1014 is formed by splitting from assay droplet 1018 may result in a certain quantity of satellite droplets 1034 being left behind upon a certain electrode 1010. Satellite droplets, such as satellite droplets 1034, may be the source of carryover (cross contamination) from one droplet to another, which is not desired. Figure 10B illustrates that a wash droplet, such as wash droplet 1022, may be transported along electrodes 1010 subsequent to the assay operations of, for example, assay droplets 1018 and 1014 in order to capture satellite droplets 1034 and transport them away prior the next assay operation occurs. In this way, electrodes 1010 are cleaned between assay operations. The cleaning process of the invention is not limited to the sequence that is shown in Figures 10A and 10B. Any sequence is possible as long the sequence includes a suitable number of wash droplets in order to suitably clean the electrodes.

#### 8.4.2 Improving Detection Operations in a Droplet Actuator

**Figures 11A, 11B, and 11C** illustrate side views of droplet actuator 1100 by which respective operations for improving the sensitivity of droplet detection may be performed. Droplet actuator 1100 includes a top plate 1110 and a bottom plate 1114 that are arranged having a gap 1118 therebetween. An arrangement of electrodes 1122, e.g., electrowetting electrodes, may be associated with bottom plate 1114 for performing droplet operations on a droplet 1126. A PMT window 1130 may be associated with top plate 1110, by which a PMT (not shown) is used to measure light (i.e., detect photons 1134) emitted from droplet 1126.

Figure 11A shows a method of improving the sensitivity of droplet detection by spreading out a droplet, such as droplet 1126, in order to increase the surface area that is exposed to PMT window 1130 and, thus, increase the number of photons 1134 that may be detected. A droplet may be spread linearly across one or more electrodes 1122 depending on the volume of the droplet. In the case of a small-volume droplet, a buffer droplet may be added to make the droplet larger, as long as losses due to dilution by the buffer droplet are offset by the increased droplet area exposed to the PMT. For example, Figure 11B shows droplet 1126 that is spread continuously across multiple electrodes 1122, which increases the number of photons 1134 that may reach PMT window 1130 and may be detected by the PMT.

Figure 11C shows a scenario wherein droplet 1126 is split up into multiple droplets 1126, such as droplets 1126a, 1126b, 1126c, and 1126d that are on multiple electrodes 1122. Again, the surface area that is exposed to PMT window 1130 is increased, which increases the number of photons 1134 that may reach PMT window 1130 and may be detected by the PMT. Alternatively and referring again to Figures 11A, 11B, 11C, the spreading of a droplet, such as droplet 1126, is not limited to linear spreading only. The droplet may be spread two dimensions, such as across a grid or array of electrodes 1122, in order to increase the surface area that is exposed to PMT window 1130. Alternatively, one or more large-area electrodes may be provided, across which one or more droplets may be spread.

**Figure 12** illustrates a top view of a droplet actuator 1200 for improving the sensitivity of droplet detection. Droplet actuator 1200 includes an arrangement of electrodes 1210, e.g., electrowetting electrodes, for performing droplet operations on multiple droplets 1214.

Additionally, droplet actuator 1200 may include a droplet detection region 1218 that has an associated PMT (not shown) for measuring light that is emitted from a certain droplet 1214 when present. In order to reduce, preferably substantially eliminate, the carryover of light from a distant droplet 1214 to droplet detection region 1218, a minimum distance  $d$  is maintained in all directions between the outer perimeter of droplet detection region 1218 and any distant droplets 1214 within droplet actuator 1200, as shown in Figure 12. The minimum distance  $d$  is sufficiently large to reduce, preferably substantially eliminate, the carryover of light from a distant droplet 1214 to droplet detection region 1218. As a result, in a droplet actuator that includes multiple droplets 1214, a spacing is maintained during detection between the target droplet 1214 that is being measured and a distant droplet 1214, such that the carryover of light from the distant droplet 1214 to droplet detection region 1218 is reduced, preferably substantially eliminated. As a specific case the distance  $d$  is an integer multiple  $m$  of a unit electrode size, and the droplet detection may be conducted on a set of electrodes which are electrically connected as an  $m$  phased bus. Alternatively, Figure 13 (described below) describes a scenario wherein the real-estate with a droplet actuator is limited and, therefore, sufficient spacing between droplets, as described in Figure 12, may not be achieved.

In an alternative embodiment, cross-over from nearby droplets is eliminated by using optical elements, such as one or more lenses, which focus only light from the droplet being interrogated onto the sensor and eliminates signal from other droplets.

**Figure 13** illustrates a side view of a droplet actuator 1300 for improving the sensitivity of droplet detection. Droplet actuator 1300 includes a top plate 1310 and a bottom plate 1314 that are arranged having a gap 1318 therebetween. An arrangement of electrodes 1322, e.g., electrowetting electrodes, may be associated with bottom plate 1314 for performing droplet operations on droplets, such as a droplet 1326 and a droplet 1330. A PMT window 1334 may be associated with top plate 1310, by which a PMT (not shown) is used to measure light (i.e., detect photons 1338) that is emitted from, for example, droplet 1326. Because the spacing between, for example, droplet 1326 at PMT window 1334 and distant droplet 1330 is not suitably sufficient to avoid the carryover of light from droplet 1330 to PMT window 1334, a mask 1342 is provided upon top plate 1310. The purpose of mask 1342 is to block light from a distant droplet from carrying over to PMT window 1334, which is the detection region of a target droplet.

Mask 1342 may be formed upon top plate 1310 via a layer of any light-absorbing material, as long as the material that is used is compatible with the electrowetting process and does not unduly interfere with the droplet actuator operations. In one example, mask 1342 may be formed by applying a layer of black paint to top plate 1310, such that one or more windows, such as PMT window 1334, are provided in selected detection regions of droplet actuator 1300. In the example shown in Figure 13, mask 1342 reduces, preferably substantially eliminates, the carry over of light from distant droplet 1330 to the target droplet 1326 at PMT window 1334. In another example the mask 1342 is formed by an opaque conductor on the side of the top plate 1310 facing the droplet. The conductor may, for example, be aluminum, chromium, copper, or platinum. The conductor may additionally serve as an electrical reference electrode.

### 8.5 Droplet Actuators with Magnet Assemblies

**Figures 14A and 14B** illustrate top views of a modular droplet actuator assembly 1400, which is a nonlimiting example of a universal assembly for orienting a magnet assembly to a droplet actuator. Modular droplet actuator assembly 1400 may include, for example, a mount 1410, a magnet assembly 1420, and a droplet actuator 1430. Figure 14A shows modular droplet actuator assembly 1400 when disassembled. Figure 14B shows modular droplet actuator assembly 1400 when assembled.

Magnet assembly 1420 may include a substrate 1424 upon which is mounted one or more magnets 1428, as shown in Figure 14A. The magnets 1428 may be permanently affixed to substrate 1424 or may be removable. Removable magnets 1428 facilitate selection by a user of magnets having desired properties, such as desired magnet strength. In one embodiment, a droplet actuator instrument is provided with a droplet actuator assembly 1400 including a mount 1410 and a magnet assembly 1420 without magnets. In another embodiment, the user is also provided with magnets having specified properties which may be affixed by the user to the magnet assembly 1420. In another embodiment, the user is also provided with sets of magnets having various specified characteristics such that the user may select a set of one or more magnets having desired properties and affix the selected set to the magnet assembly 1420.

Magnets may be marked or coded (e.g., color coded) to facilitate selection of magnets having appropriate properties, as well as marked to show the orientation of the magnet's

magnetic field (e.g., by color coding or otherwise marking the North and South faces of the magnets). Similarly, the magnet assembly 1420 may be marked to show the desired orientation of magnets inserted therein, and in some embodiments, magnets may be shaped to permit them to be affixed to the magnet assembly 1420 only in an appropriate orientation.

Moreover, in another embodiment, the user may be provided with magnet assemblies 1420 having magnets already affixed thereto, wherein the magnet assemblies 1420 each have different magnet configurations, e.g., sets of magnets having different properties. The user may select the magnet configuration having magnets having properties appropriate to the user's desired use for the instrument. Magnet assembly 1420 may be marked or otherwise color coded to facilitate selection by the user. Magnet properties may, for example, be selected based on the properties of beads selected by the user.

Droplet actuator 1430 may include a substrate 1434 upon which is an arrangement of electrodes 1438, e.g., electrowetting electrodes, as shown in Figure 14A. A second (top) substrate may also be included (not shown).

Magnet assembly 1420 is designed such that magnets 1428 substantially align with certain electrodes 1438 of interest on droplet actuator 1430. For example, in some embodiments, parallel configurations of magnets may be present for conducting parallel assay steps on droplet actuator 1430. Magnets may be configured and oriented, for example, according to the various configurations and orientations described herein.

Mount 1410 may serve as a universal platform for mounting a magnet assembly, such as magnet assembly 1420, and a droplet actuator, such as droplet actuator 1430. In one embodiment, mount 1410 is configured to accept a wide variety of magnet assemblies 1420 and a wide variety of droplet actuators 1430. Magnet assembly 1420 may include one or more magnets arranged in any of a variety of patterns and employing any of a variety of magnet properties. Figure 14 illustrates a row of magnets, but magnets may also be provided in a grid or any arrangement that places the magnets in their proper position in relation to the droplet actuator 1430 in order to facilitate desired operations on the droplet actuator.

In one example, magnet assembly 1420 and droplet actuator 1430 may be installed into mount 1410 via respective fittings 1418 and 1414. Fittings may, for example, involve slots into which the mount 1410 may be fitted, openings on the mount 1410 for accepting posts on the magnet assemblies 1420 or vice versa, openings on the mount 1410 for accepting screws on the magnet assemblies 1420, threaded posts for accepting bolts, various spring loaded mechanisms, recessed trays, complimentary fittings, and the like. Any mechanism which facilitates sufficiently secure attachment to permit the device to function for its intended purpose will suffice.

Further, the mount 1410 may include multiple fittings for multiple possible positions of magnet assembly 1420 and/or droplet actuator 1430, and/or mounting of multiple magnet assemblies 1420 and/or multiple droplet actuators 1430 in a single mount 1410. Further, mount 1410 may be configured to permit magnet assemblies 1420 to be mounted above, below and/or beside the droplet actuator 1430, i.e., in any relationship with the droplet actuator. With droplet actuator 1430 installed in modular mount 1410, any magnet assembly of interest, such as magnet assembly 1420, may be inserted into modular droplet actuator assembly 1400 via, for example, the slot.

**Figure 15** illustrates a side view of a modular droplet actuator assembly 1500, which is another nonlimiting example of a universal assembly for orienting a magnet assembly to a droplet actuator, similar to the assembly 1400 shown in Figure 14, except that mount 1410 is replaced with a mount 1510. Mount 1510 includes recessed trays into which magnet assembly 1420 and droplet actuator 1430 may be fitted in order to provide a “drop in” method of loading.

Referring to Figures 14A, 14B, and 15, an aspect of the invention is that the slots or other attachment means serve to orient the magnet substrate and the droplet actuator so as to align the magnets on the magnet assembly with the appropriate electrodes or electrode paths on the droplet actuator. In this manner, the magnets may be removed when not needed for an assay. Additionally, different magnet mounts with different distributions of magnets may be provided for different types of assays or different droplet actuator layouts.

