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(54) **MICROFLUIDIC GRADIENT GENERATOR**

(57) The invention provides an improved microfluidic device and a method for the generation of a concentration gradient of a soluble by means of a gradient network and micromixer units.

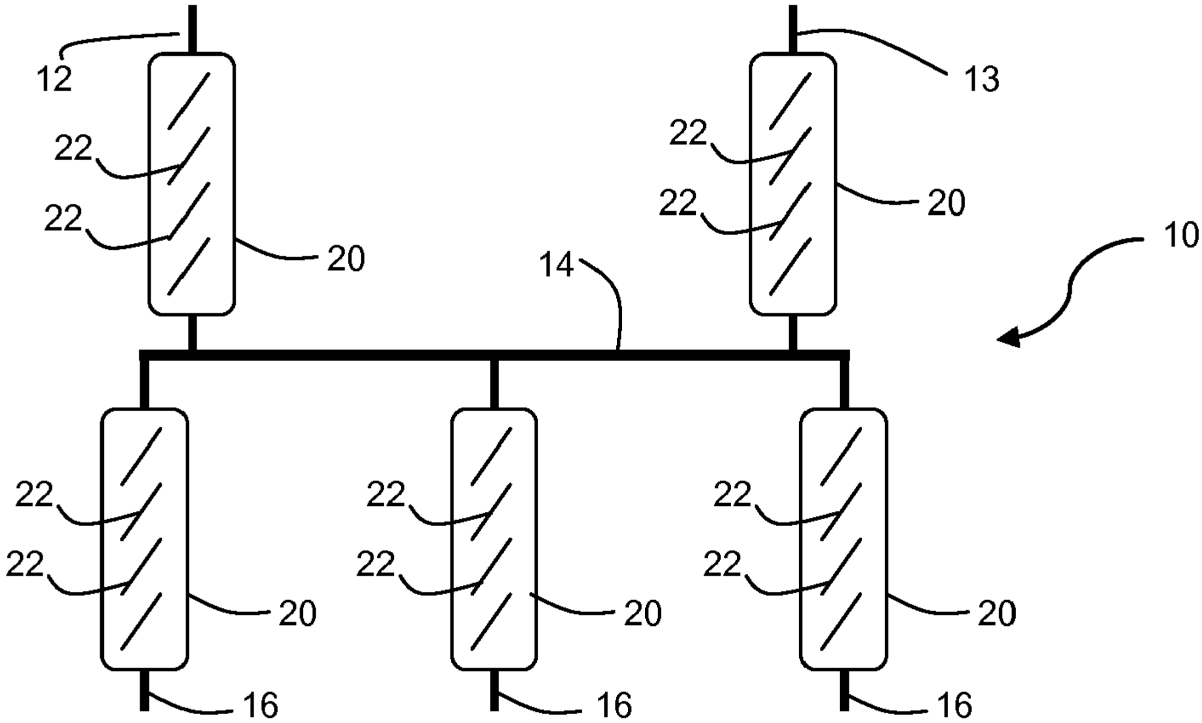

Fig. 1

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Description

[0001] The present invention relates to the field of microfluidic devices and provides improved means and methods for the continuous generation of a concentration gradient of a soluble out of at least two fluids of different concentration.

[0002] In chemistry, biochemistry, biology and related fields the concentration of solubles, for example, electrolytes, drug compounds, hormones, neurotransmitters, or cytokines, is a fundamental property. For example, living cells may even react to slight variations in concentration levels of a stimulant. In basic and applied research, precise control of concentration levels of solubles are required to assess, for instance, such fine-tuned reactions of cells, of receptor populations, or of cell populations and systems to a specific compound or stimulant. The generation of concentration gradients is of great importance, for example, for rapid and high throughput screening: In the presence of a concentration gradient of a soluble, for example, a stimulant, the effective concentration range of that stimulant can be assessed on many cultured cells in parallel, whereby each individual cell is exposed to a particular concentration along that concentration gradient. Gradients may be also employed to other applications, such as chemical synthesis, nanoparticle synthesis, electrophoresis, among others.

[0003] For the generation of concentration gradients microfluidic devices may be provided. Such devices may generate a continuous flow of a concentration gradient, for example, by means of a gradient generation network or so-called "gradient tree". However, the gradient generated by known microfluidic devices with gradient tree architecture is greatly dependent of the actual flow rate of the fluids. That is, the distribution of the discrete concentrations at the plurality of outputs of the gradient tree is greatly influenced by the actual flow rate through the microfluidic device. Alternative microfluidic devices which do not rely on gradient trees, such as know T- and H-mixers for instance, have been developed, but have other substantial drawbacks.

