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(54) Title: APPARATUS

(57) Abstract: Method and apparatus for performing controlled performance of reactions, the apparatus comprising at least one channel for receiving a substance, the channel having a first end and a second end, and at least one treatment zone intermediate the first end and second end of the channel, for performing a treatment on substance in the channel(s). Means are provided for applying a pump action to the channel(s), the pump action in use causing substance within the channel(s) to pass back and forth over the treatment zone(s).

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Apparatus

The invention generally relates to the field of life sciences, bio-technology, genetic research, DNA and RNA diagnostics. In particular it relates to a system for carrying out controlled chemical processes, including but not limited to nucleic acid amplification such as polymerase chain reactions.

When performing clinical diagnostics, a fast growing technique used by molecular laboratories is the amplification of DNA sequences, which allows the replication of a small amount of DNA into an amount that is sufficient for performing subsequent processes. Nucleic acid amplification may be used for identifying and amplifying specific elements of a genetic sequence in a sample of material. An example of nucleic acid amplification is Polymerase Chain Reaction (PCR). PCR has found widespread use as a way of performing nucleic acid amplification. PCR replicates DNA using a sequence of heating and cooling cycles. This involves using a thermal cycling system for carrying out temperature controlled processes. Such a system may be used for, but is not limited to, nucleic acid amplification such as polymerase chain reaction.

Such a procedure is currently complex and requires high-tech and expensive equipment, typically including an array of test-tubes, for instance as described in US Patent 5508197. The elapsed time between the moment the patient gives his sample and when he gets his results is also fairly long. The current challenge is to transform PCR into a real-time on-the-spot application that could be used directly by a general practitioner or by the patient himself.

There is a move away from the test-tube approach of implementing nucleic acid amplification towards the so-called miniaturised "lab on a chip" approach. Such chips tend to be of the order of a few centimetres in width and length.

There are currently two main approaches to "lab on a chip" implementation. The first one is based on macro-scale apparatus: the sample is positioned in a well on a chip and the well is heated/cooled. Such an approach is described in Northrup, M.A., et al., "DNA amplification with a microfabricated reaction chamber" Transducers '93, 1993: p. 924-926. One major drawback of this design is inertia: the cooling and heating rates are slow due to the amount of matter involved in the process. It is well documented that very fast heating/cooling rates are essential to achieve a specific DNA replication, (for instance, see Wittwer, C.T. and D.J. Garling, "Rapid cycle DNA amplification: time and temperature optimization. BioTechniques", 1991. 10(1): p. 76-83). This first approach fails to fulfil these needs.

The second approach is based on a continuous flow-through design (for instance, see Kopp, M.U., A.J. de Mello, and A. Manz, "Chemical amplification: Continuous-flow PCR on a chip". Science, 1998. 280(5366): p.1046-1048). In this paper the authors describe a single channel that meanders in a serpentine fashion over three heating zones. The sample is pumped through the channel in a continuous manner. The heating/cooling rates only depend on the flow rate. In theory, a step-like profile can be achieved. This approach greatly lacks flexibility regarding the number of PCR cycles. The design can be modified to allow the user to reduce the number of cycles (for instance as described in Obeid, P.J., et al., "Microfabrication device for DNA and RNA amplification by continuous-flow polymerase chain reaction and reverse transcription-polymerase chain reaction with cycle number selection", Analytical Chemistry, 2003. 75(2): p. 288-295), but even in this case the possible options are determined at the time of designing the chip and of finite number.

Trying to achieve high parallelism with the continuous flow-through design is anything but easy, if possible at all. This is an unacceptable option for potential users who are required to perform hundreds of PCR a day.

To summarise, the state of the art is too complex, greatly lacks flexibility and is too slow and expensive to meet the needs of the end-user.

The invention offers an excellent alternative. The simplicity of the design allows an enormous flexibility combined with high parallelism and a possible enhancement in the reaction yield.

The invention is based on a channel passing over a plurality of treatment zones, such as thermal zones. A sample is pumped back and forth over the treatment zones as many times as desired/requested, providing great flexibility to the user. This allows channels to be shorter than in known devices as the substance in the channel does not necessarily follow a forward path through the channel.

A high parallelism can be effortlessly achieved by including channel divisions. In a preferred embodiment, the chip has one channel that splits into eight separate channels, but a greater number of channels could be easily manufactured. Preferably a single portion of each channel passes over each treatment zone. This design combines a high throughput with step-like temperature profiles and is consequently a strong competitor to the macro-apparatus. One main input channel may feed into a plurality of channels. This means that the same input enters each channel and passes over the same treatment zones, so is treated the same.

Thus there is provided apparatus for the controlled automated performance of processes on nucleic acid, in particular amplification including polymerase chain reactions such as PCR, reverse PCR etc.