## 8.6 Magnets

In addition to other aspects already described, it should be noted that magnets selected for use with the invention may be permanent or electromagnets. There may be a relationship between the magnetically responsive content of the beads in the droplet and the magnetic strength/pull force. Therefore, the magnetic strength/pull force of the magnet may be selected relative to the responsiveness of the magnetic beads such that it is:

- sufficiently strong relative to the magnetic responsiveness of the beads to substantially immobilize magnetically responsive beads;
- not so strong relative to the magnetic responsiveness of the beads that it significantly magnetizes the beads and, thus, causes irreversible formation of clumps of beads;
- not so strong relative to the magnetic responsiveness of the beads that resuspension occurs poorly when the magnet field is removed; and/or
- not so strong relative to the magnetic responsiveness of the beads that the beads are pulled out of the droplet altogether.

In some embodiments, the magnet may have high magnetic strength (in Tesla) with lesser pull force (in pounds). In one example, magnet is a neodymium permanent magnet that has a surface field strength of about 1 Tesla (T). In another example, the magnet is an electromagnet that has a surface field strength of about 1 T, which may be turned on and off electronically. Where a permanent magnet is used, the magnet may be moved away from the magnetically responsive bead-containing droplet for uses in which it is desirable to remove the influence of the magnetic field. While not limited to the following ranges, it is understood that ranges of magnetic strength that generally encompasses the useful strength of the present invention can include: a broad range of 0.01 T to 100 T (pulsed) or 45 T (continuous); an intermediate range of 0.01 T to 10 T; and a narrow range of 0.1 T to 1 T (preferably 0.5 T).

## 8.7 Droplet Composition

Droplets including magnetic beads and subjected to droplet splitting operations may include any of a wide variety of samples, reagents, and buffers useful for conducting assays using the beads. For example, during washing, the droplet may include a buffer,

5 such as a phosphate-buffered saline (PBS) buffer with a surfactant that is suitable for use in magnetic based immunoassays. Preferred surfactants are those which facilitate immobilization and/or resuspension of beads following immobilization by magnetic forces. The surfactant and amount of surfactant may be adjusted to provide a substantial improvement in resuspension as compared to a control solution lacking the surfactant. In one embodiment, the droplet includes PBS buffer with about 0.01% Tween<sup>®</sup> 20.

10 A hydrophilic polymer and/or surfactant may be included in the droplet to facilitate retention and resuspension of magnetically responsive beads during splitting operation. The droplet may include a wide variety of liquids immiscible with the filler fluid. Examples of buffers include, but are not limited to, phosphate-buffered saline (PBS) buffer and Tris buffer saline. In one embodiment, the droplet fluid includes a buffer, such as the PBS buffer, and any surfactant that is suitable for use in magnetic based immunoassays.

15 Preferred hydrophilic polymers and surfactants are those which facilitate resuspension of beads following immobilization by magnetic forces. The surfactant and amount of surfactant may be adjusted to provide a substantial improvement in resuspension as compared to a control solution lacking the surfactant. Examples of surfactants that are suitable for use in magnetic based immunoassays include, but are not limited to, polysorbate 20, which is commercially known as Tween<sup>®</sup> 20, and Triton X-100. Tween<sup>®</sup> 20 may be supplied by, for example, Pierce Biotechnology, Inc. (Woburn, MA). Triton<sup>®</sup> X-100 may be supplied by, for example, Rohm & Haas Co (Philadelphia, PA). In one example, the droplet fluid within the droplet actuator is a mix of PBS with Tween<sup>®</sup> 20 in the range of from about 0.001% to about 0.1%. In another example, the droplet fluid within the droplet actuator is a mix of PBS with about 0.01% Tween<sup>®</sup> 20.

25 Other examples include pluronic surfactants, polyethylene glycol (PEG), methoxypolyethylene glycol (MPEG), poly-sorbate (polyoxyethylene sorbitan monooleates or Tween<sup>®</sup>), polyoxyethylene octyl phenyl ether (Triton X-100<sup>®</sup>), polyvinyl pyrrolidone, polyacrylic acid (and crosslinked polyacrylic acid such as carbomer), polyglycosides (nonionic glycosidic surfactants such as octyl glucopyranoside) and soluble polysaccharides (and derivatives thereof) such as heparin, 30 dextrans, methyl cellulose, propyl methyl cellulose (and other cellulose esters and ethers), dextrans, maltodextrins, galactomannans, arabinogalactans, beta glucans, alginates, agar,

carrageenan, and plant gums such as xanthan gum, psyllium, guar gum, gum traganth, gum karya, gum ghatti and gum acacia. The particular additive can be selected for maximum compatibility with a specific microfluidic sample.

## 8.8 Droplet Actuator

5 For examples of droplet actuator architectures that are suitable for use with the present invention, see U.S. Patent 6,911,132, entitled, "Apparatus for Manipulating Droplets by Electrowetting-Based Techniques," issued on June 28, 2005 to Pamula et al.; U.S. Patent Application No. 11/343,284, entitled, "Apparatuses and Methods for Manipulating Droplets on a Printed Circuit Board," filed on January 30, 2006; U.S. Patents 10 6,773,566, entitled, "Electrostatic Actuators for Microfluidics and Methods for Using Same," issued on August 10, 2004 and 6,565,727, entitled, "Actuators for Microfluidics Without Moving Parts," issued on January 24, 2000, both to Shenderov et al.; and International Patent Application No. PCT/US 06/47486 to Pollack et al., entitled, "Droplet-Based Biochemistry," filed on December 11, 2006.

15 Droplet actuator techniques for immobilizing magnetic beads and/or non-magnetic beads are described in the foregoing international patent applications and in Sista, et al., U.S. Patent Application Nos. 60/900,653, filed on February 9, 2007, entitled "Immobilization of magnetically-responsive beads during droplet operations"; Sista et al., U.S. Patent Application No. 60/969,736, filed on 20 September 4, 2007, entitled "Droplet Actuator Assay Improvements"; and Allen et al., U.S. Patent Application No. 60/957,717, filed on August 24, 2007, entitled "Bead washing using physical barriers."

Combinations of these various techniques are within the scope of this invention.

## 25 8.9 Fluids

For examples of fluids that may be subjected to droplet operations of the invention, see the patents listed in section 8.8, especially International Patent Application No. PCT/US 06/47486, entitled, "Droplet-Based Biochemistry," filed on December 11, 2006. In some 30 embodiments, the droplet is a sample fluid, such as a biological sample, such as whole blood, lymphatic fluid, serum, plasma, sweat, tear, saliva, sputum, cerebrospinal fluid, amniotic fluid, seminal fluid, vaginal excretion, serous fluid, synovial fluid, pericardial

fluid, peritoneal fluid, pleural fluid, transudates, exudates, cystic fluid, bile, urine, gastric fluid, intestinal fluid, fecal samples, fluidized tissues, fluidized organisms, biological swabs and biological washes. In some embodiments, the fluid that is loaded includes a reagent, such as water, deionized water, saline solutions, acidic solutions, basic solutions, detergent solutions and/or buffers. In some embodiments, the fluid that is loaded includes a reagent, such as a reagent for a biochemical protocol, such as a nucleic acid amplification protocol, an affinity-based assay protocol, a sequencing protocol, and/or a protocol for analyses of biological fluids.

### 8.10 Filler Fluids

As noted, the gap is typically filled with a filler fluid. The filler fluid may, for example, be a low-viscosity oil, such as silicone oil. Other examples of filler fluids are provided in International Patent Application No. PCT/US 06/47486, entitled, "Droplet-Based Biochemistry," filed on December 11, 2006.

### 8.11 Washing Magnetically Responsive Beads

For protocols making use of beads, droplets with beads can be combined using droplet operations with one or more wash droplets. Then, while retaining the beads (e.g., physically or magnetically) using magnet configurations of the invention, the merged droplet may be divided using droplet operations into two or more droplets: one or more droplets with beads and one or more droplets without a substantial amount of beads. In one embodiment, the merged droplet is divided using droplet operations into one droplet with beads and one droplet without a substantial amount of beads.

Generally, each execution of a washing protocol results in retention of sufficient beads for conducting the intended assay without unduly detrimental effects on the results of the assay. In certain embodiments, each division of the merged droplet results in retention of more than 90, 95, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99, 99.9, 99.99, 99.999, 99.9999, 99.99999, or 99.999999 percent of beads. In other embodiments, each execution of a washing protocol to achieve a predetermined reduction in the concentration and/or amount of removed substance results in retention of more than 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99, 99.9, 99.99, 99.999, 99.9999, 99.99999, or 99.999999

percent of beads. In still other embodiments, the amount of retained beads is calculated and the results are adjusted accordingly.

5 In some embodiments, beads can be washed in reservoirs in which the bead-containing droplet and wash droplets are combined, beads are retained (for example by a magnet, by physical structures, electrostatic forces), and droplets lacking beads are dispensed from the reservoir using droplet operations. For example, beads can be washed by dilute-and-dispense strategy whereby a wash buffer is added to the reservoir to dilute the contents, magnetically responsive beads are localized within the reservoir with a magnet and most of the solution is dispensed from the reservoir, and this cycle is repeated till acceptable levels of washing are achieved.

As an example, washing magnetically responsive beads may generally include the following steps:

- (1) providing a droplet comprising magnetically responsive beads and unbound substances in proximity with a magnet;
- 15 (2) using droplet operations to combine a wash droplet with the droplet comprising the magnetically responsive beads;
- (3) immobilizing the beads by application of a magnetic field;
- (4) using droplet operations to remove some or all of the droplet surrounding the beads to yield a droplet comprising the beads with a reduced concentration of unbound components and a droplet comprising unbound components;
- 20 (5) releasing the beads by removing the magnetic field; and
- (6) repeating steps (2) to (3) or (2) to (4) until a predetermined degree of purification is achieved.

25 In this manner, unbound substances, such as contaminants, byproducts or excess reagents, can be separated from the beads. Each cycle produces a droplet including the beads but with a decreased level of the unwanted substances. Step (5) is not required in each

washing cycle; however, it may be useful to enhance washing by freeing contaminants which may be trapped in the immobilized beads. Steps may be performed in a different order, e.g., steps (2) and (3) may be reversed. Steps in the washing protocol may be accomplished on a droplet actuator using droplet operations as described herein.

5 In embodiments in which magnetically responsive beads are used, the inventors have found that application of a magnetic field though useful for temporarily immobilizing beads, moving beads and/or positioning beads, sometimes results in unwanted aggregation of the beads. As already noted, in one embodiment, a hydrophilic polymer and/or surfactant is included to prevent or reduce bead aggregation. Hydrophilic  
10 polymers and surfactants should be selected and used in amounts which reduce or eliminate bead aggregation and minimize non-specific adsorption while at the same time not resulting in significant loss of target analytes or reagents from the droplet. In one embodiment, the hydrophilic polymer and/or surfactant reduces bead clumping in a droplet in a non-gaseous filler fluid and specifically does not serve to reduce molecular  
15 adsorption of droplet components to a surface of the droplet actuator.

Another approach to eliminating or reducing clumping aggregation of beads involves the use of smaller numbers of larger beads. Any number of beads which can be contained in a droplet during one or more droplet operations may be used. In some embodiments, the number of magnetically responsive beads can range from 1 to several 100,000's. For  
20 example, in one embodiment, the invention makes use of one to 100 magnetically responsive beads per droplet. For example, the invention may make use of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ... 100 magnetically responsive beads per droplet. In one embodiment, the number of magnetically responsive beads is from one to 10. Use of smaller numbers of magnetically responsive beads permits larger beads to be used. For example, in one  
25 embodiment, the invention makes use of one to 100 magnetically responsive beads per droplet, where the beads have an average diameter of about 25 to about 100 microns. In another embodiment the invention makes use of one to 10 magnetically responsive beads per droplet, where the beads have an average diameter of about 50 to about 100 microns.

## 9 Concluding Remarks

The foregoing detailed description of embodiments refers to the accompanying drawings, which illustrate specific embodiments of the invention. Other embodiments having different structures and operations do not depart from the scope of the present invention.