[0004] A major prerequisite for stimulant studies on cell cultures is that the gradient produced is invariant in time, because time-dependent gradients would introduce additional complexity to the study design or impair the obtainable results. It is of great importance that turbulence is absent in the output lines of the microfluidic device. This requires that in the gradient generating device no convective mixing is possible, but only diffusive. In known devices the generation of gradients thus not only is dependent on the flow rate, but also on the diffusion coefficients of the fluids and solubles.

[0005] It is thus desirable to provide a device in microfluidic scale that generates discrete concentration gradients on a plurality of output lines out of at least two fluids with different concentrations of at least one soluble, wherein gradient generation is substantially independent of the actual flow rate of theses fluids and of the diffusion constants of the fluids and solubles. The technical problem of the present invention is based on this desire.

[0006] The technical problem is fully solved by the provision of a microfluidic device which basic principle is based on a gradient tree comprised of two or more mixing stages or on at least one mixing stage having a at least two input lines and a plurality of output lines, which, according to the invention, comprises a microfluidic micromixer unit in each of the output lines.

[0007] Thus, in a first aspect of the invention, a microfluidic device according to claim 1 is provided, which is particularly characterized in comprising at least one mixing stage with at least two, i.e. a plurality of n fluid input lines that feed into, i.e. are in fluid connection with, a common gradient rail. These input lines are arranged along said common rail and are spaced apart from each other, in particular are at least positioned at each opposite end of the common rail. From that common gradient rail at least on more (*n+1*) fluid output lines are branching off i.e. are in fluid connection with that common gradient rail. These output lines are arranged along the rail and are spaced apart from each other and positioned in between the input lines. According to the invention, each of these output lines is comprised of a micromixer unit which is capable of thorough mixing of the fluids entering this output line from the common rail, more particular by chaotic advection, and independent of the flow rate.

[0008] It had been surprisingly found that by the use of such micromixers in each output line of a gradient generating microfluidic device a chaotic, but not turbulent, flow can be generated, which substantially decreases the diffusion distance. Hence, within the output lines mixing times down to milliseconds and even microseconds are possible. The micromixer units provide homogenous mixtures of fluids of different concentrations and hence the stable and flow rate independent generation of discrete gradients on multiple output lines of the device in a very short period and, be it at ultra-low flow rates of less than 1 μl/min or at high flow rates, for example as high as 200 μl/min. Thus, the microfluidic device of the present invention not only allows for precise, stable and thus reliable generation of a discrete gradient, but also allows for high flow rates in the order of 100 to 1000 μl/min as well as for flexible and intra-experiment changes in the flow rate of one or more of the fluids if needed and thus allows for highly efficient, albeit precise and thus cost-effective high-throughput experiments on concentration-dependent effects of one or more stimulant to biological cells or cell systems, or other applicable studies as indicated above.

[0009] In the context of the present invention a "micromixer" or "micromixer unit" is understood as a structure, commonly a passive micromixer which confers non-turbulent, but chaotic flow of the fluids entering the unit, thus reducing the diffusion length between the fluids. A particular technical effect conferred by such a micromixer is advection. The operating parameters, such as the Reynolds number Re in the flow are preferably below 2300, more preferably ranging from 0.2 to 2000, more preferably ranging from 2 to 200, for example at mixing times of 50 μs. Micromixers are known as such and in particular embodiments may also include mixing by lamination and/or injection.

[0010] In particular variants, the micromixer unit comprises a fluid channel with mixing obstacles to confer chaotic flow and thus mixing. These obstacles are preferably selected from: slanted ribs, slanted grooves, staggered-herringbone ribs, staggered-herringbone grooves and combinations thereof, and two level channel modifications that confer fluid fractionation and recombination.

[0011] In a particular variant, the micromixer unit comprises a twisted fluid channel to confer chaotic flow.

[0012] In further particular variants, the micromixer may also comprise parts or consist of an active micromixer, for example, for mixing by means of active disturbance, including acoustic disturbance, pressure disturbance, thermal disturbance, mechanical disturbance, or hydrodynamic disturbance.

