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 13100 Hämeenlinna (FI)
- (54) A component for use in a three-dimensional microfluidic device, a three-dimensional microfluidic device, and a method for manufacturing a three-dimensional microfluidic device
- (57) A component (2, 3) for use in a three-dimensional microfluidic device, characterized in that it comprises:

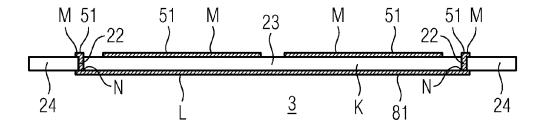
 at least one layer (1) which is manufactured by causing a hydrophobic substance (21, 22) to permeate a porous material (24) so that the hydrophobic substance (21, 22) is absorbed into the porous material (24) of the layer (1) in such a way as to delimit at least one boundary (22) of a channel (K) free of the hydrophobic substance; and

 at least one channel floor and/or ceiling area (51, 81) which is at least partially on top of, or which extends to

the top of, an above-mentioned channel (K), and which is manufactured by spreading a hydrophobic substance (51,81) on the surface of the material (24) of a mentioned layer (1) so that the floor area (L) forms at its location a barrier to the advance of fluid below the layer (1) and/or that the ceiling area (M) forms at its location a barrier to the advance of fluid above the layer (1).

The patent application also contains an independent claim for a three-dimensional microfluidic device and a method of manufacturing a component for use in a three-dimensional microfluidic device.

FIG 9



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Description

Field of the invention

[0001] The invention is related generally to three-dimensional devices for handling microfluids.

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State of the art

[0002] Various means currently exist for analyzing fluids. Microfluidic devices form one category of these means.

[0003] With microfluidic devices it is possible, when they are used in biotechnology or medicine, for example, to trigger a biochemical reaction by using a relatively small sample.

[0004] Three-dimensional microfluidic devices comprise at least two layers within which a fluid arriving in the device can travel in the plane of a layer. Characteristic of a three-dimensional microfluidic device is that the fluid can be transferred from one layer to another. The manufacture of a three-dimensional microfluidic system by using porous material requires that the porous layers are attached on top of each other.

[0005] A three-dimensional microfluidic system manufactured by patterning with a hydrophobic substance on a porous material requires a separate, non-porous layer that is impervious to fluids and that is located between the fluid-transporting porous layers that are patterned with a hydrophobic substance.

[0006] According to the solution presented in international patent application publication WO 2010/102294 A1, this non-porous layer that is impervious to fluids is manufactured using a non-porous material, such as a two-sided tape or plastic, that is impervious to fluids (the term "fluid" in this context and in what follows refers to water or to any other liquid than water intended for use in any given application and which possibly contains water). In order that a fluid is able to travel from one channel layer to another, the two-sided tape has to be perforated. During the perforating process, the adhesive on a two-sided tape tends mess up the perforating device.

[0007] The known two-sided tapes are of relatively thick material. For this reason, the fluids' ability to flow from one layer to another in a three-dimensional microfluidic device such as that presented in international patent application publication WO 2010/102294 A1 is not necessarily sufficiently good, as such, but it may be necessary at the holes to use separately prepared paper pieces for setting in the holes. The preparation of the paper pieces and their fixing to the holes further complicates the manufacture of a three-dimensional microfluidic device.

Purpose of the invention

[0008] The purpose of the invention is to simplify the manufacture of a three-dimensional microfluidic device.

[0009] This purpose can be fulfilled by means of the component according to patent claim 1 for use in a three-dimensional microfluidic device, by the three-dimensional microfluidic device according to the parallel patent claim 8 and by the method according to patent claim 9.

[0010] The dependent claims describe advantageous embodiments of the component and the method.

[0011] A component for use in a three-dimensional microfluidic device comprises:

- at least one layer manufactured by causing a hydrophobic substance to permeate a porous material so that the hydrophobic substance is absorbed into the layer's porous material in such a way as to delimit at least one boundary of a channel free of the hydrophobic substance; and
- at least one channel floor and/or ceiling area which is at least partially on top of, or which extends to the top of, an above-mentioned channel, and which is manufactured by spreading a hydrophobic substance on the surface of the material of a mentioned layer so that the floor area forms at its location a barrier to the advance of fluid below the layer and/or that the ceiling area forms at its location a barrier to the advance of fluid above the layer.

[0012] A three-dimensional microfluidic device contains at least two layers made of a porous material, each of which contains at least one channel defined using a hydrophobic substance, implemented in such a way that a fluid permeating in a channel can travel along the channel from one layer to another by means of capillary action. At least one layer of a three-dimensional microfluidic device is implemented by means of a component for use in a three-dimensional microfluidic device of the invention.