5 This specification is divided into sections for the convenience of the reader only. Headings should not be construed as limiting of the scope of the invention.

It will be understood that various details of the present invention may be changed without departing from the scope of the present invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation, as the present  
10 invention is defined by the claims as set forth hereinafter.

**What is claimed is:**

1. A droplet actuator device comprising magnet sets and electrode sets, wherein:
  - (a) each electrode set comprises a plurality of electrodes associated with a substrate, each electrode set defining a lane of electrodes;
  - (b) two or more electrode sets are:
    - (i) arranged to form an array of electrodes; and
    - (ii) configured for conducting one or more droplet operations;
  - (c) each magnet set comprises one or more magnets;
  - (d) each magnet set is arranged in sufficient proximity with an electrode set such that magnetic field(s) exerted by the magnet set restricts mobility of magnetically responsive particles within one or more droplets located on one or more of the electrodes; and
  - (e) the polar orientation of each magnet set is arranged to be opposite to the polar orientation of its adjacent magnet set(s), such that adjacent magnetic fields cancel and thereby reduce crossover of magnetic fields between adjacent magnet sets.
2. The droplet actuator device of claim 1 wherein each magnet set is oriented with a pole facing an electrode set, and each adjacent magnet set is oriented with an opposite pole facing the electrode set.
3. The droplet actuator device of claim 1 wherein each magnet set has a sufficiently strong field to immobilize magnetically responsive particles during a droplet splitting operation, but not sufficiently strong to immobilize magnetically responsive particles during a droplet transport operation.

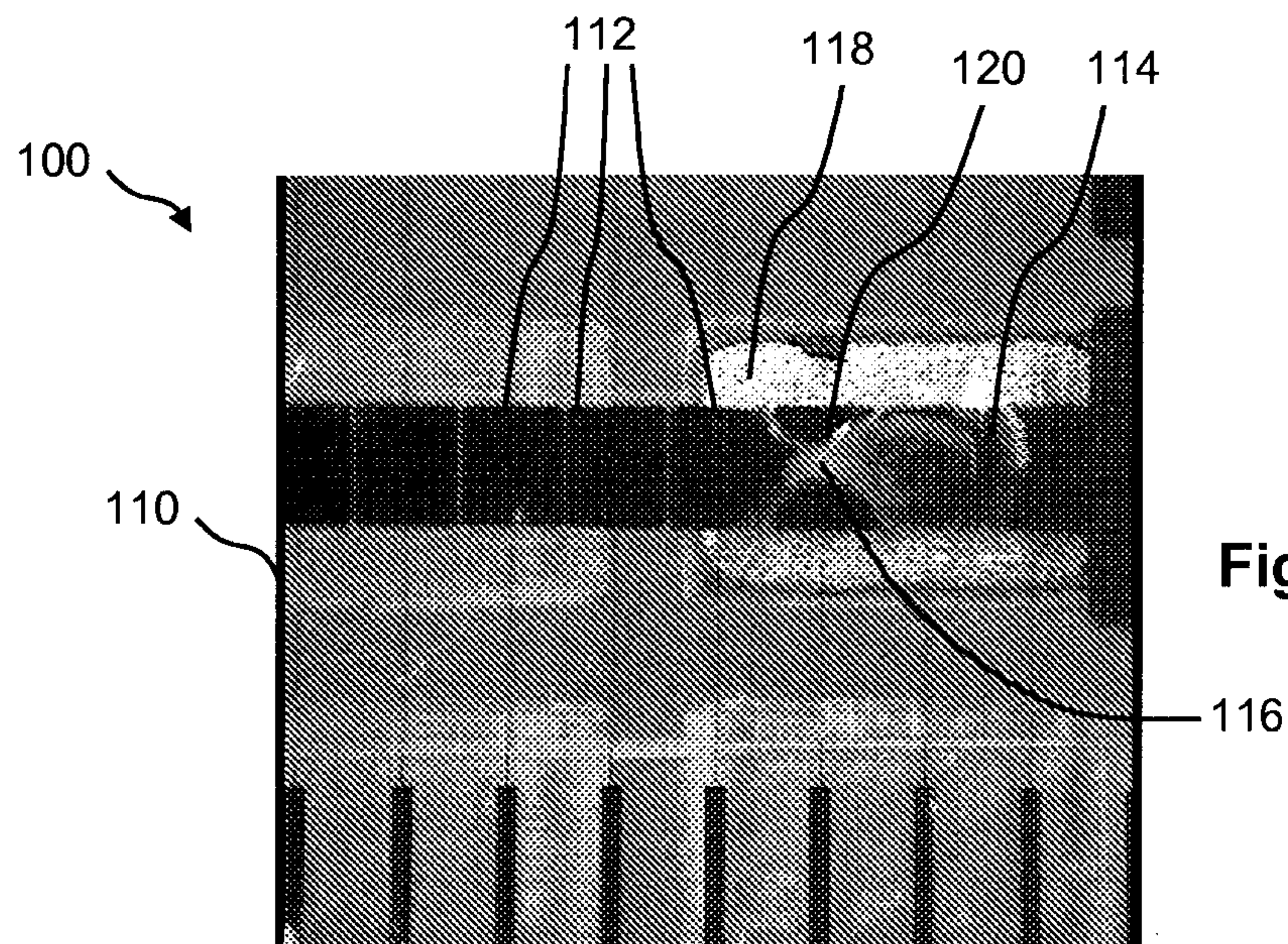


Figure 1A

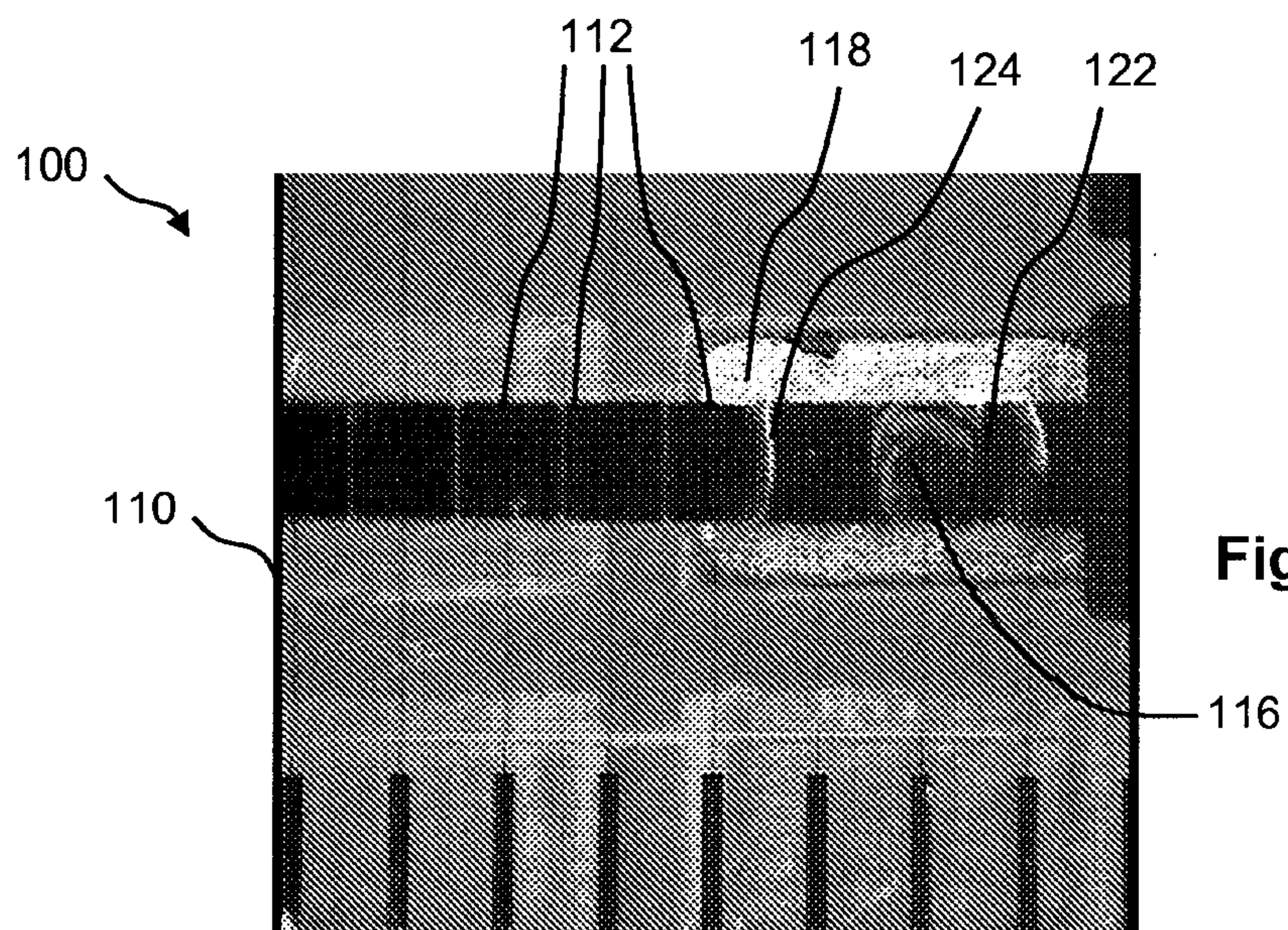
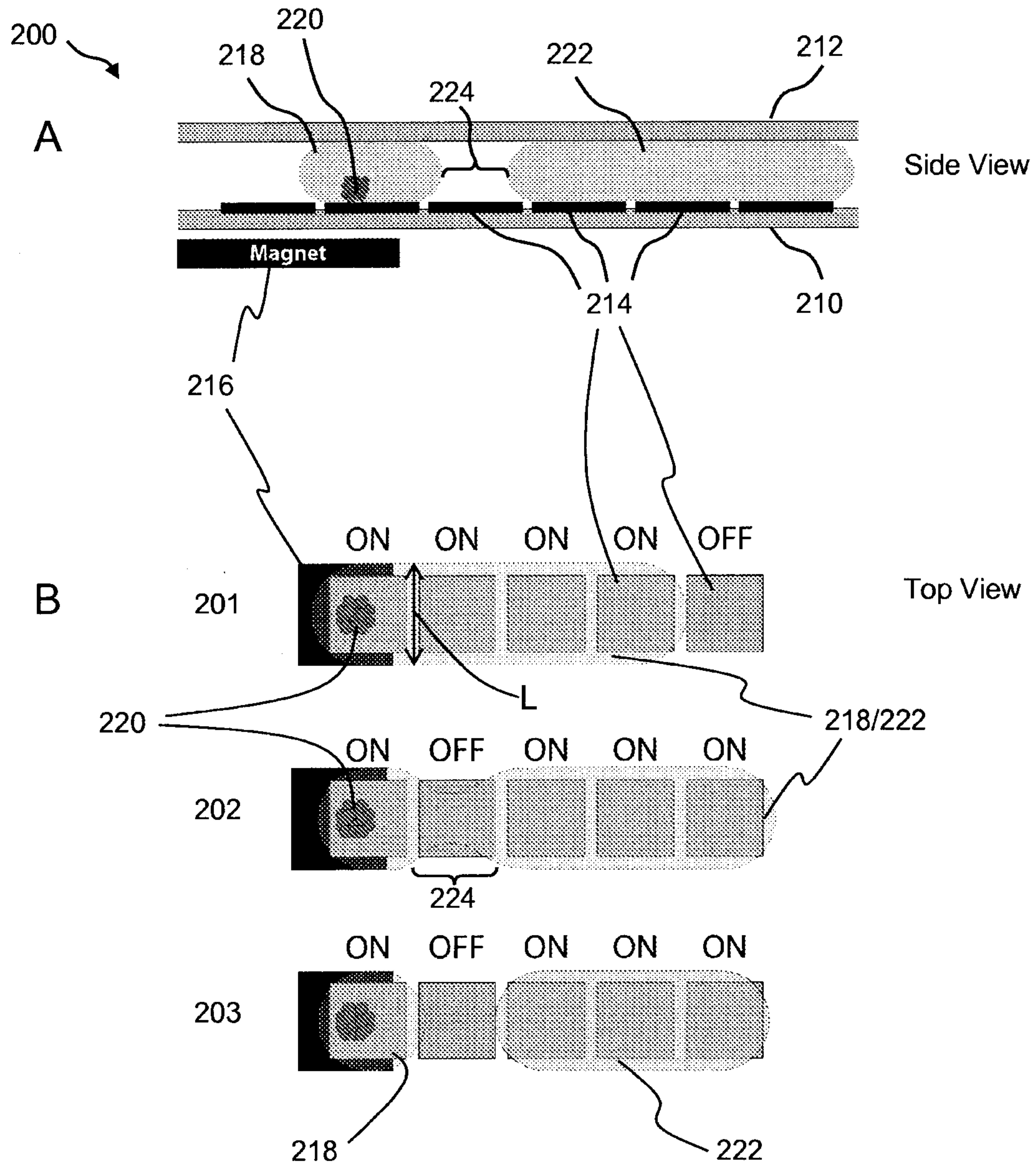


