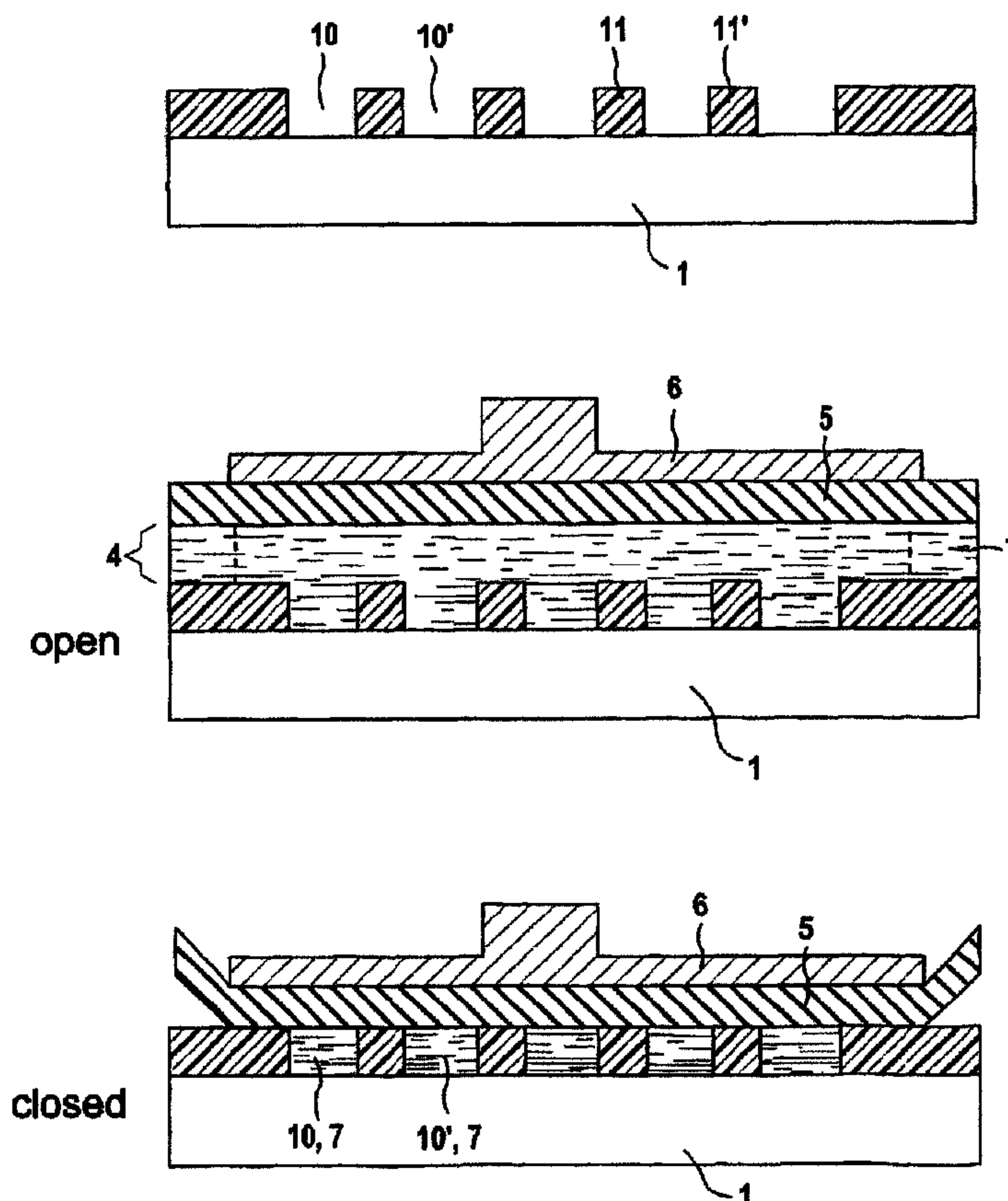




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(54) Titre : METHODE DE PREVENTION DU PHENOMENE D'INTERFERENCE CHIMIQUE DANS DES REACTIONS ENZYMATIQUES ET DISPOSITIF CONNEXE
(54) Title: METHOD FOR PREVENTING CHEMICAL CROSSTALK IN ENZYME-LINKED REACTIONS, AND ASSOCIATED ARRANGEMENT



(57) Abrégé/Abstract:

The invention relates to a method for biochemical analysis using a micro-reaction array with at least two reaction chambers for materials which react together chemically or biochemically. According to the invention, the reaction chambers are smaller than 1 µl,



(57) **Abrégé(suite)/Abstract(continued):**

said reaction chambers are filled together by throughflow, the chemical or biochemical reactions of the substances retained therein then occurs in the individual isolated reaction chambers, thus preventing an interference between the reactions in the individual reaction chambers and the reaction products remain enclosed in the relevant reaction chambers. According to the invention, in said arrangement the planar array has at least two reaction chambers for substances, whereby means are provided for closing the reaction chambers with the goal of preventing an exchange of substances.

