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(54) Titre : METHODE ET DISPOSITIFS POUR FORMER UNE MULTITUDE DE PUITS SUR UN GEL
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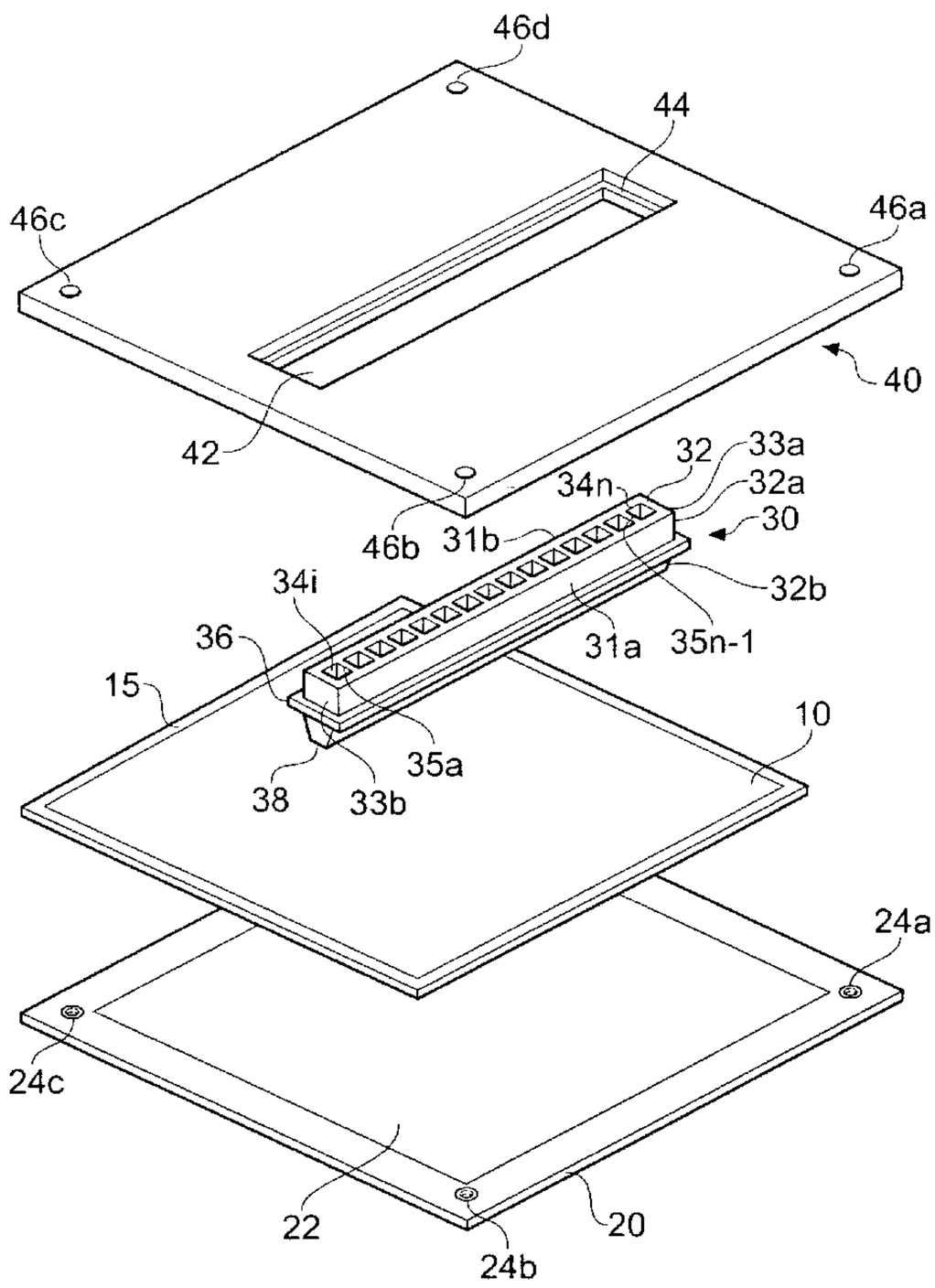

(57) Abrégé/Abstract:
The present invention relates to methods and devices for forming a plurality of wells on a gel containing an analyte. The invention further provides a system for eluting a liquid analyte reagent mixture from a gel. The invention is useful in the separation of biological molecules such as nucleic acids, carbohydrates, proteins and peptides. In particular, the invention has utility for separating and eluting peptides from isoelectric focusing gels.

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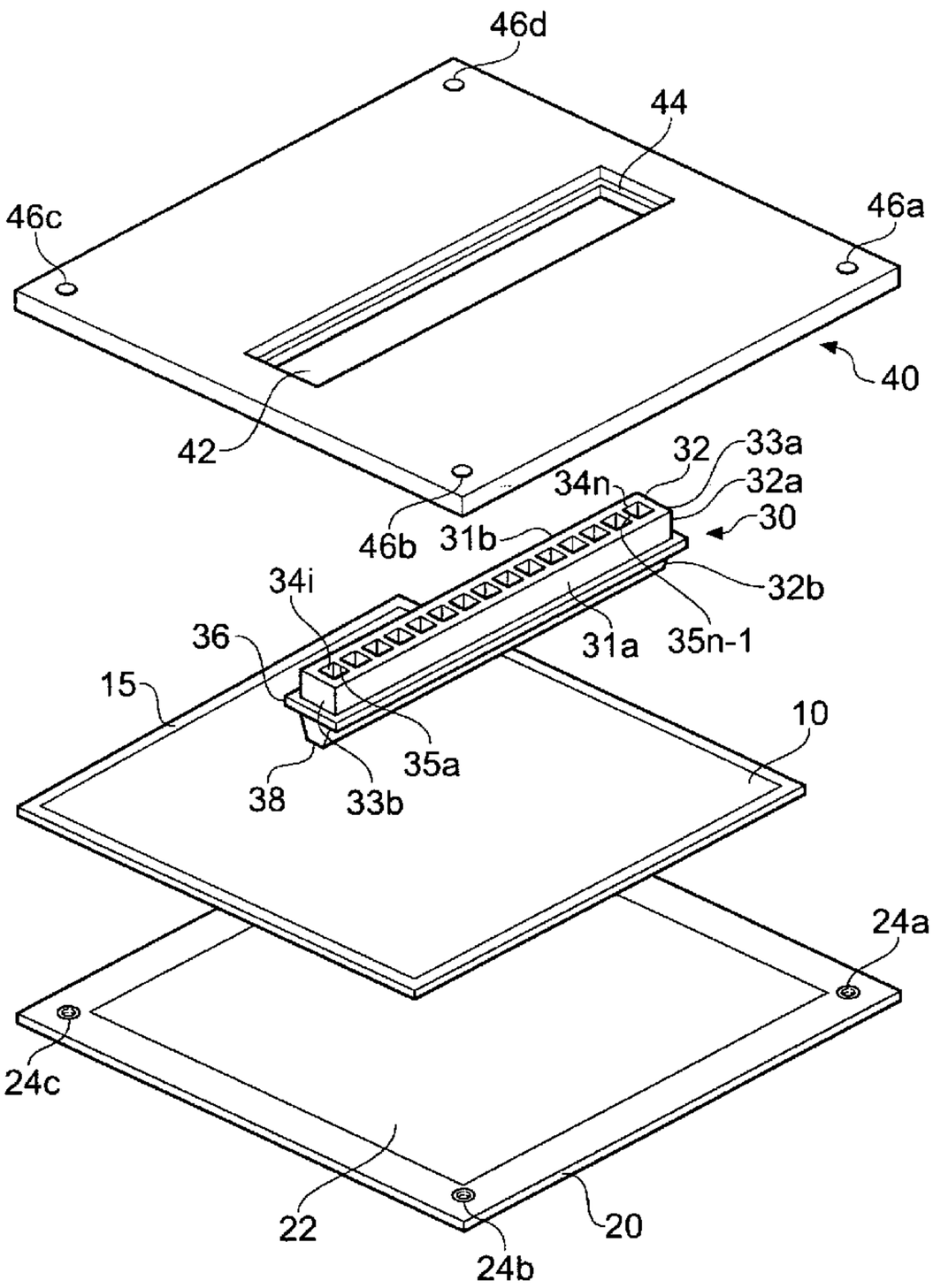
(54) Title: METHOD AND DEVICES FOR FORMING A PLURALITY OF WELLS ON A GEL



(57) Abstract: The present invention relates to methods and devices for forming a plurality of wells on a gel containing an analyte. The invention further provides a system for eluting a liquid analyte reagent mixture from a gel. The invention is useful in the separation of biological molecules such as nucleic acids, carbohydrates, proteins and peptides. In particular, the invention has utility for separating and eluting peptides from isoelectric focusing gels.

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Method and Devices for Forming a Plurality of Wells on a Gel

The present invention relates to methods and devices for forming a plurality of wells on a gel containing an analyte, in particular methods and devices for forming a plurality of wells on gels which have been used to separate biological molecules such as peptides, proteins, carbohydrates or nucleic acids.

Background to the Invention

The separation of biological molecules, such as proteins, peptides and nucleic acids, prior to or in parallel with their identification and quantification, can be achieved by a variety of techniques. Gel electrophoresis is a technique which is commonly used to separate these biological molecules on the basis of their size and/or their charge.

Mass spectrometry has today become the method of choice for the determination of the identity and composition of proteins and peptides. To allow collection of the information required a protein is in a first step cut up into peptides by either enzymatic or chemical means. The most common approach is enzymatic digestion using enzyme(s) which cut the protein at specific amino acid residues, a typical example being trypsin which hydrolyses the protein after lysine or arginine residues. It is, when tryptic digestion is carried out on a sample containing a very limited number of proteins, possible to determine the identity of the protein present from the masses of the peptides resulting from the digestion. A second approach used for identification purposes is the generation of a collision induced secondary mass spectra ion from ions separated in a primary mass spectrum. As the secondary mass spectra contains information on the masses of the amino acid residues constituting a peptide, these masses in combination with the mass of the ion selected in the primary spectrum can be used for identification of the tryptic peptide and the protein corresponding to this peptide. Evidently MS/MS spectra can be used not only for the identification and characterisation of enzymatically digested peptides, but also for peptides originally

present in the biological sample. In proteomic studies it is common to use MS or MS/MS not only for identification of protein but also for relative quantification (Aebersold et al; Nature, 2003, 422, 198-207).

A sample applied to a MALDI-MS target is only allowed to contain a limited number of peptides and similarly ESI-MS can only accept a limited number of peptides per time unit. The sample is normally a very complex mixture containing many thousand of proteins which after digestion could easily correspond to one hundred thousand to more than one million peptides. There is therefore a need for rigorous separation of the peptides prior to MS characterisation and quantification. A variety of different separation methods including electrophoretic and chromatographic methods can be used; normally multiple separation steps are required.

Separation can be conducted solely at the protein level prior to tryptic digestion. A typical example of this approach is two-dimensional (2-D) electrophoresis.

Alternatively, separation can be carried out at the protein level in the first step, followed by digestion and finally separation of the resulting peptides prior to MS. One example of this approach uses reverse phase chromatography (RPC) at the protein level followed by digestion and reverse phase chromatography separation of resulting peptides prior to ESI MS/MS. Another approach described is SDS-electrophoresis at the protein level followed by digestion and RPC (Breci et al; Proteomics, 2005, 5, 2018-2028). Finally tryptic digestion can be carried out prior to multidimensional separation at the peptide level. Approaches of this type include MudPit (Washburn et al; Nat Biotechnol., 2001, 19, 242-247), more conventional ion-exchange chromatography followed by RPC (Peng et al; Journal of Proteome Research, 2003, 2, 43-50) as well as peptide isoelectric focusing (IEF) followed by RPC (Cargile et al; Electrophoresis, 2004, 25, 936-945).

When tryptic digestion is the first step, an alternative approach is to decrease the complexity of the sample by the use of methods which allow the selection of a small

fraction of the peptides (e.g. iCAT [Aebersold et al; Proteomics, 2005, 5, 380-387] alt COFRADIC [Vandekerckhove et al; Nat Biotechnol., 2003, 21, 566-569]).