[0013] Further particular embodiments are described in the following:

In a particular embodiment the device of the invention further comprises at least one, preferably two, three or more, further mixing stages, which are located downstream the previous or initial mixing stage, wherein the further mixing stages also comprise a plurality of input lines and a plurality of output lines, wherein the input lines of the downstream mixing stage are in fluid connection with the output lines of the previous mixing stage. By that, a mixing network or gradient tree can be provided, each mixing stage adding at least one further output line to the number of output lines of the previous mixing stage, thus increasing the number of discrete gradient concentrations being generated.

[0014] In a particular embodiment the device comprises a gradient tree that is comprised of three or more consecutive mixing stages, wherein each output line of the previous mixing stage is feeding into each individual input line of the following mixing stage. Particularly each mixing stage has n input lines and at least *n+1* fluid output lines. In a particular variant each mixing stage has n input lines and at least 2*n-1* fluid output lines. For example, if the initial mixing stage has two input lines it may comprise three output lines. The consecutive mixing stage may thus comprise three input lines and may comprise five output lines. The consecutive mixing stage may thus comprise five input lines and nine output lines. The consecutive mixing stage may thus comprise nine input lines and 17 output lines, and so forth.

[0015] Such a device is capable of producing a gradient of a soluble out of line discrete concentration levels at the output lines of the third mixing stage, provided that the soluble is fed into one of the two input lines of the initial mixing stage at a given concentration.

[0016] The common rail to which the input lines feet and from which the output lines are branching of, is preferably arranged substantially perpendicular to the flow direction of the input lines and output lines. In preferred variants, input lines feed to the common rail at an angle of about 90°. In a preferred variant, output lines branch off from the common rail at an angle of about 90°. Upstream the entering point of the input lines and downstream the branching point of the output lines, respectively, input lines and output lines may follow other directions. In a preferred variant, the net flow within input lines and output lines is co-linear, and is perpendicular to the extension of the common rail joining these input and output lines.

[0017] Of course, other architectures of gradient networks are possible, and the present invention is not limited to the exemplary architectures disclosed herein. The present invention also encompasses microfluidic gradient networks which generate gradients with other distributions than linear, for example, exponential distribution, logarithmic distribution, Gaussian distribution, or combinations thereof. Such distributions can be accomplished by variation of flow rate and pressure of the input lines, but also by the geometrics and design of the input lines, the common rail of each mixing stage, the output lines, and the actual positioning of input lines and/or output lines along the common rails, but in particular by the individual design of the micromixer in the output lines. That is, by specific design of each micromixer within the output lines of the mixing stages a specific concentration gradient can be obtained. The present invention also foresees means for specific control of the hydrodynamic properties of individual mixing stages within the gradient generating network of the microfluidic device of the present invention. This allows for dynamic control of the gradient distribution or gradient profile at the individual output lines of the network.

[0018] In a further aspect, the invention also provides a method for the generation of a discrete concentration gradient of one soluble, comprising the steps of: feeding a first fluid at a first concentration C_1 of a soluble into a common gradient rail at a first input line, and concomitant feeding of a second fluid at a second concentration C_2 of said soluble, or a fluid free from that soluble into this common gradient rail at at least one further input line along the common gradient rail spaced apart from said first input line; branching off the flow of the fluids in said common gradient rail into a plurality of output lines spaced apart from each other and in between the two input lines; to obtain a plurality of discrete concentrations of that soluble at specific ratios at the output lines. The method is specifically characterized in that the fluids branching off from the common gradient rail are individually mixed in each of the output lines by means of micromixer units, preferably by chaotic advection.

[0019] More generally, a method is provided for the generation of a discrete concentration gradient of one or more solubles, comprising the steps of: feeding a first fluid at a first concentration C_1 of a first soluble into a common gradient rail at a first input line; concomitant feeding of at least one second or further fluid having at least one further concentration C_2 or C_n of said first or of a further soluble into said common gradient rail having at least one further input line spaced apart from said first input line; branching off the flow of said fluids from said common gradient rail into a plurality of output lines spaced apart from each other and in between the input lines; and obtaining a plurality of discrete concentrations of said first and/or further soluble as a specific ratio of $C_1 : C_2 ... : C_n$ at the output lines; characterized in that the fluids branching off from the common gradient rail are individually mixed by chaotic advection by means of micromixer units in each of the output lines.

[0020] In a further aspect, the invention also provides the use of a micromixer unit characterized herein for flow rate-independent generation of a discrete gradient of at least one soluble from at least two fluids in a microfluidic device having different concentrations of said soluble[s].