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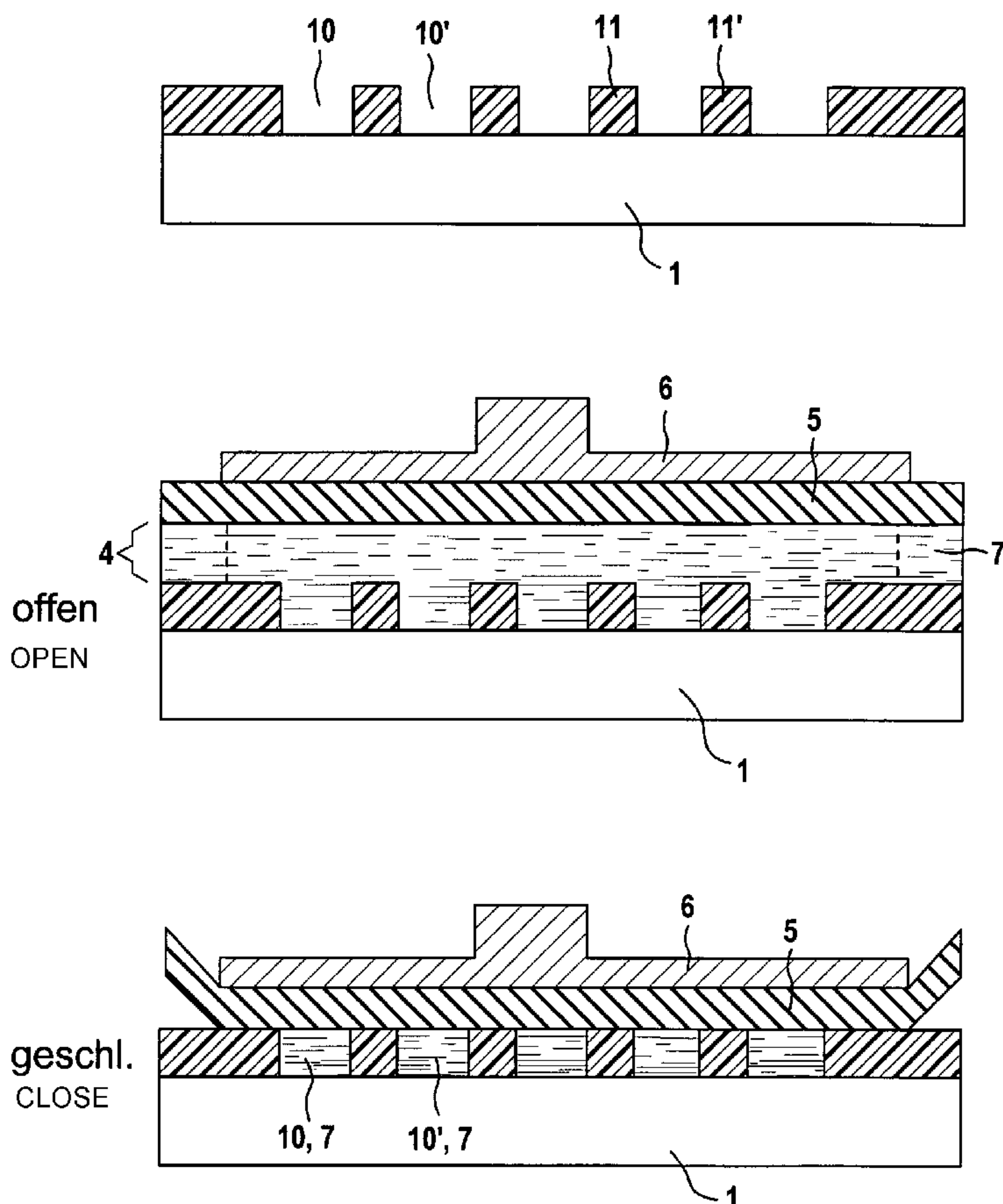
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(54) Title: METHOD FOR BIOCHEMICAL ANALYSIS AND CORRESPONDING ARRANGEMENT

(54) Bezeichnung: VERFAHREN FÜR DIE BIOCHEMISCHE ANALYTIK UND ZUGEHÖRIGE ANORDNUNG



(57) Abstract: The invention relates to a method for biochemical analysis using a micro-reaction array with at least two reaction chambers for materials which react together chemically or biochemically. According to the invention, the reaction chambers are smaller than 1 μ l, said reaction chambers are filled together by throughflow, the chemical or biochemical reactions of the substances retained therein then occurs in the individual isolated reaction chambers, thus preventing an interference between the reactions in the individual reaction chambers and the reaction products remain enclosed in the relevant reaction chambers. According to the invention, in said arrangement the planar array has at least two reaction chambers for substances, whereby means are provided for closing the reaction chambers with the goal of preventing an exchange of substances.

(57) Zusammenfassung: Bei einem Verfahren für die biochemische Analytik wird ein Mikroreaktionsarray mit mindestens zwei Reaktionsräumen zur Aufnahme von Stoffen, die miteinander chemisch bzw. biochemisch reagieren, verwendet. Gemäß der Erfindung sind die Reaktionsräume kleiner als 1 μ m, werden die Reaktionsräume gemeinsam im Durchfluss befüllt, erfolgen anschließend in den einzelnen voneinander getrennten Reaktionsräumen die chemischen bzw. biochemischen Reaktionen der dort eingeschlossenen Substanzen, wobei ein Übersprechen von Reaktionen zwischen

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- *hinsichtlich der Berechtigung des Anmelders, ein Patent zu beantragen und zu erhalten (Regel 4.17 Ziffer ii) für die folgenden Bestimmungsstaaten CA, JP, europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR)*
- *Erfindererklärung (Regel 4.17 Ziffer iv) nur für US*

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Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

den einzelnen Reaktionsräumen ausgeschlossen ist, und bleiben die Reaktionsprodukte in den jeweiligen Reaktionskammern eingeschlossen. Bei der zugehörigen Anordnung hat das planare Array wenigstens zwei Reaktionsräume zur Aufnahme von Stoffen, wobei Mittel zum Schließen der Reaktionsräume zum Zwecke des Verhindern eines Stoffaustausches vorhanden sind.

Description

Method for preventing chemical crosstalk in enzyme-linked reactions, and associated arrangement

5

The invention relates to a method for preventing chemical crosstalk in enzyme-linked reactions using a microreaction array having at least two reaction chambers for receiving substances which react
10 chemically or biochemically with other substances. In addition, the invention also relates to an arrangement for carrying out the method.

Combinatorial analysis and synthesis are nowadays in
15 increasingly widespread use for the development of new active ingredients in the life sciences industry (pharmaceuticals), food technology, agro technology (crop science), in medical diagnostics and also to solve a very wide range of objectives in general
20 biotechnology. To carry out these methods, what are known as microtitration plate techniques with reaction wells in an array structure are used, employing either 96 or even 384 wells for simultaneous reaction on an array surface of, for example, approx. 12 x 8 cm². The
25 density of these arrays will increase further in future, which means that different types of chemical reactions have to take place in reaction chambers arranged ever closer together.