Figure 1B

**Figure 2**

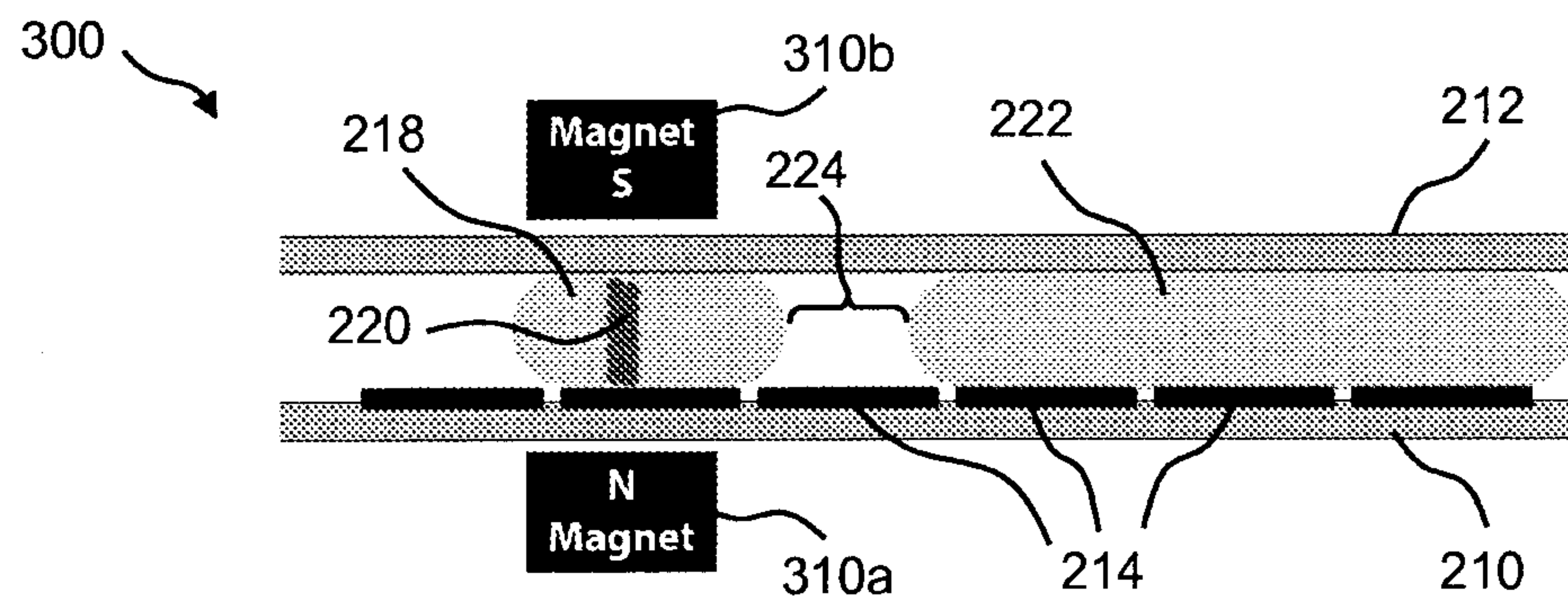


Figure 3

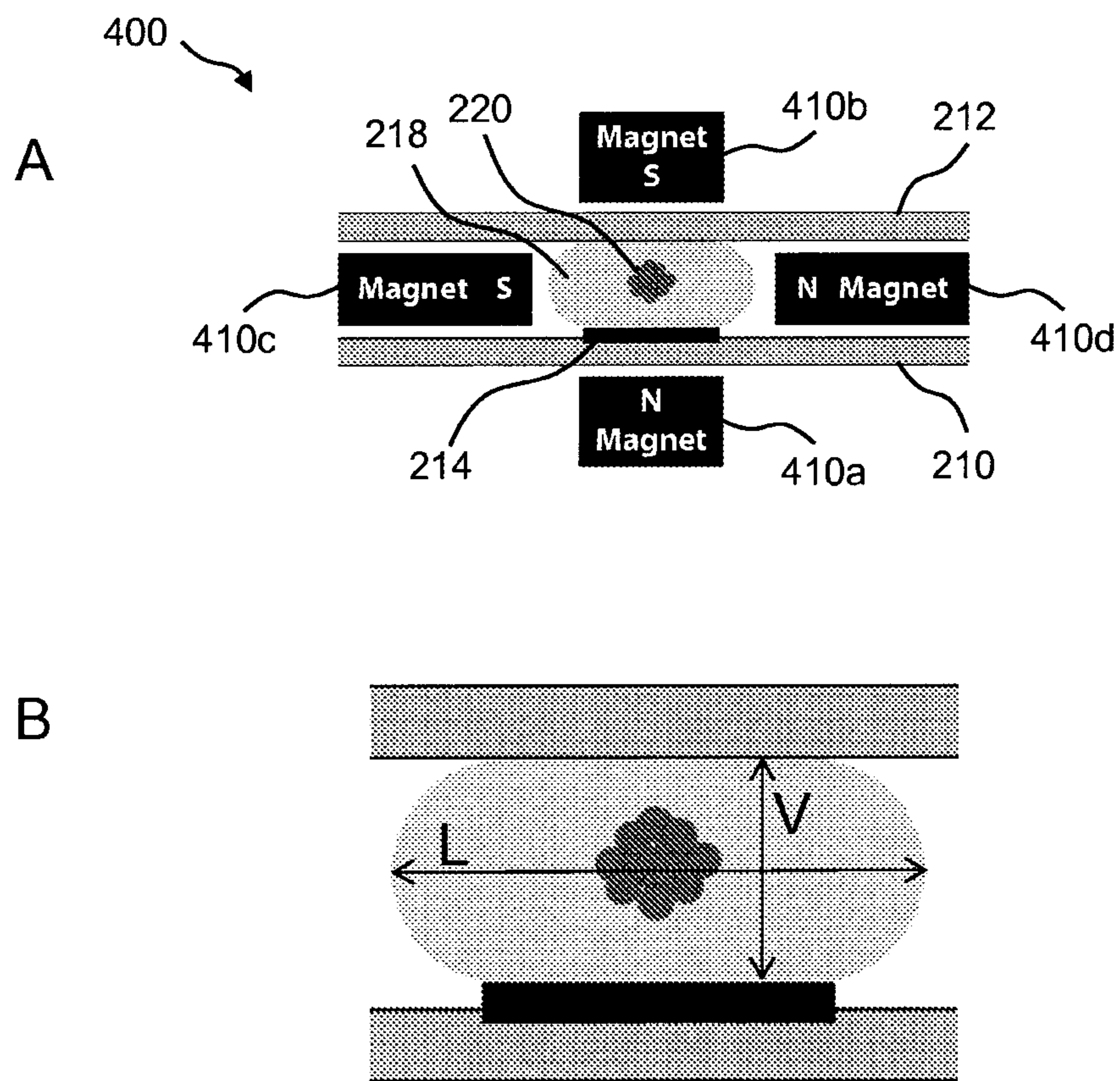


Figure 4

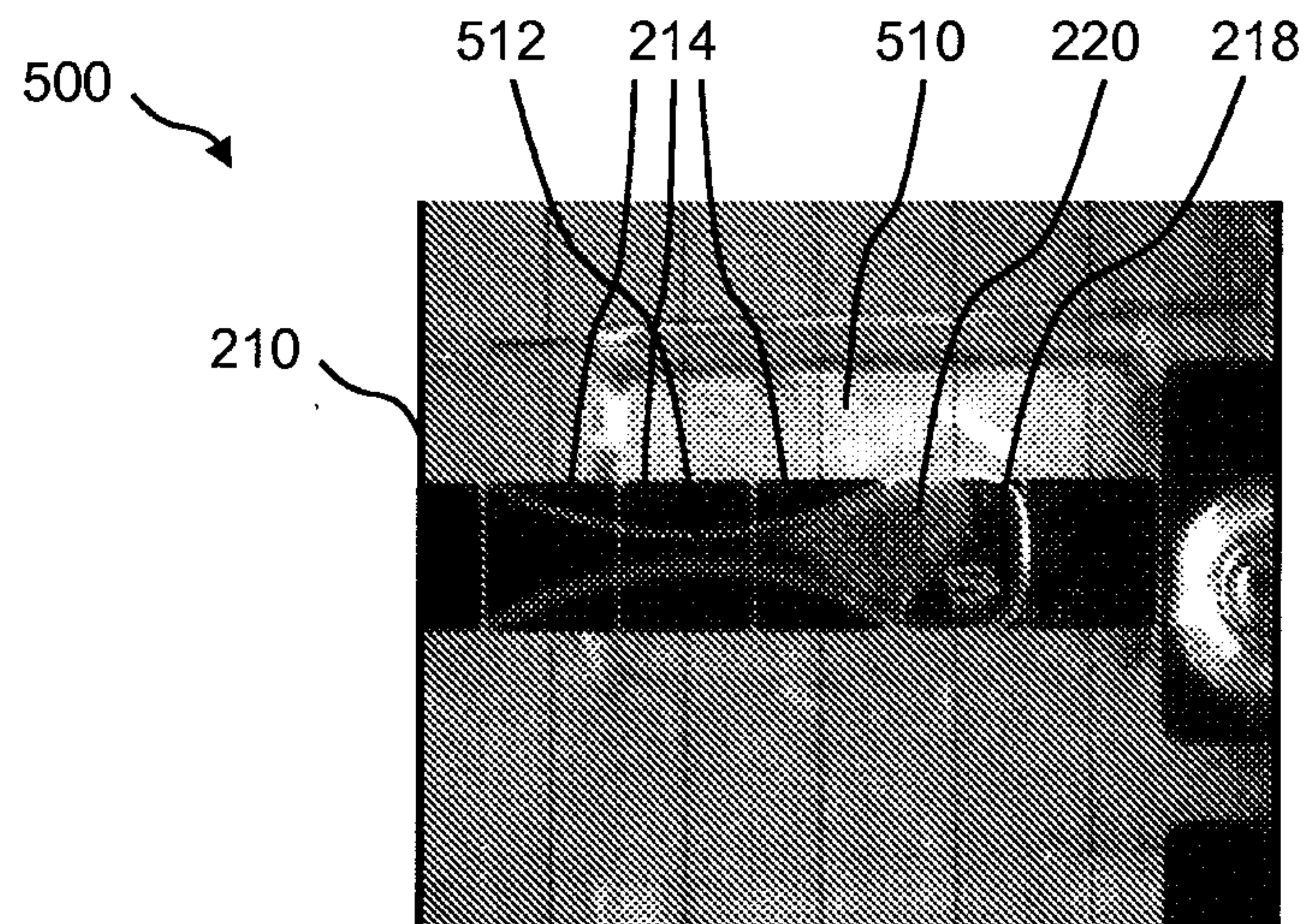