[0021] In a further aspect, the invention also provides the use of the microfluidic device characterized herein for flow rate-independent generation of a discrete gradient of at least one soluble from at least two fluids having different concentrations of said soluble[s].

[0022] The present invention is further detailed and illustrated by the following specific examples, which are not considered to be limiting the scope of the invention.

Figure 1 schematically depicts one mixing stage (10) of the microfluidic device of the invention, having a common rail (14) and two input lines (12, 13). First input line (12) and second input line (13) feed into opposite ends of the common rail (14). Three output lines (16) are arranged along the common rail (14). At least one output line (16) is arranged in between the position of the both input lines (12, 13). According to the invention, all three output lines (16) comprise or consist of a micromixer unit (20), having a plurality of obstacles (22) to confer chaotic non-turbulent mixing. In the depicted embodiment, also the two input lines (12, 13) comprise or consist of a micromixer unit (20), having a plurality of obstacles (22). The micromixer units in the initial input lines contribute to a chaotic non-turbulent flow in the common mixing rail (14) to which they feed.

Figure 2 schematically depicts one embodiment of a gradient network or gradient tree comprised of three mixing stages (10) according to figure 1.

Figure 3 schematically depicts an embodiment of figure 2, specifically designed as a working example as microfluidic device. The micromixer units are shown as dark zones (20) within the output lines (16) and input lines (12, 13) respectively.

Figure 4 is a part of a micro-photograph of an actual micromixer unit (20) within the output line (16) of the microfluidic device according to figure 3. Obstacles conferring chaotic mixing are depicted as dark zones and are specifically designed as staggered herringbone grooves.

REFERENCE LIST

[0023]

10	mixing stage
12	first fluid input line
13	second fluid input line
14	common gradient rail
16	output line
20	micromixer unit
22	obstacle

Claims

1. A microfluidic device for generation of a gradient on a plurality of output lines, comprising:

    at least one mixing stage (10), comprising

    - a plurality of n fluid input lines (12) feeding into a common gradient rail (14), the input lines (12) being arranged along said rail and spaced apart from each other, and
    - at least *n+1* fluid output lines (16) branching off the gradient rail (14), the output lines (16) being arranged along said rail and spaced apart from each other and positioned in between the input lines (12,13),

    characterized in that each of said output lines (16) is comprised of a micromixer unit (20) for chaotic advection of the fluids in said output line (16).

2. The device of claim 1, further comprising at least one further mixing stage (10), located downstream the previous mixing stage (10), the further mixing stage (10) comprising input lines (12,13) in fluid connection with the output line (16) of the previous mixing stage (10).

3. The device of claim 1 or 2, wherein the micromixer unit (20) comprises a fluid channel with mixing obstacles (22) to confer chaotic flow.

4. The device of claim 3, wherein the obstacles (22) are selected from: slanted ribs, slanted grooves, staggered-herringbone ribs, staggered-herringbone grooves, two level channel modifications that confer fluid fractionation and recombination, and combinations thereof.

5. The device of any one of the preceding claims, wherein the micromixer unit (20) comprises a twisted fluid channel to confer chaotic flow.

6. The device of any one of the preceding claims, wherein the the common gradient rail (14) is arranged substantially perpendicular to the input lines (12,13) and to the output lines (16)

7. The device of any one of the preceding claims, wherein each of the initial fluid input lines (12,13) of the first mixing stage (10) is comprised of a micromixer unit (20) for chaotic advection of the fluids in said initial input line (12,13).

8. The device of any one of the preceding claims, wherein each mixing stage (10) has n input lines (12,13) and at least *n+1* fluid output lines (16).

9. The device of any one of the preceding claims, having a gradient tree (30) that is comprised of three or more of said mixing stages (10).

10. A method for generation of a discrete concentration gradient of at least one soluble, comprising the steps of:

- feeding a first fluid at a first concentration C_1 of a first soluble into a common gradient rail (14) at a first input line (12),
- concomitant feeding of at least one second or further fluid at least one further concentration C_2 or C_n of said first or of a further soluble into said common gradient rail (14) at least one further input line (13) spaced apart from said first input line (12),
- branching off the flow of said fluids from said common gradient rail (14) into a plurality of output lines (16) spaced apart from each other and in between the input lines (12,13), and
- obtaining a plurality of discrete concentrations of said first and/or further soluble as a specific ratio of $C_1 : C_2 ... : C_n$ at the output lines (16),

the method **characterized in that** the fluids branching off from said common gradient rail (14) in each of the output lines (16) are individually mixed by chaotic advection by means of micromixer units (20).