30 US 6,143,496 A has disclosed a PCR (Polymerase Chain Reaction) method, in which, in an array having a multiplicity of reaction chambers, individual reactions take place next to one another at elevated temperatures. In this array, there are suitable means
35 for isolating specimen chambers, which can also be achieved, for example, by means of a displacement liquid. In particular, it is important to reduce the specimen volume or to prevent evaporation of water. The same problem in connection with a PCR method is

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discussed in WO 01/34842 A2, which claims an earlier priority but was not published before the priority date of the present application.

- 5 The situation is in principle different with what are known as DNA chips, as are known from various publications. The situation is taken to extremes, for example, with an array of different DNA probe molecules which are arranged at a spacing of only a few tens of
10 micrometers and with a density of, for example, a few hundred positions per few mm² on a planar substrate, known as the DNA chip. If molecules which can move freely are involved in the analytical detection of, for example, unknown DNA, chemical crosstalk occurs with
15 such dense arrays.

For a number of reasons, for example on account of the high specificity and the low detection limit,

biochemical analysis often uses enzyme-linked detection methods. By way of example, what are known as ELISA (Enzyme-Linked ImmunoSorbent Assay) tests are in widespread use in medical diagnostics and in the research sector. (Literature reference c.f. B. Alberts et al. (eds.), Molekularbiologie der Zelle [Molecular biology of the Cell] (1997), 3rd edition, page 216, VCH Weinheim). Methods using enzyme markers in a known redox (re)cycling are also employed for applications in the field of the DNA chip (A.v.d.Berg, P. Bergveld (eds.) Proceedings of the μ TAS '94 Workshop (1994), pp. 249 to 254, Kluwer Academic Publishers Dordrecht).

In all cases mentioned in the specialist literature, the enzyme is not free in the liquid phase of the arrangement, which is also known as an assay, but rather is bonded and therefore, as an "enzyme label" marks the primary substance to be detected. In this case, the bonding of the enzyme molecules to the substance to be detected is always stoichiometric. Amplification occurs in that the enzyme converts added substrate molecules at high speed. This conversion is quantified, for example, optically or electrochemically, depending on the substrate used and/or the product formed. For this purpose, irrespective of the method used, in particular the increase in concentration of the product P, i.e. the time-dependent function $dc(P)/dt$, is monitored.

If assays of this type are carried out in an array, as described in detail in the prior art, reaction products which can move freely and are formed by the enzyme can also reach adjacent enzyme-free array positions, where they may simulate the presence of the enzyme label. This phenomenon is known as crosstalk, which leads to measurement errors and may therefore give false results.

Working on the basis of the above, it is an object of

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the invention to provide methods and associated arrangements which, compared to the prior art, ensure increased reliability by avoiding

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crosstalk and thereby ruling out "false positive" results. An increased accuracy is intended to produce improvements in particular in the effectiveness of the measurements.

In one aspect, the invention relates to a method
5 for preventing chemical crosstalk in enzyme-linked detection reactions, using a microreaction array with at least two reaction chambers for receiving substances which chemically or biochemically react with one another, comprising the following measures: locally delimited reaction chambers are
10 used as a first volume, the reaction chambers are connected to one another via a second volume, known as the supply volume, enzyme-linked reactions take place in the individual reaction chambers differentiated by species and/or quantitatively, the mass transfer between reaction chambers
15 and the supply volume is permitted or prevented in one or both directions as required, wherein the mass transfer is prevented by a housing top part that closes off the reaction chambers by means of a mechanical ram.

In another aspect, the invention relates to the
20 arrangement as described above, wherein the reaction chambers have a volume of less than 1 μ l. Refinements of the arrangement are given in the dependent device claims.

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In the method according to the invention, locally delimited reaction chambers are used as a first volume, and the reaction chambers can be connected to one another via a second volume, known as the supply
5 volume, and in the individual reaction chambers chemical or biochemical reactions take place differentiated by species and/or quantitatively. Reactions differentiated by species are understood as meaning qualitatively different processes. In this
10 case, equally, mass transfer between reaction chambers and the supply volume is permitted or prevented in one or both directions as required.

A significant advantage of the invention is that,
15 despite the fact that the reaction chambers are closely adjacent, disruptive crosstalk, which may distort the measurement results, is rendered impossible, and the selectivity is thereby improved. Moreover, the invention also increases the detection sensitivity,
20 i.e. the detection limit is shifted toward smaller quantities.

For practical realization of the detection sensitivity increase, it is appropriate for the change in
25 substrate/product concentration over the course of time to be increased as far as possible. In the method according to the invention, this is achieved by a targeted reduction in the reaction volume to significantly less than 1 μm ,

in particular to the region of 1 nanoliter (1 nl), and an associated increase in the substrate product concentration changes.

5 The arrangements according to the invention are in each case arrays of more than two positions, and typically a few hundred positions, on a few square millimeters, preferably 1 to approx. 10 mm², arranged on a planar substrate. The array is in each case designed as an
10 array of reaction chambers or reaction spaces and advantageously forms part of a vessel with a supply volume which is jointly accessible to the reaction chambers. A supply volume of this type can be produced, for example, by embedding the reaction chamber array in
15 a flow cell, via which the overall fluid handling of the chemical/biochemical substances required for the detection or synthesis reaction can be performed.

In a first preferred embodiment of the invention, by
20 pressing a mechanical device onto the substrate, the reaction chambers formed by the individual array positions can be separated from one another by means of an elastic membrane or layer which lies opposite the planar substrate in the flow cell and may consist, for
25 example, of silicone rubber, so that crosstalk is effectively prevented. A device of this type may, for example, be in the form of a cover, a ram or a sealing membrane, by means of which the cavities formed by the reaction chambers are closed off. Closing off the
30 cavities also causes the volume of the liquid spaces above the individual array positions to be reduced, so that the change in concentration of the substrate/product which is initiated by the chemical/biochemical reactions is increased.
35 Consequently, therefore, the detection sensitivity is also advantageously increased.

