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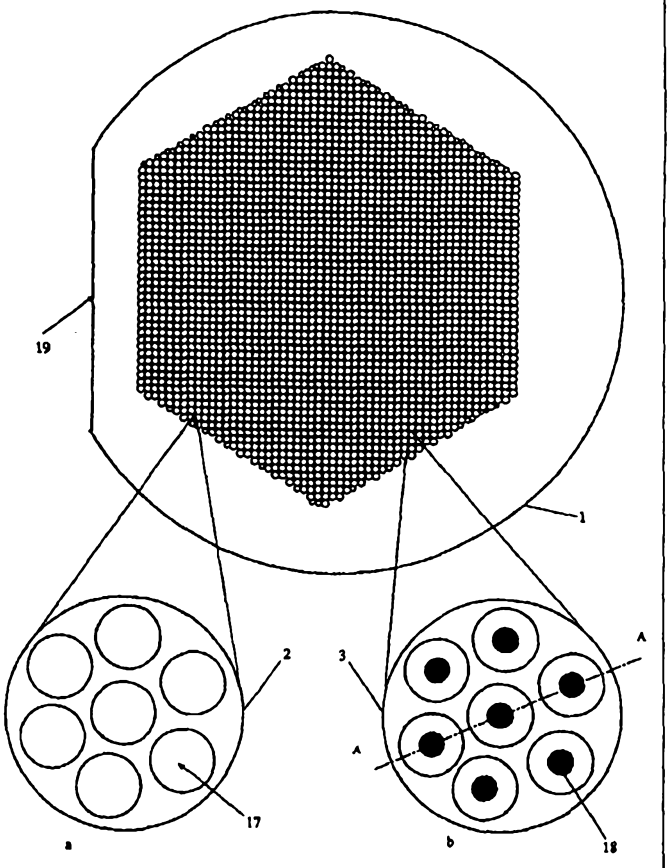
(54) Bezeichnung: KOMPAKTZELLKULTURSCHEIBE

(57) Abstract

An arrangement for cultivating cells and/or other biological samples comprises a plurality of very small chambers or compartments (17) preferably covered by transparent foils which are separate from one another and can be sealed off from the environment by a wall of given permeability.

(57) Zusammenfassung

Eine Anordnung zum Kultivieren von Zellen und/oder von anderen Bioproben umfasst eine Vielzahl von Kleinstkammern bzw. von mit vorzugsweise transparenten Folien überdeckten Weihern (17), welche voneinander getrennt sind und welche durch eine Wandung von wählbar definierter Permeabilität von der Umgebung verschliessbar sind.



Compact Cell Culture Disk

The present invention relates to an arrangement of habitats, separated from one another, of microscopic dimensions, a method for producing such arrangement as well as methods for investigations and measurements with the aid of this arrangement.

The arrangement described in the present invention of habitats of microscopic dimensions, laid out with microscopic precision on a planar support and separated from one another, is especially suited for the aseptic culture and observation of single cells and their monoclonal descendants as well as also for the culture and study of other small colonies such as bacteria, human, animal or plant cell populations, tissue cultures, mammalian or plant embryos, insects or nematodes at different developmental stages.

To do so, the cells or the other biological samples cited above are placed individually or in relevant combinations into a "well" disposed on a flat, precisely prepared base, for example a semiconductor, quartz, glass, synthetic or metal base, and covered with a thin semipermeable sheeting, which well is thin and of extremely small dimensions and which thus forms a sterile microlaboratory. Into each such well which forms a boxlike closable microchamber, concentrations, adapted to the particular emplaced culture, are added of, for example, buffer components, oxygen, nutrient substances, protective antibiotics, active substances such as pharmacological agents, analytically useful reagents such as for example fluorescent colouring agents etc, by means of penetration of culture media through the semipermeable closable cover sheeting. The supply of, for example, growth factors or other macromolecular medium-specific substances, depending on the selected pore size of the sheeting, can be ensured either before or after the closing of the chamber respectively the well. It is therein advantageous, for example to place patient sera and expensive biomolecule-preparations into the wells before closing it in order to minimise its use and to be able to control the concentration in the chambers individually.

The described arrangement provides good conditions for the individual handling and the microscopic observation of the cultures generated therein with respect to their individual properties for example for the controlled exposition in the presence of physical, chemical or other agents and for the optical and physicochemical monitoring and characterisation as well as for the final intravital or postmortal storage. This storage can take place for example through deep-freezing or after chemical fixing.

In genetics, microbiology, immunology, oncology, radiobiology, pharmacology, embryology, zoology and botany the need exists for the individual culture of individual cells or multicell samples, in order to observe the growth and the properties of the cultures developing therein, to document them or to preserve them alone or fixed. The invention therefore comprises, in addition to the cited arrangement, respectively its preparation, further the adaptation of the described structures and corresponding measuring parameters in view of embedding the new technology in the current biomedical and biotechnical research and development, as well as for the needs of the intended important applications in clinical diagnostic and therapeutic planning, for the "gene-technology engineering" and applied toxicology or environmental hygiene or pharmacology.

Because the cover sheeting of the wells can be impermeable to bacteria, viruses and other biological contaminants the microchambers present can be used as sterile laboratories for biological cells. A problem presents itself in the exchange of nutrients and other substances which is only limited via the cover sheeting. With the suggested construction and the tiny cell mass of the individual cultures this exchange problem can, however, be eliminated or reduced.

It is an object of the present invention to create an arrangement by means of which, to the highest extent independently and simultaneously a multiplicity of biological samples can be cultured and simultaneously be observed in an extremely simple manner. In the case of monoclonal cell populations chamber-like microlaboratories are created which permit especially favourable conditions for culture and analysis. Therewith, for example in the presence of genetic uniformity, the advantage is attained that from a tumour sample comprising a few hundred thousand or million cells, the reliable characterisation of a representative selection of cloned cells is made possible. By creating discrete, separate, individual places for at least approximately 1000 clones the object according to the invention ought to, for example, be solved in clinical oncology by creating the preconditions for the desired representativeness of the cited selection. It is understood that it should also be possible to apply the arrangement for the culture, study and storage of smaller quantities of cell populations down to a single cell in a miniature laboratory.

Further objects comprise being able to provide the arrangement in extremely simple manner with the individual cells and, during the culture and observation of the growth and the cell properties, to eliminate any undesired external influences on the uniformity of the growth conditions in the microchambers respectively in the discrete "wells".

A further object, on the other hand, comprises creating suitable measuring methods in order to record or evaluate the arrangements created according to the invention respectively the cells or biological cultures disposed therein during the growth phase.

The objects posed according to the invention are solved in particular by means of an arrangement for culturing cells and/or other biosamples, characterised by a multiplicity of microchambers formed by wells covered with films, which are separated one from the other and which can be closed against the environment by a wall of selectively defined permeability, wherein the microchambers or the wells covered by the wall are disposed on a disk-like planar, at least nearly smooth, base, and wherein the microchambers or wells are defined or formed by a sandwich structure of congruent disks, sheet-like elements or walls, matched with one another or adapted to each other, wherein for the formation of the microchambers or wells one or several of the disks, sheeting or walls have a honeycomb or matrix-like structure with perforations..

According to the invention an arrangement for culturing cells is proposed which comprises a multiplicity of microchambers or "wells" which are separated from one another and are disposed with microscopic precision for example in the form of honeycombs on a disk-like planar base. The microchambers are covered by a semipermeable wall or sheeting, ie. against biological contaminations of the environment. The chamber structure is typically a sandwich structure of parallel planar thin plates and/or in combinations selected for different application purposes. Plates or films disposed centrally in the sandwich structure are implemented such that they are perforated in

partially net-like or honeycomb-like manner for the formation of the microchambers. The base which can also be part of the "sandwich structure" is for example a disk formed by a thin silicon, quartz, glass or synthetic material structure. Depending on the number and size of the microchambers to be disposed on the base, the surface diameter of the base can be 5 to 20cm. With the selection of a circular base a possible embodiment variant of the arrangement is attained which can best be compared to a compact disk used in the audio and video field, and which can be scanned by means of, for example, microscopic or spectroscopic analysis of the microchambers.

In particular the covering of the microchambers for cell culture with defined permeability which can be selected or with accurately predetermined, selective permeability, is not described in the prior art. The covering should therefore be permeable not only to oxygen, as described for example in FR-2 693 739, but to many different kinds of materials, for example fluids or low-molecular constituents dissolved in solutions, should be able to be transported through the covering, in order to be capable of acting upon a cell culture in a controlled manner. In FR-2 693 739, the cells as well as all constituents in the chamber responsible for cell growth are fed in at the beginning, and the covering is to be merely oxygen-permeable, cell culture thus being facilitated anyway.

The microchambers, disposed for example in the form of honeycombs, can typically, however not necessarily, be implemented circularly with a diameter of approximately 0.5 to 5mm. Between the honeycomb structure defining the chamber geometry, which also determines the distribution, form and size of the chambers, and the disk-like base can, if advantageous, be disposed a thin transparent film or a thin layer of a transparent gel lacking any cell affinity, such as, for example, an agarose layer. It is understood that for the formation of this layer another suitable material can be used which is extremely inert with respect to cells or their cultures or has other properties.