Generally electrophoretic techniques like IEF and SDS electrophoresis give, when used at the protein level in gel, much better resolution and protein yields than chromatographic alternatives. 2-D electrophoresis based on the combination of these two techniques, IEF and SDS, is also a commonly used approach when separation of very complex samples is conducted at the protein level. The disadvantages with electrophoretic techniques are however that they are labour intensive, often demand craftsmanship and that they are hard to automate. Problems can also be encountered extracting the analyte from gel.

The processing of gel fractions containing peptides, proteins, carbohydrates or nucleic acids from electrophoretic gels in order to facilitate further separation or to enable analyte analysis presents significant difficulties to the operator. Where the gel is present on a glass or plastic plate, individual bands or fractions must be blotted or scraped from the plate, typically with a spatula or sharp knife, and carefully transferred either to a second gel or a reaction vessel for further analysis. In the situation where the gel is supported on a plastic sheet, as with an IPG strip, the strip must be carefully cut with scissors or a sharp blade into a series of pieces which can then be transferred to another gel or reaction vessel for further processing/analysis.

Automatic sampling systems are known for removing bands or spots from gels, such as those described in WO 02/071072. In fact, 2-D electrophoresis frequently employs automatic spot pickers in which gels are generally stained to detect the protein or peptide samples. However, these systems usually involve aspiration of the gel into a pipette which leads to losses due to gel sticking to the outside or inside of the pipette. Furthermore, these systems are labour intensive and time consuming, involving protein/peptide staining and careful use of the apparatus to avoid losses and contamination.

It will be understood by the skilled person that the process of removing bands or fractions of gel manually from a plate or strip is time consuming as painstaking care must be taken in order to ensure that the gel is divided evenly into the appropriate number of fractions, that there is quantitative recovery of the analyte from the gel, and that cross-contamination from 'dirty' instruments used in the transfer process is avoided. The problem of cross-contamination is particularly significant where the analyte has been separated using IPG strips and scissors or a scalpel is used to cut the strip into bands for further processing/analysis, as the blades of these instruments must be thoroughly cleaned before the next band of gel is excised from the strip. Furthermore, such processes generally involve the additional step of pre-staining the gel in order to detect peptides or proteins, such systems are extremely labour intensive.

It will also be understood by the skilled person that the problems described above experienced in removing and transferring gel bands from a plate or IPG strip to a second gel or reaction vessel for further processing will be exacerbated with an increasing number of bands or fractions. Thus, for example, where an IPG strip has to be divided into some 50 pieces and each of the 50 pieces transferred to another gel or a reaction vessel, there is an increasing likelihood of cross-contamination and poor recoveries.

To avoid the problem with sample extraction from gels, isoelectric focusing separation can be carried out in liquid phase (Zuo et al; Methods Mol Biol., 2004, 244, 361-75). The equipment used by Zuo et al. comprises a series of chambers separated by membranes titrated to specific pH-values. However, one disadvantage of this approach is that peptides and proteins have low solubility in the vicinity of their isoelectric points; the resulting precipitation and aggregation can lead to problems of poor resolution of the peptides and proteins during the isoelectric focusing.

Michel et al. (Electrophoresis, 2003, 24, 3-11) describe a technique which allows the fractionation of complex biological samples according to their isoelectric point (pI) as well as the direct recovery of the compounds for further analysis. The technique, termed 'off-gel IEF', involves dividing IPG strips into a series of wells using a multiwell device which is open at both ends, adding protein sample in an IPG buffer and then conducting electrophoresis to separate the protein mixture. The content of each well is then removed for protein analysis by mass spectrometry and the technique shown to effect a resolution of 0.1 pH units. However, as in the approach of Zuo et al. discussed above, the proteins are present in liquid phase during focusing which increases the risk of precipitation and aggregation. With the geometry resulting from the approach of Michel et al., the proteins will be present in a region with much lower electric field than would be the case if the focusing was done solely in the gel in the absence of any solution added in the multiwell device. Compared to conventional gel focusing the result is lower resolution and a demand for longer focusing times.

The same group (Heller et al.; Electrophoresis, 2005, 26, 1174-1188) has recently reported the use of 'off-gel IEF' for the separation and identification of proteins and their isoforms by use of a two-stage process, the first involving separation of the proteins and their isoforms on the basis of their pI's and the second the separation and identification of the trypsinized peptide fragments.

IEF can also be carried out in configurations where separated proteins are collected in solution in chambers separated with membranes (Righetti et al; J. Biochem. Biophys. Meth., 1987, 15, 199-206). This approach is also limited by the fact that proteins close to their isoelectic point tend to aggregate and precipitate.

Other systems have been disclosed which describe methods for processing proteins in gels wherein gel fragments containing proteins are isolated from the gel, subjected to proteolytic digestion and then the cleavage peptides produced are identified. Such an automated system is described in WO 02/071072, in which isolated protein-gel

fragments are directly transferred to a corresponding number of reaction vessels of a first microtitre plate by a robotic arm device, the base of the microtitre plate having a hydrophobic filter membrane, and incubated with a protease. Following hydrolysis, the peptide products are filtered through the hydrophobic filter membrane into a second microtitre plate and concentrated for subsequent analysis.

A number of groups have used electroelution to transfer biomolecules from a gel. Thus Buzas et al. (Proteomics, 2001, 1, 691-698) describe a system for transferring proteins present in a gel directly to mass spectrometry by the application of an electric field which passes vertically through the protein band located on a horizontal minigel. Yefimov et al. (Electorphoresis 2001, 22, 2881-2887) and Gombacz (Electrophoresis, 2001, 21, 846-849) describe the use of fluorescently labelled proteins and dyes as size markers to locate the position where electroelution takes place. US 2004/0178073 discloses an apparatus for preparative electrophoresis and recovery of target molecules that have been electroeluted out of a gel sample. EP 0382426 describes a micro-preparative gel electrophoresis apparatus or column which is used for electroelution of samples. An apparatus and process for electroelution of a gel containing charged macromolecules is described in US 5,840,169. An electroelution device for the elution of macromolecules from a support, such as a gel or membrane, is disclosed in US 5,340,449.

A basic requirement of electroelution is that an electric current or voltage must be applied to the support such as a gel or membrane. Several problems are associated with this technique, including the need for the support (including, for example, any backing materials as can be the case with gels) to be electrically conductive, the generation of heat by the electric current, and the requirement for the additional step of electroelution to transfer a sample from the gel.

Thus while electrophoretic separation in gel provides outstanding resolution, as discussed above, problems with sample transfer from the gel to liquid phase are often encountered and this, in turn, makes sample transfer a difficult process to automate.

It is therefore an object of the present invention to provide methods and devices which facilitate the preparation of gel fractions and enable the further processing and manipulation thereof while ameliorating the problems encountered in the prior art. Another object of the invention is to provide such methods and devices without the need to pre-stain gels for the detection of such analytes. A further object of the present invention is to provide methods and devices for adding reagents to gel fractions and for eluting analyte, either prior to or following chemical or enzymatic modification, from a gel.

Summary of the Invention

According to a first aspect of the present invention, there is provided an apparatus for forming a plurality of wells on a gel containing an analyte comprising,

(i) a multiwell template comprising a body having a plurality of open-ended chambers, each said chamber being defined by one or more walls; and

(ii) a top plate for securing said multiwell template to the gel comprising one or more openings for receipt of the multiwell template.

Suitably, the gel is supported on a sheet.

Suitably, the apparatus additionally comprises a base plate for positioning the gel or said sheet or a retainer for the gel or the sheet thereon. Preferably, the base plate

comprises fastening means for affixing the base plate to said top plate in a predefined position.

Suitably, the top plate additionally comprises fastening means for affixing the top plate to the base plate in a predefined position.

Suitably, the base plate additionally comprises one or more recesses and/or protusions on a single surface for locating the sheet or retainer for the sheet on said surface.

Suitably, the apparatus additionally comprises a retainer for holding the gel or the sheet, said retainer comprising one or more recesses or protusions on one surface for receipt of the gel or sheet thereon. Preferably, said one or more recesses or protusions of the retainer additionally comprises locating means for positioning the sheet thereon. Most preferably, the retainer is an electrophoresis manifold.

Suitably, the apparatus additionally comprises a securing strip for affixing the multiwell template to the top plate, said strip comprising a plurality of openings corresponding to the positions of the open-ended chambers in the template. Preferably, the strip additionally comprises fastening means for affixing the strip to the base plate in a predefined position.

Preferably, said fastening means comprises a threaded screw bore in the base plate and an opening suitable for a screw in the top plate and the securing strip.

Preferably, the sheet is composed of a low fluorescent plastic or low fluorescent glass.

Preferably, the gel is composed of polyacrylamide. More preferably, the gel is a SDS gel or an isoelectric focussing gel. Most preferably, the gel is an Immobiline™ DryStrip gel (GE Healthcare).

Suitably, the body of the multiwell template is divided into a first portion and a second portion, said first portion being shaped for insertion into the opening in the top plate and said second portion being tapered to a base for compressing the gel. Preferably, the first and second portion are separated by a flange for supporting the multiwell template within the opening in the top plate. More preferably, the base of the second portion comprises one or more notches for locating the multiwell template on the retainer in a predefined position.

Optionally, the multiwell template and the top plate are an integral unit.

According to a second aspect of the present invention, there is provided a system for eluting an analyte or a modified analyte from a gel by non electroelution means comprising an apparatus as hereinbefore described and a liquid dispensing and eluting device. By "electroelution means" is meant any method which involves the application or use of an electric current or field or voltage to elute an analyte or modified analyte from a support such as gel or membrane.

Preferably, said dispensing and eluting device is automatically controlled. More preferably, the dispensing and eluting device is an automatic liquid handling device under the control of a computer.

Suitably, the analyte is selected from the group consisting of nucleic acid, carbohydrate, protein and peptide. Preferably, the analyte is a peptide.

According to a third aspect of the present invention, there is provided a method for forming a plurality of wells on a gel which contains an analyte, said method comprising the steps of

i) inserting a multiwell template into an opening in a top plate, said template comprising a body having a plurality of open-ended chambers, each said chamber being defined by one or more walls;

ii) moving the multiwell template onto the gel to form a plurality of wells between the gel and the one or more walls; and

iii) optionally, affixing a securing strip over the end of the multiwell template located within the top plate, said strip comprising a plurality of openings corresponding to the positions of the open-ended chambers in the template.

Optionally, step ii) of the method is carried out prior to step i) of the method.

Suitably, the gel is supported on a sheet.

Suitably, the method additionally comprises the step of positioning the gel or said sheet either directly onto a base plate or, wherein the gel or sheet is within a retainer, positioning said retainer onto said base plate in a predefined position. The base plate could, for example, correspond to a cooling plate of an horizontal electrophoretic apparatus.

Preferably, the base plate additionally comprises one or more recesses and/or protusions on a single surface for locating the gel or the sheet or retainer for the gel or the sheet on said surface.

Suitably, wherein said retainer comprises one or more recesses or protusions on one surface for receipt of the gel or the sheet thereon. Preferably, said one or more recesses or protusions of the retainer additionally comprises locating means for positioning the sheet thereon.

Preferably, the base plate and/or the top plate and/or the securing strip additionally comprise fastening means for positioning the plurality of wells formed on the gel in a predefined position relative to the base plate and the top plate. Such fastening means may include, for example, clasps, clamps, snap-fasteners, pins and holes. More preferably, said fastening means comprises a threaded screw bore in the base plate and an opening suitable for a screw in the top plate and the securing strip.

Preferably, the gel is a polyacrylamide gel. More preferably, the gel is a SDS gel or an isoelectric focusing gel.

Suitably, the body of the multiwell template is divided into a first portion and a second portion, said first portion being shaped for insertion into the opening in the top plate and a second portion being tapered to a base for moving onto the gel. Preferably, the first and second portions are separated by a flange for supporting the multiwell template within the opening in the top plate.