In another preferred embodiment of the invention, the same effect can be achieved by positioning a layer of barrier liquid on top. As soon as a suitable barrier liquid which cannot mix with the liquid in the reaction
5 cavities fills the flow channel, the same effects are achieved as with the array cavities being closed up by means of a silicone ram. The barrier liquid is, for example, silicone oil. In an advantageous variant of this embodiment, the reaction chambers are filled with
10 hydrogel, in order in this way to impart mechanical stability to the water-containing reaction chambers when the barrier liquid enters the flow channel. The hydrogel used may, for example, be polyacrylamide, which has the required properties with respect to
15 silicone oil.

In a refinement of the claimed method which is independently inventive, it is also possible to make use of different chemical solubility characteristics of
20 the substances and materials involved. In this embodiment of the arrangement according to the invention too, the reaction chambers are advantageously filled with a hydrogel. Different solubility characteristics between hydrogel reaction chamber and a
25 suitable liquid phase in the flow channel of the supply volume ensures that reaction starting materials from the liquid phase enter the hydrogel phase but reaction products can no longer leave the hydrogel phase. An example of a reaction starting product of this type is
30 the enzyme substrate.

Further details and advantages of the invention will emerge from the following description of figures illustrating exemplary embodiments with reference to
35 the drawing in conjunction with the patent claims. In the figures:

Figure 1 diagrammatically depicts a measurement structure according to the invention,

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illustrating the measuring method, on the one hand, and the disruptive crosstalk, on the other hand,

Figure 2 diagrammatically depicts, in three substeps, an example of an arrangement for mechanically closing the cavities,

5 Figure 3 diagrammatically depicts, in the form of three substeps, a corresponding arrangement for closing the cavities by means of barrier media, and

10 Figure 4 diagrammatically depicts, in the form of three substeps, a third arrangement in which the cavities are closed off by utilizing different solubility characteristics of the media involved.

15

In the figures, parts which are identical or have a similar action are denoted by identical or corresponding reference numerals. The figures are in part described jointly in the text which follows.

20

In Figure 1, 1 denotes a substrate with a planar surface which is formed, for example, by the crystallographic surface of a silicon chip. An array of optical/electrical detectors 2, 2', ... is produced on the substrate 1 at array positions 8, 8', ... and can be used to carry out bioanalytical tests using enzyme-linked reactions, for which purpose probe molecules, on the one hand, and analyte molecules, on the other hand, are used. On the array position 8, 8', ... there are different probe molecules 110, 120, ..., so that different analyte molecules can be detected on each specific array position.

35 In detail, in Figure 1, for a method for bioanalytical testing, a first probe molecule is denoted by 110 at array position 8 and a second probe molecule is denoted by 120 at array position 8', an analyte molecule is denoted by 200 and an enzyme label is denoted by 300. By way of example, the probe molecule 110 reacts

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specifically with a complementary analyte module 200 and thereby immobilizes an enzyme label 300 in a position-specific manner in the array. An enzyme substrate 400 which is then added as starting material
5 is converted into a product 500 by the catalytic effect of the enzyme label 300.

In Figure 1, therefore, the analyte molecule 200 can react only with the probe molecule 110 but not with the probe molecule 120. The increase/decrease in substrate/product can be measured at each array position 8, 8', ... of the wafer 1 with the aid of the optical or electrical detector 2, 2', ... located there. In particular electrical detectors have metrological advantages.

- 10 In accordance with the prior art, it is endeavored to keep the array positions 8, 8', ... and the distances between them as small as possible. A problem in the prior art is that what is known as chemical crosstalk may occur between the individual positions 8, 8', ...
- 15 This means that either enzyme substrate 400, which has been defined above as the starting material, or the reaction product 500 may move from a first array position 8 to a second array position 8'. If a neighboring position is reached, a false signal is
- 20 generated, simulating a positive result. In practice, this is also referred to as a "false positive" signal.

In Figures 2 to 4, for different alternatives individual reaction chambers 10, 10', ... with an individual volume of in each case less than 1 μ l are arranged in an array configuration. The reaction chambers 10, 10', ... are operationally separated from one another.

- 30 Figure 2 illustrates three substeps describing the actuation of an arrangement in which the reaction chambers 10, 10', ... are separated by walls 11, 11', ... The walls 11, 11' can be produced in a particular geometric embodiment by means of photopatterned,
- 35 circular polymer rings with an internal diameter of, for example, 150 μ m, an external diameter of, for example, 180 μ m and a height of, for example, 50 μ m. The reaction chambers 10, 10', ... are filled, for

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example, with reaction starting material, e.g. an enzyme substrate, dissolved in an electrolyte 7,

the electrolyte 2 being supplied to the individual reaction chambers via a supply volume 4.

In Figure 2, the reaction chambers 10, 10', ... can be closed off by a housing top part 5 by means of a mechanical ram 6. In the open state, a supply volume 4 holding a liquid electrolyte is located above the cavities. In Figure 2, the reaction spaces 10, 10', ..., as chambers which are open when the housing top part 5 is removed, are filled with an electrolyte/starting material 7 flowing through them, the reservoir for the electrolyte 7 not being shown in detail in this figure. After the reaction cavities 10, 10', ... have been filled with electrolyte/starting material 7, the housing top part 5, which may comprise, for example, a silicone membrane, is placed onto the walls 11, 11', ..., which, as mentioned above, consist of polyimide, by means of the ram 6. In this way, the reaction spaces 10, 10', ... are closed off, so that mass transfer is then prevented.