Figure 5A

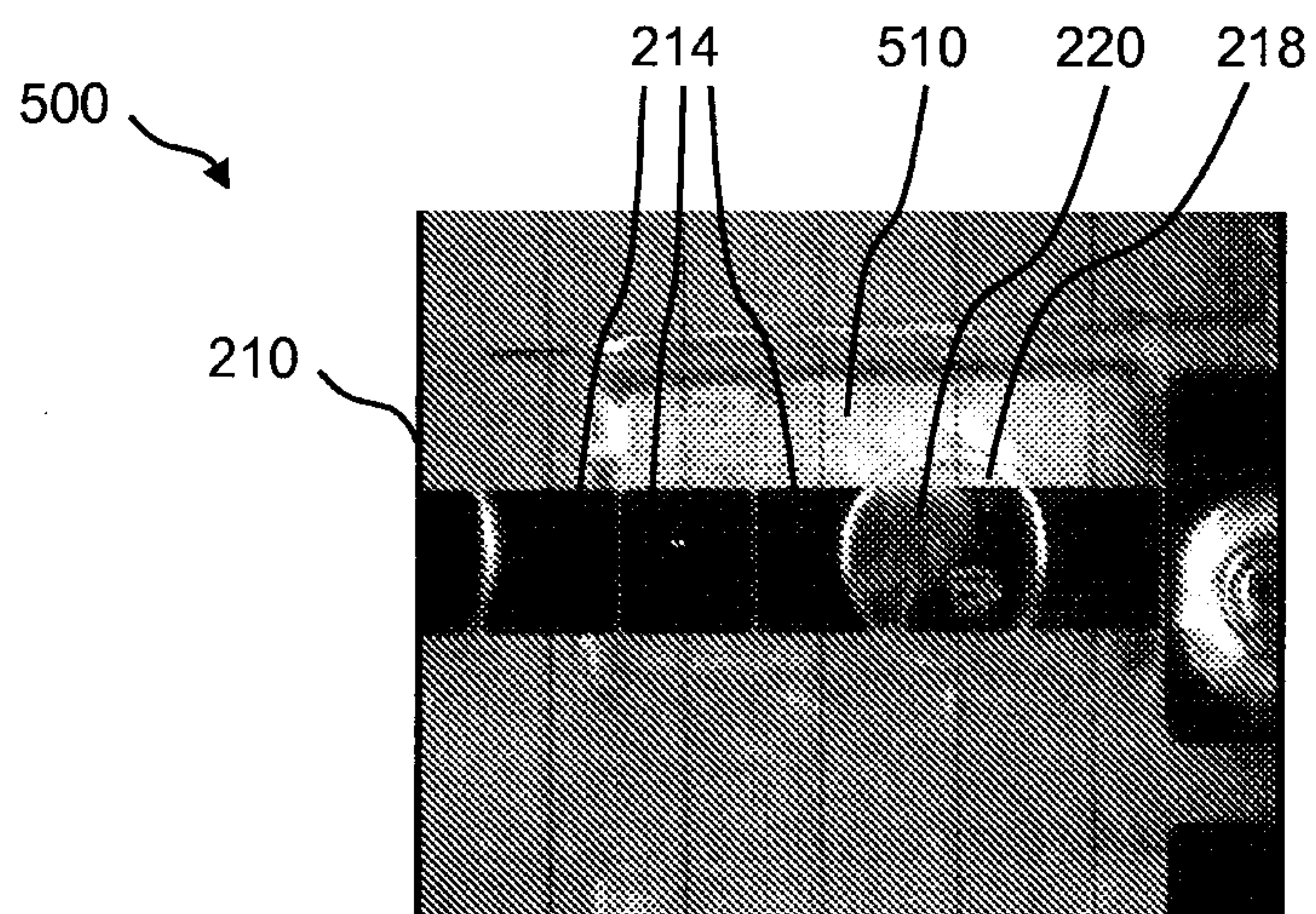
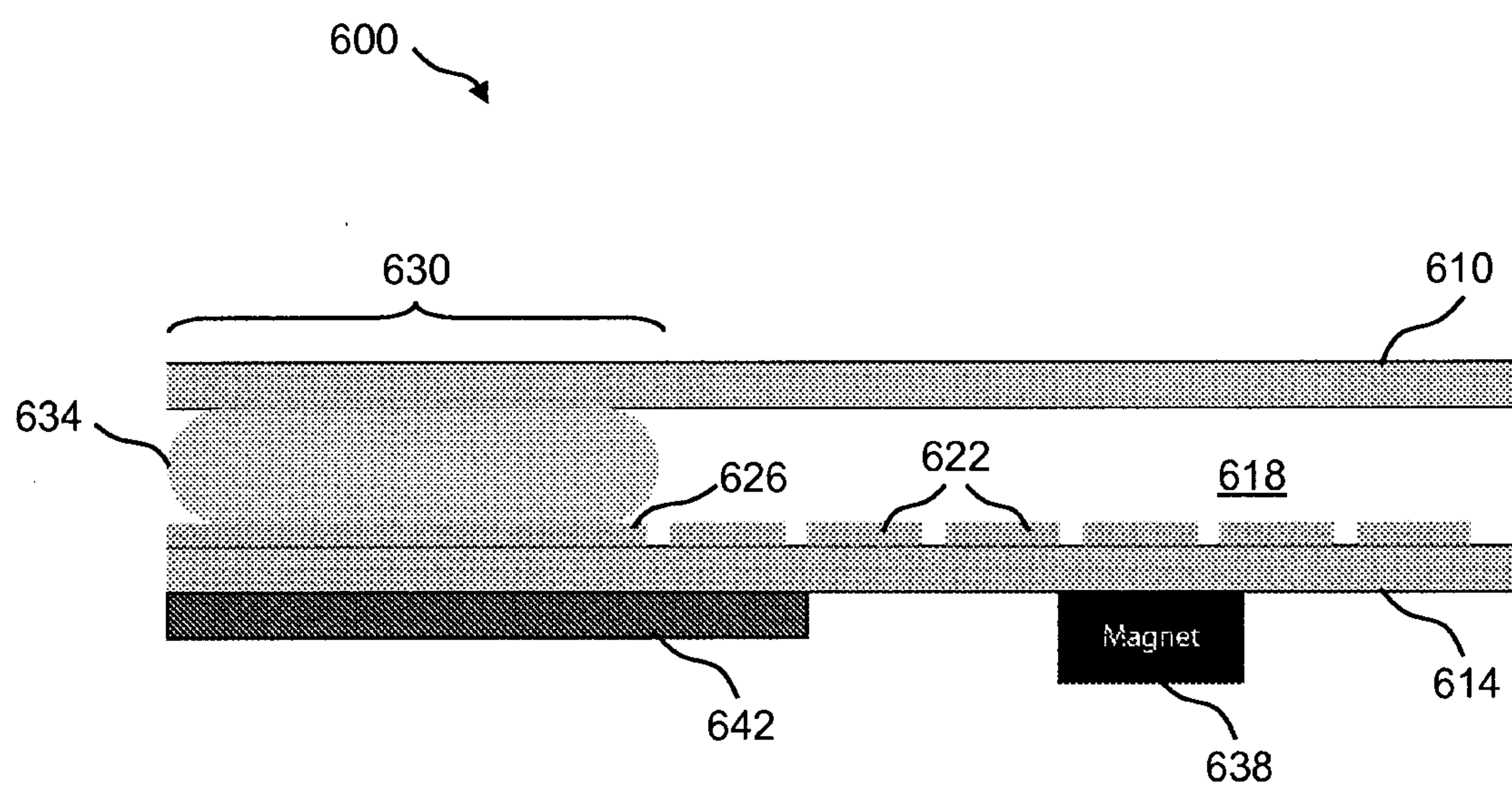
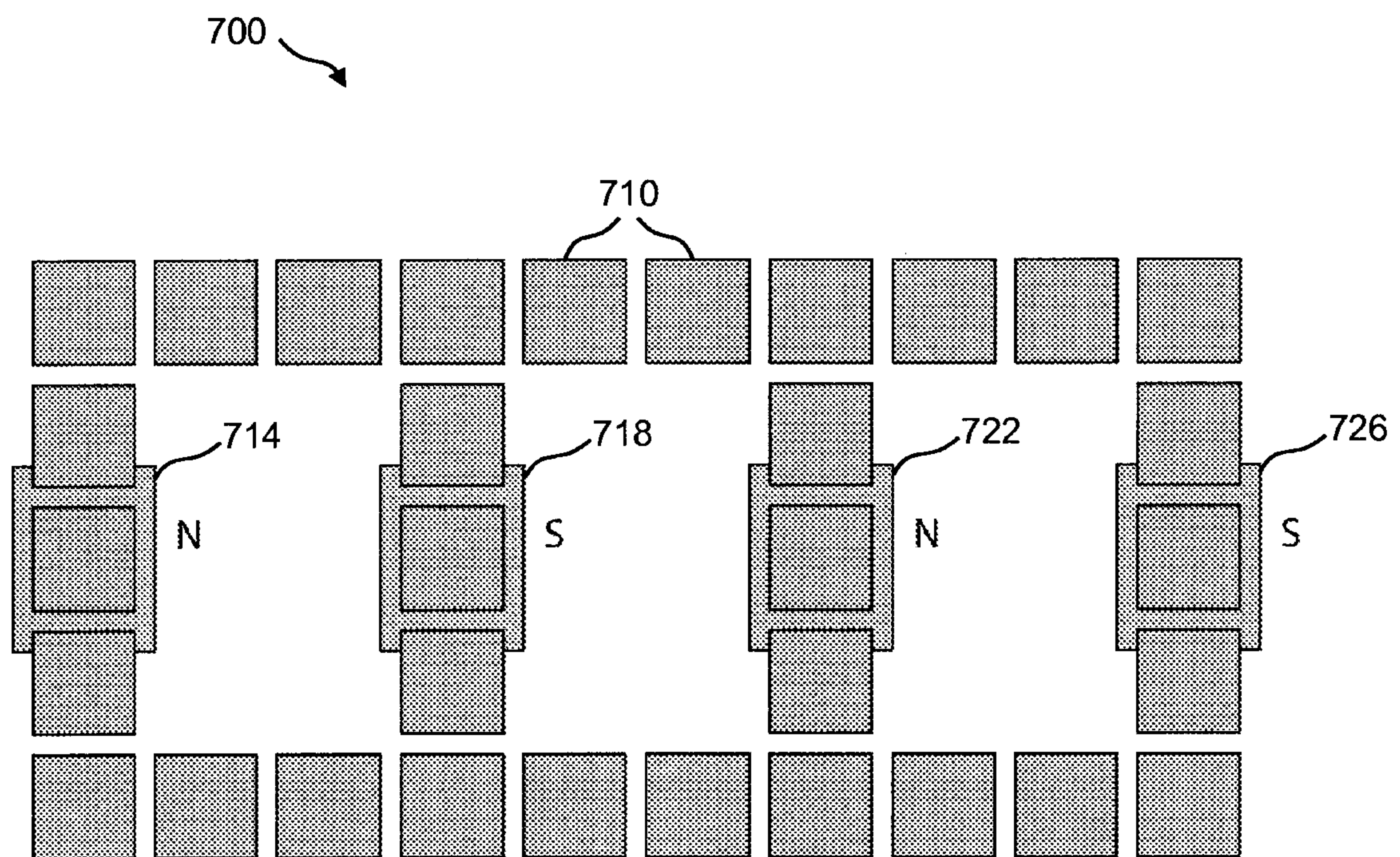