Suitably, said analyte is a peptide, protein, nucleic acid or carbohydrate. Preferably, the analyte is a peptide or protein.

Suitably, the analyte has been subjected to a chromatographic or electrophoretic separation prior to moving the multiwell template onto the gel to form a plurality of wells between the gel and the one or more walls.

Suitably, the analyte is subjected to a chromatographic or electrophoretic separation following moving the multiwell template onto the gel to form a plurality of wells between the gel and the one or more walls.

Preferably, the method further comprises the step of adding a liquid reagent to one or more of the plurality of wells so formed to form a liquid analyte reagent mixture.

Suitably, the liquid reagent is added by manual or automated means. An example of manual means includes manually operated pipettes, whilst examples of automated means include automated or programmable liquid handling devices.

Suitably, said reagent can solublise the analyte or chemically modify the analyte or its environment. The reagent can include, for example, a buffer, acid, alkali, organic solvent, enzyme or chemical reactant which can modify the analyte. Preferably, the reagent is a protease enzyme.

Preferably, the method further comprises the steps of transferring the liquid analyte reagent mixture to a second vessel by either manual or automatic means. An example of manual means includes manually operated pipettes, whilst examples of automated means include automated or programmable liquid handling devices. Suitably, the second vessel is a well in a microtitre plate, such as a 96 well microtitre plate.

Preferably, the reagent is added by an automatic liquid handling device and/or the liquid analyte reagent mixture is transferred by an automatic liquid handling device. Most preferably, said automatic liquid handling device is under the control of a computer.

Brief Description of the Invention

Figure 1 shows an apparatus according to the invention for forming a plurality of wells on a SDS gel.

Figure 2 shows an apparatus according to the invention for forming a plurality of wells on an isolectric focusing gel which is in the form of an IPG strip.

Figure 3 is a plan perspective of the apparatus of Figure 2.

Figures 4a & b illustrate different features of a multiwell template according to the invention wherein Figure 4a is an underside view showing the base of the template featuring a plurality of open-ended chambers and Figure 4b is the same view but with an IPG strip in position on the base of the template.

Figure 5 shows a top plate according to the invention.

Figures 6a is a plan view giving details of a top plate and securing strip according to the invention. Figure 6b is an underside view of a top plate with the multiwell template positioned within it.

Figure 7 shows an automatic eluting system according to the present invention.

Figures 8a & b are fluorescence intensity scans of an IPG strip which has been used to separate fluorescently labelled peptides *before* (Figure 8a) and *after* (Figure 8b) elution of the gel by the method according to the invention. Additional fluorescence scans of the IPG strip before and after extraction are seen in Figure 8c, together with a scan of a microtitre plate containing the fractions eluted from the strip.

Figure 9 is a graphical illustration of the distribution of identified peptides present in only one or several fractions extracted from a gel using the method of the invention.

Detailed Description of the Invention

A diagram of an apparatus for forming a plurality of wells on a gel which is supported on a sheet is shown in Figure 1. The gel (10), such as an SDS gel, is present on the surface of the sheet (15). The sheet (15) is positioned on a base plate (20), made of a plastic or metal material, which has a recess (22) for locating the sheet in a predefined position relative to the plate (20). Fastening means, in the form of threaded screw bores (24 a-c, 24d not shown), are located at each corner of the plate (20) to allow affixing by screws (not shown) of the base plate (20) to a top plate (40) in a predefined position. It will be understood that other forms of fastening means can be used (e.g. clasps, clamps, pins and holes, snap fastening).

A plurality of wells is formed on the gel by means of a multiwell template (30) which may be made of any suitable material such plastic, a metal, ceramic or composite material. The multiwell template (30) consists of an elongated body (32) having two elongated side walls (31a, 31b) joined at their ends by two end walls (33a, 33b). A plurality of open-ended chambers (34 i-n) are arranged along the longitudinal axis of the body (32), side chambers being separated from their neighbour(s) by intermediate wall (35a-35n-1), each of which extends from side wall 31a to side wall 31b. Each chamber (34) may take any appropriate shape, for example circular, oval, polygonal, square or rectangular (as shown).

The body (32) of the multiwell template (30) is divided by a flange (36) into a first (32a) and second (32b) portion; the first (32a) portion being shaped for insertion into an opening (42) in the top plate (40) and a second portion (32b) being tapered to a base (38) for compressing the gel. It will be understood that when the template (30) is lowered or pushed onto the gel (10) and contacts, or comes into close proximity with,

the sheet (15) supporting the gel (10), each chamber (34) forms a well with the gel or the sheet forming a base and the walls of the chamber (not shown) defining the walls of the well. In this way a plurality of wells are created on the gel. The multiwell template (30) may be inserted into an opening (42) in a top plate (40), which is composed of a plastic or metal (e.g. stainless steel) material, either before or after it has been lowered or pushed onto the gel to form a plurality of wells thereon. In the example shown, the flange (36) supports the template (30) on the ledge (44) of the top plate (40). It should be noted that the template does not cut the gel but rather compresses it to form a plurality of wells. The wells are held in a predefined position relative to the base plate (20) and the top plate (40) by affixing the top plate (40) to the base plate (20) by fastening means in the top (46 a-d) and base (24 a-d) plates. In the embodiment of Figure 1, screws (not shown) are used to secure the top plate (40) to the base plate (20) by insertion through openings in the top plate (46 a-d) and into the screw bores (24a-d) in the base plate (20). In this way the plurality of wells formed in the gel is held in a predefined position relative to the top and bottom plate.

Figure 2 is a perspective view of another embodiment of an apparatus according to the invention. The apparatus shown in Figure 2 is suitable for use with isolectric focusing gels, in particular IPG strips such as Immobiline^{TM} DryStrip gels. The IPG strip (not shown), consisting of a plastic base sheet supporting a coating of polyacrylamide gel (110), is placed within a recess (152) of a retainer (150) which is an electrophoresis manifold. The retainer (150), which is typically made of a plastic material, may consist of a plurality of recesses (152 i – n), twelve being shown in the embodiment of Figure 2, such that a plurality of IPG strips may be processed at the same time. Following electrofusing of an analyte in the IPG strip, the retainer (150) together with the strip is located in a predefined position within a recess (122) in the top surface of the base plate (120). The base plate may be made of a plastic or metal material. A multiwell template (130), similar in construction to that described above with respect to the first embodiment of the present invention, comprises a plurality of

open-ended chambers (134i-n) and is inserted in an opening (142) in a top plate (140) such that it supported by its flange (136) on a ledge (not shown) surrounding the opening (142). The multiwell template (130) and the top plate (140) are typically made of a plastic material but may be made of other materials such as a metal. It will be understood that a plurality of multiwell templates (130) may be positioned in the top plate (140) in the manner described; thus, for example, in the embodiment shown, twelve multiwell templates (130) can be positioned within the top plate (140).

Once it is positioned within the top plate (140), the multiwell template (130) is lowered or moved onto the surface of the gel (110), such that the tapered portion (132b) of the body of the template compresses the gel (110) such that the base (138) of the template comes into close proximity to the plastic sheet supporting the gel (110). In this position, a well is defined with the gel or the plastic sheet forming the base and the walls of the template defining the walls of the well.

It will be understood that with different embodiments of the invention, the multiwell template (130) may be lowered or moved onto the gel (110) to form a plurality of wells thereon before the template (130) is inserted into the top plate (140). The multiwell template can then be secured into position relative to the top (140) and bottom (120) plates by use of the fastening means in the top (146 a-d) and bottom (124 a-d; d not shown) plates; for example, in Figure 2, screws (not shown) could be used to affix the plates together.

In the preferred embodiment shown in Figure 2, a securing strip (160) is positioned over the top of the multiwell template (130) such that the openings (164i- n) in the strip (160) overlap and correspond to the positions of the open ended chambers (134 i-n) in the template (130). The securing strip (160) may then lock the template (130) into a predefined position by affixing it to the top plate (140) by use of the fastening

means in the strip (166 a–b) and the top plate (148 l and n); such fastening means may take the form of openings in the securing strip (166a-b), screw bores in the top plate (148 l & n) and the use of one or more screws of appropriate bore. Alternatively the securing strip may be formed integrally with a multiwall template.

Figure 3 is a plan perspective of the apparatus of Figure 2, where each of the component parts has the same features as described above for Figure 2. Thus the apparatus consists of a base plate (220) having a recess (222) and fastening means (224 a-d, d not shown). A retainer (250) in the form of an isoelectric focussing manifold holds a number of IPG strips (not shown) within a series of recesses (252 i-n) consisting of a plastic sheet supporting a polyacrylamide gel (210). The top plate (240), made of a plastic material, consists of a plurality of openings (242 i-n) corresponding to the positions of the IPG strips within the retainer (210). Fastening means (246 a-d; and 248 i & n), corresponding to those present in the base plate (224 a-d) and securing strip (260), respectively, are present in the top plate (240). The multiwell template (230) comprises a plurality of open ended chambers (234 i-n). The securing strip (260) consists of a number of openings (264 i-n) corresponding to the position of the open-ended chambers (234i-n) in the template (230) and fastening means (266 a & b).

The apparatus of Figure 3 may be used in the same way as described above in connection with Figure 2 to obtain a plurality of wells on a gel.

Figure 4a is a view of the base (338) of a multiwell template (330) according to the invention. The open-ended chambers (334 i-n) are defined by a series of walls (333) throughout the body (332) of the template (330). Recesses or notches (337) on the base of the template (330) are used to place the template (330) onto protrusions in the retainer (not shown) which holds the IPG strips, and thus to locate the template (330) in a predefined position relative to the IPG strip.

Figure 4b shows the plastic sheet (315) of an IPG strip positioned on the base (338) of the multiwell template (330). In the perspective view shown, the gel cannot be seen because it is on the underside of the sheet (315) and is in contact with the base (338) of the template (330). In this position, the base of the sheet (315) within each chamber (334) or the gel forms the base of a well and the walls of the chamber act as the walls of a well.

Figure 5 shows a plan perspective of a top plate (440) according to the invention which is made of steel. The openings (442 i-n) for receipt of the multiwell template (shown in position), together with fastening means for affixing to the base plate (446 a-d) and for affixing to the securement strip (448 i-n) are illustrated in the diagram.

Figure 6a is a plan view showing details of a top plate (540) of the invention in which the securing strip (560) has been positioned to affix the multiwell template (not shown) to the top plate (540). The fastening means (566), in the form of openings, are shown and co-locate with those of retainer (not shown) in the top plate (see 448 i-n in Figure 5).

Figure 6b is an underside view showing details of the arrangement given in Figure 6a. The base (538) of the tapered second portion of the multiwell template, which protudes from the lower surface of the top plate (560), is seen clearly from this angle. It is this base (538) which compresses the gel, each open-ended chamber (534i-n) forming a well with the base sheet (not shown) of the gel.

Figure 7 shows an automatic eluting system according to the present invention. Following electrophoresis of a sample on a gel, for instance an IPG strip, a plurality of wells is formed using the method of the invention as described above. The gel in each well is then eluted with buffer to extract the analyte (such as a peptide) and the

resulting eluant transferred to a reaction vessel for further processing/analysis. Figure 7 shows an eight channel eluting probe (670) in the process of transferring eluant from the wells present in the top plate (640) of the apparatus of the invention to wells (682 i-n) in a microtitre plate (680). The system is under the control of a computer (not shown). The number of wells formed in the IPG strip typically correspond to the number of wells across the length or breadth of the microtitre plate (e.g. they are a multiple of 8 or 12 for a 96 well microtitre plate) or a fraction of these numbers (e.g. 2, 3, 4, 6).