In Figure 3, the lower region is of similar construction to that shown in Figure 2. In a particular embodiment, which is not visible in the drawing presented in Figure 3, the walls 11, 11', ... may be specially produced by photopatterned, circular polymer rings with an internal diameter d ($d=2r$) of, for example, $d=150\text{ }\mu\text{m}$, an external diameter of, for example, $D=180\text{ }\mu\text{m}$, a height h of, for example, $h=5\text{ }\mu\text{m}$. The reaction cavities which result from dimensions of this type, with a filling volume of approximately 0.1 nl ($r^2\pi h = (75\text{ }\mu\text{m})^2 \cdot 3.14 \cdot 5\text{ }\mu\text{m}$) are in this particular embodiment filled with a hydrogel 3 with a high capacity to take up water, e.g. polyacrylamide. Then, a probe DNA for specific DNA detection can be introduced in immobilized form into the hydrogel 3.

To carry out the assay, the reaction chambers 10, 10', ... are once again supplied with buffer, reagents and

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ultimately enzyme substrate via the common supply
volume 4. After the hydrogel 3 of each reaction chamber
10,

10' has been brought into equilibrium with buffer containing enzyme substrate and the enzymatic conversion has commenced, the supply volume 4 is flooded with a barrier liquid, e.g. silicone oil. The result of this is that the liquid above the reaction chambers is displaced by silicone oil. The hydrogel structure is responsible for the mechanical stability of the reaction chambers. Since enzyme product is insoluble in silicone oil, it is prevented from diffusing out of the hydrogel toward neighboring reaction chambers. Therefore, the reaction product can increase greatly in the reaction chambers without reaching the neighboring reaction chambers. Therefore, high sensitivity and high selectivity are equally present.

In both exemplary embodiments as shown in Figures 2 and 3, it is significant that the individual reaction cavities 10, 10', ... are first of all filled with the electrolyte 7 passing through them from the supply volume 4 and then a material, for example a silicone oil 9, which forms phase boundaries with the electrolyte 7, is applied. The phase boundary ensures that mass transfer is then no longer possible and disruptive distortions are prevented.

In the specific variant of the embodiment shown in Figure 3, the reaction chambers 10, 10', ... are filled with hydrogel 3, e.g. polyacrylamide, in order, in this way to impart mechanical stability to the water-containing reaction chambers 10, 10', ... when the barrier liquid 9, e.g. silicone oil, enters the flow channel.

In terms of its structure, Figure 4 once again substantially corresponds to Figure 2. In a corresponding way to Figure 2 and Figure 3, the reaction chambers 10, 10' are filled from the supply volume 4 by liquid passing through. In this case,

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however, the reaction starting materials, which are denoted here by E, have the ability, on account of their specific solubility characteristics, to penetrate into the

electrolyte 7 located in the reaction chambers 10, 10',
... after they have been filled.

In the arrangement as shown in Figure 4, the reaction
5 in the reaction chambers then takes place in the same
way as has already been described above. On account of
the specific solubility characteristics of the reaction
product which forms and which is denoted here by P,
however, mass discharge of P is not possible in the
10 reaction. Therefore, the disruptive crosstalk is once
again prevented. In this embodiment, too, in a
corresponding way to Figure 3, the reaction chambers
are advantageously filled with a hydrogel 3.

15 The process described and the associated arrangements
can be used particularly successfully in medical
diagnostics and biotechnology. The prevention of
crosstalk as a significant source of errors which is
now achieved makes it possible to obtain more accurate
20 results than has hitherto been possible.

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CLAIMS:

1. A method for preventing chemical crosstalk in enzyme-linked detection reactions, using a microreaction array with at least two reaction chambers for receiving substances which chemically or biochemically react with one another, comprising the following measures:
- locally delimited reaction chambers are used as a first volume,
 - the reaction chambers are connected to one another via a second volume, known as the supply volume,
 - enzyme-linked reactions take place in the individual reaction chambers differentiated by species and/or quantitatively,
 - the mass transfer between reaction chambers and the supply volume is permitted or prevented in one or both directions as required,
 - wherein the mass transfer is prevented by a housing top part that closes off the reaction chambers by means of a mechanical ram.
2. The method as claimed in claim 1, wherein at a first time (t_1), a defined mass transfer takes place between the reaction chambers and the supply volume, and, at a second time (t_2), mass transfer between the reaction chambers is as far as possible suppressed or prevented.
3. The method as claimed in claim 1, wherein mass transfer in one direction is selectively prevented by means of different solubility characteristics of chemical substances in different liquids in the reaction chambers and the supply volume.

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4. The method as claimed in claim 2, wherein the reaction chambers are closed by displacement of the supply volume.

5. The method as claimed in claim 2, wherein for the purpose of temporally defined mass transfer, the phase boundaries between the substances located in the reaction chambers and the supply volume are temporarily made permeable.

6. The method as claimed in claim 4, wherein the closing of the phase boundaries is effected by displacement of the supply volume by means of a barrier medium.

7. The method as claimed in claim 6, wherein the liquid in the supply volume is displaced by a gas, or by an immiscible liquid.

8. The method as claimed in claim 3, wherein the phase boundaries between the substances located in the reaction chambers and the supply volume become impermeable as a result of different dissolution characteristics of the reaction partners.

9. The method as claimed in claim 3, wherein starting materials for a chemical reaction which takes place in a hydrogel layer diffuse out of the supply volume into the hydrogel layer, but at least one product of the chemical reaction does not diffuse out of the hydrogel layer.

10. The method as claimed in claim 1, wherein a combinatorial analysis and/or synthesis of the substances takes place in individual reaction chambers which are separate from one another.

11. The method of any one of claims 1 to 10, characterized by its use in Redox recycling.

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12. The method of any one of claims 1 to 11, characterized by its use in enzyme-linked reactions.

13. An arrangement for preventing crosstalk in enzyme-linked detection reactions in a method as claimed in claim 1 or any one of claims 2 to 12 using a planar array, wherein the planar array (8, 8', ...) has at least two reaction chambers (10, 10', ...) for receiving substances from a supply volume (4), there being means for closing the reaction chambers (10, 10', ...).