On the bottom of each microchamber of the honeycomb structure or resting on the base or on the cited film or gel layer can be arranged a typically concentrically implemented surface with cell affinity, comprising a thin metal or molecule layer suitable for the culture of cells and having cell affinity, such as for example a concentrically implemented "palladium island", or comprising another functionally similar surface-forming material. The honeycomb structure itself or the chamber walls can be fabricated of a dimensionally stable material, such as for example synthetic material, metal, ceramic or a composite material. The semipermeable membrane, lastly, comprises advantageously a material, at least nearly transparent to light, such as Teflon, polycarbonate or polystyrene.

A preferred arrangement is characterised in that the base is formed by a thin plane-parallel disk of optically high and uniform quality which is preferably smooth. At least a portion of the chambers of wells is formed by a honeycomb-like grid structure or matrix resting on a base and fixedly connected with it, wherein the openings opposite to the base of chambers or of the grid interspaces are coverable or closable by means of a preferably semipermeable membrane or film, which membrane forms the wall with selectively defined permeability. The discrete chambers or wells are typically implemented circularly with a diameter of approximately 0.2 to 5mm and that at least a portion of the chambers is disposed in the form of a honeycomb structure. Preferably, the base is covered by means of a preferably transparent gel or polymer layer lacking cell affinity, on which the wells are

disposed. It is preferred that on the chamber bottom, resting on the base or the gel or polymer layer, one spot each implemented in the form of a point or island, comprising a thin layer suitable for the culture of cells is disposed. The grid structure or the chamber walls preferably comprise a rigid and dimensionally stable material such as polycarbonate, silicon, a metallic material, ceramic or a composite material. It is preferred that the wall of selectively defined permeability comprises a polymer material such as Teflon, polystyrene or polycarbonate, with selectively permeability properties. The circular disk preferably has a diameter of approximately 5 to 20cm with a central perforation, in order to dispose the disk on a holder of a microscope, measuring device or working device. The arrangement or structure is preferably held together in the manner of a sandwich by means of a magnet configuration and is disposed in a container such as a special steel box with openings transparent to light on one or both sides, in order to carry out measurements with the arrangement disposed in the container.

The individual process steps for the production of the arrangement according to the invention will be described in further detail with reference to the Figures described in the following.

In a further embodiment, the invention provides a process for producing an arrangement for culturing cells or other biosamples, characterised by the steps:

- formation of a grid or honeycomb-like structure comprising as interspaces or perforations the open culturing spaces for the individual cells or biosamples, ie. for the formation of the microlaboratories or of the wells
- application and connection of the grid or honeycomb-like structure on a semipermeable film or covering layer,
- after filling the discrete interspaces or perforations closed in the downward direction by the semipermeable film or covering layer with nutrients, sera, active agents and/or other liquid or dissolved or soluble components as well as placing the cells or biosamples into the discrete interspaces or perforations or chambers, application of a disk-like plate as base in order to close the perforations or interspaces opposite to the semipermeable film or covering layer, and rotation by 180° of the arrangement formed.

It is preferred that the semipermeable wall is fixedly connected with the honeycomb or grid-like structure by means of a silicon derivative or a UV-curing material and that the disk-like base is fabricated of silicon, quartz or glass, subsequently coated by means of a preferably transparent gel or polymer layer such as an agarose layer lacking cell affinity and subsequently provided, geometrically congruent with the interspaces or perforations of structure, with dot-form or island-form spots whereupon subsequently the base is connected congruently with the structure such that the spot in each instance comes to lie substantially centrally in the interspace or the perforation. The base and the structure are preferably fixedly connected one with the other by means of a aliphatic substance such as aliphatic silicone. It is preferred that the covering layer is placed onto a metal plate or foil provided with perforations, with the perforations corresponding geometrically to the interspaces or perforations of the structure, and subsequent to the application of the structure and the base a magnet is applied onto the base, opposite to the structure, in order to hold together the arrangement

due to the attraction of the metal plate or foil through the magnets, whereupon lastly the arrangement is rotated by 180°.

The present invention or arrangement, also referred to as Compact Cell Culture Disk (CCCD) permits the microscopic, molecular biological, biochemical and physicochemical studies of individual cells or cell or multicell structures, in small as well as in large number, ie. from a single cell to typically a few 1000 cells under physically, chemically or biologically optimised conditions. Implanted into the suggested cell culture arrangement together with biological materials a microlaboratory is created which permits the control, observation and analysis of the individual cultures or their formation, with the control of the environment being possible separately or *in toto*. The entire arrangement as well as also the individual chambers, or miniature microlaboratories can be kept under sterile or nonsterile conditions for short periods of time as well as also for long periods of time as well as also for permanent storage, transport or for observation purposes, for example under a microscope or with spectroscopic methods, for example by utilising fibre-optic waveguides, photomultipliers or photodiodes.

Lastly, a process is suggested for measuring or recording, evaluating and potentially for storing information regarding the cells or cell culture clones implanted or deposited in the arrangement, defined according to the invention, and other above listed biosamples. These are preferably automatic, electronic data processing-supported processes which can increase the implantation and analysis capacity as well as the speed, in connection with manual and visual techniques.

The invention will now be described in further detail by example and with reference to the enclosed Figures. Therein depict:

Fig. 1 an example of a disk-like arrangement according to the invention in top view,

Fig. 1a, b enlarged detail from the arrangement of Figure 1,

Fig. 2 schematically and enlarged different, typically used, individual elements or components of a sandwich-like arrangement according to the invention in cross section,

Fig. 2a in cross section a possible embodiment variant of an arrangement in the assembled state as well as in exploded representation,

Fig. 2b a further embodiment variant of an arrangement according to the invention in cross section assembled as well as in exploded representation,

Fig. 2c again a further possible embodiment variant of an arrangement according to the invention in cross section, assembled as well as in exploded representation,

Fig. 3a depicted schematically, a possible *in situ* measuring method applied to an arrangement according to the invention in cross section,

Fig. 3b depicted schematically, a further measuring method of killed material and with covered arrangement in cross section,

Fig. 4a depicted schematically in cross section, an embodiment variant of the arrangement according to the invention,

Fig. 4b depicted schematically in cross section, a further embodiment variant of an arrangement according to the invention held together by magnetic fields,

Fig. 5a depicted schematically in top view a disk-like circularly implemented arrangement according to the invention,

Fig. 5b a further embodiment variant in top view implemented analogously to a compact disk, and

Fig. 6 depicted schematically in perspective a possible application of the arrangement according to the invention for investigating the biological effectiveness of various types of radiation into an exposed body as a function of the coordinates in a sample system.

Figure 1 shows in top view an example of a "compact cell culture disk", or cell culture plate with microchambers in two embodiment variants shown in Figures 1a and 1b. The structure of this disk 1 will be explained in further detail with reference to the succeeding Figures 2 and following. In the centre region of the disk 1 are disposed the microchambers or microlaboratories 17 or 18, defined according to the invention, each comprising a well closable toward the outside, and in each microchamber or in each well can be arranged centrally a cell or a cell culture to be cultured. For reasons of manufacturing technology the disk 1 lastly comprises in the margin region a recess or notch 19, the purpose of which is also evident in the following description.

The arrangement of the microchambers 17 depicted in Figure 1 represents only an example and it is understood that it is possible to arrange the microchambers 11 in any other desired manner on disk 1 as will become evident from the Figures 5a and 5b described in the following.

In the two Figures 1a and 1b in a detail of disk 1 of Figure 1 are shown a number of microchambers 17 and in the microchambers in Figures 1b surfaces 18 are provided in the centre having cell affinity, which are absent in the microchambers according to Figure 1a. Such surfaces of different type, location and implementation and at different positions or other cell-affinitive structures, such as free flowing particles, can also be used.

With reference to Figure 2 as well as 2a to 2c different embodiment variants of possible arrangements according to the invention are shown in cross section with Figure 2 showing schematically the components or individual elements designated for the structure of the described arrangements. For the structure of the arrangements are to be provided, as shown in Figure 2, a metal, ceramic or synthetic material sheeting, implemented for example to be perforated honeycomb-like or hole-like, as well as a semipermeable wall 5 which can comprise for example Teflon, polystyrene or any other suitable material preferably transparent to light. For the formation of the microchambers or microlaboratories proper a grid-like or honeycomb-like matrix 6 is used, which is formed of a dimensionally stable material such as for example synthetic material, metal, ceramic or a composite material or reinforced duromers. But this matrix can also be fabricated of a silicon disk treated by means of oxidation or etching, such as denoted in Figure 2 by the reference number 8. In this case the oxidised or etched silicon matrix is preferably arranged with a transparent quartz layer.