[0013] A method of manufacturing a component for use in a three-dimensional microfluidic device comprises the following steps:

- causing a hydrophobic substance to permeate a porous material so that the hydrophobic substance is absorbed into the component's porous material in such a way as to delimit the boundary of a channel free of the hydrophobic substance; and
- manufacturing at least one channel floor and/or ceiling area which is at least partially on top of, or which extends to the top of, an above-mentioned channel, by spreading a hydrophobic substance on the surface of the porous material so that the resulting floor area forms at its location a barrier to the advance of fluid below the hydrophobic substance and/or that the resulting ceiling area forms at its location a barrier to the advance of fluid above the hydrophobic substance.

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Advantages of the invention

[0014] By means of the component and the method, a three-dimensional microfluidic device can be implemented without a separate non-porous intermediate layer that is impervious to fluids, such as a two-sided tape. As a separate non-porous intermediate layer that is impervious to fluids is no longer separately added, and as a hydrophobic substance is now only spread in manufacturing the component, a clear advantage is achieved in manufacturing. When a hydrophobic substance is spread on the surface of the porous material, the hydrophobic substance tends to penetrate much deeper into the surface layer of the porous material, unlike a two-sided tape. On the other hand, compared with taping, one difficultto-control and messy work phase can thus be avoided, which significantly simplifies the manufacturing process. From the perspective of the invention, the most economical spreading method is printing or pressing, especially with solid ink technology or mask or screen printing technology.

[0015] When, in the method or the component, the hydrophobic substance that is spread on the floor or ceiling area and the hydrophobic substance that is caused to permeate the porous material are together arranged to seal at least one floor or ceiling corner of at least one above-mentioned channel, the travel of a fluid from a channel in the component to an upper or lower layer of a three-dimensional microfluidic device can be better controlled, because, in this way, an unwanted and unintended arrival of a fluid in a different layer at the location of a floor or ceiling corner can be better prevented.

[0016] In a certain advantageous embodiment of the method and the component, the hydrophobic substance that is caused to permeate a porous material is wax or contains wax, especially printing wax.

[0017] In an advantageous embodiment of the method and the component, the hydrophobic substance that is spread to form the floor or ceiling area is wax or contains wax.

[0018] The behavior of wax as a substance delimiting the channel of a three-dimensional microfluidic device has been studied so much that practitioners can easily accept, for a three-dimensional microfluidic device, an embodiment that is based on wax or contains wax.

[0019] In an advantageous embodiment of the method and component, the hydrophobic substance permeating the porous material is caused to permeate the porous material by mask or screen printing technology or by heating, especially in the form of a pattern.

[0020] In an advantageous embodiment of the method and component, the hydrophobic substance spread to form a floor or ceiling area is spread on the surface of the porous material by printing or pressing, especially printing with solid ink technology or mask or screen printing technology, and most advantageously in the form of a pattern

[0021] It is possible, by printing with solid wax technol-

ogy and through mask or screen printing technology, to achieve a component manufacturing result that is very even in quality. Similarly, the use of modern production technology thus becomes possible. Heating is, in terms of production technology, a manufacturing phase that can be very simply implemented.

[0022] In an advantageous embodiment of the method and component, at least one protein or other test zone is arranged in connection with at least one channel. This is done most advantageously before the compilation of the three-dimensional microfluidic device, during compilation or after compilation.

[0023] In an advantageous embodiment of the method, when causing a hydrophobic substance to permeate a porous material, a change in the surface area of the porous material and/or the hydrophobic substance, especially the relative expansion of the hydrophobic substance and/or the relative shrinkage of the porous material, is compensated so that the size of the hydrophobic substance spread to implement the floor and/or ceiling area of the porous material and the alignment in relation to the surface of the porous material will better correspond to the changed size of the hydrophobic substance that permeates the porous material. Especially in the case that the hydrophobic substance is caused to permeate the porous material by using heating technology, any insufficient sealing caused by a transformation of the hydrophobic substance or the component can be compensated. The resulting three-dimensional microfluidic device will be less susceptible to leaks than one in which the transformation of components is not taken into account during manufacture.