10 14. The arrangement as claimed in claim 13, wherein the reaction chambers (10, 10', ...) have a volume of less than 1 µl.

15 15. The arrangement as claimed in claim 13, wherein the planar array (8, 8', ...) is applied to a silicon substrate (1).

16. The arrangement as claimed in claim 13, wherein the reaction chambers (10, 10', ...) are separated from one another by a polymer layer (11) applied to silicon.

20 17. The arrangement as claimed in claim 13, wherein the reaction chambers (10, 10', ...) are introduced into the silicon by microstructuring technology.

25 18. The arrangement as claimed in claim 2, wherein the reaction chambers (10, 10', ...) are liquid-filled cavities which are open for the purpose of mass transfer, so that they are in contact with a supply volume (4) and can therefore be filled simultaneously.

19. The arrangement as claimed in claim 13, wherein the mass transfer between the liquid-filled reaction cavities (10, 10', ...) and the supply volume (4) is

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suppressed by closure, in which case no further medium, can reach the cavities (10, 10', ...).

20. The arrangement as claimed in claim 13, wherein a closure layer is provided for the purpose of closing the
5 reaction chambers (10, 10', ...).

21. The arrangement as claimed in claim 20, wherein the closure is effected by a planar, partially elastic polymer layer (5).

22. The arrangement as claimed in claim 21, wherein
10 the closure of the opening of the reaction chambers (10, 10', ...) is effected by displacement of the supply volume (4).

23. The arrangement as claimed in claim 22, wherein gas, or an immiscible liquid (9) is present for the purpose
15 of displacing the supply volume (4).

24. The arrangement as claimed in any one of claims 13 to 23, wherein the reaction chambers (10, 10', ...) are formed by gel-filled cavities (3) which have a gel/supply volume phase boundary for the purpose of mass transfer.

20 25. The arrangement as claimed in claim 24, wherein mass transfer between the gel-filled reaction chambers (10, 10', ...) can be suppressed by closure of the phase boundary.

26. The method according to claim 7, wherein the gas
25 is air.

27. The method according to claim 7, wherein the immiscible liquid is silicone oil.

28. The arrangement according to claim 19, wherein the medium is air.

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29. The arrangement according to claim 21, wherein the planar, partially elastic polymer layer is silicone rubber.

30. The arrangement according to claim 23, wherein the gas is air.

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PATENT AGENTS

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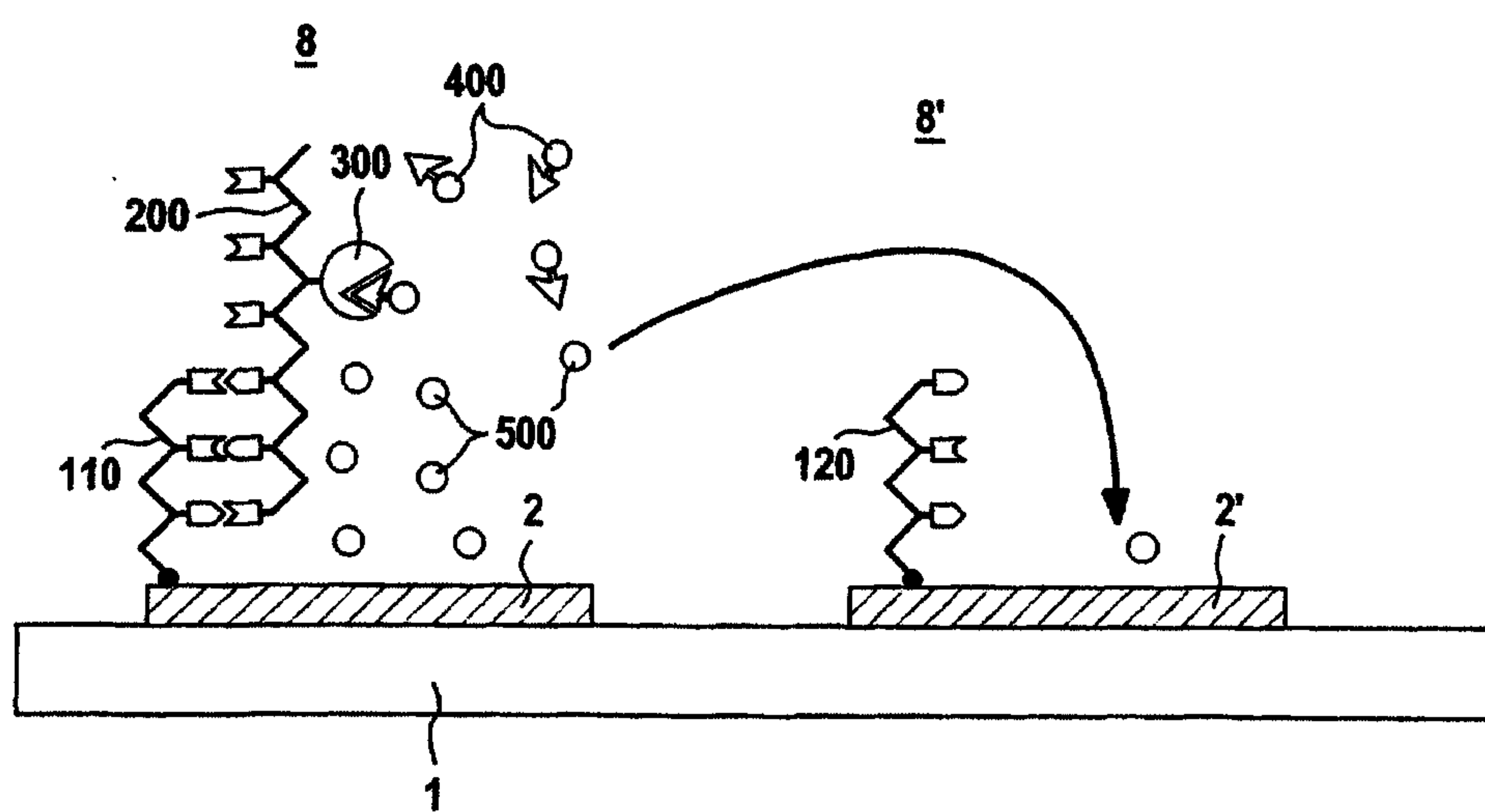