As base for the matrix 6 can be used for example a silicon disk 7 which is provided with cell-affinitive surfaces 18 on which the cells to be cultured can be disposed. This base can, in turn, be part of a silicon disk which is treated by means of oxidation and etching, as, for example, the disk in Figure 2 denoted by 8 already mentioned above. But this base 7 can, instead of from silicon, also be produced of quartz or glass or of another suitable material. This silicon base is preferably covered

with a thin agarose polymer layer, resting on which agarose layer the cell-affinitive spots 18 are disposed, comprising, for example, palladium. In contrast to palladium, agarose does not have any cell affinity. The cited palladium spots are in particular suitable for the culture and cloning of individual cells. The palladium island technique will not be further discussed here since it is well known within relevant prior art for the optimum culture of individual cells. Reference is only made to the literature site: U. Amaldi, B. Larsson, Hadrontherapy in Oncology, Proceedings of the First International Symposium on Hadrontherapy, Como, Italy, 18 - 21 October 1993, Excerpta Medica, International Congress Series 1077, 1994, Elsevier, pp. 735. It is understood that other similar processes can also be used for this purpose.

Depending on the arrangement selected it is possible to hold the sandwich structure together magnetically, which is the reason for providing a magnet disk 9. This magnet disk can, as denoted by reference symbol 9, be flat or perforated in the form of a honeycomb or hole, as indicated at 10. The latter magnet disk is necessary if, for example, through this magnet disk optical analyses are being performed.

For the secure storing, handling and subsequent transport of the sandwich-like arrangement or analysis structure according to the invention, a container 11 is provided which, for example, can be a special steel box with observation windows provided on at least one side.

In Figure 2a a container 11, comprising an arrangement 12, is shown in cross section. For better understanding, the arrangement 12 is shown in exploded view, which clearly shows the individual components used from Figure 2. The structure or arrangement 12 correswells to the section along line A-A from Figure 1b.

The structure or arrangement 12 comprises primarily a silicon disk in the manner of a honeycomb or perforated, which can be combined with a quartz sheeting or quartz disk 8. The perforated openings of the silicon disk 8 can be obtained through oxidation or etching from a silicon disk. The quartz surface is preferably covered with an agarose polymer layer which material does not have any affinity for cells. The structure according to Figure 2a comprises further a membrane 5 covering the honeycombs as well as a nickel foil 4 implemented in the form of a honeycomb, wherein the honeycomb structure is congruent with that of the silicon disk 8. In order to hold together the structure or arrangement according to the invention, opposite to the silicon honeycomb on the quartz layer a magnet disk 10 is disposed which, again, is provided with corresponding openings congruent with the silicon honeycomb, Through the arrangement of the metal foil and the magnet disk the arrangement is held together by magnetic forces. For the secure storing, lastly, the arrangement 12 is inserted into a container 11, for example comprising special steel, and stored therein. This special steel box can be provided on one side or both sides with window-like openings which, again, are congruent with the silicon honeycomb structure. These window-like openings serve for the purpose of being able to analyse or observe the arrangement or structure or the cells or cell cultures disposed therein through suitable analytical instruments 21 or 21a. As shown in particular through the assembled structure as example of a cell-affinitive structure, the discrete chambers comprise on the quartz surface in the centre a palladium island 18.

On this palladium island can adhere a single cell 23 whereupon the culture of the cell commences. The discrete chambers are mixed with various liquids, such as sera, nutrient substances, active agents, etc. Since the structure is covered or closed by means of the semipermeable wall 5, it is possible to add further substances to the chambers during the culture process. This semipermeable wall comprises for example Teflon, polystyrene or another suitable transparent material.

In Figure 2b again, assembled as well as also in exploded view, a further arrangement according to the invention is shown schematically in cross section and in detail. Again, the detail corresponds approximately to section A-A from Figure 1b, however, in Figure 2b no cells are placed into the structure. In the arrangement or structure according to Figure 2b onto a silicon disk 7 a matrix 6 is placed comprising, for example, polycarbonate. Again, the matrix is covered by a membrane 5, and the entire arrangement is held together by means of magnetic forces through a metal foil and magnet disk. For the storage of the arrangement again a container 11 is provided, however, in the example according to Figure 2b, the analytical instruments are provided from above through the cover of the container 11 by means of, for example, a measuring device 21. In the present example it is advantageous if, again, the silicon disk is provided by means of an agarose layer. Again, on the agarose layer are provided palladium islands 18 for emplacing the corresponding cells.

It is understood that it is possible, in the case of structure 12 according to Figure 2a as well as also in the structure 13 according to Figure 2b, to select highly different dimensions in the realisation of the discrete microchambers, however, the following dimensions have proven to be for example suitable:

Diameter of the silicon disk:	ca. 10-20cm;
Thickness of the silicon disk:	0.3-1mm;
Thickness of agarose layer:	<10µm;
Inner diameter of the microchambers 11, preferably implemented circularly:	ca. 0.5-5mm;
Wall thickness of honeycomb structure between discrete chambers:	ca. 0.2mm;
Height of walls of honeycomb structure:	ca. 0.1-2mm;
Diameter of palladium island:	ca. 0.3mm;
Thickness of polymer cover layer or semipermeable membrane, comprising for example Teflon:	<10µm.

In Figure 2c is again shown schematically a detail in cross section of a further possible embodiment variant of an arrangement 14 according to the invention, provided for example for carrying out measurements, for observation or for analytical measuring methods through the bottom of the container 11. The structure comprises again a silicon quartz disk 8, a metal foil 4 as well as a membrane 5 disposed on the bottom of the container 11. The structure is covered by a magnet disk 9 which simultaneously forms the cover of the container or of the special steel box 11.

The compact cell culture arrangements or structures 12 to 14, depicted according to the invention, comprising the chamber structures described in detail in Figures 2a to 2c, are especially suitable for the investigations of tumours containing a multiplicity of different cells. Therein, first the cell mass of a tumour sample (biopsy) is divided, dispersed into single cells. Practice has shown that it is necessary to investigate a relatively large number of these single cells. Such sample comprises as a rule approximately 100 000 to 1 000 000 single cells, which is why it is desirable to study at least

several 1000 of a discrete sample in order to obtain a significant view of the tumour biopsy. The separation and dispersion of such a cell biopsy is well known.

The individual cells, obtained through the cited dispersion, are now placed into the discrete, not yet closed, wells 17. Lastly, the honeycomb structure is closed toward the outside by means of the semipermeable wall 5, and specifically toward the cited palladium island 18. After filling and closing chambers 17, as a rule, standardised low-molecular growth components or nutrients are added through the cover sheeting. The advantage of the arrangement or structure described according to the invention lies therein that it comprises a multiplicity of compact, biologically sterile microlaboratories which offers the following feasibilities:

1. The implantation of any desired number of single cells to be cultured (from a sample of several 1000) into discrete self-contained chambers which can subsequently be observed and analysed visually as well as with instruments.

2. Morphological or spectroscopic investigations of the cultures and the media enveloping them under optimum optical conditions by means of upright or inverted, conventional or confocal laser scanning microscopy.

3. The capability of adding standardised low-molecular medium components, such as buffer, nutrients or antibiotics.

4. Elimination of waste products, produced by the living and growing cell through the semipermeable wall.

5. Supplying the discrete chambers with respiratory or other gases, again through the semipermeable wall.

6. Continuous or temporary addition of low-molecular active substances such as pharmacological agents or toxic environmental factors.

7. Control of biochemical and, if relevant, of the microbiological environment individually for each culture at the time of the implantation or at any time by means of microinjection through the cover sheeting.

8. Jointly or differentially influencing the nonchemical test modalities, such as ionising or nonionising radiation at any time by means of external exposure.

9. Joint or differentiable temperature control.

10. Sterile conditions for the cultures during the storage, transport, handling, during the analyses and the observation in nonsterile environments.

In Figures 2a to 2c is shown schematically a microscope or other optical device 21 or 21a in order to demonstrate the way in which cells in a discrete chamber can be observed or analysed during the investigation. It was found for example that healthy cells form "monolayers" which can be observed in simple manner with conventional microscopy while transformed cells ("tumour cells") can form a "multilayer" structure which can be observed or analysed better by means of a confocal microscope.

The realisation of the compact cell culture plate defined according to the invention is based on a novel construction principle of cell culture microchambers and the configuration of such chambers with which limited as well as also large-scale manual or automatic isolation, culture, characterisation

and archiving of individual living cells, cell populations or structured systems of cell, such as small multicellular organism or tumour or tissue explants, become possible.

The compact cell culture plate described according to the invention, constructed in the manner of a sandwich on simple disk-like components, entails enormous application and handling advantages. For one, it is relatively small and compact, and, for another, an extremely great multiplicity of information is stored on it, which can be analysed or called up with high precision in an extremely simple way. For example, such cell culture plate or disk can be inserted analogously to a compact disk into an analytical device or processing device, and a specific cell chamber can be precisely analysed or influenced specifically by means of precisely defined coordinates. In this way the many microlaboratories can be scanned relatively rapidly, in any desired way or systematically, for example, by the stepwise radial and azimuthal movement of the disk.

A multiplicity of applications is evident for the compact cell culture plate claimed according to the invention, such as for example in molecular or cellular genetics, in microbiology, in tumour biology, toxicology, pharmacology and radiobiology. The "compact cell culture disk" (CCCD) is suitable for automated routine applications in biotechnology, clinical medicine or environmental technology and is applied, for example, in the field of teaching. The strongly increased capacity, precision, reproducibility and asepsis permits specifically in manual as well as also computer-controlled processes carrying out "cell cloning", ie. isolation, culture, characterisation and archiving of individual clones.