11. Use of a micromixer unit (20) **characterized in** any one of claims 1 to 7 for flow rate-independent generation of a discrete gradient of at least one soluble from at least two fluids in a microfluidic device having different concentrations of said soluble[s].

12. Use of the microfluidic device according to any one of claims 1 to 9 for flow rate-independent generation of a discrete gradient of at least one soluble from at least two fluids having different concentrations of said soluble[s].

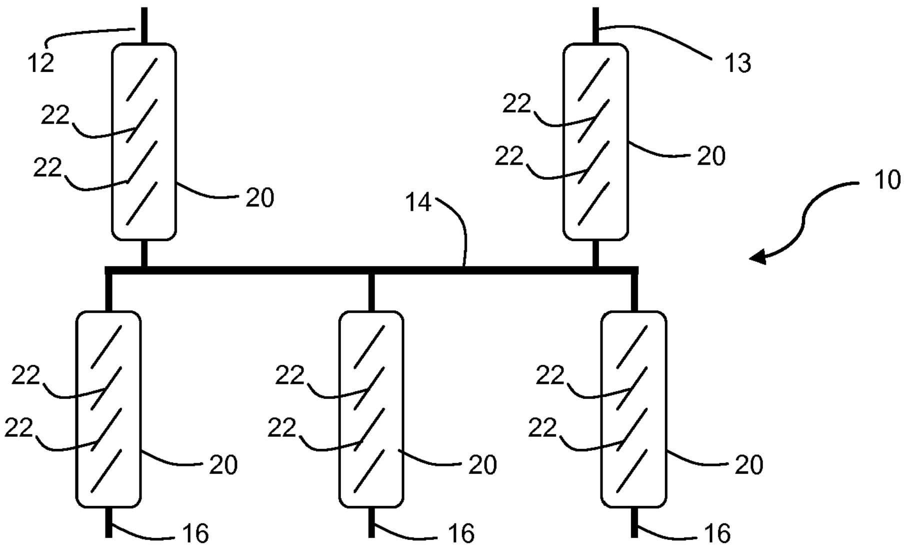
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Fig. 1

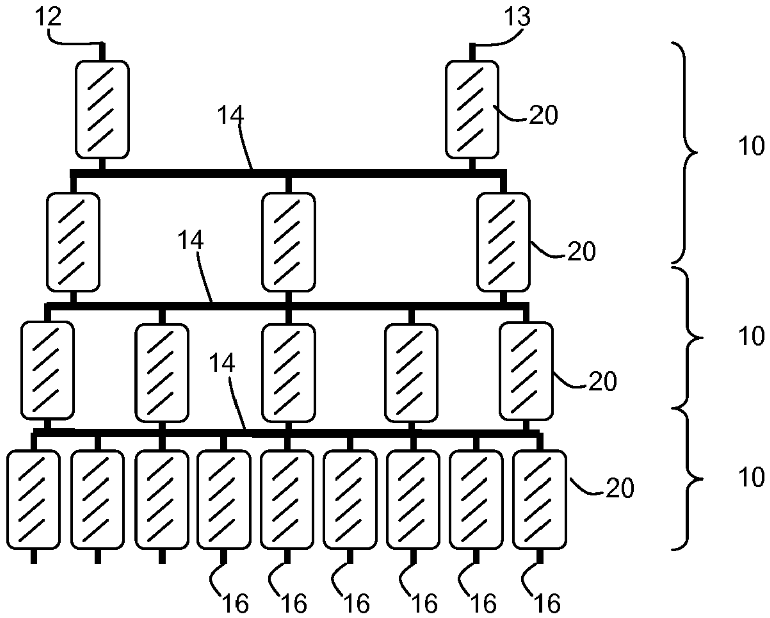
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Fig. 2