List of drawings

[0024] In the following we present a device and a method of the invention, illustrated by the examples in the accompanying drawings:

40	FIG 1	a first sheet	printing	nattern:
, ,	1101	a mot sneet	printing	pattern,

- FIG 2 a single model of the first sheet printing pattern:
- 45 FIG 3 a cross-section III-III of one model of a component billet;
 - FIG 4 a second sheet printing pattern;
- 50 FIG 5 a single model of the second sheet printing pattern;
 - FIG 6 a cross-section VI-VI of the first single component;
 - FIG 7 a third sheet printing pattern;
 - FIG 8 a single model of the third sheet printing pat-

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tern;

- FIG 9 a cross-section IX-IX of the second single component;
- FIG 10 the component billet made for the sheet after printing the first sheet printing pattern;
- FIG 11 the component billet of FIG 10 after the hydrophobic substance has permeated the component billet;
- FIG 12 a component for use in a three-dimensional microfluidic device manufactured using the first component billet presented in FIG 11; and
- FIG 13 a component for use in a three-dimensional microfluidic device manufactured using the second component billet presented in FIG 11.

[0025] The same reference numbers refer to the same technical features in all drawings.

Detailed description of the invention

[0026] FIG 1 presents a first sheet printing pattern 10. Sheet printing pattern 10 contains many single models 11, one of which is presented in more detail in FIG 2.

[0027] FIG 10 presents sheet 1a, on which sheet printing pattern 10 is printed. Wax is brought to sheet 1a by printing on it the desired patterns of hydrophobic substance 21, 22 with a Xerox Corp. Solid Ink technology printer - a Xerox Phaser 8560 or 8860 printer, for example, preferably with the "fine" print setting. As shown in FIG 10, the patterns printed on one face of the sheet can be seen faintly on the other face, at least if viewed against the light.

[0028] FIG 11 presents component billet 1 formed from sheet 1a presented in FIG 10. Component billet 1 is manufactured from sheet 1a by causing the hydrophobic substance printed on it to permeate sheet 1a. In practice this can be implemented so that sheet 1a is put in an approximately 150°C oven for about two minutes. Under this influence, the wax used in the printing will melt. In that case, the wax will be absorbed into the sheet through the thickness of sheet 1a.

[0029] FIG 3 presents a cross-section of a single component billet. A sheet of component billets is presented in FIG 11, which sheet thus contains many component billets. The component billet of FIG 3 shows how hydrophobic barriers are formed in porous material 24 of component billet 1 at cross-section III-III of model 11 presented in FIG 2, which barriers are thus formed at those locations where hydrophobic substance 22 (i.e. the printing wax) is absorbed through the thickness of sheet 1.

[0030] A hydrophobic barrier is formed from the printed wax lines, the target width of which is at least 300 pm. Lines that are thinner than this width do not contain

enough wax to allow a hydrophilic barrier to be formed through the entire thickness of the sheet.

[0031] The hydrophobic barrier formed by means of hydrophobic substance 22 defines the boundaries of channel K. The porous material 24 of the channel's interior 23 remains entirely or mostly free of hydrophobic substance.

[0032] During the heat treatment the width of the wax lines presented in FIG 10 is increased, so that a line that is 300 μm in width becomes 850 $\mu m \pm 50$ pm. This is the minimum width for a functional hydrophobic barrier with the paper and wax used.

[0033] In order to form the hydrophilic channel K, the wax prints defining its boundary should be at a distance of at least 1100 μm from each other. In this case the width of channel K will be about 560 μm due to the heat treatment. A hydrophilic channel can preferably be even thicker; most important is only that the fluid advances in channel K by capillary action.

[0034] The filter papers Whatman NO. 1, Ahlstrom grade 601 and Hahnemuehle Grade FP595 have proven to be very good as sheet materials. The use of other paper grades is also possible, but the filter paper grades presented here have a pore size that is especially well suited for the absorption of fluids. Similarly the basis weight (g/m2) of the mentioned filter papers and the form of the fiber matrix are advantageous for the intended purpose and allow the implementation of a device for handling microfluids, which device can be used without an expensive external pump.

[0035] The required wax line width is chosen in accordance with the sheet material to be used. The above-presented wax line width (at least 300 μ m) works with Whatman NO. 1 paper, but the other paper grades may require the use of a thicker line width.

[0036] FIG 4 presents the second sheet printing pattern 40. The second sheet printing pattern 40 contains many single models 41, one of which is presented in more detail in FIG 5.

[0037] In accordance with the principle apparent in FIG 12, sheet printing pattern 40 is printed on component billet 1 presented in FIG 11. During printing, hydrophobic substance 51 is spread on component billet 1. Thus, sheet 2 of components for use in three-dimensional microfluidic devices is created. The printing is implemented most simply by using the above-described printing arrangement.