FIG 1

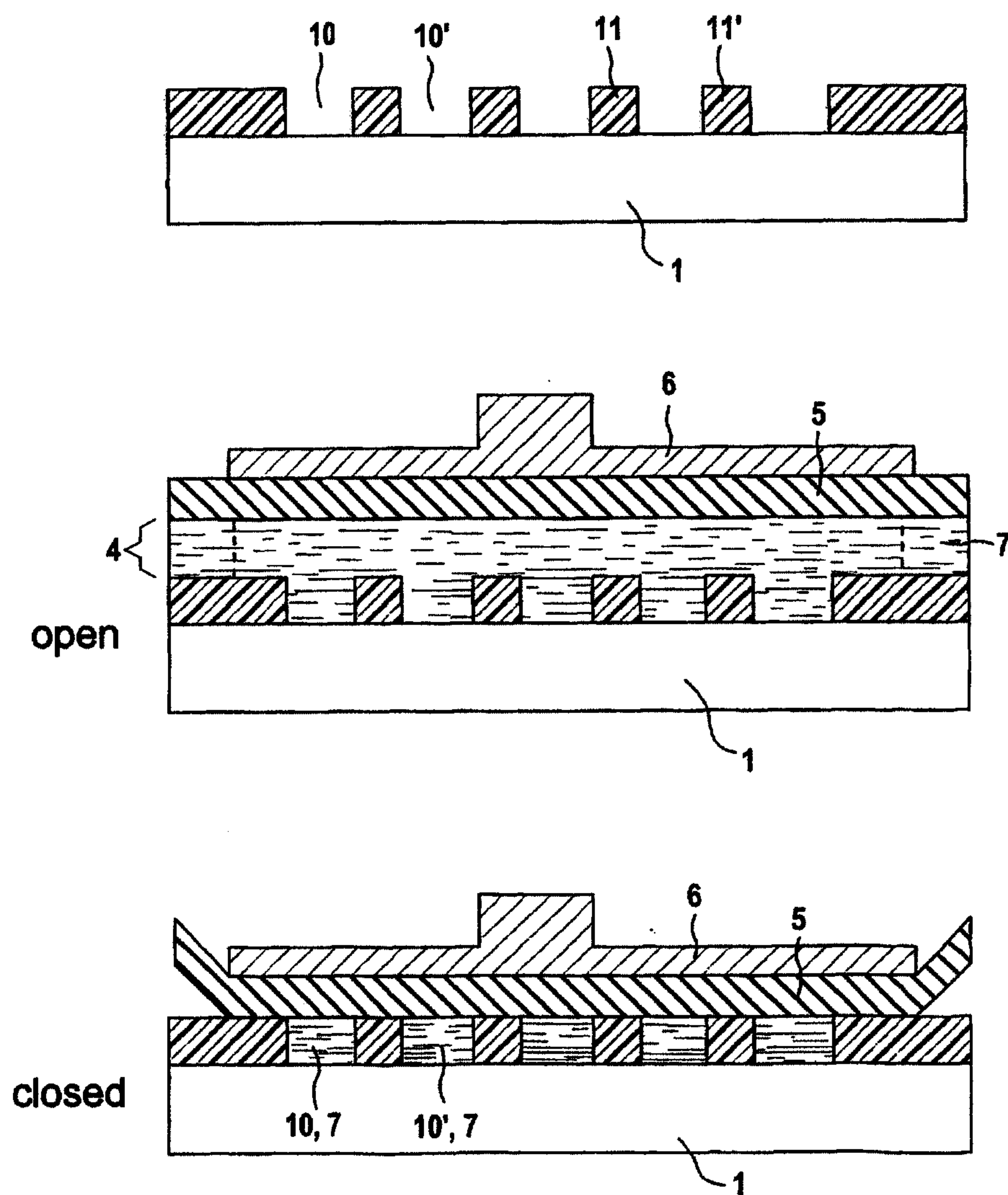


FIG 2

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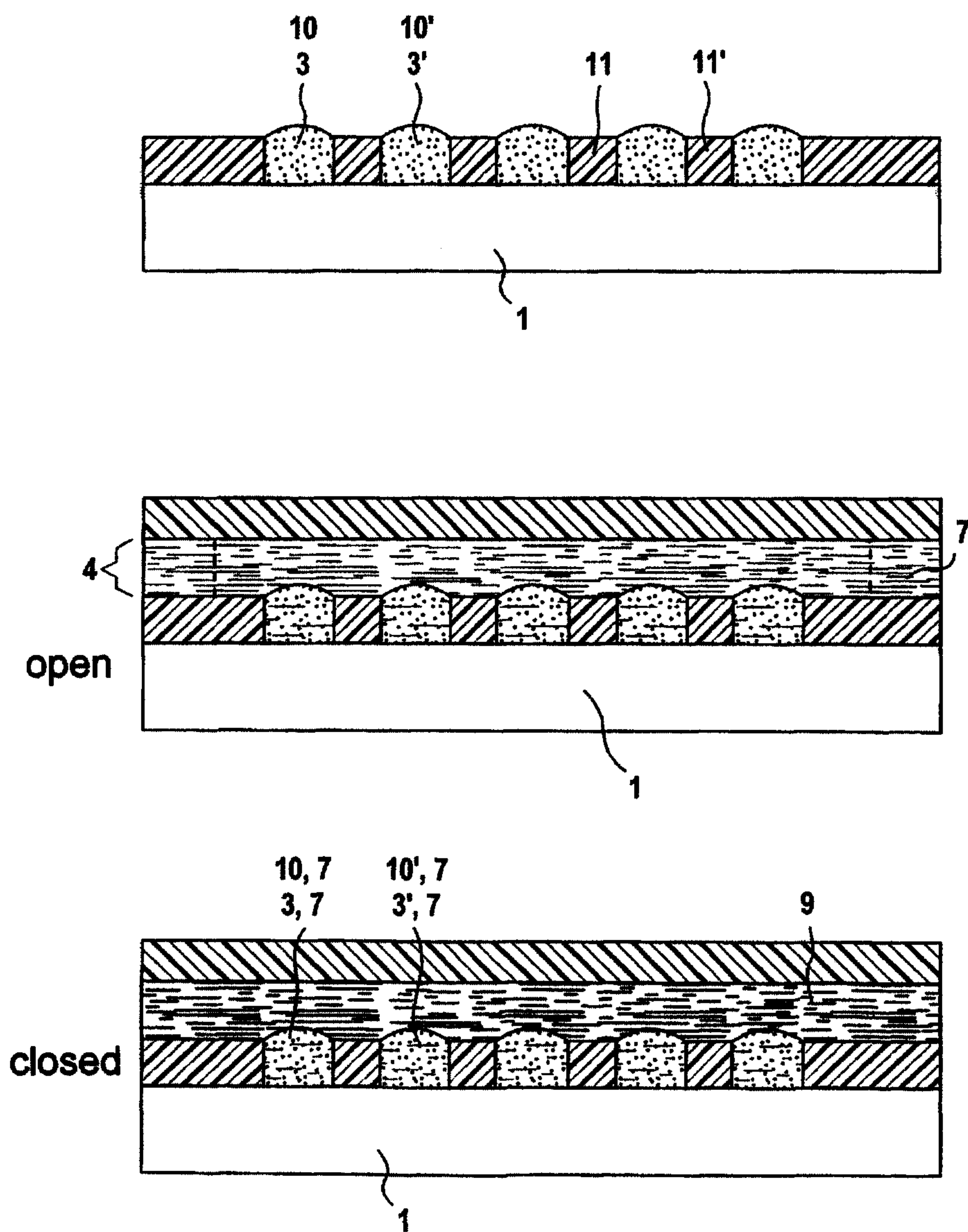