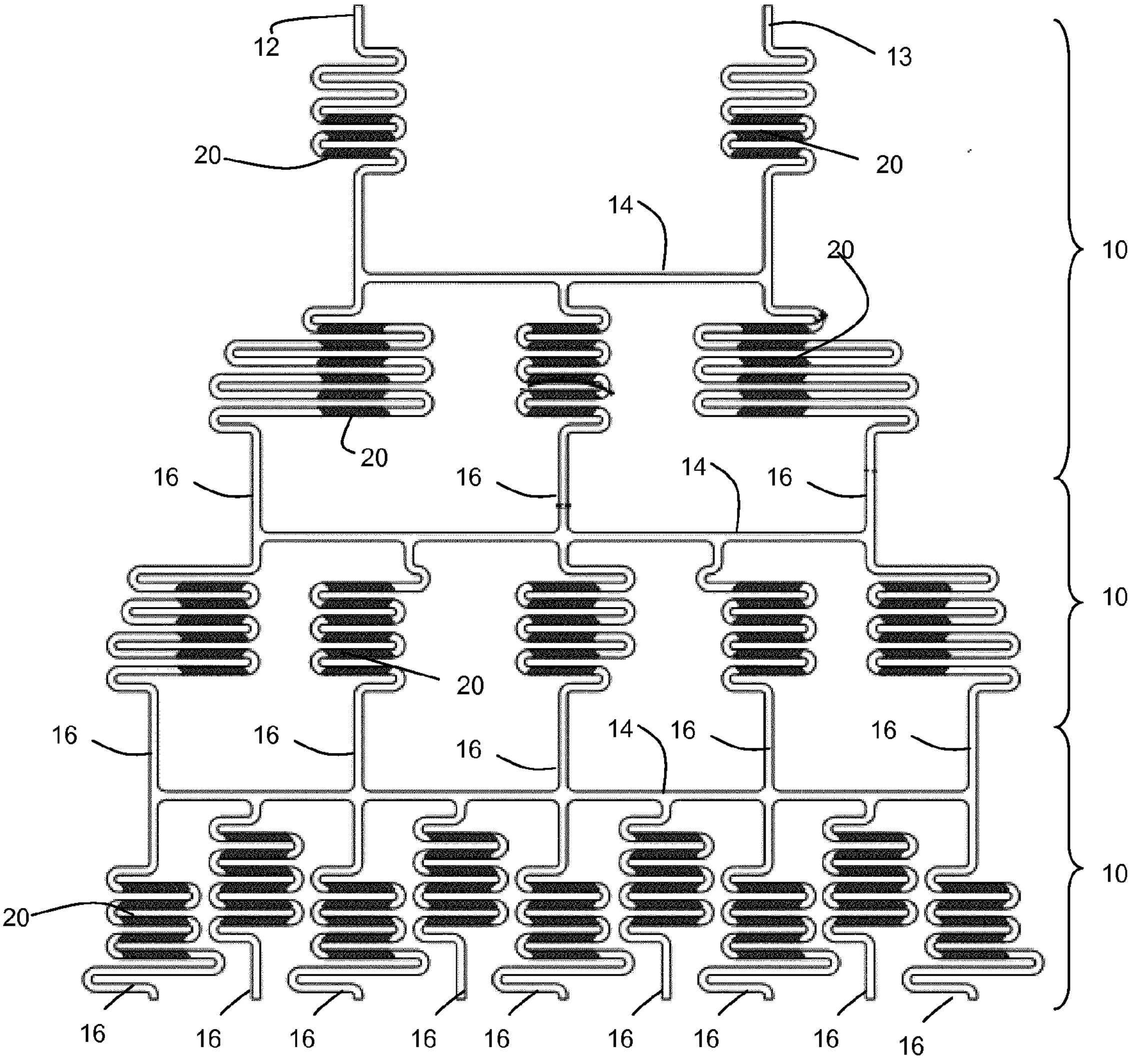
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Fig.3

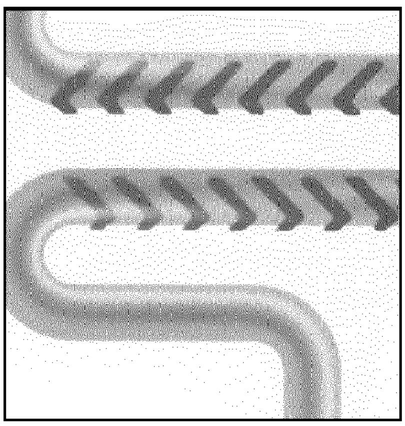

Fig. 4

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EUROPEAN SEARCH REPORT

Application Number
EP 17 17 7540

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The present search report has been drawn up for all claims

1

Place of search	Date of completion of the search	Examiner
The Hague	15 September 2017	Ruiz-Echarri Rueda

CATEGORY OF CITED DOCUMENTS

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