[0038] FIG 6 shows a cross-section of a single component formed on sheet 2. A sheet of components is presented in FIG 12, from which sheet a single component is thus presented in FIG 6. Compared with the cross-section presented in FIG 3, it is apparent from FIG 6 that hydrophobic substance 51 has been added to the surface of the component on one side. This hydrophobic substance 51 comes from model 41 presented in FIG 5.

[0039] FIG 7 presents a third sheet printing pattern 70. The third sheet printing pattern 70 also contains many single models 71, one of which is presented in more detail

in FIG 8.

[0040] In accordance with the principle apparent in FIG 13, sheet printing pattern 70 is printed on sheet 2 presented in FIG 12. During printing, hydrophobic substance 81 is spread on the surface of sheet 2. Thus, sheet 3 of components for use in three-dimensional microfluidic devices is created. The printing is implemented most simply by using the above-described printing arrangement.

[0041] FIG 9 shows a cross-section of a single component formed on sheet 3. A sheet of components is now presented in FIG 13, one of which is thus presented in FIG 9. Compared with the cross-section presented in FIG 6, it is apparent from FIG 9 that hydrophobic substance 81 has been added to the surface of the component on the other side, too. This hydrophobic substance 81 comes from model 71 presented in FIG 8.

[0042] The inventor has noticed that, in heating the wax pattern printed on sheet 1a in order to cause the hydrophobic substance to permeate sheet 1a in order to form component billet 1, the tendency is for the sheet to shrink or for the wax patterning to expand. This therefore contributes to complicating the alignment of the following print (sheet 2) or the following prints (sheet 3) to be performed on sheet 1.

[0043] One solution to the problem is to heat-treat the sheet before the first printing (in other words, before printing sheet printing pattern 10 in order to form sheet 1a). Heat treatment is implemented most advantageously in the above-described way (150°C, 120 seconds). The idea behind this is that, by so doing, the oven heating process between printings would no longer cause shrinkage of the sheet due to drying it, for example, since shrinkage would complicate the alignment of the prints.

[0044] Another solution to the problem is to scale sheet printing pattern 10 (i.e. the sheet printing pattern printed before the oven heating process) to be larger or sheet printing patterns 20, 30 (i.e. the sheet printing patterns printed after the oven heating process) to be smaller, in which case the effects of shrinkage of the paper due to the oven heating process can be minimized.

[0045] Hydrophobic substance 51 of printing pattern 20 forms the ceilings M for the channel network. Hydrophobic substance 81 of printing pattern 30 forms the floors L for the channel network.

[0046] The inventor has noticed that channel K works better solely with floor layer L. According to the inventor's observations, the side on which floor layer L is printed relative to the first printing has a significant influence. It has significance especially when three-dimensional microfluidic devices (which, by definition, comprise many layers) are made by this technique combined with gluing or folding and pressing.

[0047] If component 2 for use in a three-dimensional microfluidic device is made so that solely floor layer L is printed, the print must be made on the same side of the printed sheet as the printing has been done in FIG 10. Then component 2 for use in a three-dimensional microfluidic device will work better.

[0048] The invention is presented with the aid of the above exemplary embodiments. The exemplary embodiments are not meant to limit the scope of the patent protection applied for, but the scope of protection can vary and differ from the exemplary embodiments within the framework of the attached claims and their legal equivalents.

[0049] For example, insect waxes, vegetable waxes, mineral waxes, petroleum waxes, microchrystalline waxes, synthetic waxes or combinations thereof may be used instead of, or in addition to, Xerox Corp.'s wax-based ink. Candle wax may also be used. Hydrophobic area 51, 81 may consist of or contain glue.

[0050] Each test zone P can include, in particular, one or more of the following: a protein assay, a cholesterol assay, a glucose assay and a bioassay.

[0051] For a protein assay, a priming solution (0.20 pL, 250-mM citrate buffer, pH 1.9, prepared in 92% water and 8% ethanol by volume) can be spotted in the protein test zone using a micro-pipette (VWR) and allowed to dry for 10 minutes at ambient temperature. A reagent solution (0.20 μ L, 9-mM tetrabromophenol blue prepared in 95% ethanol and 5% water by volume) is spotted on top of the priming solution and dried for 10 minutes at ambient temperature.

[0052] More detailed instructions for preparing protein and other assays can be found in patent application publication WO 2010/102294 A1 and in particular in the documents referred to therein.