In Figures 3a and 3b are schematically indicated two possible measuring or analytical methods according to which the cell or cell cultures to be cultured or having been cultured can be observed or analysed. Figure 3a shows schematically in cross section an arrangement 1 according to the invention with the microchambers or laboratories 17 accessible for measurements from above. The observation or analysis of the discrete cell spots 23 consequently takes place *in situ* during the culture process, for example by means of an optical device 21, and it is understood that other analytical devices can also be used. This measuring method is advantageously applicable during the culture process since it can be repeated any number of times. The entire compact cell culture plate or disk 1 is therein scanned in order to scan a specific selection or all chambers 17 provided in the arrangement. In Figure 3b, in contrast, for conclusive highly precise measurement or for the clearing of the material, the entire covering of the original structure has been removed and the cell cultures or the cultured biosamples are present in the form of dead, fixed, optionally freeze-dried or incinerated material 25. Measurement or analysis of the killed material takes place by means of photon or neutron radiation incident in the direction of the indicated arrow. The great advantage of this measuring method lies therein that it provides, on the one hand, highly precise results and, on the other hand, due to the nearly parallel impingement of the rays on the material to be investigated, no scattering onto the subjacent base, such as for example the silicon disk, takes place. Measurements by means of photons of other particle radiation or X-ray radiation or thermal neutrons is well known within prior art.

In contrast to the microchamber structures or arrangements 12 to 14 depicted sectionally in Figures 2a to 2c, which are held together in the form of a sandwich by means of magnetic forces, it is

understood that it is also possible to produce such arrangements or structures in conventional ways by means of affixing them by adhesion. This is in particular necessary whenever, as shown in section and schematically in Figure 4b, the magnetic fields occurring therein are perceived as a disturbance to the culture of cells. It can potentially also be of disadvantage if the sandwich structure is held together by means of magnetic fields and the requirement potentially exists for that reason of producing a compact cell culture plate or an arrangement or structure according to the invention, as shown schematically in Figure 4a, ie. without any magnetic fields being present at all. Such compact cell culture plate according to the invention can preferably be produced according to the process steps described in the following:

1. Creation of the honeycomb-like structure or matrix 6 of a relatively rigid material, such as for example polycarbonate, silicon, ceramic or Teflon-coated nickel with preferably circular perforations which are provided for the formation of the microchambers 17. High packing density is preferably selected therein so that on minimum space the largest possible number of microchambers 17 can be formed.

2. This grid- or honeycomb-like structure or matrix is affixed by adhesion on a thin polymer film 5 (membrane), for example comprising Teflon or polystyrene, and as the substance for adhesion, a silicone derivative or a UV-curing polymer can be used for example. The polymer film forms the above described, preferably semipermeable, covering 5 of desired constitution of the discrete chambers 17.

3. Filling of the discrete wells 17 with nutrient fluids, sera, active agents etc.

4. Placing the individual cells 13 into the chambers 11 open toward the top.

5. Onto a silicon wafer or a glass plate, for example having a diameter of approximately 10cm, initially a thin polymer layer, for example comprising agarose, is placed. In agreement with the honeycomb structure, on the silicon wafer or on the agarose layer the palladium islands or other structures can be applied or placed.

6. The silicon wafer produced in this way is placed onto the above cited honeycomb structure, and the honeycomb structure as well as the silicon wafer comprise a marking or a notch 19 (Figure 1) so that the silicon wafer is placed congruently onto the honeycomb structure. The affixing by adhesion of the silicon wafer with the honeycomb structure takes place for example by means of aliphatic silicone.

7. Lastly, the arrangement or compact cell culture plate according to the invention produced in this way is rotated by 180° into the position according to Figure 3 so that the silicon wafer serves as a base or support 23.

Alternatively, the possibility exists that, instead of affixing, on the one hand, synthetic material film with the honeycomb-like structure with the silicon disk, by adhesion, the magnets already cited are used. For this purpose during the production of the honeycomb structure, still open on one side, according to Figure 2 the polymer film, forming the semipermeable wall, is placed onto a grid-like nickel or special steel foil, which, in agreement with the honeycomb perforations also comprises perforations. Again, it can be ensured through a lateral notch 19 in the metal foil and the polycarbonate honeycomb structure that the discrete chambers 17 are visible through the nickel or

metal foil, for example for the purposes of analysis. After applying the silicon disk 7 onto the rear side of the silicon disk, a magnet plate 9 is emplaced such that the arrangement or compact cell culture sandwich structure formed in this way is held together by means of magnetic forces. Lastly, again the compact cell culture plate formed in this way is rotated by 180° whereby the discrete chambers 17 become visible through the perforations in the foil for the purposes of analysis or processing. Alternatively or in addition to the magnetic foil it is also possible to produce the honeycomb-like structure, instead of a polymer, for example of nickel or another magnetic material.

In Figure 5a a further example of a "compact cell culture disk" is shown wherein, with the exception of a relatively narrow margin portion, the entire plate is provided with the honeycomb-like structure or with discrete microchambers. In this way it becomes, for example, possible to dispose more than 8000 microchambers on a disk having a diameter of 10cm and discrete microchambers having a diameter of 0.8mm.

Figure 5b shows, in turn, a further embodiment according to the invention of a "compact cell culture disk", in particular suitable for storing, culturing and analysing cells or cell cultures or other biosamples of different test series. It is for example possible to add cells from a first sample to the region of structure 33 while cells from a second sample have to be added to the discrete chamber or wells of the structure or the region 35, etc. In the intermediate regions 34 or 44 disposed between these main regions it is possible to place reference cells but it is also possible to culture and observe cells for the culture of which only a relatively small number is necessary.

In Figure 6 lastly an application of the arrangements described according to the invention is shown, in particular suitable in radiation research and radiation medicine. Into a model structure 31, for example filled with water, are placed the discrete compact cell culture disks 1 produced according to the invention, as shown in Figure 6. It is now possible to investigate the biological effectiveness as a function of the coordinates of the various sample systems or samples on the different compact cell culture disks. This takes place thereby that the model structure 31, comprising the compact cell culture disks, is exposed to electromagnetic fields, X-ray, proton, neutron or any other radiation 30. Depending on where in the system of coordinates of the model structure 31 a discrete microchamber 17 is disposed, a different exposure to the waves conducted through the structure 31 results.

It is understood that the cell culture arrangements, ie. "compact cell culture disks" depicted in Figures 1 to 6 and defined according to the invention are only examples which can be changed, modified and supplemented in any desired way. It is in particular also possible to use, instead of the cited dimensions, other dimensions and, instead of the materials used, other suitable materials. Thus it is for example also possible to use, instead of palladium, another suitable inert metal, other organic substances or chemically fixed ligands. Instead of polycarbonate, another suitable, preferably transparent, hydrophobic polymer, a metal, a semiconductor or insulating material can be used. As the semipermeable cover layer can also be used, instead of Teflon or polystyrene, other materials such as are used generally for the production of fully permeable, semipermeable or non-permeable walls. The use of agarose on the silicon wafer is in particular suitable since agarose is inert and is avoided by cells during their growth. But it is also understood that it is possible to use for the coating of the silicon wafer other materials which have suitable properties. Lastly, instead of the silicon disk, a

transparent disk can be used (quartz or another glass) or again a disk comprising, for example, polycarbonate or another polymer which has a low absorption of water, is thermally stable, dimensionally stable and has high impact strength. Various composite material structure are also relevant, such as for example a honeycomb structure of silicon with provided perforations which is equipped with quartz windows.

With respect to the possible application of the arrangement defined according to the invention also no restrictions exist. In addition to the described application feasibilities it is in particular also possible to use such compact cell structure disks in aeronautics. It is for example possible to subject cells or cell cultures, disposed on a compact cell culture disk defined according to the invention, to space conditions in order to study in this way, for example, effects of zero gravity conditions, of cosmic radiation, etc. on the individual cells during their culture.

It is essential that in the selection of the various materials an arrangement for the culture of cells can be obtained whereby it is feasible to dispose a multiplicity of microchambers on a base, which can be separated one from the other, are closed by a preferably semipermeable wall against the environment, and can be observed through suitable window openings.

The claims defining the invention are as follows:

1. An arrangement for culturing cells and/or other biosamples, characterised by a multiplicity of microchambers formed by wells covered with films, which are separated one from the other and which are covered or closed against the environment by a semipermeable film/covering layer, wherein the microchambers or the wells covered by the semipermeable film/covering layer are disposed on a disk-like planar, at least nearly smooth, base, and wherein the microchambers or wells are defined or formed by a sandwich structure of congruent disks, sheet-like elements or walls, matched with one another or adapted to each other, wherein for the formation of the microchambers or wells one or several of the disks, sheeting or walls have a grid or honeycomb-like structure comprising interspaces or perforations.

2. An arrangement according to claim 1, characterised in that the base is formed by a thin plane-parallel, disk of optically high and uniform quality.

3. An arrangement according to claim 1 or claim 2 characterised in that the base is formed by a smooth disk.

4. An arrangement according to any one of claims 1 to 3, characterised in that at least a portion of the chambers or wells is formed by a honeycomb-like grid structure or matrix resting on a base and fixedly connected with it, wherein the openings opposite to the base of chambers or of the grid interspaces are covered or closed by means of a semipermeable membrane or film.