FIG 3

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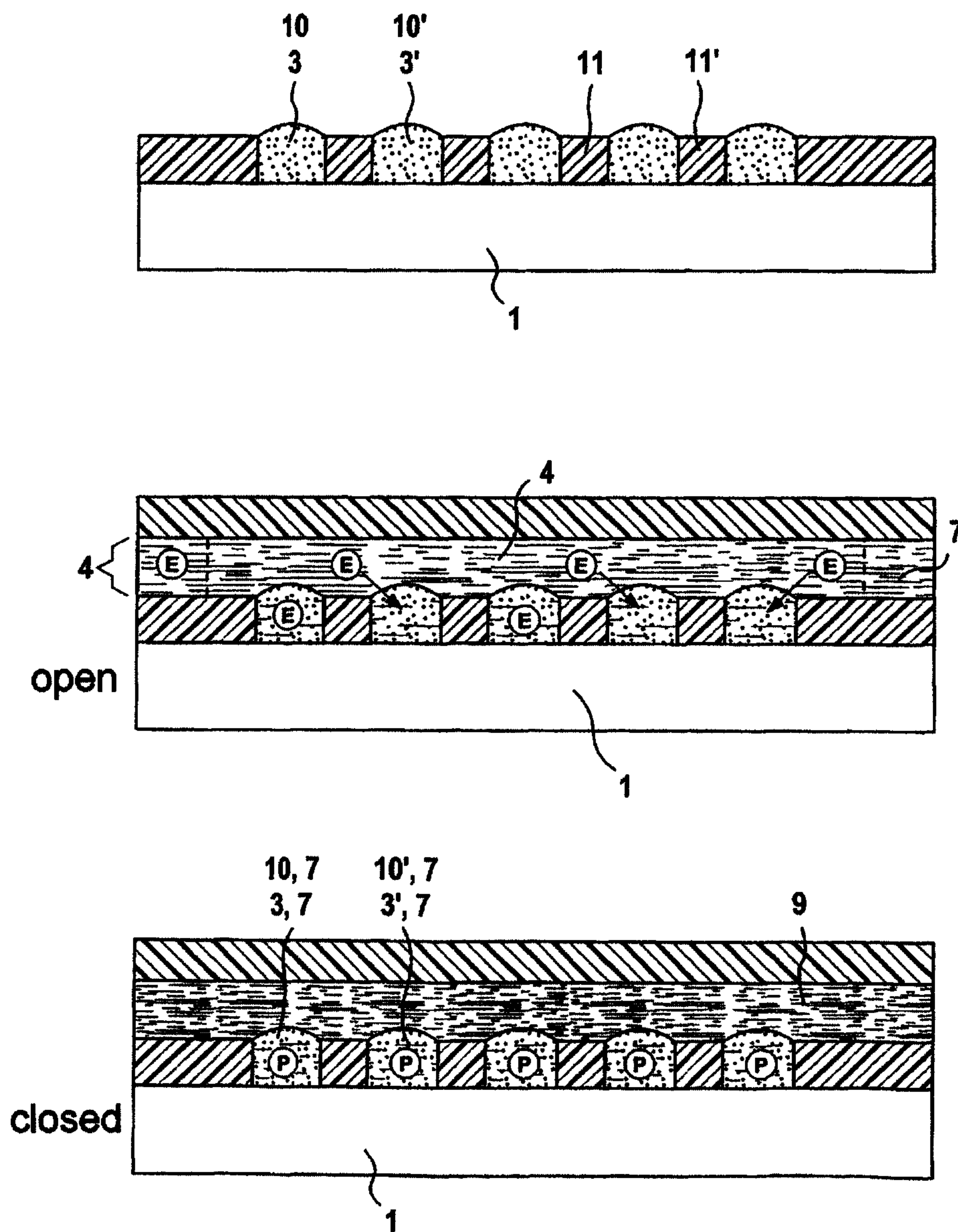


FIG 4