Claims

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- A component (2, 3) for use in a three-dimensional microfluidic device, characterized in that it comprises:
 - at least one layer (1) which is manufactured by causing a hydrophobic substance (21, 22) to permeate a porous material (24) so that the hydrophobic substance (21, 22) is absorbed into the porous material (24) of the layer (1) in such a way as to delimit at least one boundary (22) of a channel (K) free of the hydrophobic substance; and
 - at least one channel floor and/or ceiling area (51, 81) which is at least partially on top of, or which extends to the top of, an above-mentioned channel (K), and which is manufactured by spreading a hydrophobic substance (51, 81) on the surface of the material (24) of a mentioned layer (1) so that the floor area (L) forms at its location a barrier to the advance of fluid below the layer (1) and/or that the ceiling area (M) forms at its location a barrier to the advance of fluid above the layer (1).
- 2. A component (2, 3) for use in the three-dimensional

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microfluidic device according to claim 1, in which the hydrophobic substance (51, 81) that is spread on the floor or ceiling area (L, M) and the hydrophobic substance (21, 22)) that is caused to permeate the porous material (24) are together arranged to seal at least one floor or ceiling corner (N) of at least one above-mentioned channel (K).

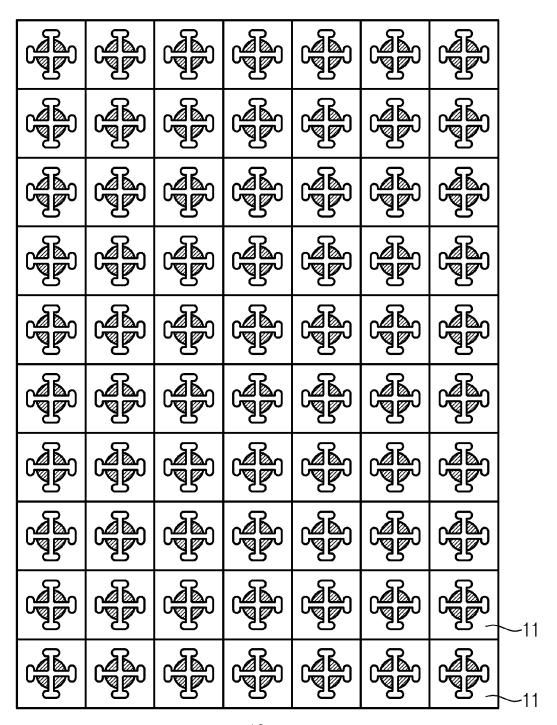
- 3. A component (2, 3) for use in the three-dimensional microfluidic device according to claim 1 or 2, in which the hydrophobic substance (21, 22) that is caused to permeate the porous material (24) is wax or contains wax, especially printing wax.
- 4. A component (2, 3) for use in the three-dimensional microfluidic device according to any one of claims 1 3, in which the hydrophobic substance (21, 22) permeating the porous material (24) is caused to permeate the porous material (24) by mask or screen printing technology or by heating, especially in the form of a pattern (10, 11).
- 5. A component (2, 3) for use in the three-dimensional microfluidic device according to any one of claims 1 4, in which the hydrophobic substance (51, 81) that is spread to form the floor or ceiling area (L, M) is wax or contains wax.
- 6. A component (2, 3) for use in the three-dimensional microfluidic device according to any one of claims 1 5, in which the hydrophobic substance (51, 81) spread to form the floor or ceiling area (L, M) is spread on the surface of the porous material (24) by printing or pressing, especially by mask or screen printing technology or by digital printing, and most advantageously in the form of a pattern (40, 41, 70, 71).
- 7. A component (2, 3) for use in the three-dimensional microfluidic device according to any one of the preceding claims, in which at least one protein or other test zone (P) is arranged in connection with at least one channel (K).
- 8. A three-dimensional microfluidic device,
 - which contains at least two layers made of a porous material, each of which contains at least one channel (K) delimited using a hydrophobic substance, implemented in such a way that a fluid permeating in a channel (K) can travel along the channel (K) from one layer to another by means of capillary action; and
 - at least one layer of which is implemented by means of a component (2, 3) for use in a three-dimensional microfluidic device according to any one of claims 1 7.