5. An arrangement according to any one of claims 1 to 4, characterised in that the discrete chambers or wells are typically implemented circularly with a diameter of approximately 0.2 to 5mm and that at least a portion of the chambers is disposed in the form of a honeycomb structure.

6. An arrangement according to any one of claims 1 to 5, characterised in that the base is covered by means of a gel or polymer layer lacking cell affinity, on which the wells are disposed.

7. An arrangement according to claim 6 characterised in that the gel or polymer layer is transparent.

8. An arrangement according to any one of claims 1 to 7, characterised in that on the chamber bottom, resting on the base or the gel or polymer layer, one spot each implemented in the form of a point or island, comprising a thin layer suitable for the culture of cells is disposed.

9. An arrangement according to any one of claims 1 to 8, characterised in that the grid structure or the chamber walls comprise a rigid and dimensionally stable material.

10. An arrangement according to claim 9 characterised in that the dimensionally stable material is polycarbonate, silicon, a metallic material, ceramic or a composite material.

11. An arrangement according to any one of claims 1 to 10, characterised in that the semipermeable film/covering layer comprises a polymer material with selective permeability properties.

12. An arrangement according to claim 11 characterised in that the polymer material is ispolytetrafluorethylene, polystyrene or polycarbonate.

13. An arrangement according to any one of claims 1 to 12, characterised in that the disk-like planar base has a diameter of approximately 5 to 20cm with a central perforation, in order to dispose the disk on a holder of a microscope, measuring device or working device.

14. An arrangement according to any one of claims 1 to 13, characterised in that the arrangement or structure is held together in the manner of a sandwich by means of a magnet configuration.

15. An arrangement according to any one of claims 1 to 14, characterised in that the arrangement is disposed in a container, with openings transparent to light on one or both sides, in order to carry out measurements with the arrangement disposed in the container.

16. An arrangement according to claim 15 characterised in that the container is a special steel box.

17. An arrangement for culturing cells and/or other biosamples, substantially as hereinbefore described with reference to the accompanying drawings.

18. A process for producing an arrangement for culturing cells or other biosamples, characterised by the steps:

- formation of a grid or honeycomb-like structure comprising as interspaces or perforations the open microchambers for the individual cells or biosamples, ie. for the formation of the microlaboratories or of the wells;
- application and connection of the grid or honeycomb-like structure on a semipermeable film or covering layer; and
- after filling the discrete interspaces or perforations closed in the downward direction by the semipermeable film or covering layer with nutrients, sera, active agents and/or other liquid or dissolved or soluble components as well as placing the cells or biosamples into the discrete interspaces or perforations or chambers, application of a disk-like plate as base in order to close the perforations or interspaces opposite to the semipermeable film or covering layer, and rotation by 180° of the arrangement formed.

19. A process according to claim 18, characterised in that the semipermeable wall is fixedly connected with the honeycomb or grid-like structure by means of a silicon derivative or a UV-curing material.

20. A process according to claim 18 or claim 19, characterised in that the disk-like base is fabricated from silicon, quartz or glass, subsequently coated by means of a gel or polymer layer lacking cell affinity, and subsequently provided, geometrically congruent with the interspaces or perforations of structure, with dot-form or island-form spots whereupon subsequently the base is connected congruently with the structure such that the spot in each instance comes to lie substantially centrally in the interspace or the perforation.

21. A process according to claim 20, characterised in that the gel or polymer layer is transparent.

22. A process according to claim 20 or claim 21, characterised in that the gel or polymer layer is an agarose layer.

23. A process according to any one of claims 18 to 22, characterised in that the base and the structure are fixedly connected one with the other by means of an aliphatic substance.

24. A process according to claim 23, characterised in that the aliphatic substance is aliphatic silicone.

25. A process according to any one of claims 18 to 24, characterised in that the covering layer is placed onto a metal plate or foil provided with perforations, with the perforations corresponding geometrically to the interspaces or perforations of the structure, and subsequent to the application of the structure and the base a magnet is applied onto the base, opposite to the structure, in order to hold together the arrangement due to the attraction of the metal plate or foil through the magnets, whereupon lastly the arrangement is rotated by 180°.

26. A process for producing an arrangement for culturing cells or other biosamples, substantially as hereinbefore described with reference to the accompanying drawings.

27. Use of an arrangement according to any one of claims 1 to 17, for carrying out measurements, characterised in that the arrangement or the chamber structure is disassembled such that the cultured material can be exposed and killed, whereupon measurements are carried out on the killed material by means of photon radiation or neutrons.

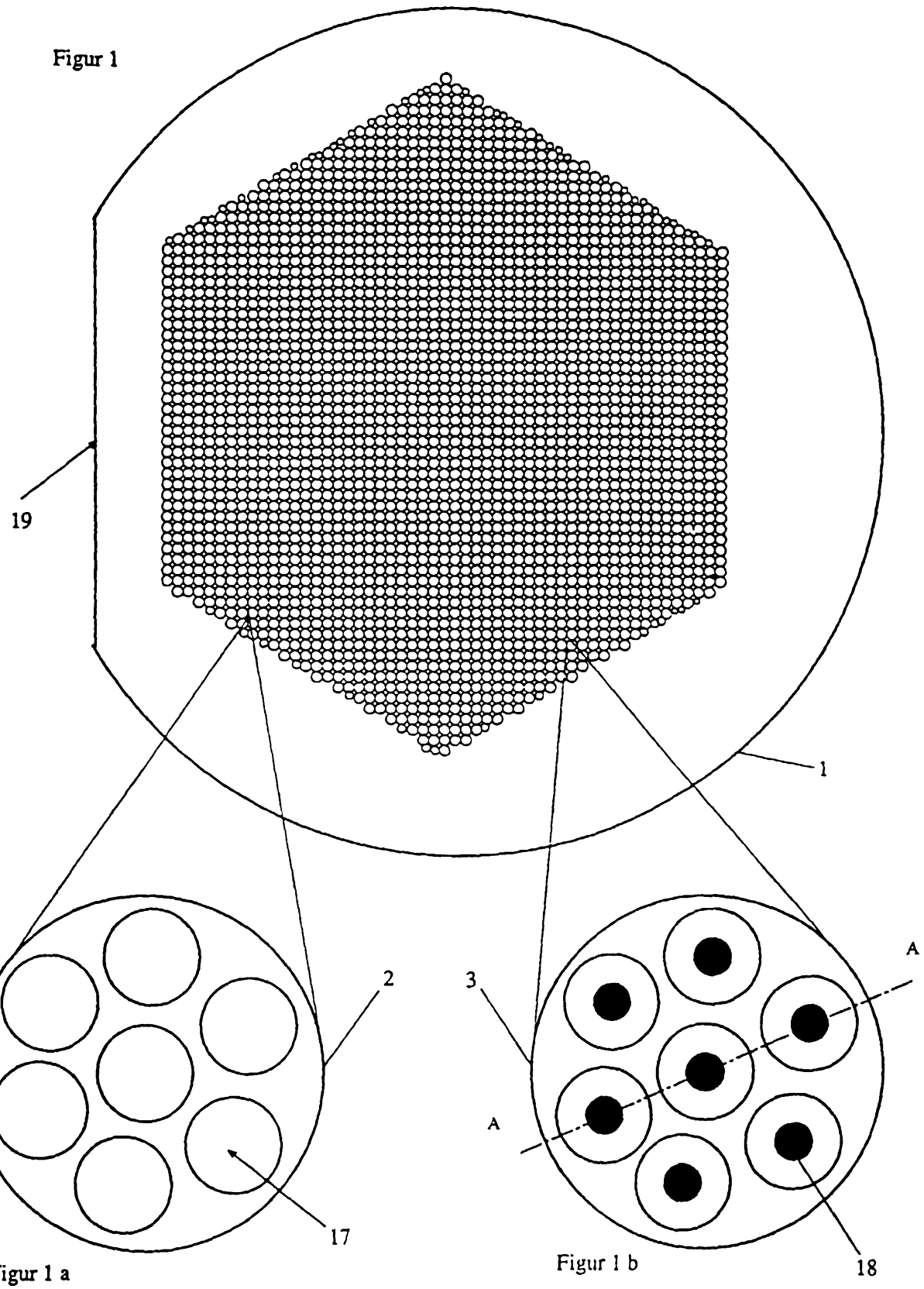
28. Use of an arrangement according to any one of claims 1 to 17 for carrying out measurements, characterised in that the arrangement or the chamber structure is disassembled such that the cultured material is exposed for biochemical or molecular biological investigative methods.

29. Use according to claim 28, characterised in that the investigative methods are electrophoresis or other separation methods.