Specific Examples

Isoelectric focusing, fluorescence analysis and extraction of peptides

0.5mg of a tryptic digest sample from *Saccharomyces cerevisiae*, Type II, was mixed with 5µg of each of the pI-markers '3.73', '4.25' and '4.54'. A 'pI-marker' is a fluorescently labelled peptide with known isoelectric point that can be detected by fluorescence scanning. The fluorescent label used was Cy5™ (available from Amersham Biosciences AB; Sweden) which emission spectrum is taken at ~660nm (Ettan DIGE System - User Manual, Amersham Biosciences AB, Sweden). A 24cm IPG peptide strip (pH 3.4-4.8) was rehydrated overnight (~15 hours, room temperature) in 350µl of 8M urea and sample solution. The rehydrated strip was transferred to an Ettan™ IPGphor™ manifold and isoelectric focusing was run using the following program: Gradient 500 V 1 minute, Gradient 4000 V 1.5 hours, Gradient 6000 V 1.5 hours, Gradient 10000 V 1.5 hours, Step 10000 V 12 hours (total ~150kVhrs). Ettan IPGphor II was used as the focusing unit and the focusing was performed at 20°C.

After focusing, the IPG strip was scanned in a fluorescence scanner (Typhoon 9400 scanner, Amersham Biosciences, Sweden) at 660 nm, to determine the exact position

of the fluorescent pI-markers. The Typhoon pictures were evaluated in ImageQuant and fluorescence intensity graphs established.

After scanning, the peptides in the strip were extracted from the gel into liquid fractions using the multiwell template of the invention. Thereby the pH gradient is divided into a series of discrete fractions along the strip. In this manner, the IPG strip was divided into 72 fractions at about 3 mm intervals. 50µl water was added to each of the 72 wells, incubated at room temperature for 60 minutes and extracted peptides were then transferred to a microtitre plate in an automated manner. The elution process was repeated three times to ensure extraction and transfer of all peptides from each well. After extraction, the multiwell template was removed from the IPG strip and the device can be reused following cleaning in consecutive experiments. In the described experiment, the IPG peptide strip was once more scanned in a Typhoon scanner and the pictures were evaluated in ImageQuant.

Figure 8 shows the fluorescent intensity of the IPG strip before (Fig 8a) and after (Fig 8b) extraction. Figure 8c shows the scanned microtitre plate with extracted peptide samples and the strips before and after extraction, demonstrating high and low levels of fluorescence, respectively. From the Figures it is clear that the peptides have been effectively extracted from the IPG strip and are now present in the wells of the microtitre plate.

Figure 9 shows the result of a comparison between all identified peptide sequences in seven fractions next to each other on the basic end of the IPG strip. Of a total of 719 identified peptides in the seven compared fractions, 82% of the peptides were present in only one fraction and 16% in two fractions. The results of this experiment not only underline the high resolution in the IPG strip but also that there is no problem with leakage between the wells formed using the multiwell template of the invention.

Claims

1. An apparatus for forming a plurality of wells on a gel containing an analyte comprising,

(i) a multiwell template comprising a body having a plurality of open-ended chambers, each said chamber being defined by one or more walls; and

(ii) a top plate for securing said multiwell template to the gel comprising one or more openings for receipt of the multiwell template.

2. The apparatus according to claim 1, wherein said gel is supported on a sheet.

3. The apparatus according to claim 1 or 2, additionally comprising a base plate for positioning the gel or said sheet or a retainer for the gel or the sheet thereon.

4. The apparatus according to claim 3, wherein said base plate comprises fastening means for affixing the base plate to said top plate in a predefined position.

5. The apparatus according to any preceding claims, wherein the top plate additionally comprises fastening means for affixing the top plate to the base plate in a predefined position.

6. The apparatus according to any of claims 3 to 5 wherein the base plate additionally comprises one or more recesses and/or protusions on a single surface for locating the sheet or retainer for the sheet on said surface.

7. The apparatus according to any of claims 1 or 6, wherein the apparatus additionally comprises a retainer for holding the gel or the sheet, said retainer

comprising one or more recesses or protusions on one surface for receipt of the gel or sheet thereon.

8. The apparatus according to claim 7, wherein said one or more recesses or protusions of the retainer additionally comprises locating means for positioning the sheet thereon.

9. The apparatus according to claim 7 or 8, wherein the retainer is an electrophoresis manifold.

10. The apparatus according to any of claims 1 to 9, additionally comprising a securing strip for affixing the multiwell template to the top plate, said strip comprising a plurality of openings corresponding to the positions of the open-ended chambers in the template.

11. The apparatus according to claim 10, wherein the strip additionally comprises fastening means for affixing the strip to the base plate in a predefined position.

12. The apparatus according to any of claims 3 to 11, wherein said fastening means comprises a threaded screw bore in the base plate and an opening suitable for a screw in the top plate and the securing strip.

13. The apparatus according to any of claims 2 to 7, wherein the sheet is composed of a low fluorescent plastic or low fluorescent glass.

14. The apparatus according to any of claims 1 to 13, wherein the gel is composed of polyacrylamide.

15. The apparatus according to any of claims 1 to 14, wherein the gel is a SDS gel or an isoelectric focussing gel.

16. The apparatus according to any of claims 1 to 15, wherein the gel is an Immobiline™ DryStrip gel.

17. The apparatus according to any preceding claim, wherein the body of the multiwell template is divided into a first portion and a second portion, said first portion being shaped for insertion into the opening in the top plate and said second portion being tapered to a base for compressing the gel.

18. The apparatus according to claim 17, wherein the first and second portion are separated by a flange for supporting the multiwell template within the opening in the top plate.

19. The apparatus according to either of claims 17 to 18, wherein the base of the second portion comprises one or more notches for locating the multiwell template on the retainer in a predefined position.

20. The apparatus according to any preceding claim, wherein the multiwell template and the top plate are an integral unit.

21. A system for eluting an analyte or a modified analyte from a gel by non electroelution means comprising an apparatus according to any preceding claim and a liquid dispensing and eluting device.

22. The system of claim 21, wherein said dispensing and eluting device is automatically controlled.

23. The system according to claim 22, wherein the dispensing and eluting device is an automatic liquid handling device under the control of a computer.

24. The system according to any of claims 21 to 23, wherein the analyte is selected from the group consisting of nucleic acid, carbohydrate, protein and peptide.

25. The system according to claim 24, wherein the analyte is a peptide.

26. The system according to claim 25, wherein said detector is a fluorescence detector.

27. A method for forming a plurality of wells on a gel which contains an analyte, said method comprising the steps of

i) inserting a multiwell template into an opening in a top plate, said template comprising a body having a plurality of open-ended chambers, each said chamber being defined by one or more walls;

ii) moving the multiwell template onto the gel to form a plurality of wells between the gel and the one or more walls; and

iii) optionally, affixing a securing strip over the end of the multiwell template located within the top plate, said strip comprising a plurality of openings corresponding to the positions of the open-ended chambers in the template.

28. The method according to claim 27, wherein step ii) is carried out prior to step i)

29. The method according to claim 27 or 28, wherein the gel is supported on a sheet.

30. The method according to any of claims 27 to 29, additionally comprising the step of positioning the gel or said sheet either directly onto a base plate or, wherein the gel or sheet is within a retainer, positioning said retainer onto said base plate in a predefined position.

31. The method according to claim 30, wherein the base plate additionally comprises one or more recesses and/or protusions on a single surface for locating the gel or the sheet or retainer for the gel or the sheet on said surface.

32. The method according to any of claims 27 to 31, wherein said retainer comprises one or more recesses or protusions on one surface for receipt of the gel or the sheet thereon.

33. The method according to claim 32, wherein said one or more recesses or protusions of the retainer additionally comprises locating means for positioning the sheet thereon.

34. The method according to any of claims 27 to 33, wherein the base plate and/or the top plate and/or the securing strip additionally comprise fastening means for positioning the plurality of wells formed on the gel in a predefined position relative to the base plate and the top plate.

35. The method according to claim 34, wherein said fastening means comprises a threaded screw bore in the base plate and an opening suitable for a screw in the top plate and the securing strip.

36. The method according to any of claims 27 to 35, wherein the gel is a polyacrylamide gel.

37. The method according to any of claims 27 to 36, wherein the gel is a SDS gel or an isoelectric focusing gel.

38. The method according to any of claims 27 to 37, wherein the body of the multiwell template is divided into a first portion and a second portion, said first portion being shaped for insertion into the opening in the top plate and a second portion being tapered to a base for moving onto the gel.

39. The method according to claim 38, wherein the first and second portion are separated by a flange for supporting the multiwell template within the opening in the top plate.

40. The method according to any of claims 27 to 39, wherein said analyte is a peptide, protein, nucleic acid or carbohydrate.

41. The method according to any of claims 27 to 40, wherein the analyte has been subjected to a chromatographic or electrophoretic separation prior to moving the multiwell template onto the gel to form a plurality of wells between the gel and the one or more walls.

42. The method according to any of claims 27 to 40, wherein the analyte is subjected to a chromatographic or electrophoretic separation following moving the multiwell template onto the gel to form a plurality of wells between the gel and the one or more walls.

43. The method according to any of claims 27 to 42, further comprising the step of adding a liquid reagent to one or more of the plurality of wells so formed to form a liquid analyte reagent mixture.

44. The method according to claim 43, wherein said liquid reagent is added by manual or automated means.

45. The method according to either of claims 43 or 44, wherein the reagent can solublise the analyte or chemically modify the analyte or its environment.

46. The method according to any of claims 43 to 45, wherein the reagent is a protease enzyme.

47. The method according to any of claims 43 to 46, further comprising the steps of transferring the liquid analyte reagent mixture to a second vessel by either manual or automatic means.

48. The method of claim 47, wherein said second vessel is a well in a microtitre plate.

49. The method according to any of claims 43 to 48, wherein the reagent is added by an automatic liquid handling device and/or the liquid analyte reagent mixture is transferred by an automatic liquid handling device.

50. The method according to claim 49, wherein said automatic liquid handling device is under the control of a computer

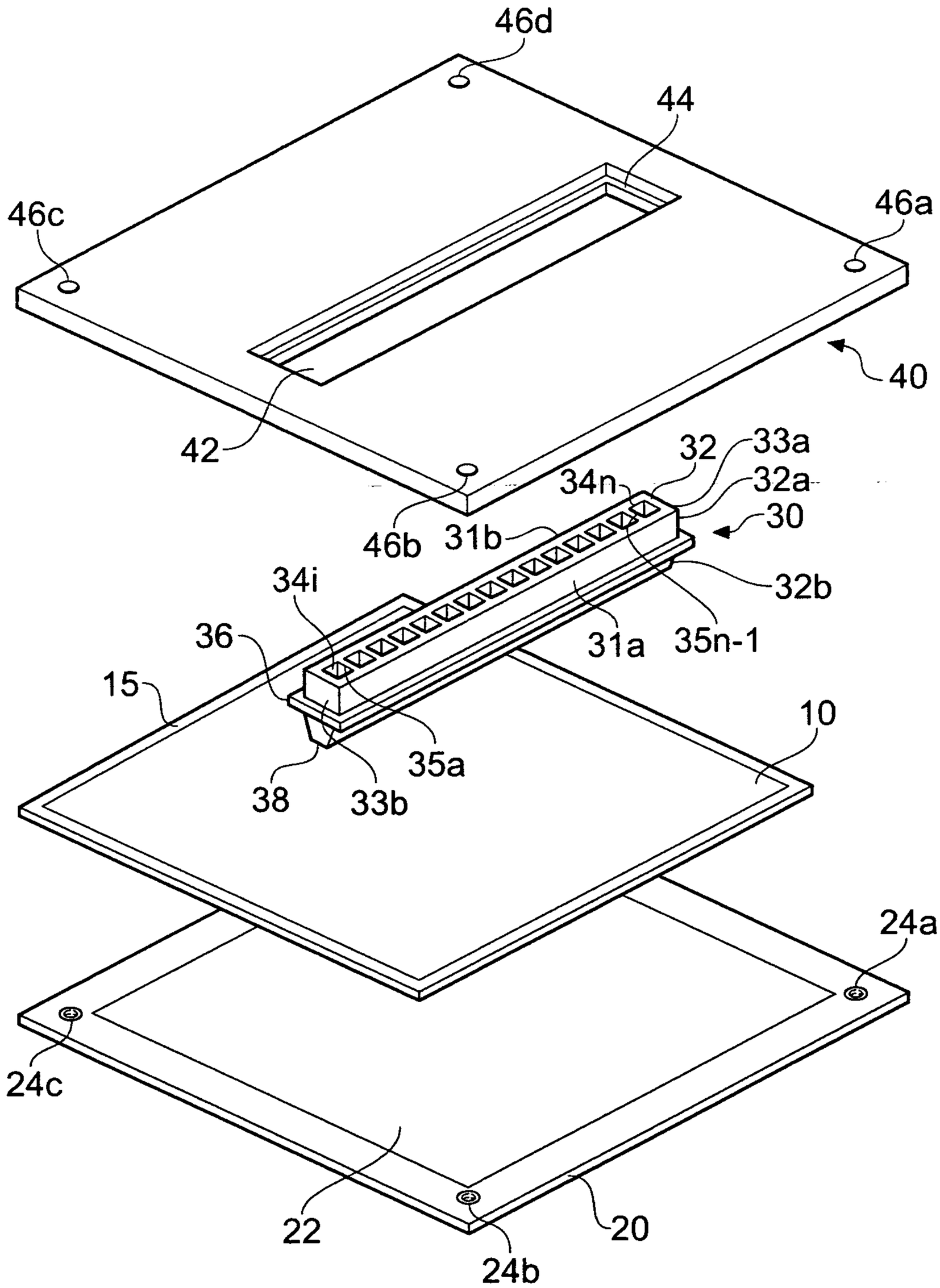
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Fig. 1

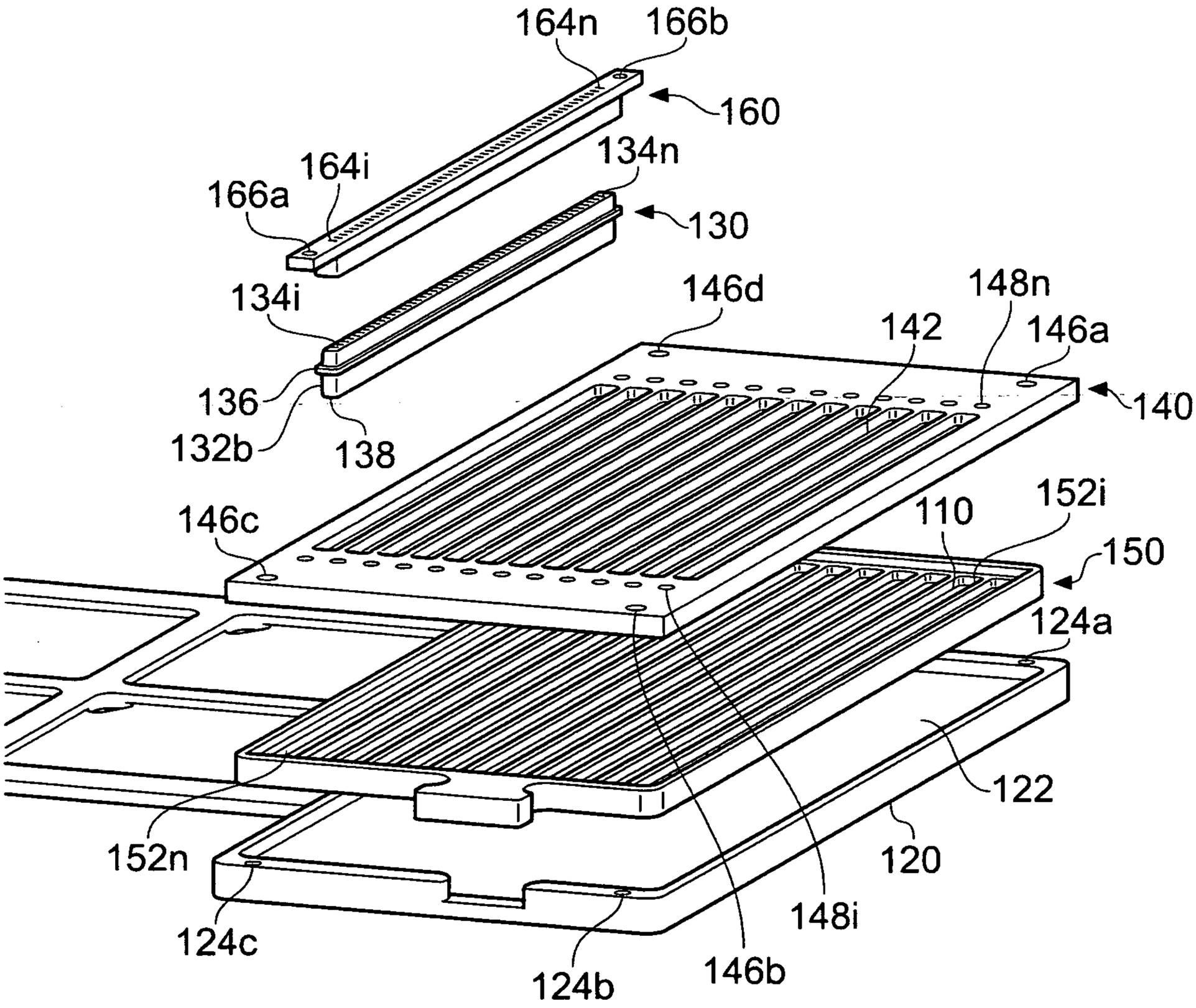
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Fig. 2

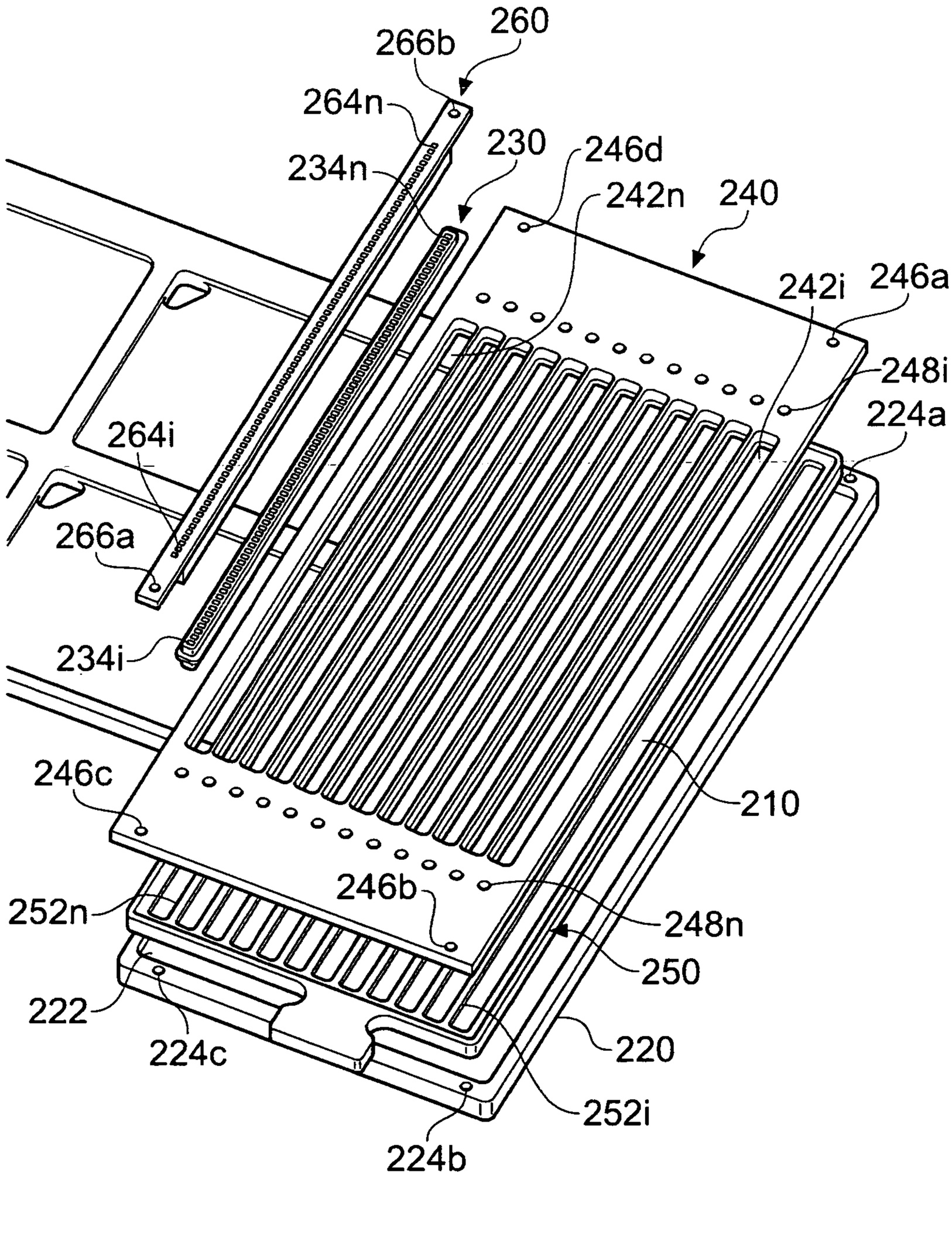
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Fig. 3

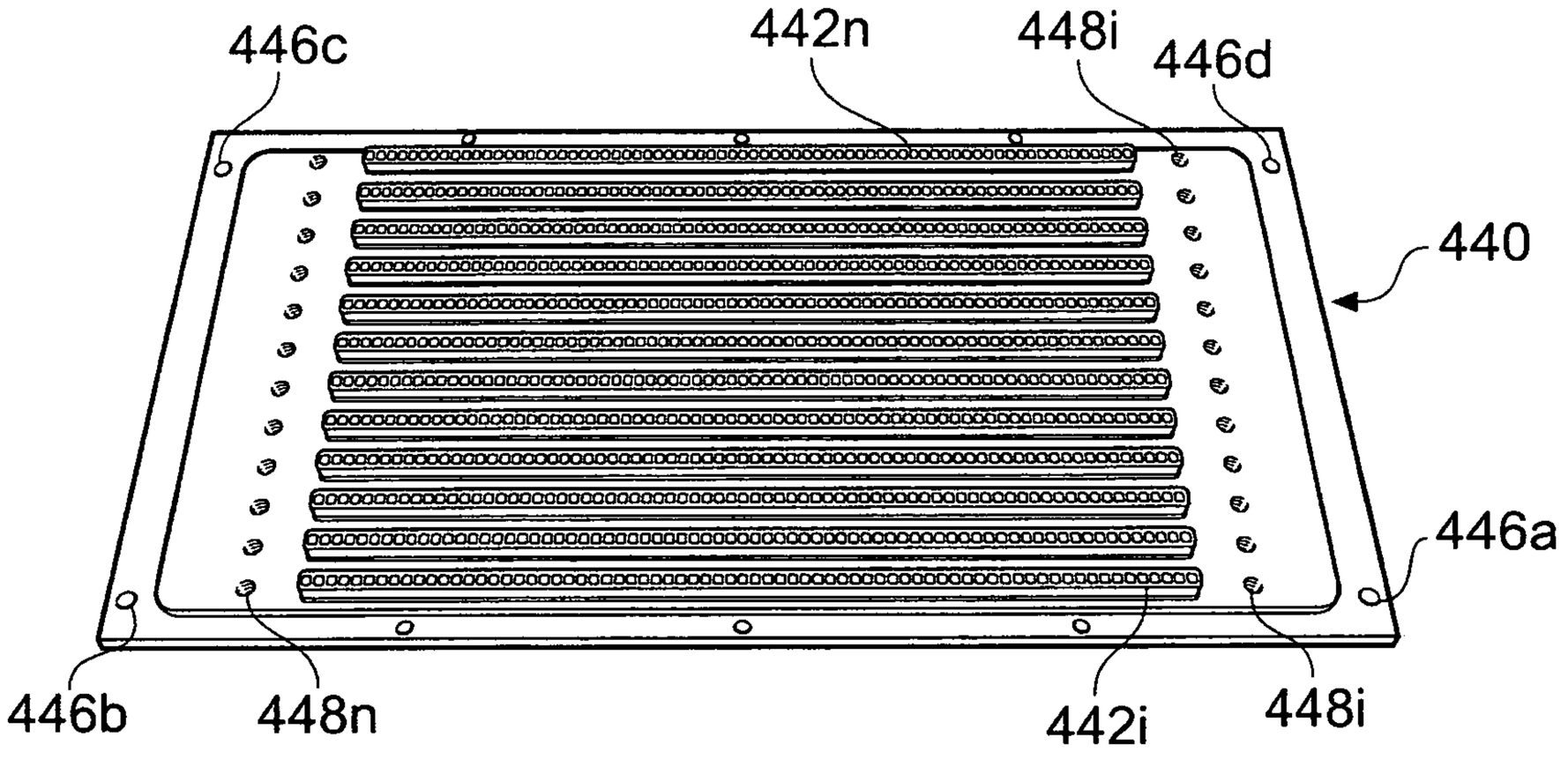


Fig. 5

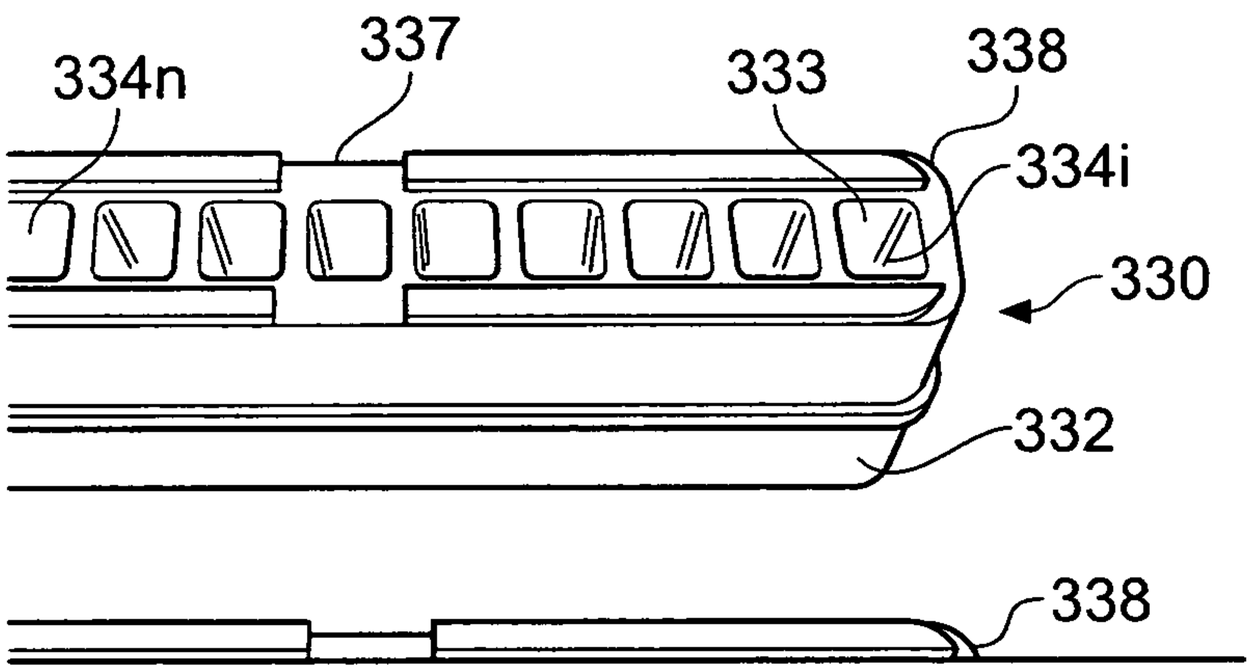


Fig. 4a

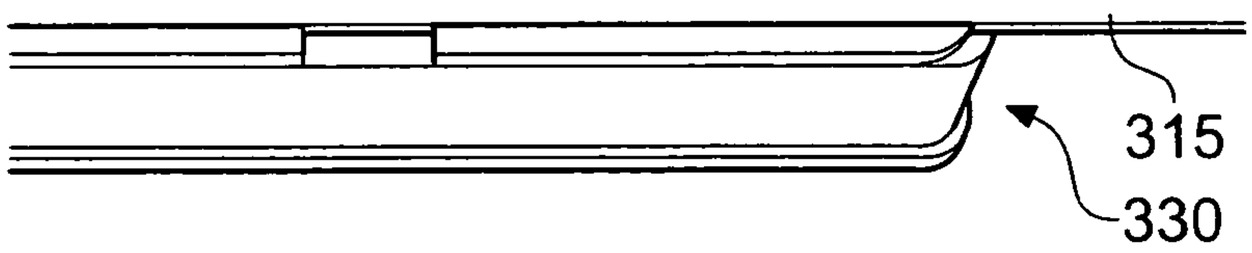


Fig. 4b

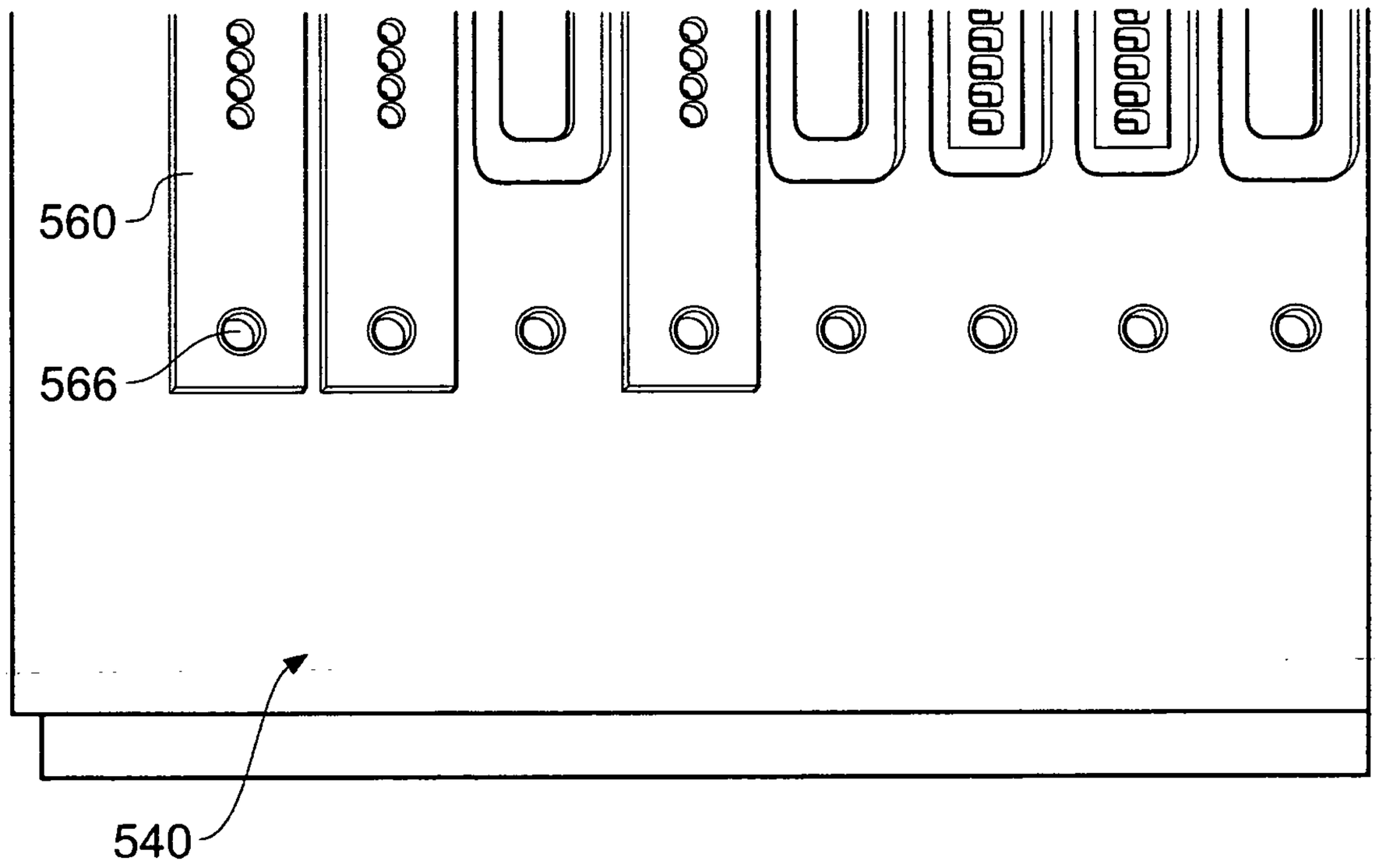


Fig. 6a

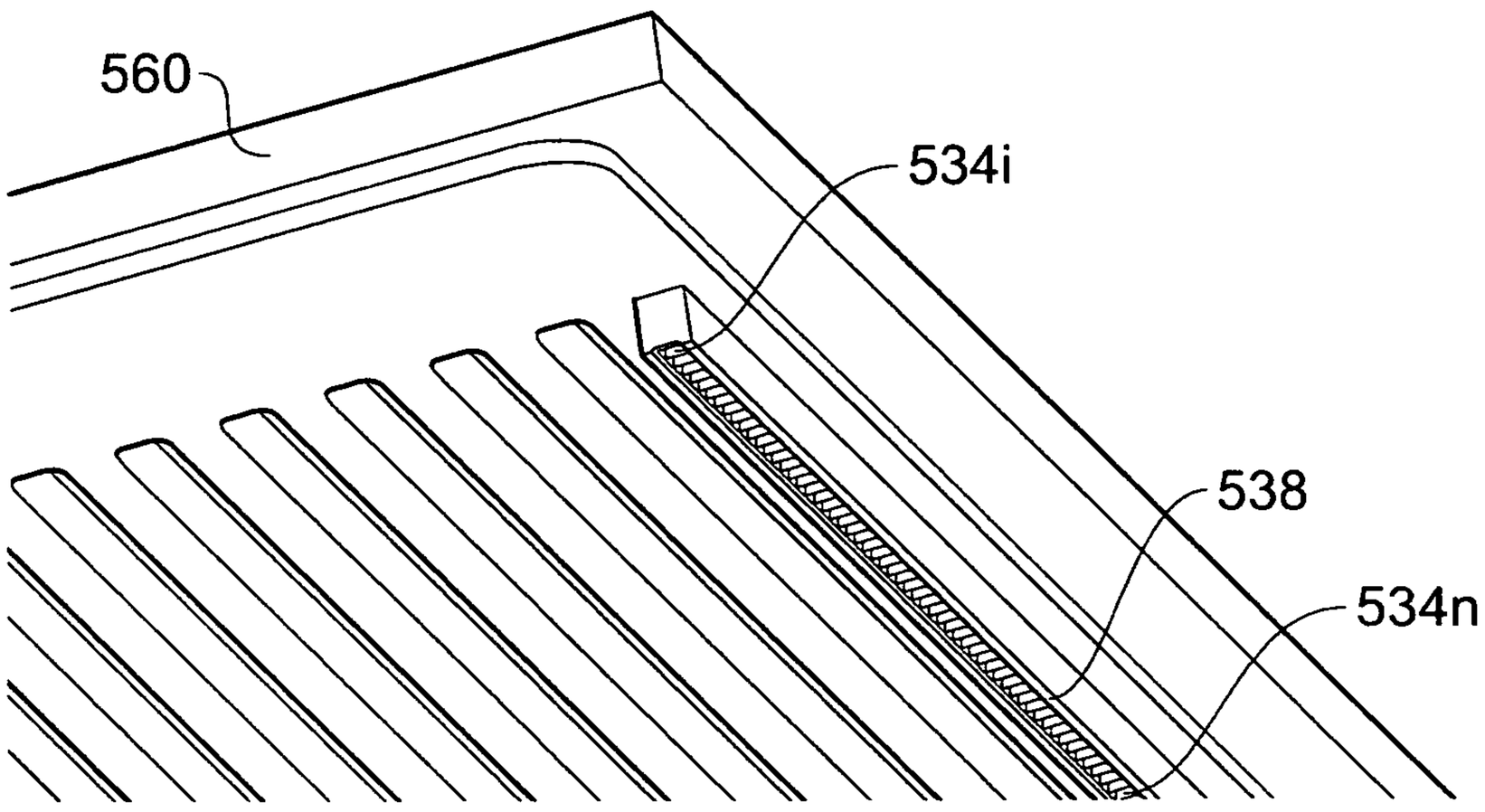


Fig. 6b

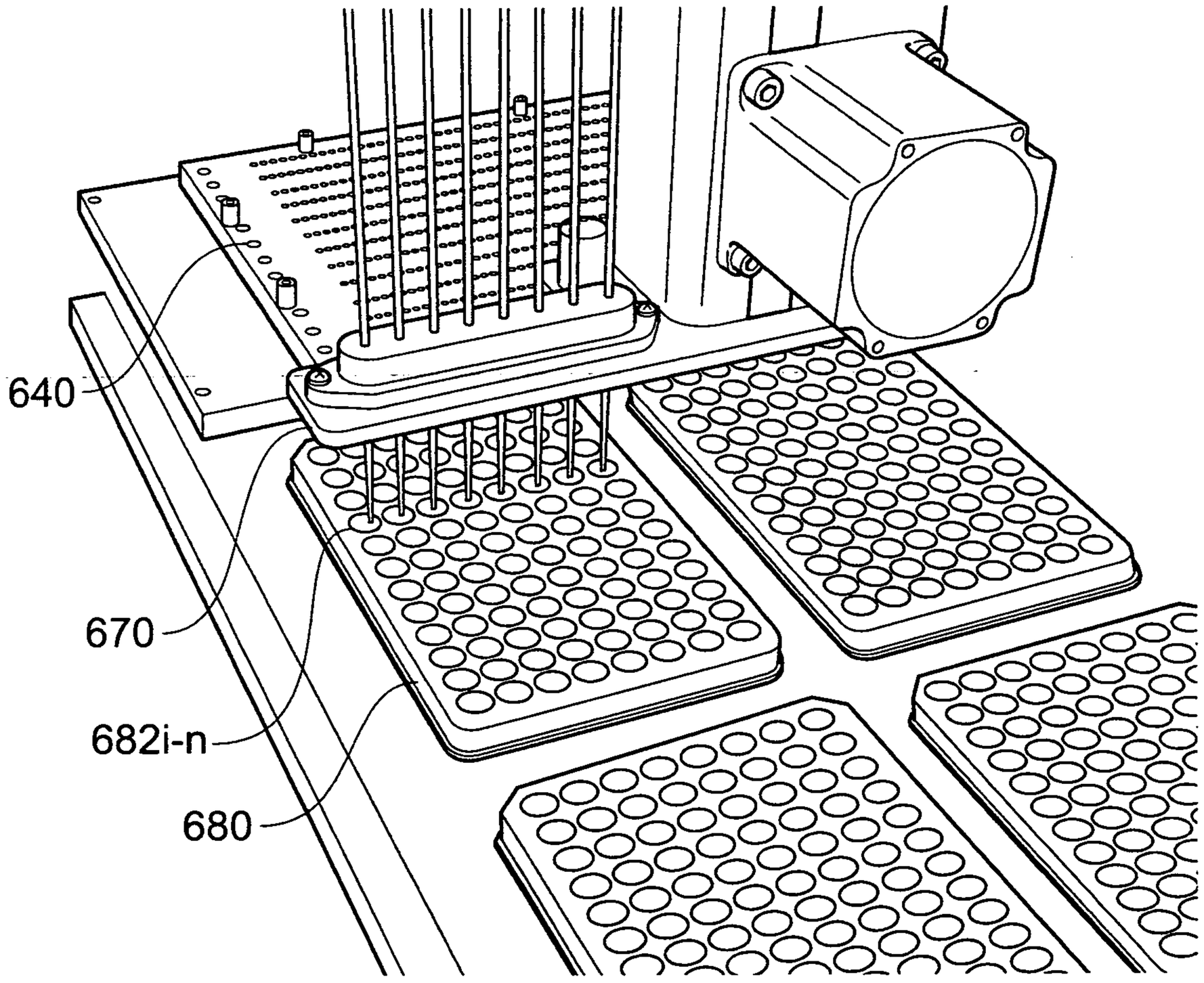
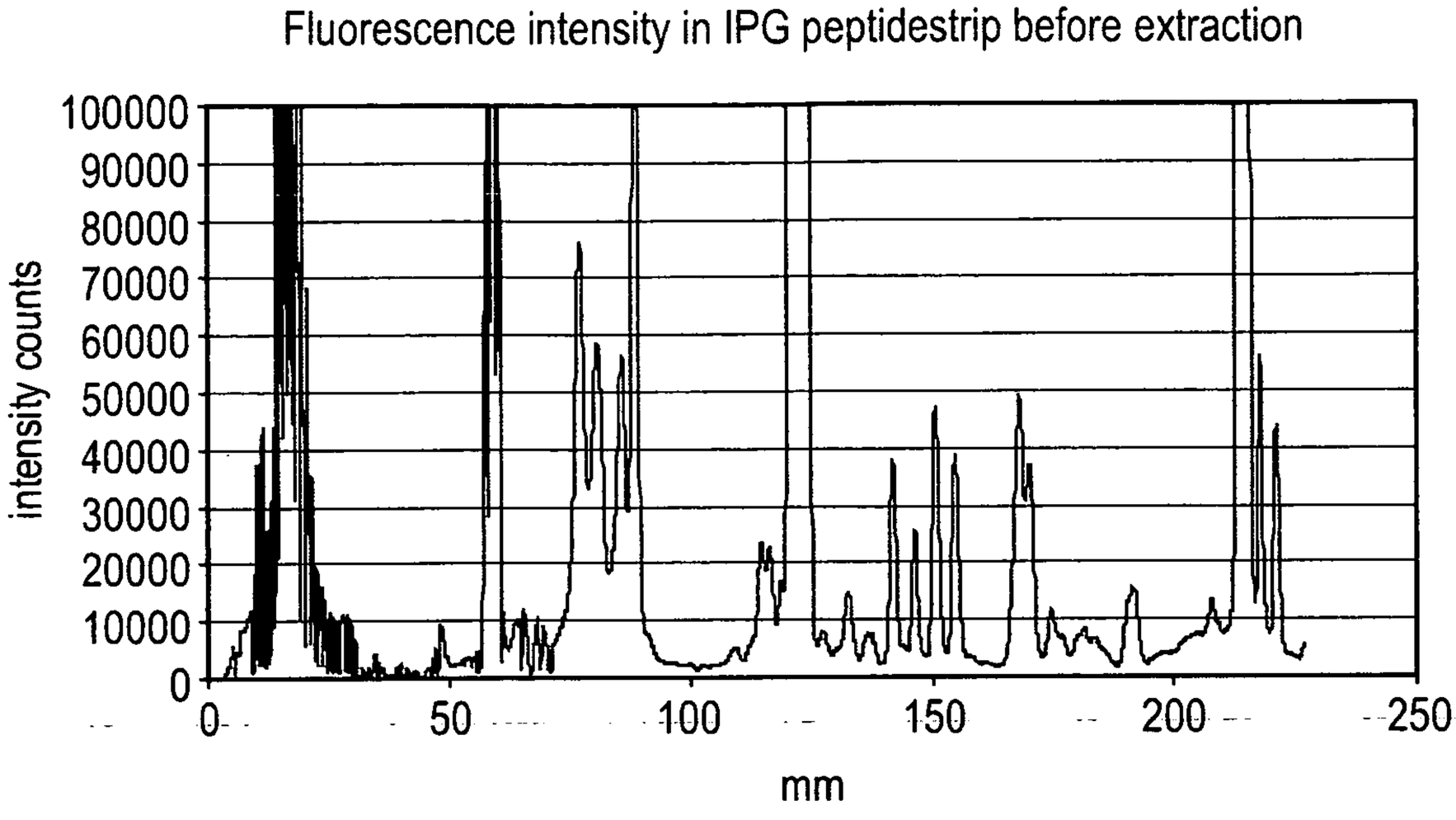


Fig. 7


Fluorescence intensity in IPG peptidestrip before extraction
intensity counts
100000
90000
80000
70000
60000
50000
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30000
20000
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50
100
150
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250
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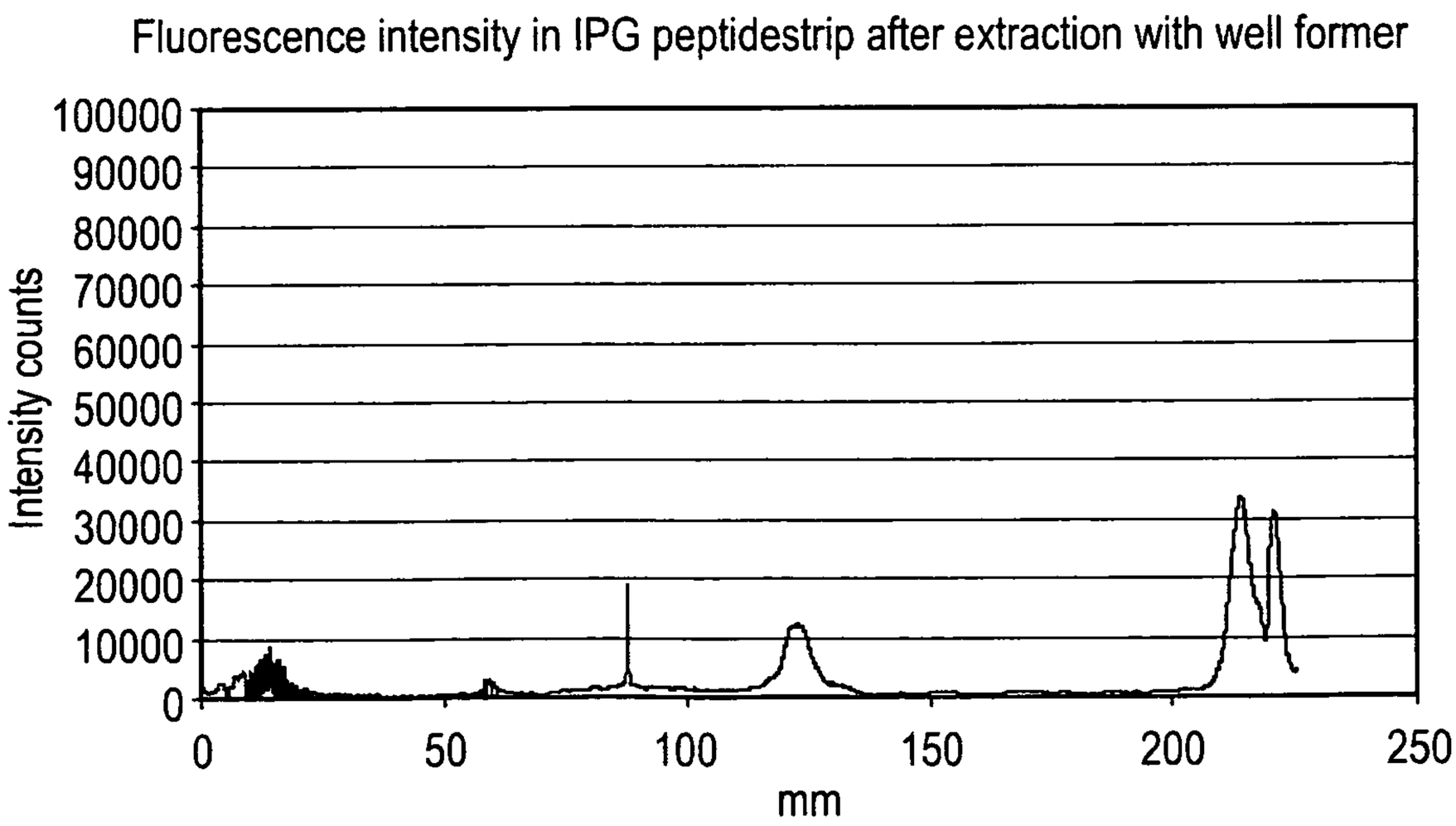
Fig. 8a


Fluorescence intensity in IPG peptidestrip after extraction with well former
Intensity counts
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50000
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Fig. 8b

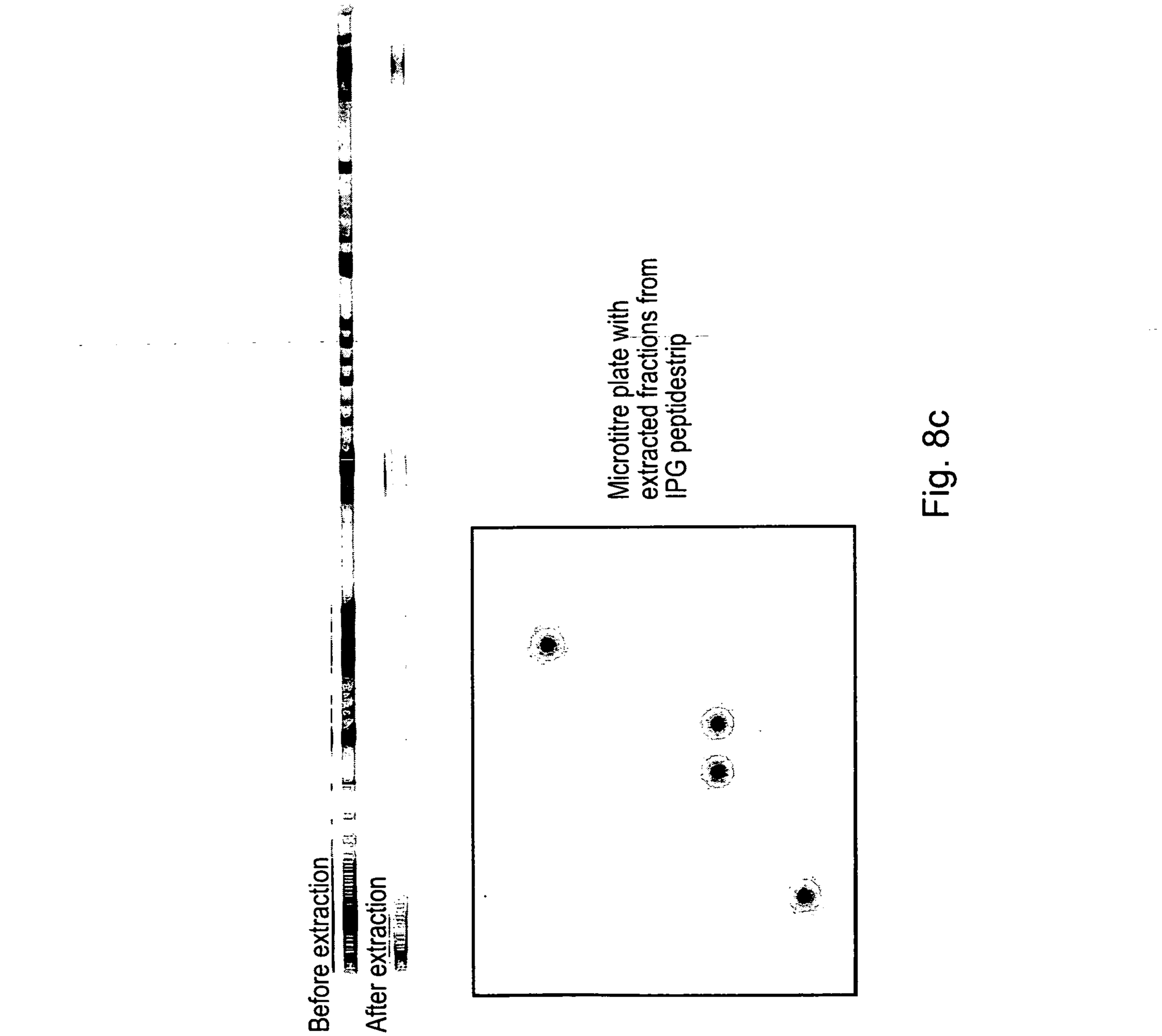
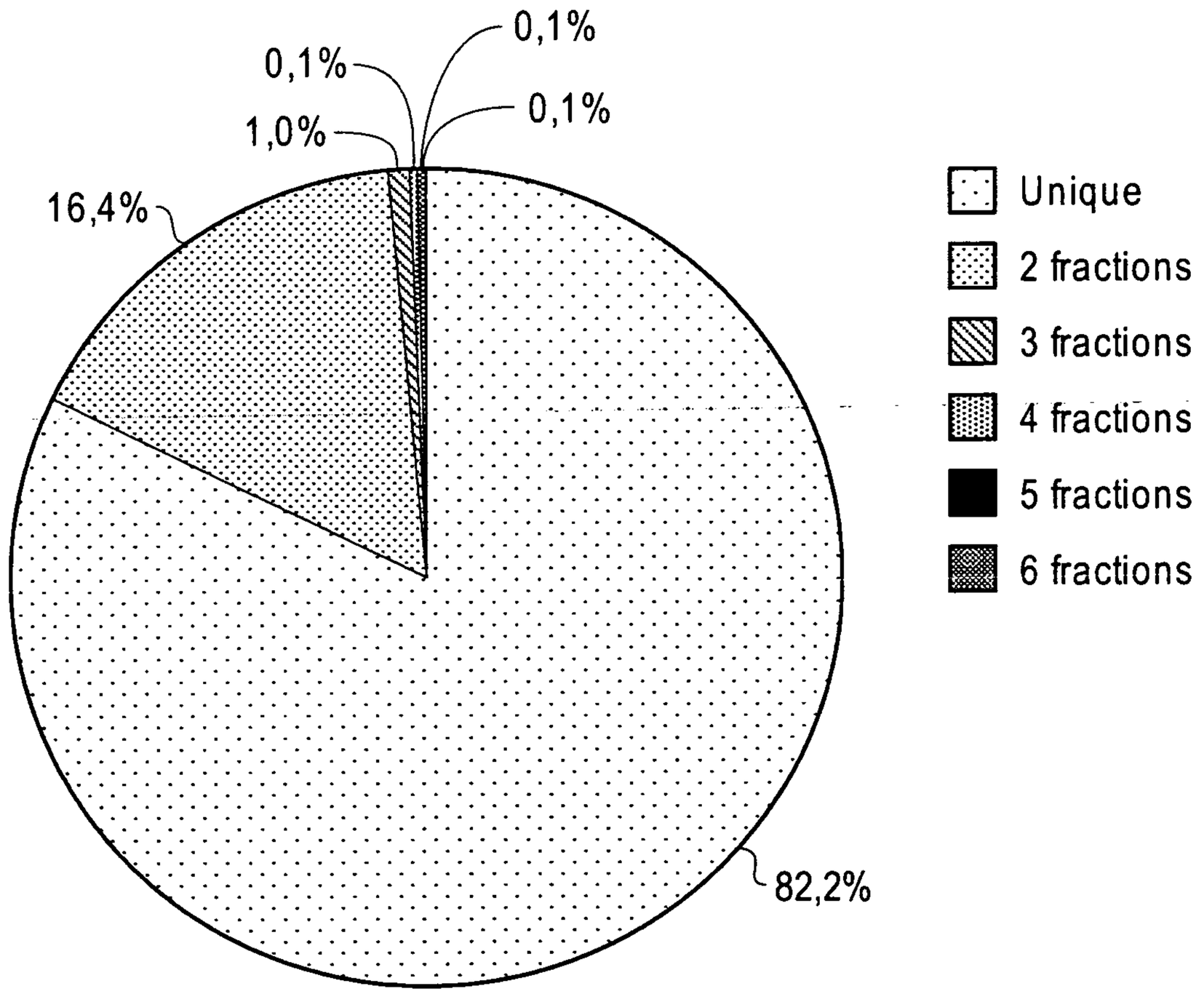

Microtitre plate with
extracted fractions from
IPG peptidestrip
Before extraction
After extraction

Fig. 8c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Unique
2 fractions
3 fractions
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5 fractions
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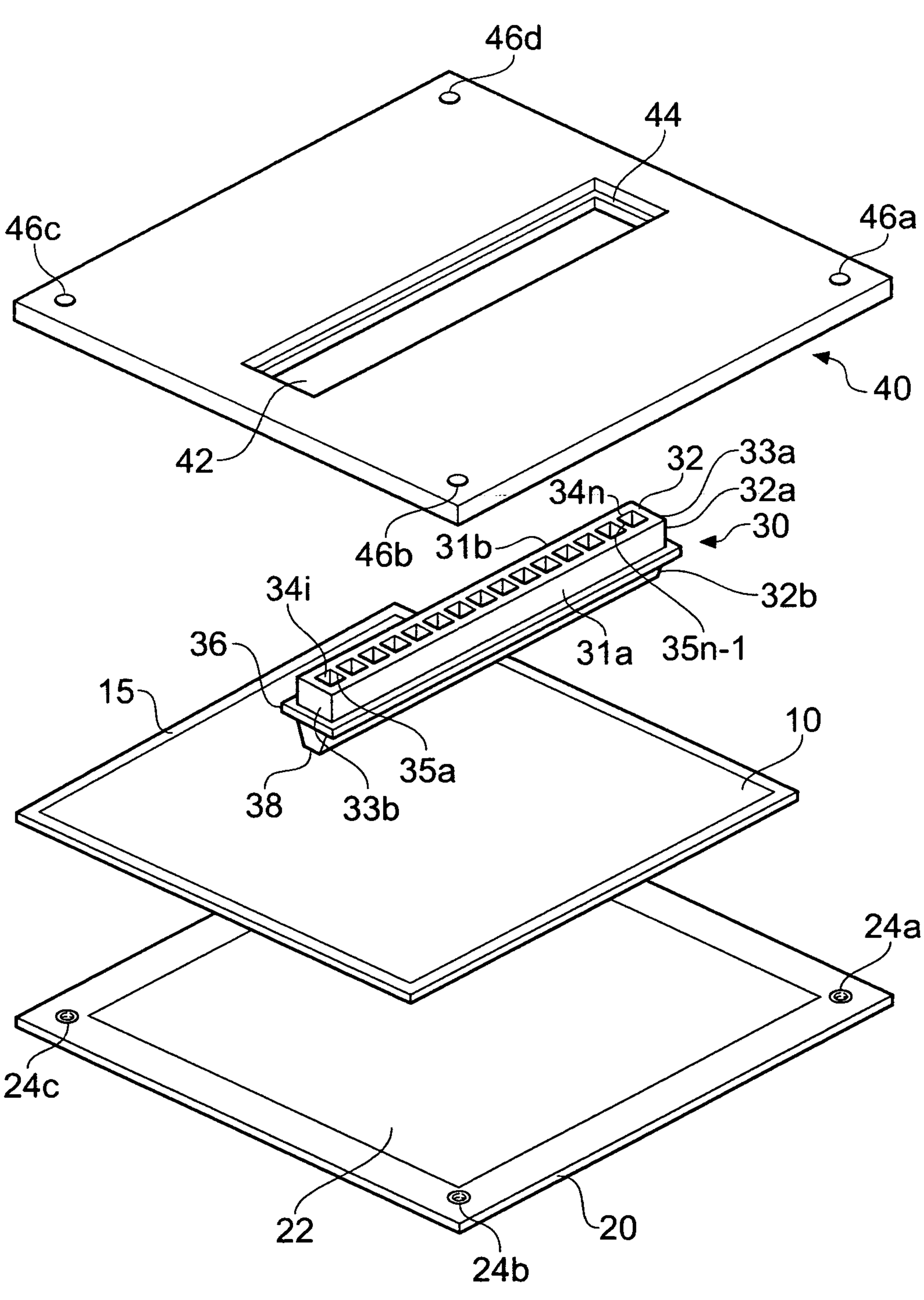
Fig. 9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