Figure 5B

**Figure 6**

**Figure 7**

800

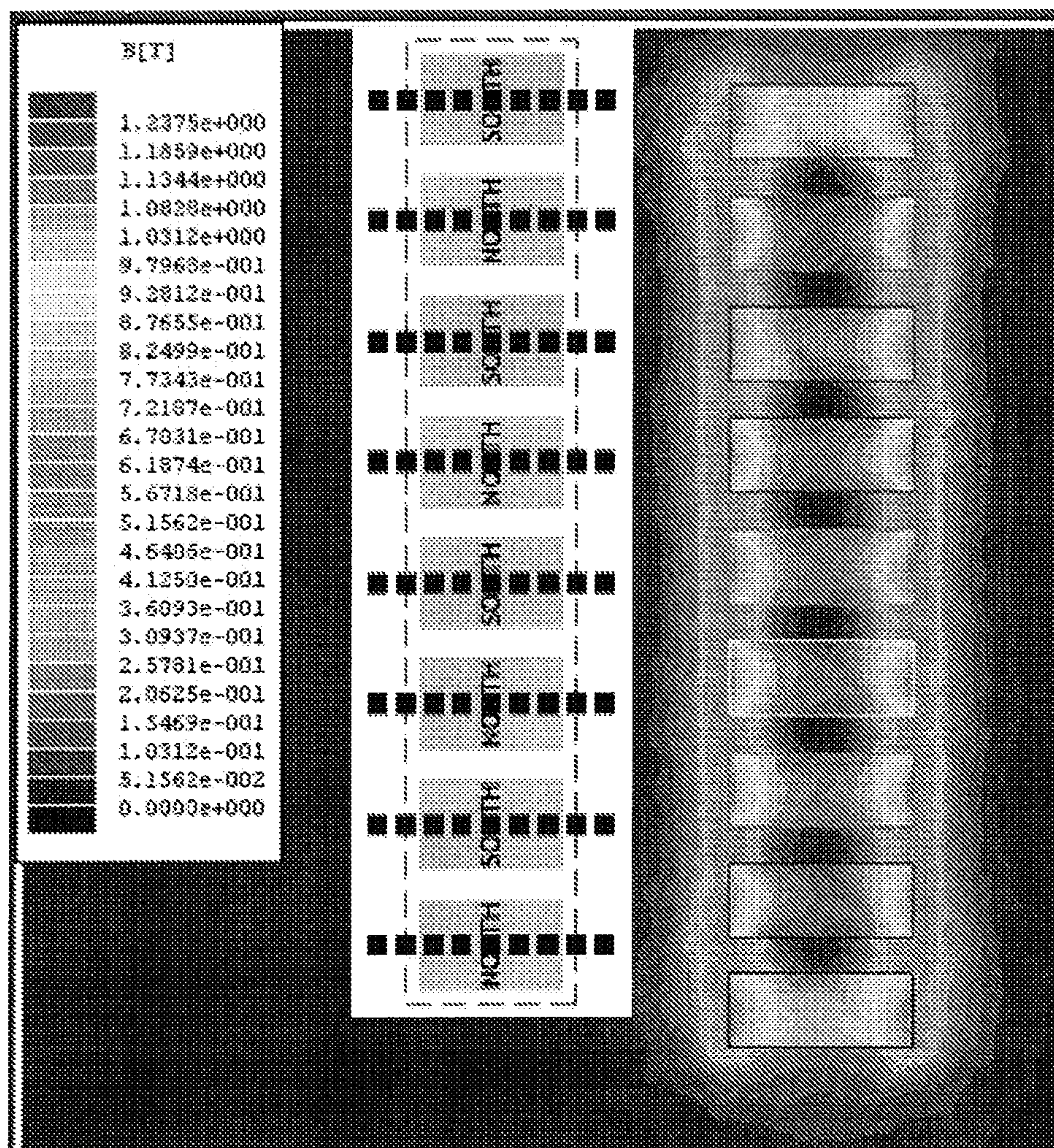
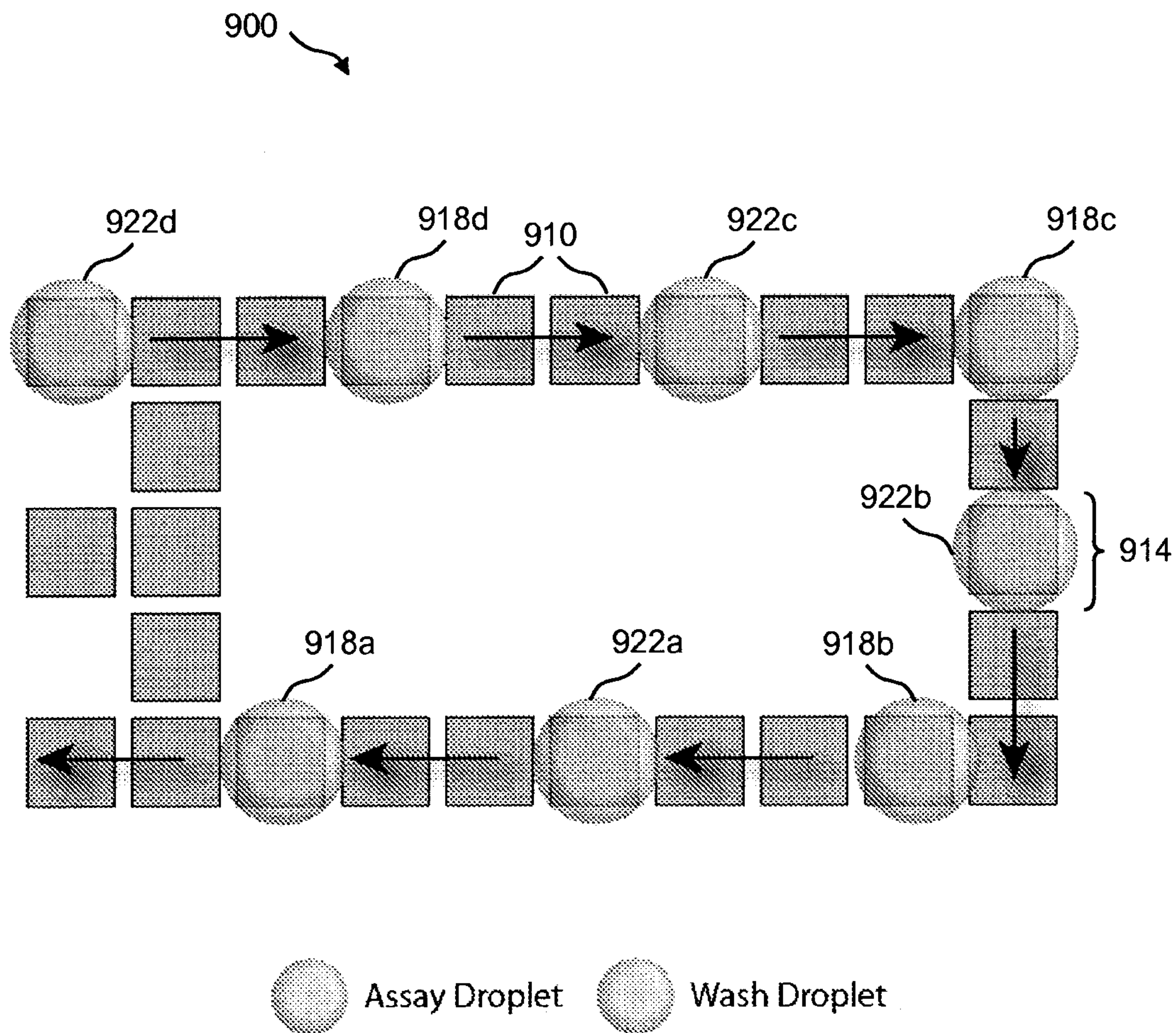