Dated 7 June, 1999
MICROCLONING CCCD AB

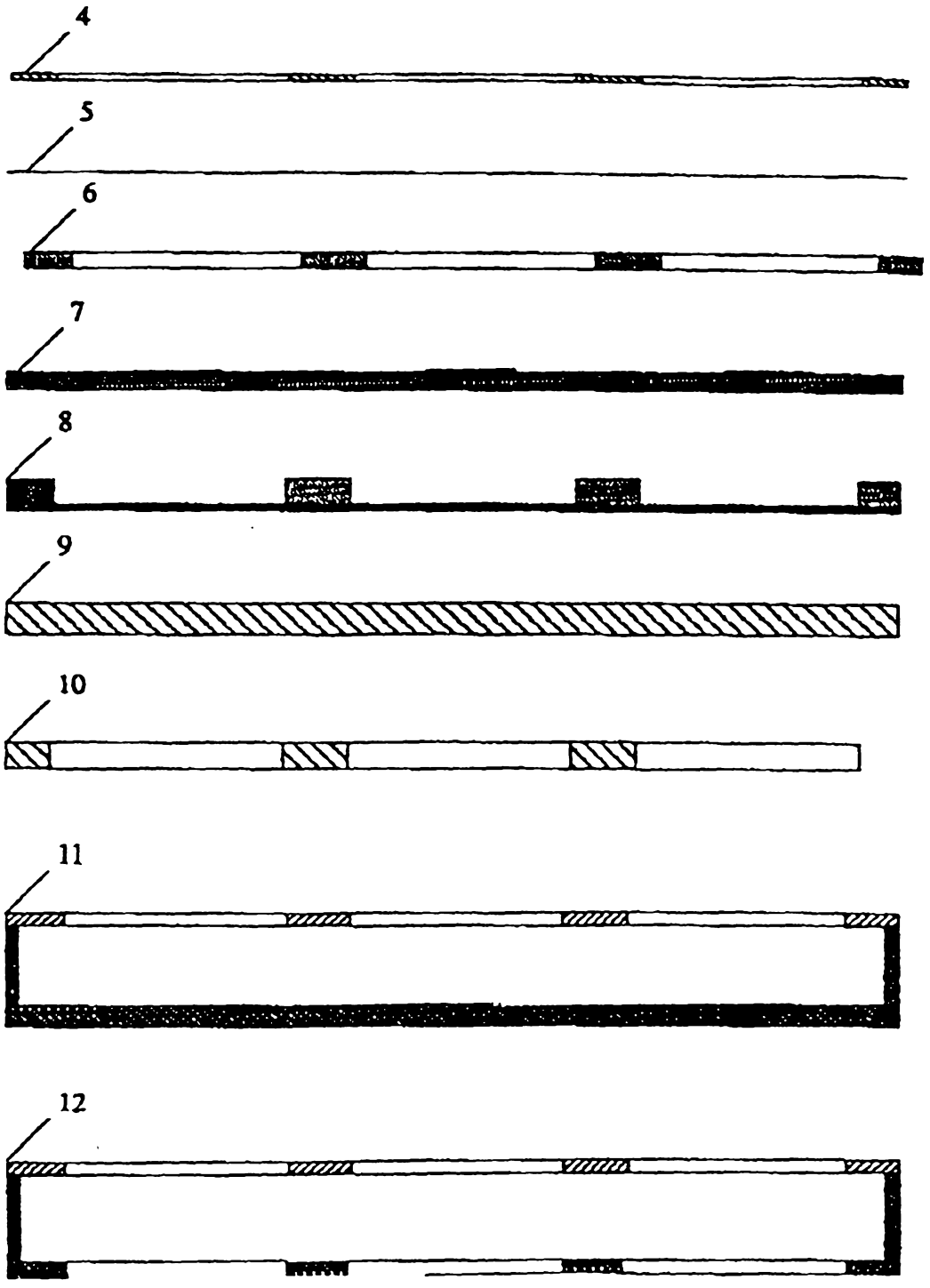
Patent Attorneys for the Applicant/Nominated Person
SPRUSON&FERGUSON