The apparatus may incorporate mixing stations. This unique approach should introduce some turbulence and enhance the PCR yield. The material used is also transparent which will permit real-time on-chip detection.

The invention will now be described further, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a schematic drawing of a first embodiment of the channel layout of apparatus according to the invention;

Figure 2 is more detailed view of a portion of the channel layout of the apparatus shown in Figure 1;

Figures 3 a-e show other examples of channel layout of apparatus;

Figures 4 a-c show examples of sample and treatment zones;

Figure 5 is an image showing a first embodiment of the mixing stations; and

Figures 6a and 6b show alternative mixing stations.

In overview the invention provides a reactor for performing reactions in a controlled manner. A channel passes over at least one treatment zone. A sample in the channel is pumped over the treatment zones in discrete steps as many times as desired/requested, providing great flexibility to the user.

In the following description, an automated thermal cycling system is described for carrying out temperature controlled processes, including but not limited to nucleic acid amplification such as polymerase chain reactions. However the system is also suitable for an automated cycling system for carrying out other controlled processes.

The system is implemented in the form of a "lab on a chip". The chip comprises a 2mm PMMA base layer onto which is spin-coated 10μm thick layer of SU8. A further layer of SU8 is spin-coated onto this; the thickness of this layer determines the depth of the pattern, and in the example described is 50μm. The pattern of the channels (e.g. that shown in Figure 1) is formed in this layer, for instance photo-lithographically. A 3mm PMMA lid is provided onto which is spin-coated a 10μm thick layer of SU8. Any drilling required is carried out prior to assembly. The construction is completed by bringing the two together (SU8 to SU8) and using the bonding properties of SU8 to seal the structure. Bonding is achieved with a combination of time, temperature and load.

Figure 1 is a schematic drawing of one example of a channel layout of apparatus according to a preferred embodiment. Figure 2 is an enlarged view of the part of Figure 1 within the dashed line. The apparatus shown is symmetrical and has at least one inlet for the introduction of substances and at least one outlet for extraction. In the embodiment shown, there are two inlets 20a, 20b and two outlets 22a, 22b. The inlets connect with a common input channel 24a. The outlets connect with a common outlet channel 24b. The input channel 24a is connected to a plurality of channels 26 to form an intermediate channel portion 25. Figure 1 shows eight such channels 26. In practice there may be more or less channels, for example up to tens of thousands, but for simplicity only eight have been shown.

In practice, the width of each channel 26 is under 500μm and generally around 1μm to 200μm, with around 150μm being a preferred width. When three treatment zones are provided, it has been found that a channel length of around 40mm is suitable for the channels 26.

Figure 1 shows two inlets 20a and 20b. Input 20a may be used to introduce the a substance such as the sample and input 20b may be used to apply a pump action. In PCR the input substance comprises the nucleic acid sample to be amplified and one or more nucleic acid primers and nucleic acid poymerase. These substances then enter the input channel 24 and subsequently enter each channel 26.

As the diffusion time of the reactants in a channel is proportional to the diffusion distance squared, splitting each main channel into n channels of similar widths decreases mixing times by a factor of n^2. After mixing, the channels 26 are then subsequently combined in a reverse network until all partial flows of the channels 26 are united in a common outlet channel 24b.

Each channel 26 traverses a plurality of treatment zones 28. In the embodiment shown in Figure 1, a plurality of channels 26 traverse the treatment zones 28 in a parallel manner. Each treatment zone 28 provides a distinct set of conditions e.g. temperature, pH, chemical reaction, optical illumination etc. For instance, a treatment zone may be a thermal zone providing a fixed temperature. Additionally or alternatively, a treatment zone may be a chemical reaction zone. A chemical reaction zone may be implemented by providing reagent molecules immobilised on the surface of the channels to react with sample molecules in a solution or by providing sample molecules immobilised on the surface of the channels with reagent molecules in solution, or a combination. In the embodiment shown in Figure 1, three treatment zones 28a, 28b, 28c are provided. However there may be as many treatment zones as a designer considers necessary for the reaction process being performed.

In this particular embodiment, each treatment zone represents a thermal zone that is maintained at a fixed temperature. Each thermal zone heats or cools the substance in the portion of the channel 26 traversing the thermal zone. For instance, considering PCR with DNA samples with three heating zones, zone 28a may be maintained at a temperature of 95°C to enable melting of double-stranded DNA (dsDNA). Zone 28b may be maintained at a temperature of 60°C to enable binding of specific primers to their target sites and zone 28c may be maintained at a temperature of 60°C to enable the extension of the primers with thermostable DNA polymerase.

Pump action is provided to the channels, the pump action causing substance within the channel 26 to be driven in discrete steps over the treatment zone(s) 28. In use, the pumping action is controlled to provide, in the intermediate channel portion 25, the required cycles for substance in the areas of the treatment zones 28. This pumping profile may be altered easily whenever a new pumping profile is required for a particular process. The substance may be pumped back and forth over the treatment zones.

Typically, the pump action is provided by connecting a pump to one of the inlets 20 or outlets 22.

Typically the pump comprises a syringe attached to a motor that drives the plunger of the syringe within the body of the syringe. When the plunger of the syringe is inserted further into the body of the syringe, a positive pressure is exerted on the fluid within the channels. When the plunger of the syringe is extracted further out of the body of the syringe, a negative pressure is exerted on the fluid within the channels. Consider the case in which a pump is connected to inlet 20b and the other inlet 20a is closed. Positive pressure at inlet 20b will cause the fluid within the channels to move towards the outlet 22

and a negative pressure at inlet 20b will cause the fluid within the channels to move towards the inlet 20.

Another type of pump suitable for use with charged particles is electrophoretic/electro-osmotic pumping. In electrophoretic/electro-osmotic pumping, when voltage is applied, the molecules in the channel move both by electro-osmosis (movement of the whole fluid) and by electrophoresis (movement of the charged particles in the fluid). The electrophoresis movement is in addition to the movement caused by electro-osmosis. Thus the molecules in solution are separated when they move one way and, when the voltage is reversed, the molecules return to their starting positions (and are re-united there). Reagent A may be introduced from one end of a channel 26 and reagent B from the other and the sample is then electrophoretically moved between the two. The amount of movement depends on the electrophoretic mobility of the sample i.e., the charge and size of the molecule and the applied voltage.

The pump is used to drive fluid through the channels 26 in discrete steps. The sample is pumped over the heating zones 28 in discrete steps as many times as desired/requested. The pump action causes the contents of the channels 26 to traverse the treatment zones in a manner defined by the pressure and time-profile of the pump. The rate and number of cycles depends on the fluids under consideration but some enzymes may require around 30-40 cycles to be conducted. Clearly, the pumping profile may be changed at any time without requiring any change of the chip design.

In the embodiment as shown in Figure 1 and considering the example of PCR, typically the pumping action causes a sample within the channel to be moved in a discrete step to the region of treatment zone 28a (the 95°C zone) for 15

seconds and then to be moved in a discrete step to the region of the treatment zone 28b (60°C) for 1 minute.

Figure 3 shows further examples of channel layouts which may use the pumping action as described above. For simplicity, the treatment zones 28 are not shown but a reader will appreciate that one or more treatment zones 28 may be provided intermediate the ends of the channel(s) 26.

In Figure 3a, the channel layout has a plurality of channels 26 each of which has one or more inlets 20. The channels 26 then connect with a main channel 24 which leads to one or more outlets 22. The substance that is input via an inlet 20 traverses the associated channel 26 and any treatment zones located in this region. The contents of each channel 26 are then collected in a common outlet 22. In figure 3b, at least one common inlet 20 is provided which leads, via a common inlet channel 24, to a plurality of channels 26 each of which has at least one outlet 22. Thus, in use, the fluid is introduced via the inlet 20, passes through a common channel 24 and then separates into individual channels 26 which again pass over any associated treatment zones. The contents of the channels 26 are then extracted from individual outlets 22. In Figure 3c, each channel 26 has one or more dedicated inputs 20 and one or more dedicated outputs 22. In such an embodiment there may be a single channel (as shown in solid lines) or a plurality of such channels (as indicated by the dotted lines). Thus, in use, substance is introduced via inlet 20, traverses the associated channel 26 and any treatment zones and is extracted from outlet 22.

In the embodiments discussed so far, each treatment zone 28 is traversed by only one portion of each channel 26. However other channel configurations are suitable. For instance, at least one of the treatment zones may be traversed

by two or more portions of a channel. Figure 3d shows an embodiment in which a single channel is provided, multiple portions of which traverse the treatment zone(s). The substance is introduced at input 20 and extracted from outlet 22 and the channel 26 meanders across the treatment zones (not shown). Of course, it is possible for a channel of any of the embodiments shown in Figures 3a, 3b or 3c to meander in this manner. An example of such an embodiment is shown in Figure 3e in which a plurality of channels 26 pass over the treatment zone(s), with three portions of each channel traversing the treatment zone. In this embodiment, each channel 26 is connected to a common input channel 24a and a common outlet channel 24b. It will be clear to a person skilled in the art that an implementation that includes more than one channel 26 does not require all the channels 26 to be identical. Thus the apparatus may include a variety of channel configurations.

Figure 4a shows some examples of the contents of channels and treatment zones to illustrate how these may be implemented and how a pumping profile may be designed. Figure 4a shows an embodiment suitable for carrying out DNA hybridisation, immunoassay and protein-protein binding, for example. One channel 26 is shown for simplicity although of course the example applies to other channel arrangements as well. In Figure 4a, four treatment zones 28a, 28b, 28c and 28d are provided. Numeral 40 indicates a sample introduced to the channel 26. Treatment zones 28a and 28b each include a reagent A and B respectively, the molecules of which are immobilised in that region, usually on the inner surface of the channel 26 at the positions shown. Treatment zones 28c and 28d may also be chemical reaction zones similar to regions 28a and 28b as described. Alternatively treatment zones 28c and 28d may have other properties, e.g. thermal properties or optical properties. The sample 40 in the channel is moved from left to right as indicated by the arrows in Figure 4a under the influence of a pumping action provided either by changing pressure

(for example using the syringe method described earlier) or by means of voltage (using an electrophoresis/electro-osmotic implementation as described earlier). Thus the sample 40 may be moved by the pumping action across the treatment zones as required. For instance the sample may be moved to the region of zone 28a for a time t_1, moved to zone 28b for a time t_2, moved back to zone 28a for a time t_3, moved to zone 28c for a time t_4, moved back to zone 28b for a time t_5, moved to zone 28d for a time t_6 etc.

A program may be provided for controlling the means for providing the pumping action. Such a program defines the amount of pumping action needed, the amount of time this pumping action is needed for, the time when the pumping action is absent etc.

Figure 4b shows an example suitable for pyrosequencing, for example. In this embodiment, the treatment zone 28 comprises molecules of the sample which are immobilised in the treatment zone. The contents of the channel comprise a number of reagents A, B, C, D which are provided in solution as "plugs" of reagent separated by plugs of buffer. The contents of the channel are then moved over the treatment zone 28 in discrete steps by means of the pump action, in a manner similar to that described with reference to Figure 4a.

In Figure 4c, an implementation suitable for DNA amplification is shown. The channel 26 is shown at time t=0 and $t=t_1$. In this implementation, plugs of reagent A and B are provided in the channel and a plug of a sample 40 is provided, each separated by a buffer. The electro-mobility of the molecules of the sample 40 is greater than that of reagent A or reagent B. Thus, when a voltage is applied to the solution in the channel at t=0, the plug of the sample 40 will move faster than the plug of the reagents A or B. On application of voltage, both the sample 40 and the reagents A and B are moved over the

treatment zones, but at different velocities. Thus, at $t=t_1$, the plug of the sample 40 has moved as shown and the plugs of the reagents A and B have also moved but to a lesser extent then the sample 40.

Each channel 24 and/or each channel 26 may include a mixing station 30 at either end of the channel or along the length of the channel or at intermediate points along the channels. These mixing stations serve to mix the substances introduced to the channels. A preferred embodiment of the mixing stations comprises protuberances with a multi-faceted cross-section (for example a star-like cross-section as shown in Figure 5) that will split the flow and induce turbulence.

Figures 6a and 6b show alternative embodiments of mixing stations. As shown in Figure 6a, a mixing station 30 is provided between the channel 26 and the inlet and/or outlet channel 24. This mixing station 30 comprises an open chamber 32 into which substances from the main channel enter. The chamber 32 causes turbulence within the path of the substance and therefore causes mixing between the reagents. In an alternative embodiment, as shown in Figure 6b, the mixing station comprises a chamber 32 which includes a central protuberance 34. This mixing station operates in a similar manner to that described with reference to Figure 6a. However, the protuberance 34 introduces extra turbulence and hence mixing into the chamber.

The different applications are numerous and any area where DNA, RNA or PCR is involved has potential application, such as defence, diagnostics, forensics, environment, and pharmaceuticals etc. The system also finds application in the fields of DNA sequencing, compound library generation, DNA hybridisation, Sanger DNA sequencing reactions (linear amplification), pyrosequencing reactions, enzymatic cleaving of DNA, enzymatic cleaving of

peptides and proteins (e.g., for tryptic digest of a protein to perform its identification), etc.

Whilst the invention has been described with particular attention to the field of DNA and PCR, the invention may also find application in other areas, in particular other thermal cycling systems that require a plurality of thermal zones. Such applications may include chemical synthesis in which reagents are moved between thermal regions of differing temperatures. The device may also find application in the area of nanoparticle manufacture. The thermal cycling system is applicable to thermally controlled biochemical or biological molecular processes.

The foregoing description of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise forms described, and obviously many other modifications are possible in light of the above teaching. The embodiments were chosen in order to explain most clearly the principles of the invention and its practical applications, thereby to enable others in the art to utilise most effectively the invention in various other embodiments and with various other modifications as may be suited to the particular use contemplated. The invention is defined as set out in the accompanying claims, the features of which may be implemented singularly or in combination.

Claims

1. Apparatus for performing controlled performance of reactions, the apparatus comprising:

at least one channel for receiving a substance, the channel having a first end and a second end;

at least one treatment zone intermediate the first end and second end of the channel, for performing a treatment on substance in the channel(s), and

means for applying a pump action to the channel(s), the pump action in use causing substance within the channel(s) to pass back and forth over the treatment zone(s).

2. Apparatus according to claim 1 further comprising a plurality of channels traversing the treatment zone(s).

3. Apparatus according to claim 2 further comprising an inlet for the introduction of a substance to the channels, the inlet being connected to a main input channel, which is connected to the first end of each channel.

4. Apparatus according to claim 2 or 3 further comprising an outlet for the introduction of a substance to the channels, the outlet being connected to a main outlet channel, which is connected to the second end of each channel.

5. Apparatus according to any preceding claim wherein each treatment zone is traversed by only one portion of each channel.

6. Apparatus according to any preceding claim wherein the apparatus further includes a pump for applying a pump action to the channels, the pump

having a pumping profile to cause substance within the channels to pass back and forth over the treatment zone(s).

7. Apparatus according to any preceding claim wherein the apparatus is for performing nucleic acid amplification.

8. Apparatus according to any preceding claim wherein the apparatus is for performing Polymerase Chain Reaction.

9. Apparatus according to any preceding claim wherein a treatment zone comprises a thermal zone for heating and/or cooling substance within the channels(s) and/or wherein a treatment zone comprises a chemical reaction zone.

10. Apparatus according to any preceding claim wherein the reaction is a chemical reaction.

11. Apparatus according to any preceding claim wherein the width of a channel is less than 500μm.

12. Apparatus according to any preceding claim further including at least one mixing device for mixing substance introduced into the apparatus.

13. Apparatus according to claim 12 wherein the mixing device comprises one or more protuberances in the channel.

14. Apparatus according to claim 13 wherein the protuberances are generally multi-faceted in cross-section.

15. Apparatus for performing controlled reactions comprising

at least one channel for receiving a substance, the channel having a first end and a second end;

at least one treatment zone intermediate the first end and the second end; and

at least one mixing device for mixing substances introduced into the apparatus.

16. A device according to claim 15 wherein the mixing device comprises one or more protuberances in the channel.

17. A device according to claim 16 wherein the protuberances are generally multi-faceted in cross-section.

18. A method of performing nucleic acid amplification comprising:

introducing a substance to be amplified into at least one channel for receiving a substance, the channel having a first end and a second end;

providing at least one treatment zone intermediate the first and second ends of the channel(s), and

applying a pump action to the channels, the pump action causing substance within the channel(s) to pass back and forth over the treatment zone(s).

19. A method according to claim 18 further comprising providing a plurality of channels traversing the treatment zone(s) and introducing substance to be amplified into the channels.

20. A method according to claim 19 further comprising providing an inlet for the introduction of a substance to the channels, the inlet being connected to a main input channel, which is connected to the first end of each channel.

21. A method according to claim 19 or 20 further comprising providing an outlet for the introduction of a substance to the channels, the outlet being connected to a main outlet channel, which is connected to the second end of each channel.

22. A method according to claim 18, 19, 20 or 21 wherein each treatment zone is traversed by a single portion of a channel.

23. Apparatus for performing nucleic acid amplification, the apparatus comprising

at least one channel for a substance to be amplified, each channel having a first and a second end;

at least one thermal zone intermediate the first and second ends of the channel(s) for heating and/or cooling substance within the channel(s); and

means for applying a pump action to the channel(s), the pump action causing substance within the channels to pass back and forth over at least one thermal zone.

24. Apparatus according to claim 23 further comprising a plurality of channels traversing the treatment zone(s).

25. Apparatus according to claim 23 or 24 wherein each treatment zone is traversed by only one portion of each channel.

26. Apparatus according to claim 23, 24 or 25 wherein the nucleic acid amplification comprises Polymerase Chain Reaction.

27. A thermal cycling system comprising:

at least one channel for receiving a substance, the channel having a first end and a second end;

at least one thermal zone intermediate the first and second end of the channel(s) for heating and/or cooling the substance in a channel in the region of the thermal zone(s), and

pump means for applying a pump action to the channel(s), the pump action causing substance within the channel(s) to pass back and forth over the treatment zone(s).

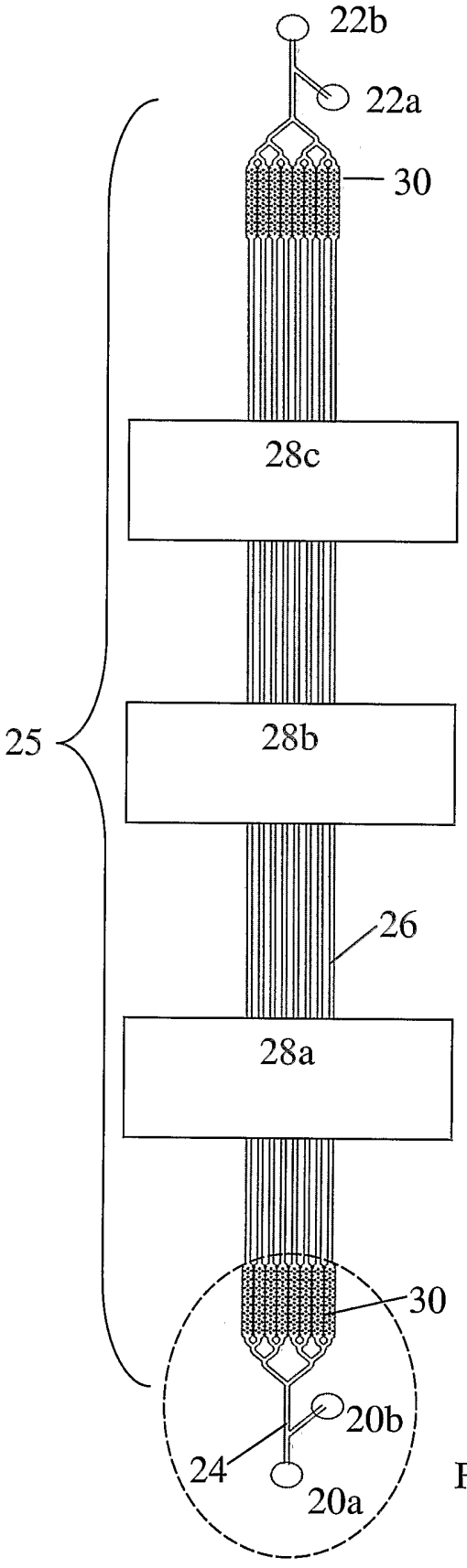

Fig 1

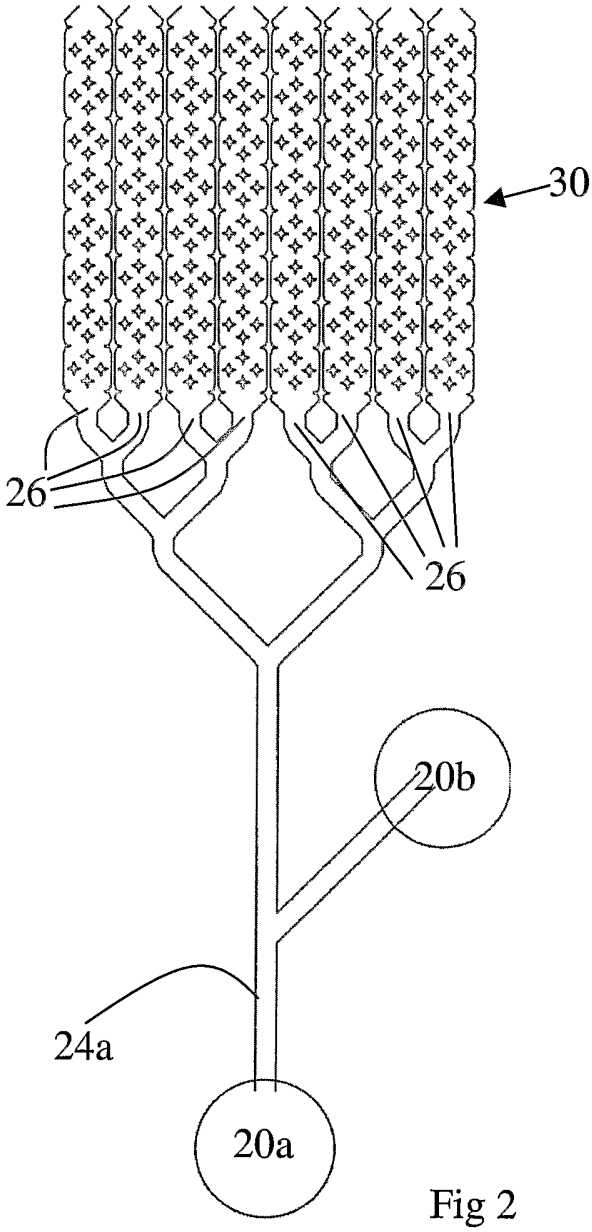

Fig 2

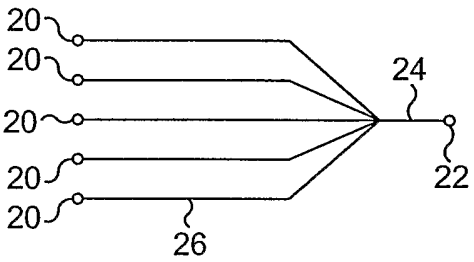

FIG. 3a

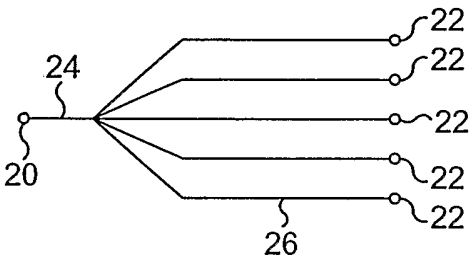

FIG. 3b

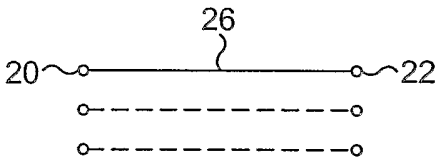

FIG. 3c

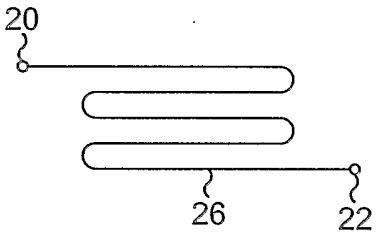

FIG. 3d

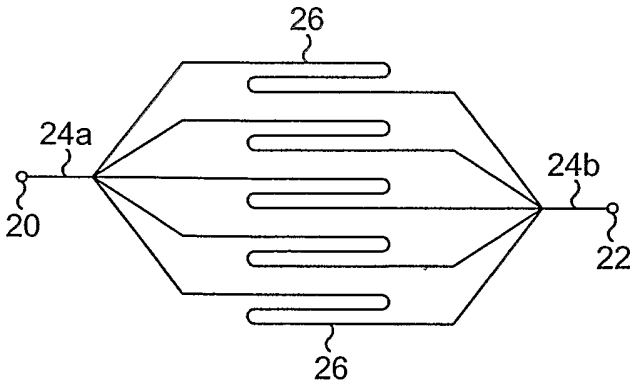

FIG. 3e

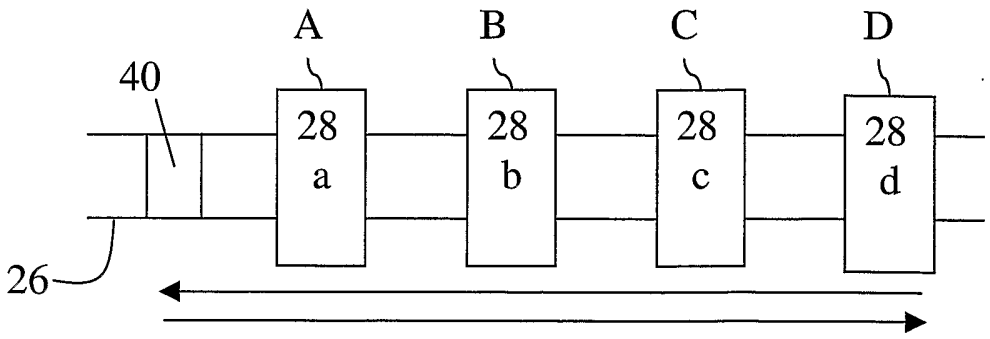

Fig 4a

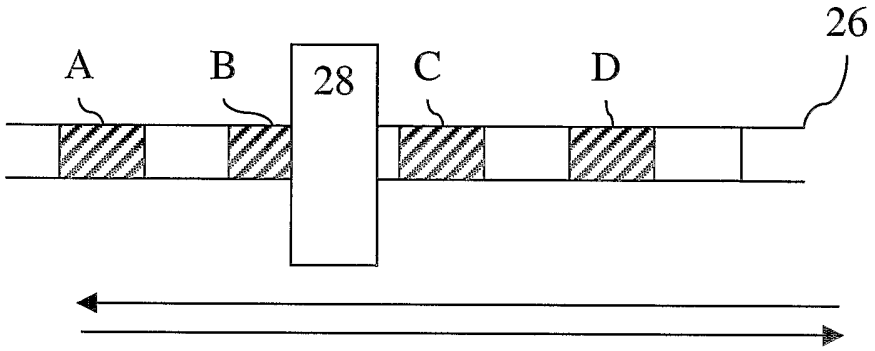

Fig 4b

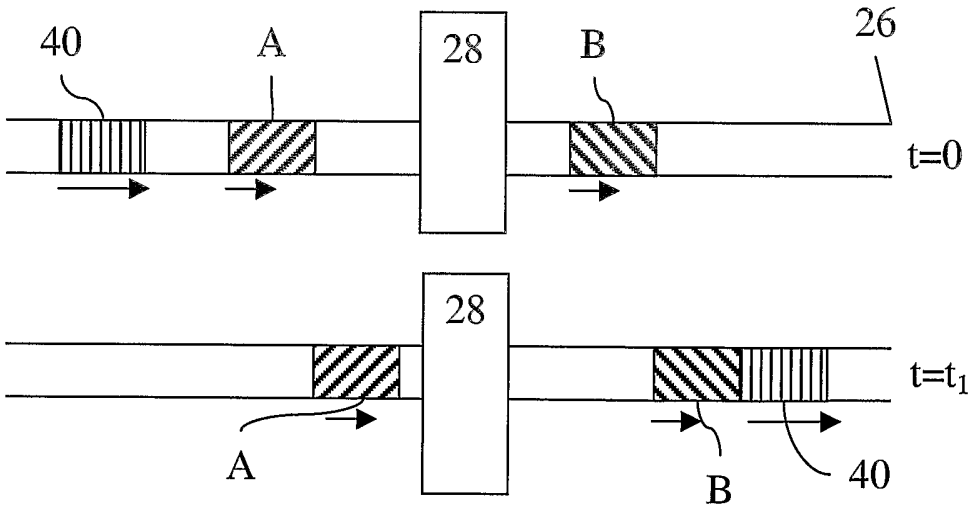

Fig 4c

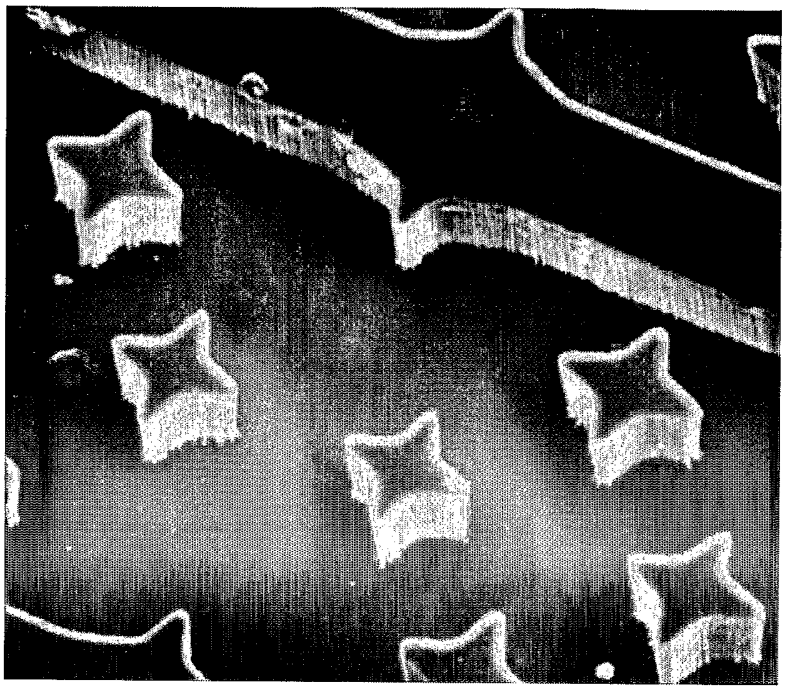

Fig. 5

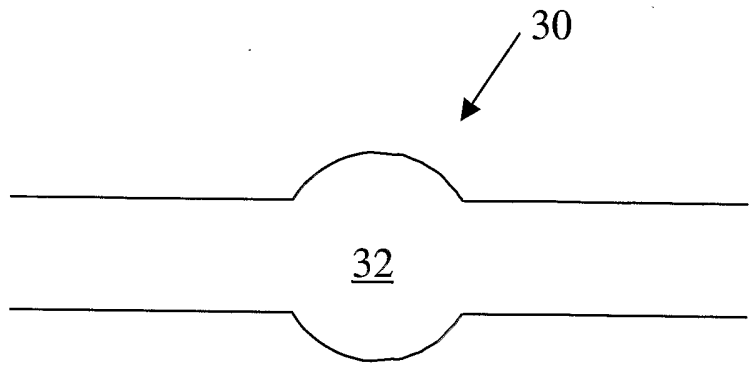

Fig 6a

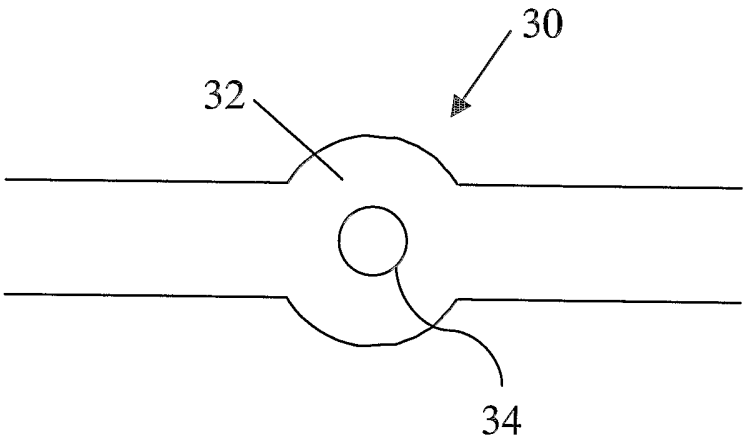

Fig 6b