- 9. A method of manufacturing a component (2, 3) for use in a three-dimensional microfluidic device, which method is characterized in that it comprises the following steps:
 - causing a hydrophobic substance (21, 22) to permeate a porous material (24) so that the hydrophobic substance (21, 22) is absorbed into the porous material (24) of the component (2, 3) in such a way as to delimit the boundary (22) of a channel (K) free of the hydrophobic substance; and
 - manufacturing at least one channel floor and/or ceiling area (L, M) which is at least partially on top of, or which extends to the top of, the abovementioned channel (K), by spreading a hydrophobic substance (51, 81) on the surface of the porous material (24) so that the resulting floor area (L) forms at its location a barrier to the advance of fluid below the hydrophobic substance (21, 22) and/or that the resulting ceiling area (M) forms at its location a barrier to the advance of fluid above the hydrophobic substance (21, 22).
- 10. A method according to claim 9, in which the hydrophobic substance (51, 81) is spread on the floor and ceiling area (L, M) so that it forms, together with the hydrophobic substance (21, 22)) that is caused to permeate the porous material (24), a seal for at least one floor or ceiling corner (N) of at least one mentioned channel (K).
- 11. A method according to claim 9 or 10, in which the hydrophobic substance (21, 22) that is caused to permeate the porous material (24) is wax or contains wax, and/or in which a mask or screen printing method or heating is used to cause the hydrophobic substance (21, 22) to permeate the porous material (24).
- 40 12. A method according to any one of claims 9 11, in which the hydrophobic substance (51, 81)) that is spread to form the floor or ceiling area (L, M) is wax or contains wax.
- 45 13. A method according to any one of claims 9 12, in which the hydrophobic substance spread to form the floor or ceiling area (L, M) is spread on the surface of the porous material (24) by pressing or printing, especially by mask or screen printing technology or by printing with solid wax technology, and most advantageously in the form of a pattern (40, 41, 70, 71).
 - 14. A method of any one of the preceding claims 9 13, in which at least one protein or other test zone (P) in connection with at least one channel (K) is added to a component (2, 3) before the compilation of the three-dimensional microfluidic device, during compilation or after compilation.

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15. A method of any one of the preceding claims 9 - 14, in which, when causing the hydrophobic substance (21, 22) to permeate the porous material (24), a change in the surface area of the porous material (24) and/or the hydrophobic substance (21, 22), especially the relative expansion of the hydrophobic substance (21, 22) and/or the relative shrinkage of the porous material, is compensated so that the size of the hydrophobic substance (51, 81) spread to implement the floor and/or ceiling area (L, M) of the porous material (24) and the alignment in relation to the surface of the porous material better corresponds to the changed size of the hydrophobic substance (21, 22) that permeated the porous material (24).

FIG 1



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FIG 2

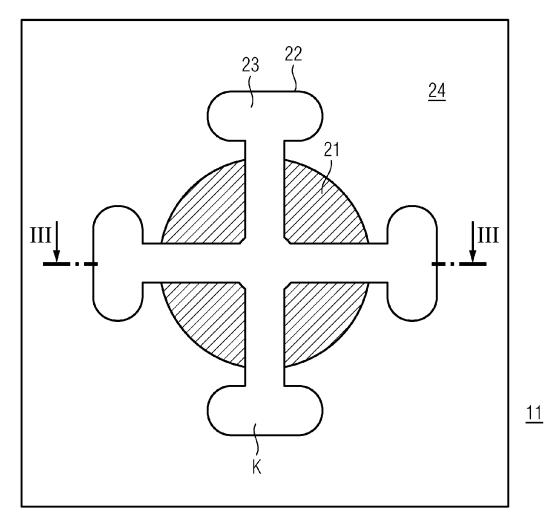
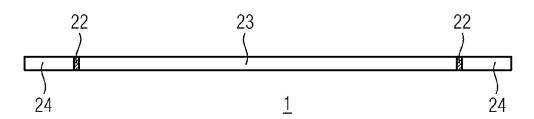


FIG 3



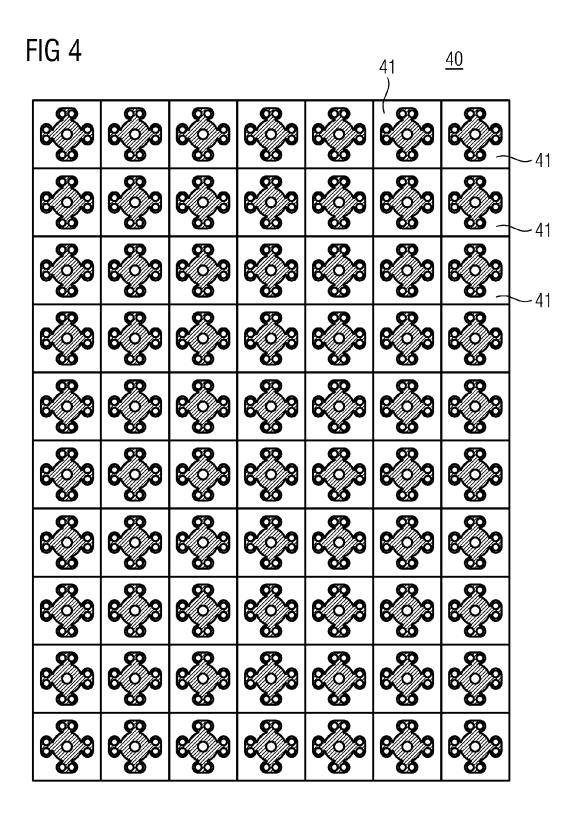
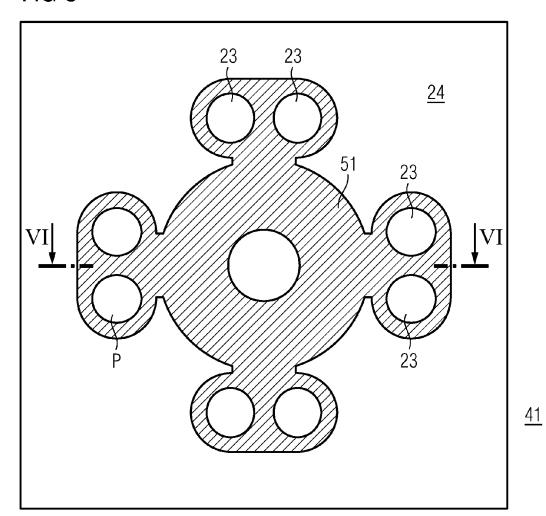
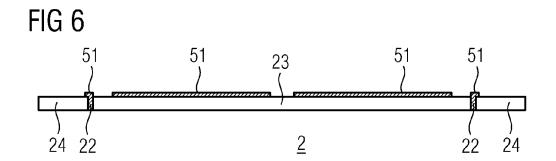


FIG 5





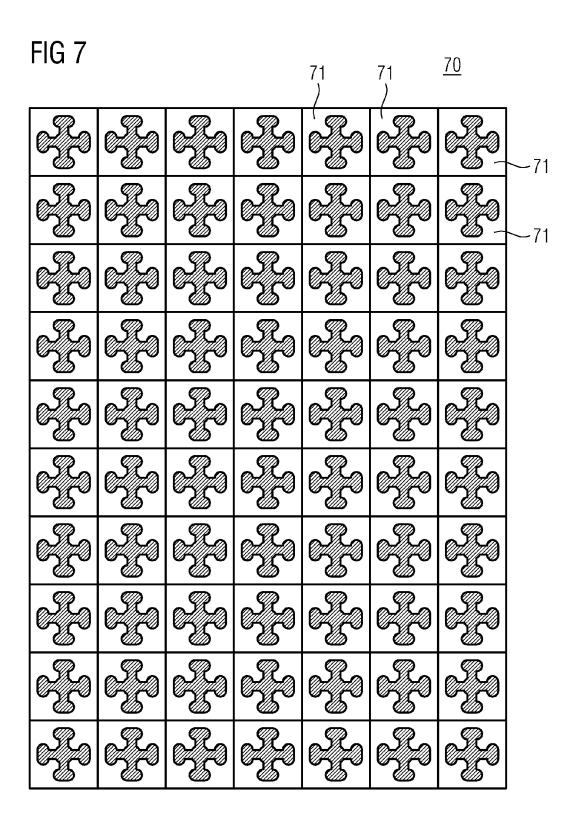


FIG 8

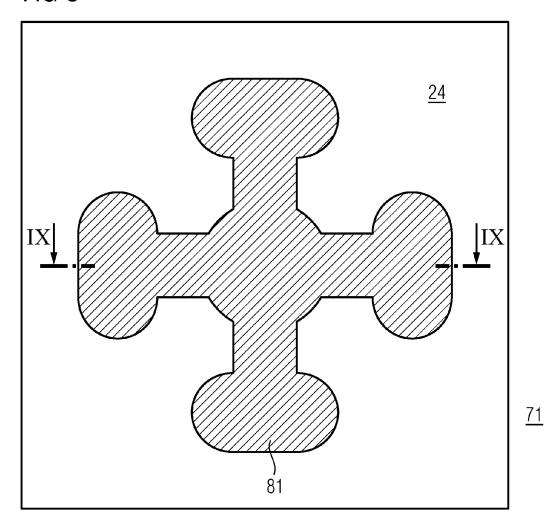


FIG 9

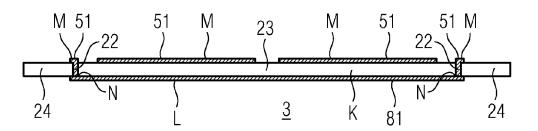


FIG 10

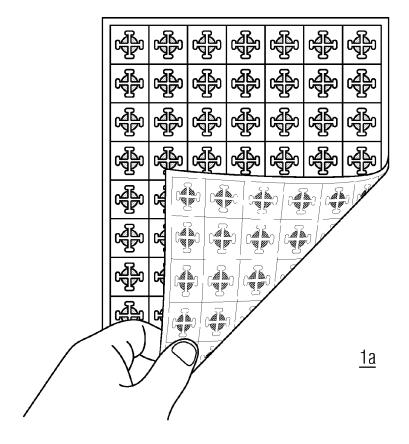


FIG 11

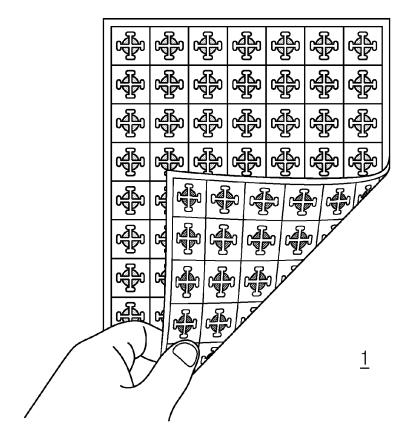


FIG 12

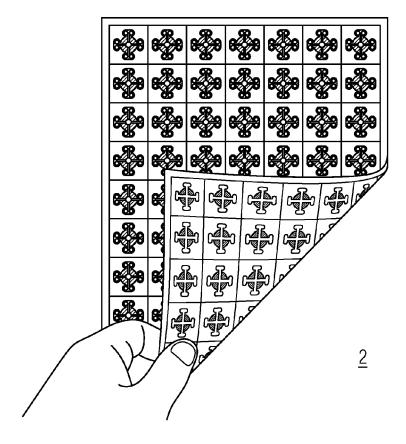
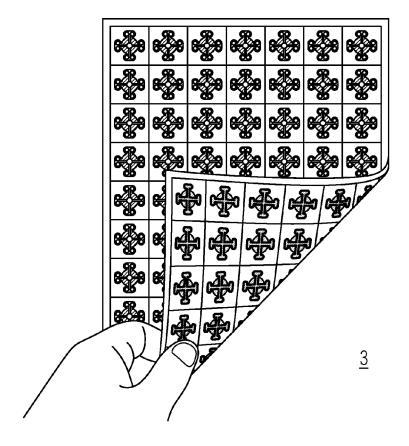


FIG 13





EUROPEAN SEARCH REPORT

Application Number EP 11 18 7407

Category	Citation of document with ir of relevant passa	ndication, where appropriate, ages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
Х	WO 2011/073519 A1 (TUTKIMUSKESKUS VTT [FI]; OLKKONEN JUU) 23 June 2011 (2011-	[FI]; LEHTINEN KAISA	1,2,4, 8-10,13, 15	INV. B01L3/00
Υ		page 4, line 10; claims	3-8, 11-15	
Y,D	CARRILHO EMANUEL [Ù	HARVARD COLLEGE [US]; (S]; MARTINEZ ANDRES Weer 2010 (2010-09-10)	3-8, 11-15	
Υ	[US] ET AL) 3 Decem	WHITESIDES GEORGE M ber 2009 (2009-12-03) claims 1-5,11,13 *	3-8, 11-15	
Y	MARTINEZ ANDRES W [HARVARD COLLEGE [US]; [US]; PHILLIPS SCOTT T by 2010 (2010-02-25) claims	3,5, 11-13	TECHNICAL FIELDS SEARCHED (IPC)
Υ			3,5, 11-13	B01L
Υ			3,5, 11-13	
Y	US 2011/105360 A1 (AL) 5 May 2011 (201 * claims 44,45,66,6		3,5, 11-13	
	The present search report has l	peen drawn up for all claims		
	Place of search	Date of completion of the search		Examiner
	Munich	28 March 2012	de	Biasio, Arnaldo
	ATEGORY OF CITED DOCUMENTS	T : theory or principle E : earlier patent doc	ıment, but publis	
Y : part	icularly relevant if taken alone icularly relevant if combined with anotl Iment of the same category	after the filing date	the application	

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