Figur 1

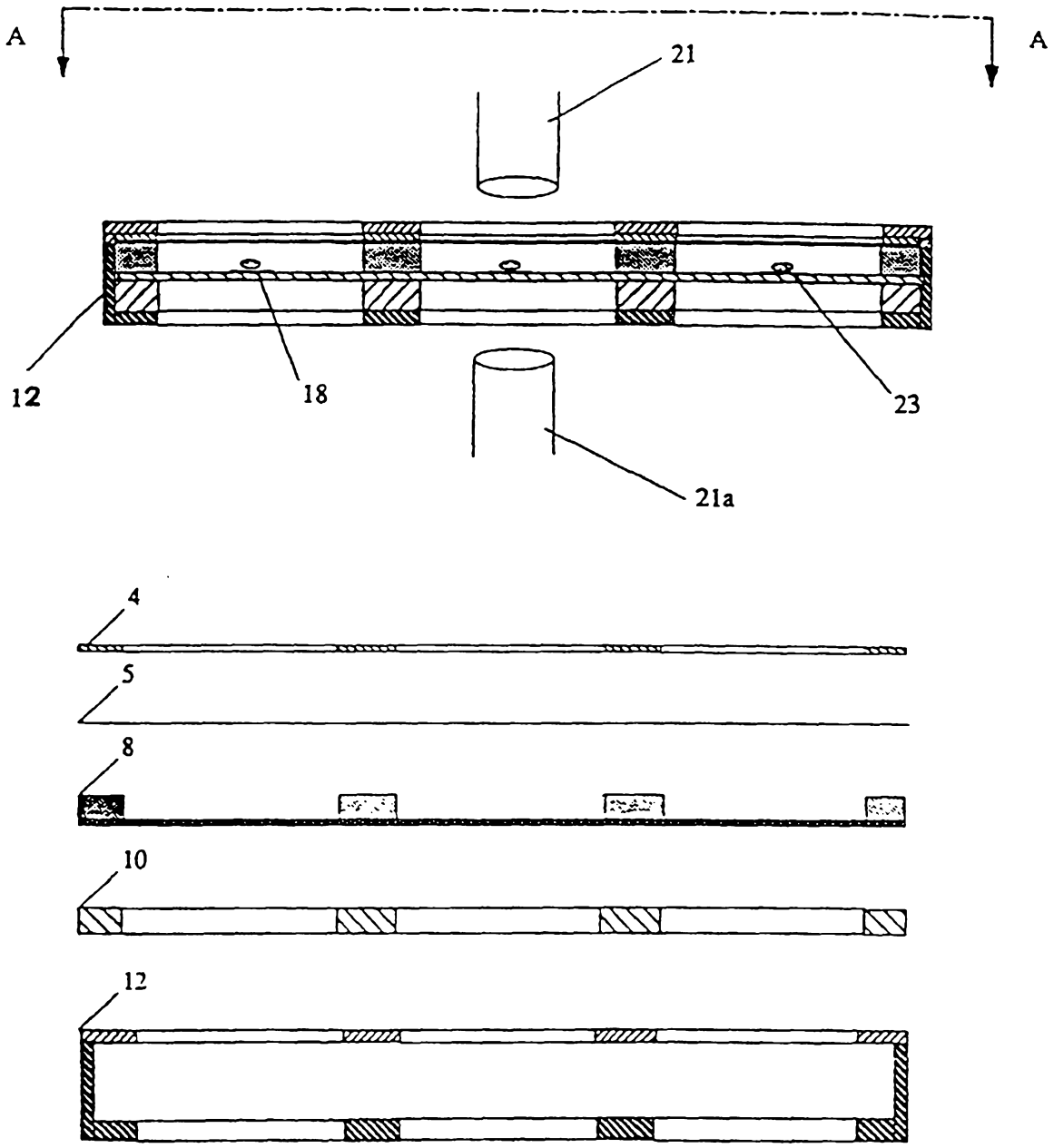


Figur 1 a

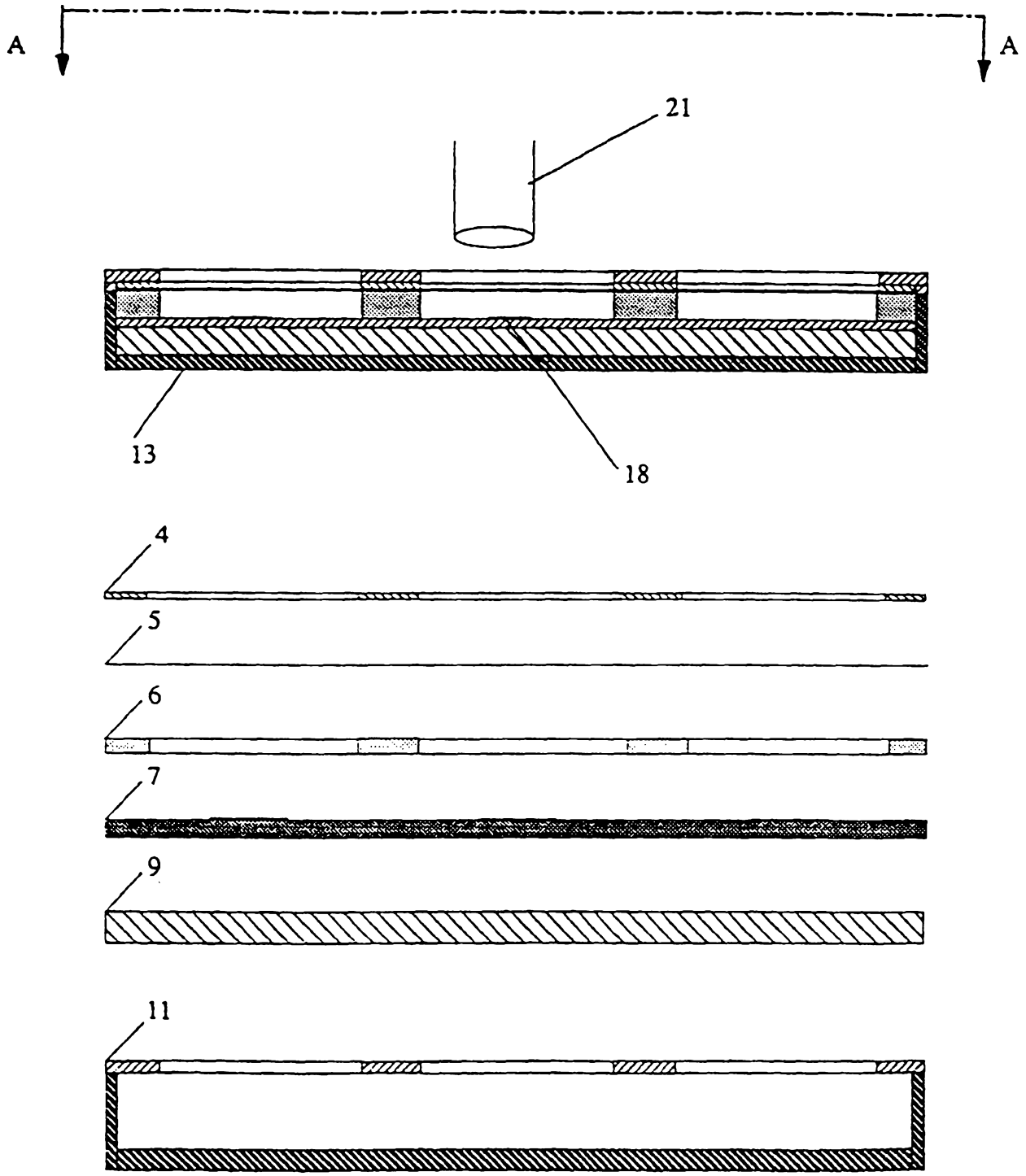
Figur 1 b



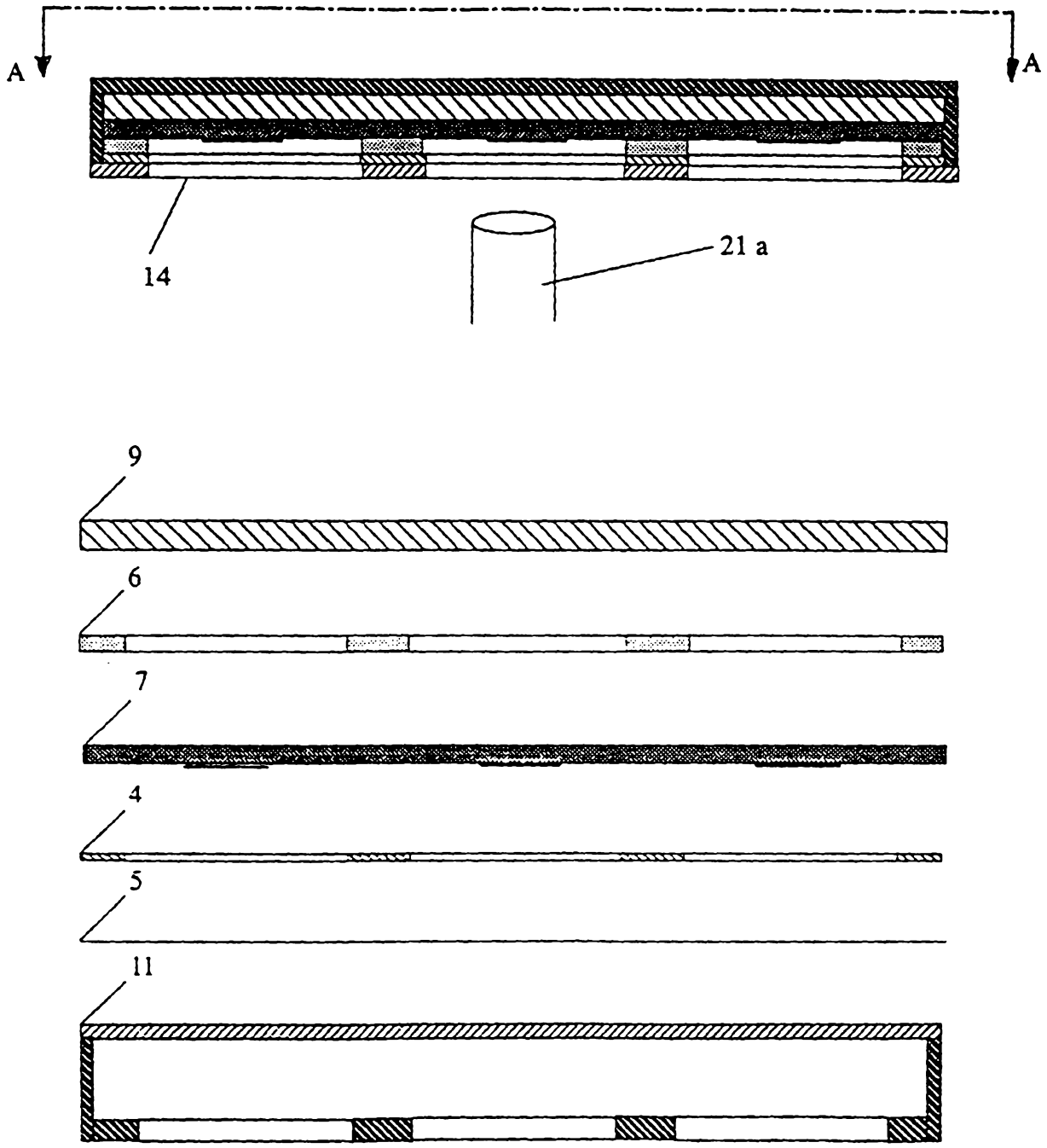
Figur 2



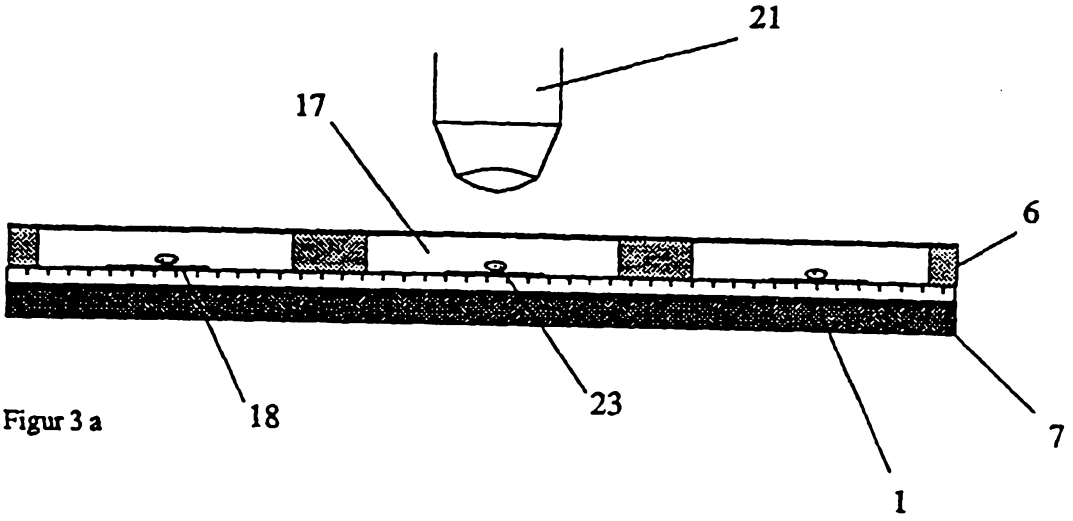
Figur 2a



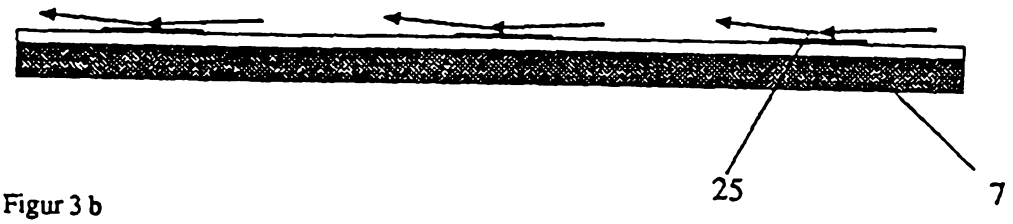