Figure 8

**Figure 9**

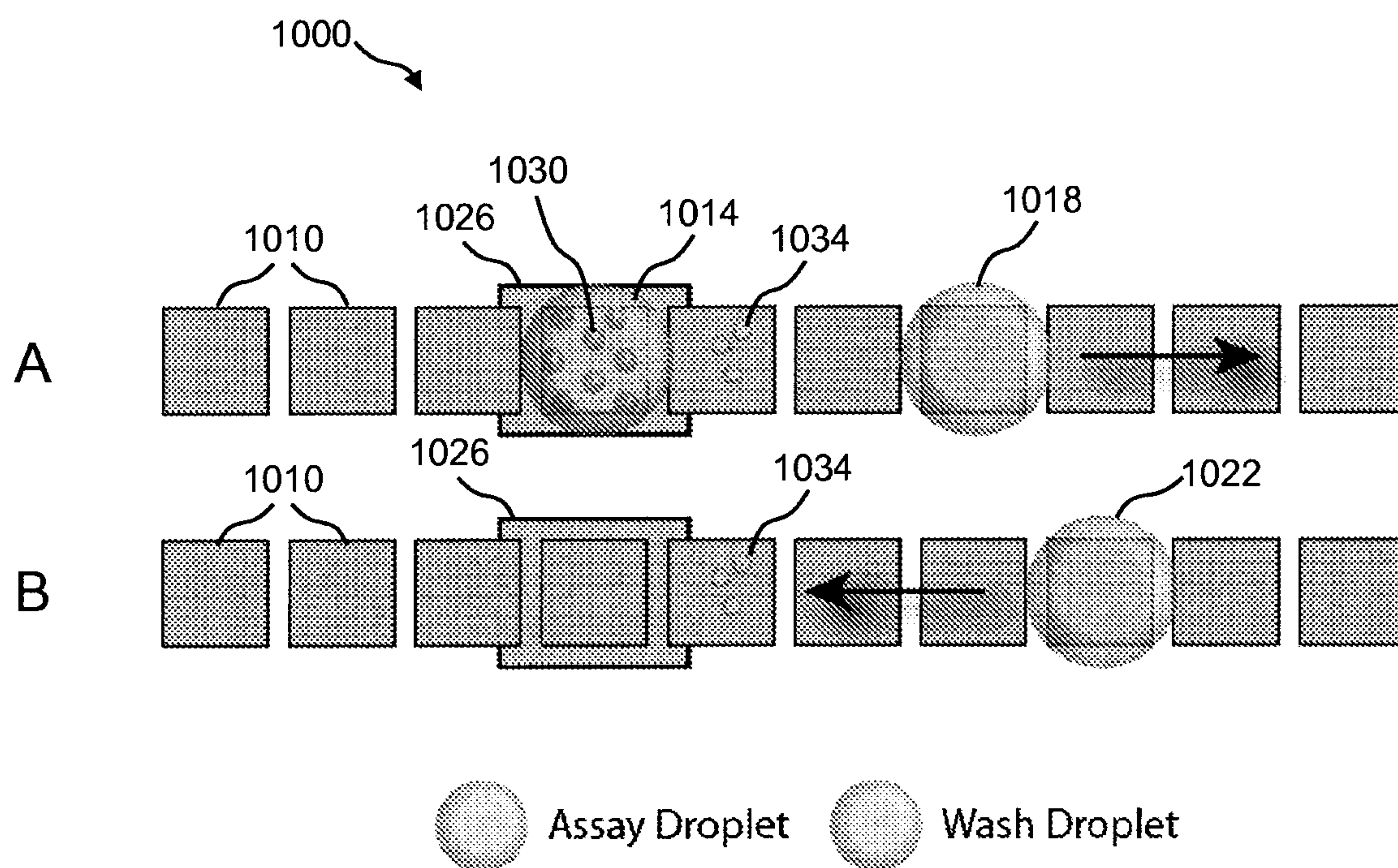


Figure 10

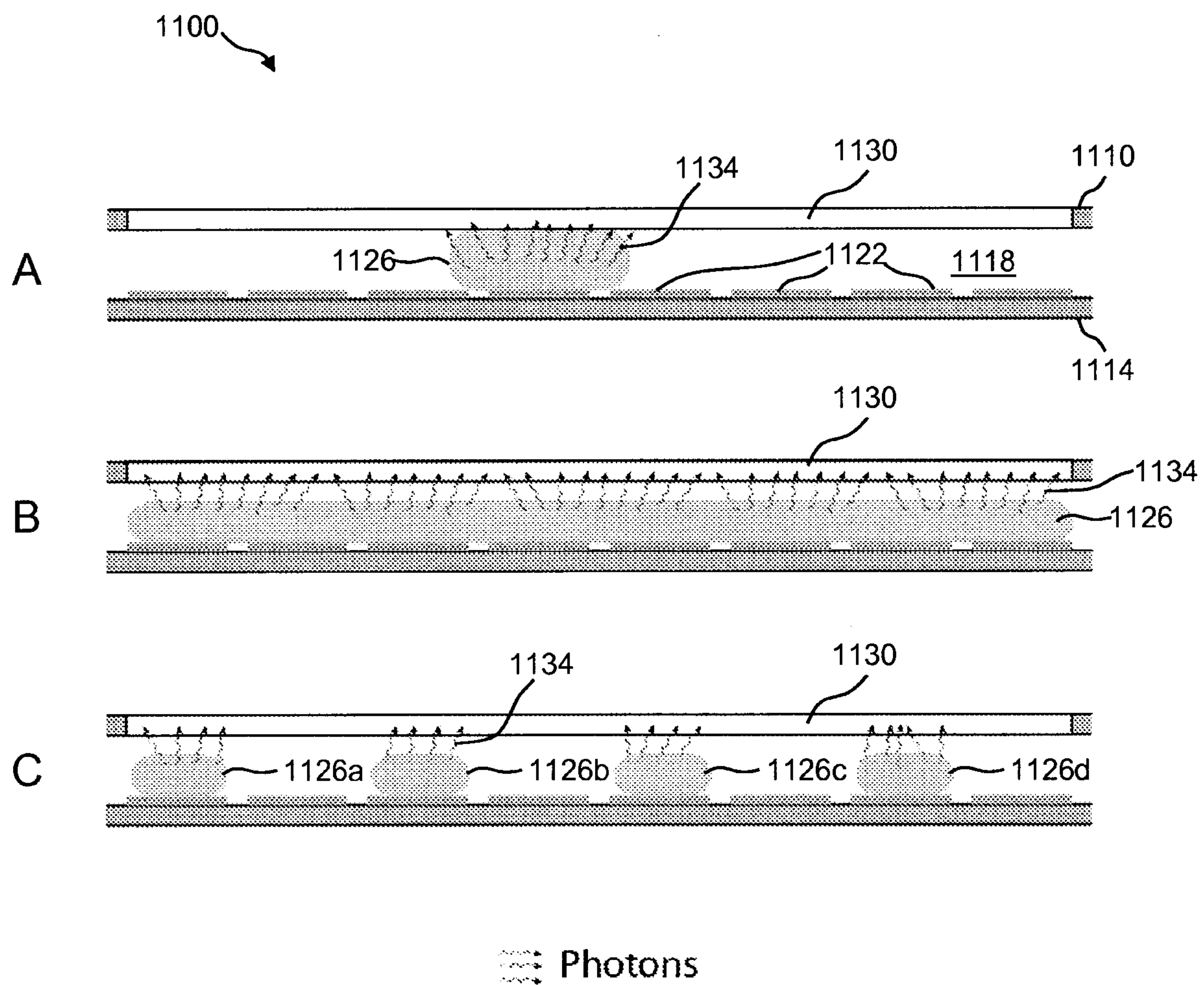


Figure 11

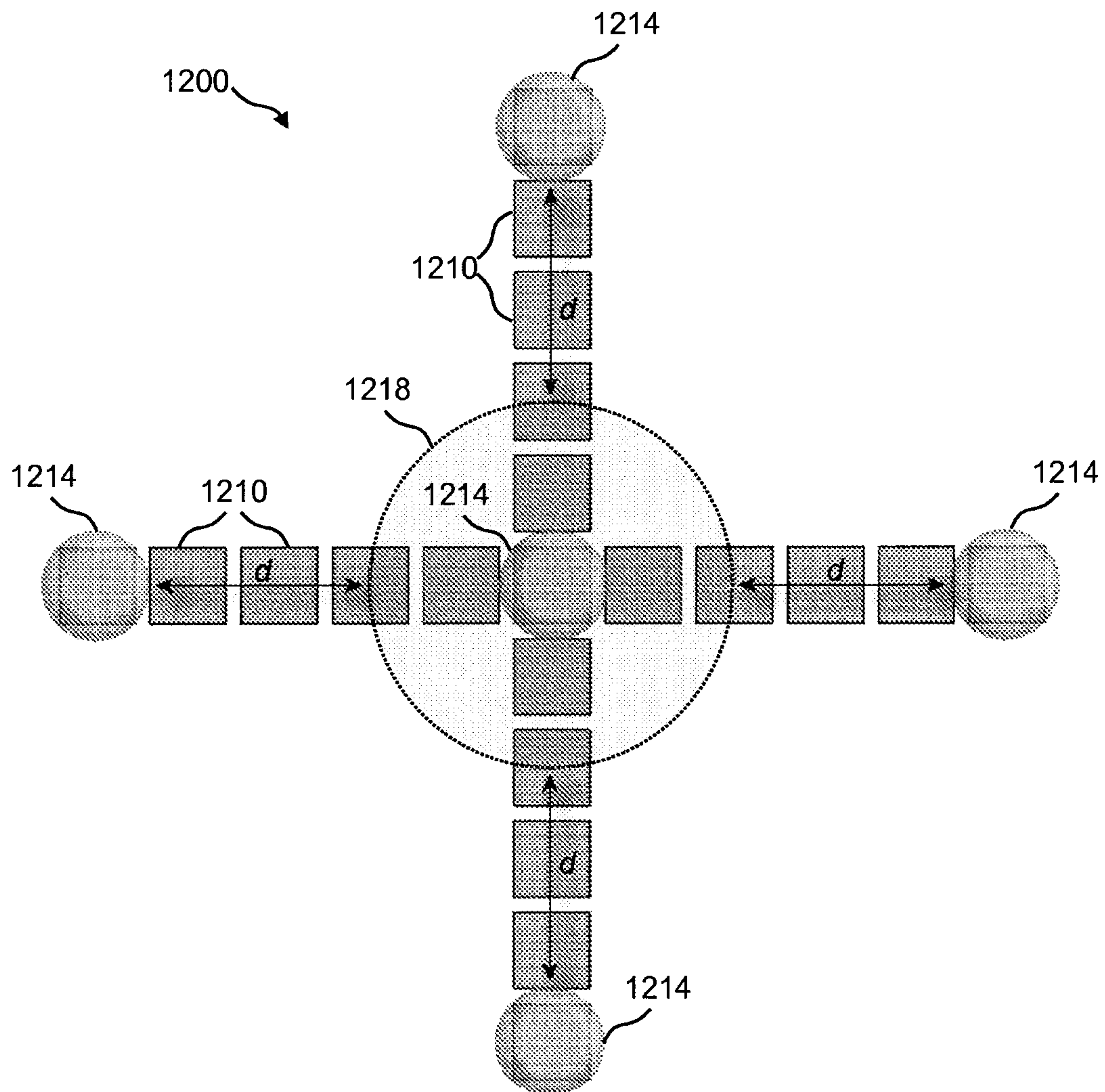
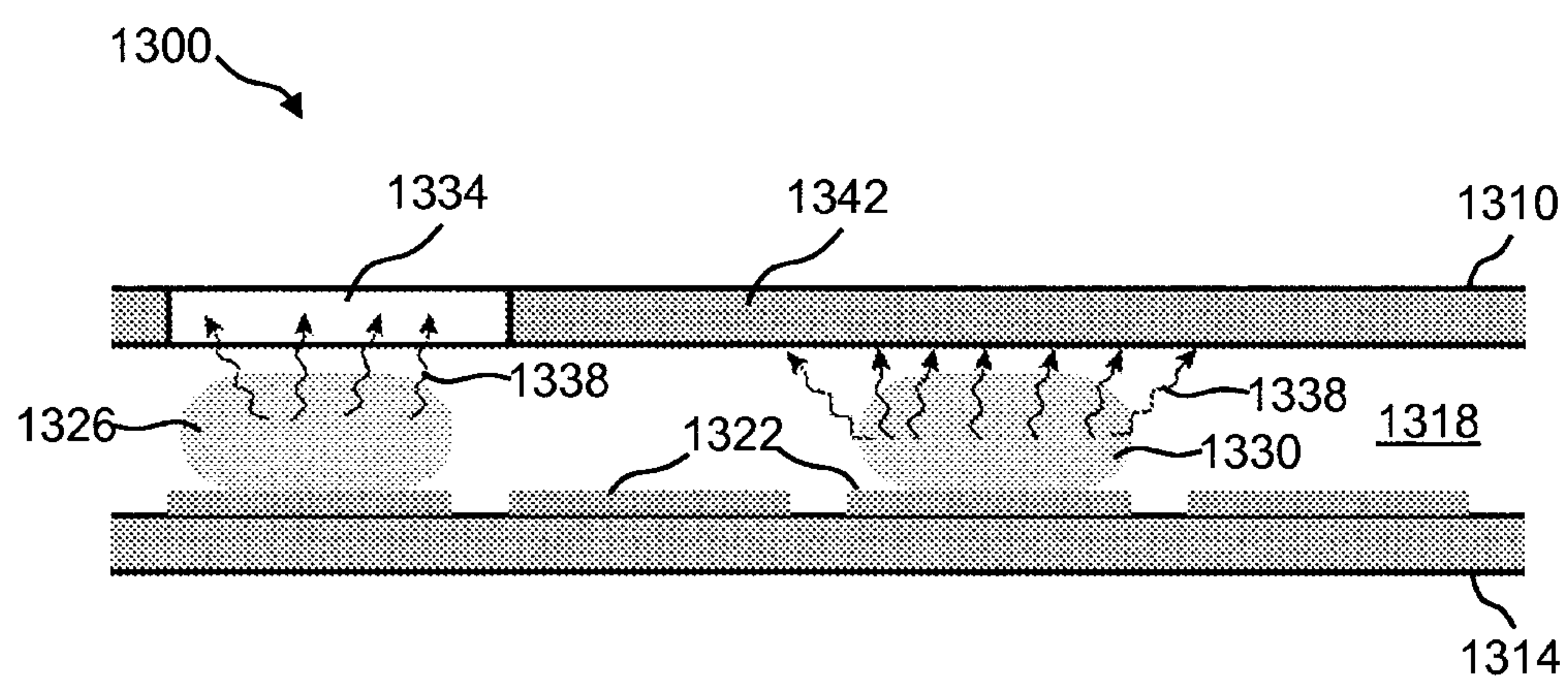
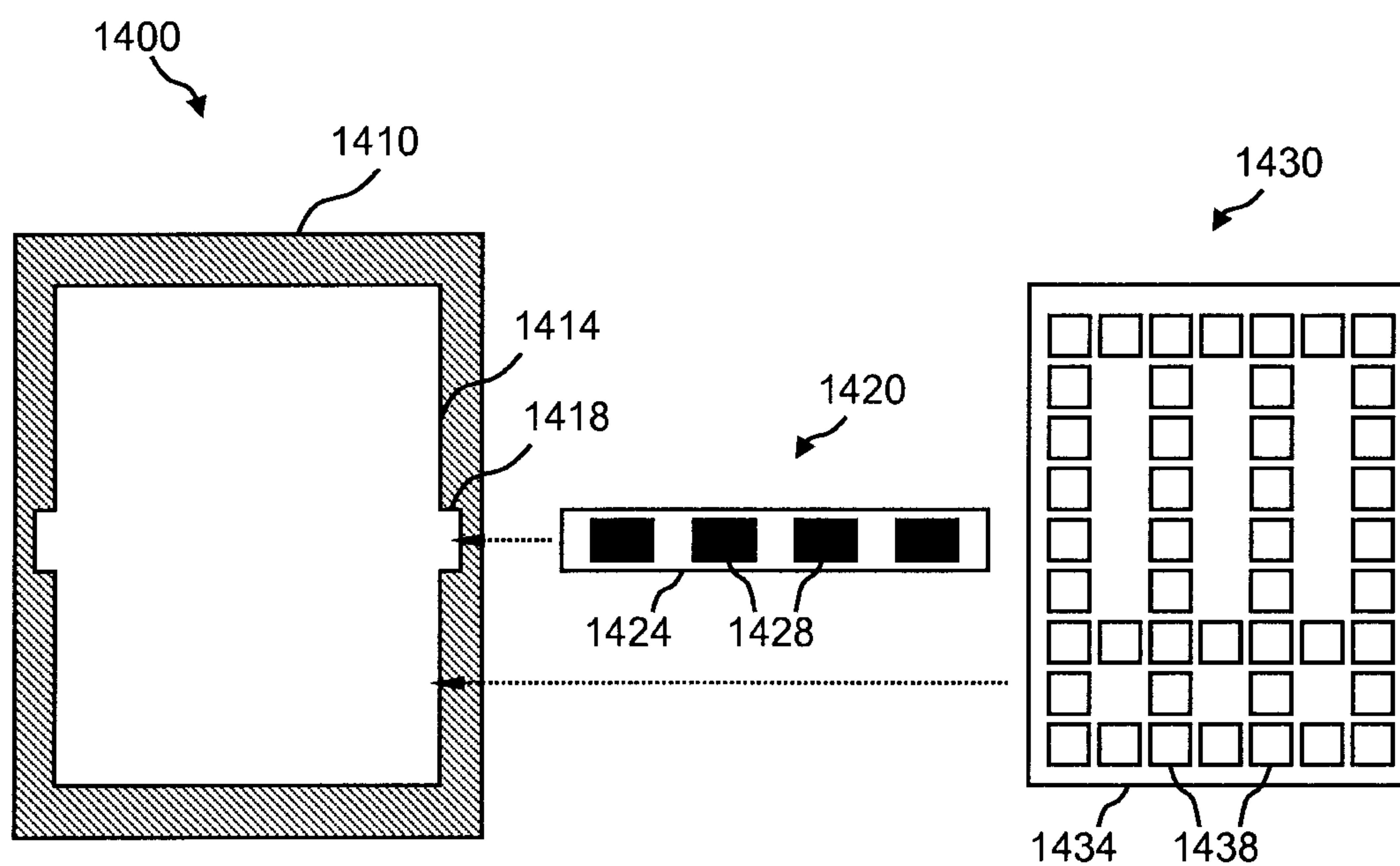
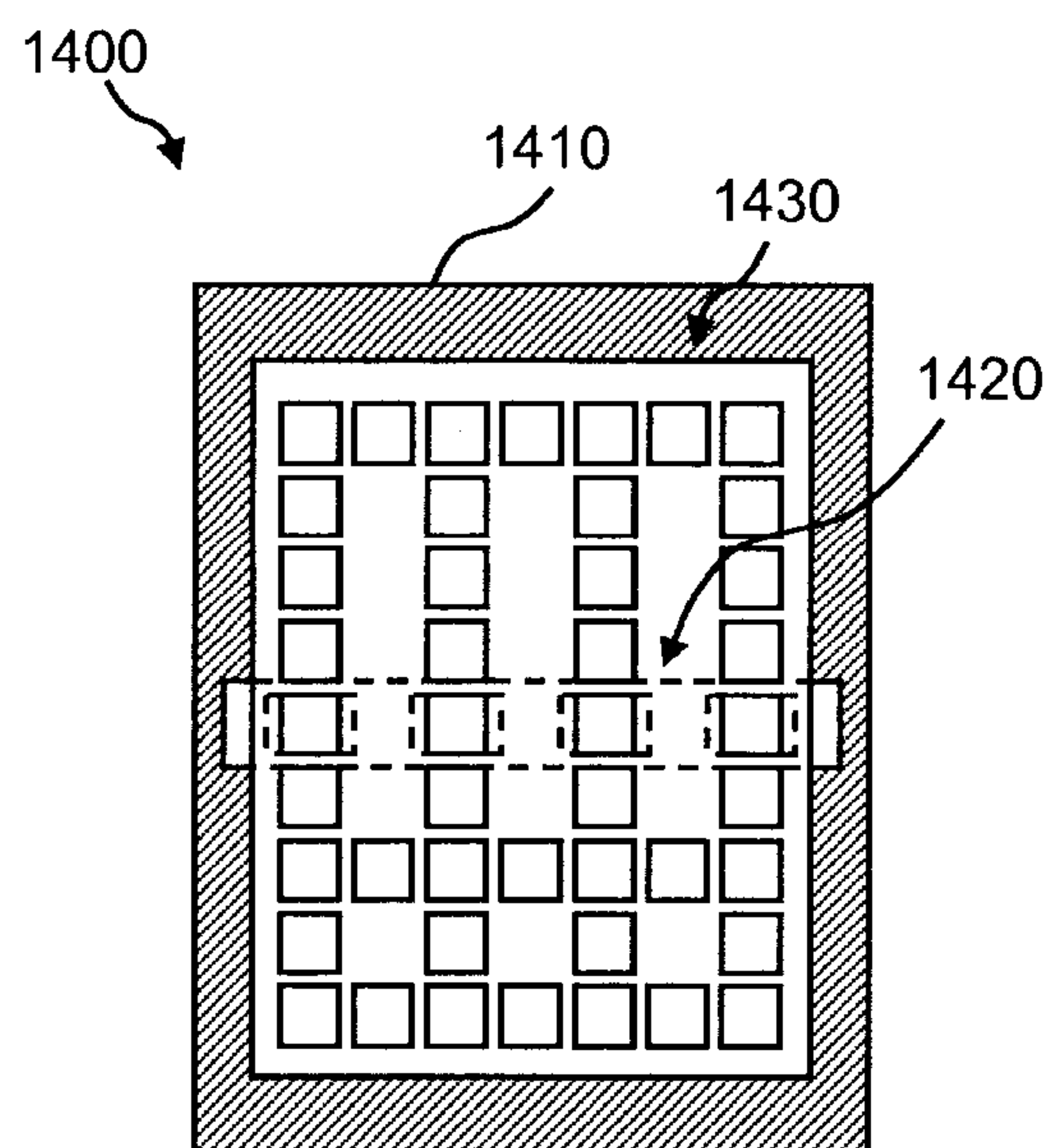


Figure 12



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~~~~~ Photons  
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Figure 13

**Figure 14A****Figure 14B**

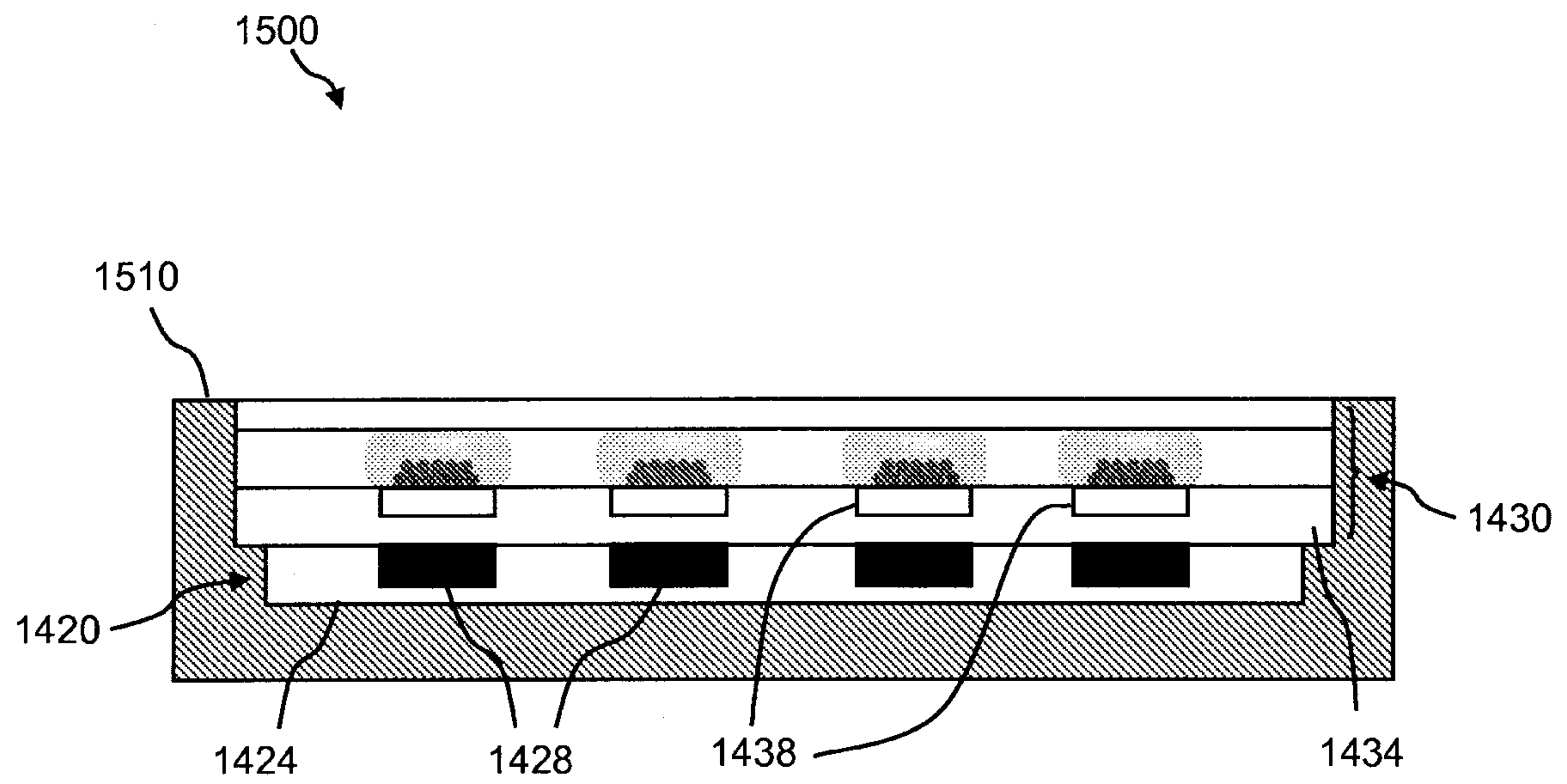


Figure 15