Figur 2b



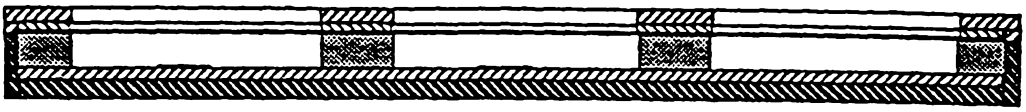
Figur 2c



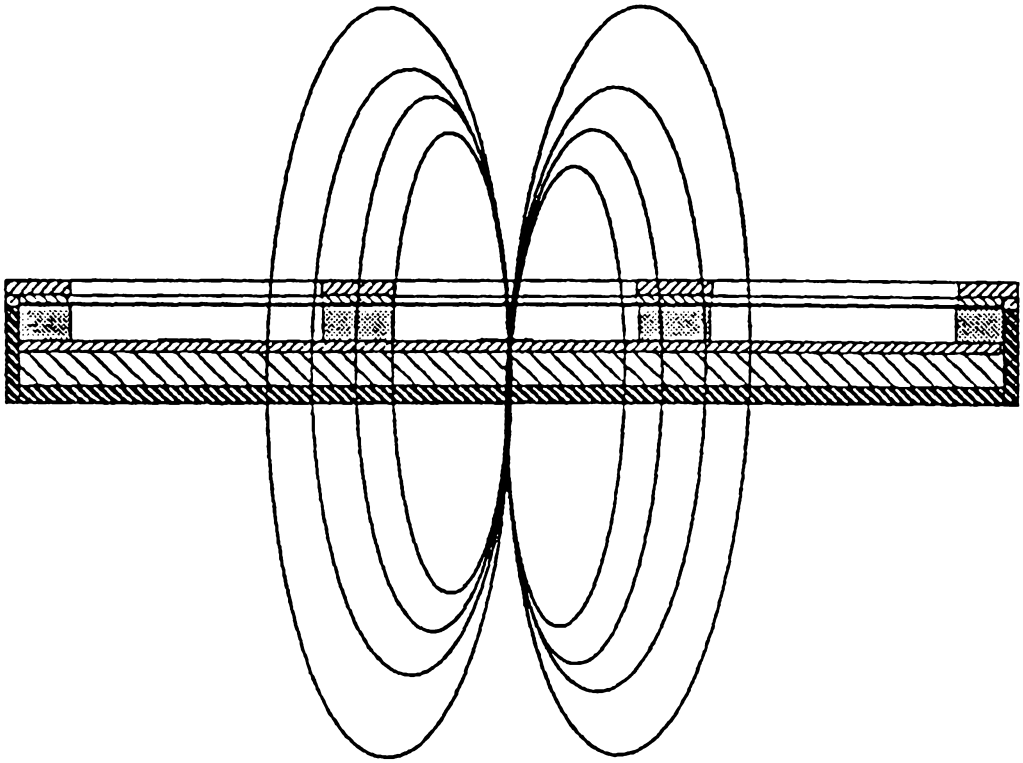
Figur 3 a



Figur 3 b



Figur 4 a



Figur 4 b

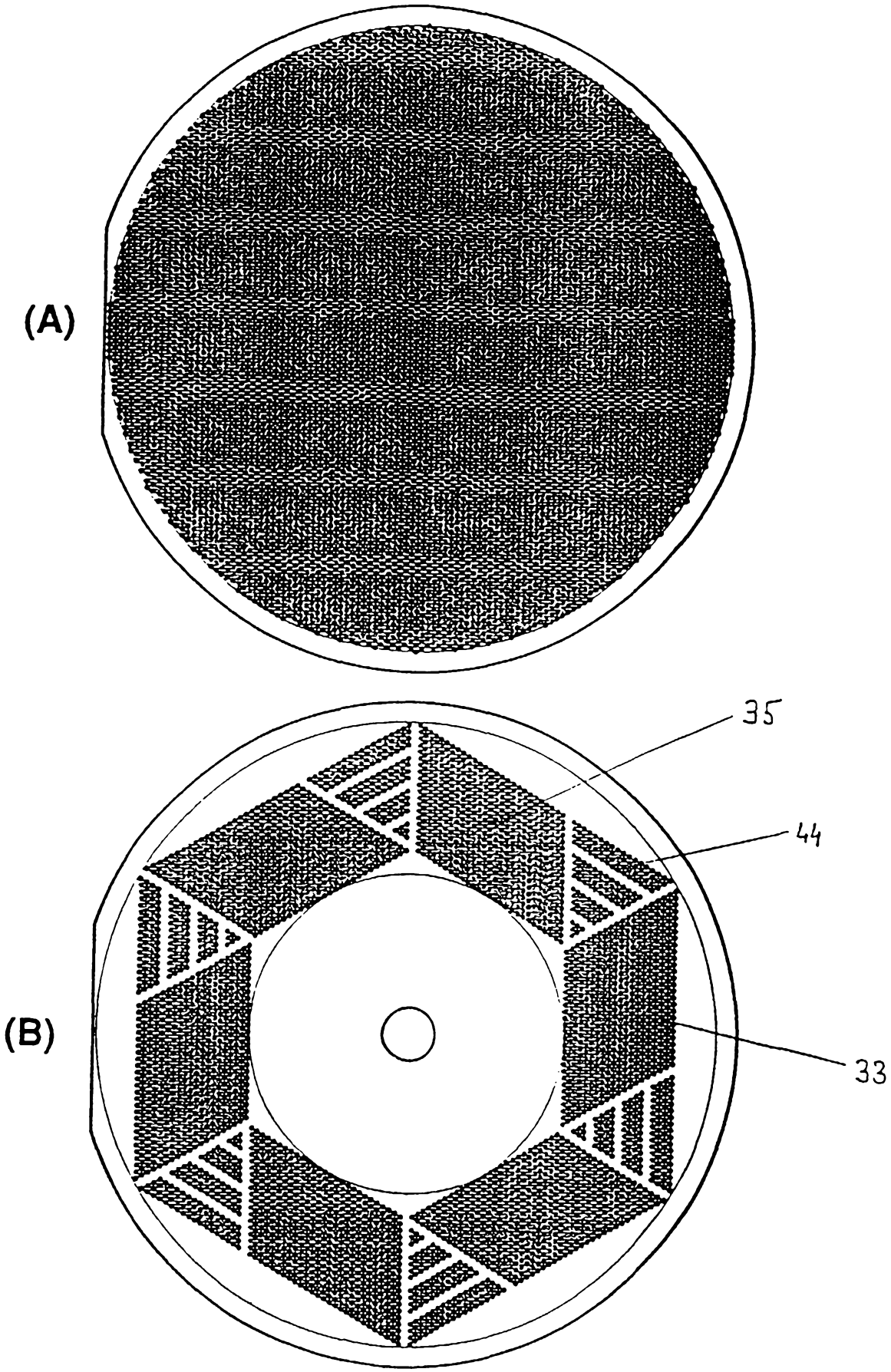


Figure 5:

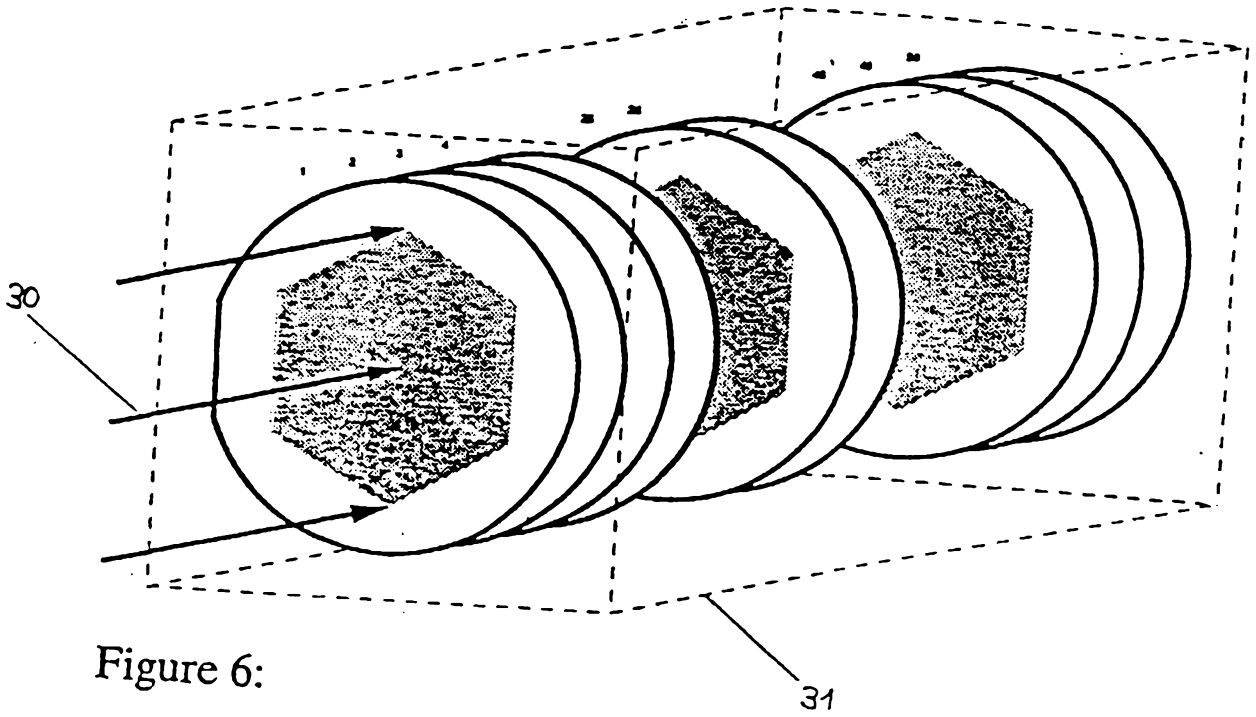


Figure 6: