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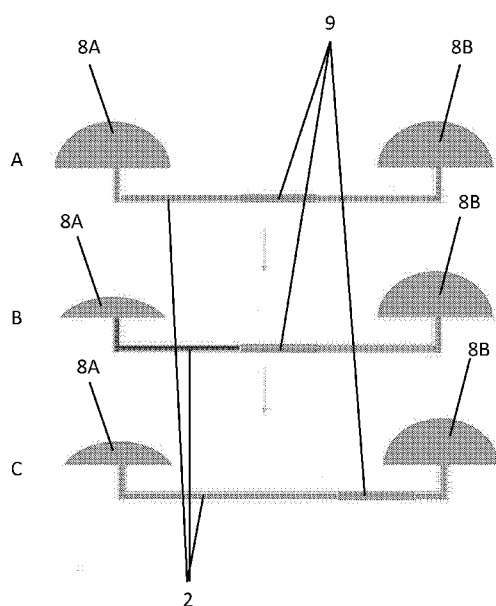


Figure 2

(57) Abstract: The present invention relates to microfluidic devices, such as cassettes for diagnostics, which allow for on-cassette process-
ing including amplification by polymerase chain reaction (PCR), and
systems and methods of using the same, wherein microfluidic cassette
is pressurised prior to amplification of nucleic acid in said sample in
an amplification/PCR section of the device.

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Improved microfluidic devices, systems and methods**Technical Field**

5 The present invention relates to microfluidic devices, such as cassettes, for diagnostics, which allow for on-cassette processing including amplification by polymerase chain reaction (PCR), and systems and methods of using the same, wherein microfluidic cassette is pressurised prior to amplification of nucleic acid in said sample in an amplification/PCR section of the device.

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Background

In recent years there have been ongoing efforts to develop and manufacture microfluidic devices, such as cassettes, that are able to perform various chemical and biochemical analyses and syntheses on device. The goal is to provide devices that allow previously laboratory-based activities to be performed at the point of care (POC) in a timely and efficient manner. Such microfluidic devices can be adapted for use with automated systems, thereby providing the additional benefits such as cost reductions and decreased operator errors.

20 In many cases it is desirable that such microfluidic devices are capable of carrying out molecular techniques including the amplification of nucleic acids. Amplification of DNA by polymerase chain reaction (PCR) requires reaction mixtures be subjected to repeated rounds of heating and cooling (thermocycling) whilst travelling through microfluidic channels present on the cassette, which can include holding the reaction mixtures in one or more static chambers present on the cassette. The temperature of the reaction mixture within the cassette therefore must be varied during a PCR cycle, and in fact varied many times during a PCR experiment, which typically requires a relatively high number of cycles to obtain a suitable amplification of the nucleic acid from the sample. The variation of the temperature within the cassette does bring technical challenges. For example, denaturation of DNA typically takes place at greater than 90 degrees, and often close to 98 degrees C. The temperature then needs to drop as annealing a primer to the denatured DNA is typically performed at around 45 degrees to 70 degrees C. Finally, the temperature is raised again as

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the step of extending the annealed primers with a polymerase is typically performed at around 70 degrees to 75 degrees C. Various mechanisms to allow for this have been described, with systems employing heated zones being utilised, or means for rapidly changing the temperature within microfluidic channels being employed. Such devices and systems are known.

One challenge associated with the rapid cycling of temperatures is that, particularly at the higher temperatures, bubbles coming out of the sample fluid can decrease the PCR efficiency and also make accurate positioning of the sample less predictable.

It would be beneficial to obviate or mitigate some of the problems associated with the prior art.

The term 'Shuttle flow' or 'Shuttle PCR' refers to techniques in which thermocycling is performed by shuttling small plugs of PCR mixture back and forth between temperature zones. In this way temperature variations occur spatially.

Throughout this document reference to "microfluidic" means with at least one dimension less than 1 millimetre and/or able to deal with microlitre or less portions of fluid.

Throughout this document reference to "cassette" or "chip" means an assembled unit comprising one or more substrates with channels or chambers therein through which fluid can flow. Such cassettes may include different regions or zones in which activities such as sample mixing, filtering, PCR amplification, identification and/or visualisation can occur and may include on-board reagents. The cassettes are typically designed to be received by a diagnostic instrument such as a point-of-care (POC) instrument which incorporates additional functionality to allow a diagnostic test, or part of such a test, to be automated.

Summary of the Invention

According to a first aspect of the present invention there is provided a microfluidic device comprising;

a fluidic channel having a polymerase chain reaction (PCR) section, said PCR section configured to allow the temperature changes required for polymerase chain reaction (PCR)

5 to occur therein,

a fluidically isolatable portion of the device (or portion of the fluidic channel) comprising at least the portion of the fluidic channel having a PCR section,

at least one closure means actuatable to fluidically isolate the fluidically isolatable portion from the external environment, and

10 at least one means to increase the pressure in said isolatable portion when it is isolated.

A PCR section of the channel is a portion of the channel that has been configured to allow thermocycling of a sample passing therethrough to occur. The PCR section of the channel is specifically adapted to allow a liquid sample travelling therethrough to be heated and cooled

15 in a cyclical manner to temperatures that allow for denaturing of DNA, annealing of DNA and amplification of DNA. The PCR section of the channel is heatable either by an integral heat source within the channel or the wall of the device, or more typically by being brought into the proximity of an external heat source which applies heat to microfluidic device (such as a microfluidic cassette) in a manner that allows heat to transfer into the PCR section of the

20 channel.

Importantly, the at least one means to increase the pressure is adapted to increase pressure within the PCR section of the channel prior to amplification of a sample by thermocycling within the PCR section.

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The microfluidic device is suitable for receiving a liquid sample. Preferably it is a microfluidic cassette for insertion into a point of care (POC) diagnostic instrument.

The fluidic channel is for transporting said liquid sample and has an inlet end and at least one

30 second end that are in fluid communication with each other.

Preferably the fluidic channel is a microfluidic channel.

Most preferably the circumference of the channel is fluid-tight.

Throughout this document, the term 'fluid-tight' means a sealing that prevents the passage of liquids, such as water, and/or gases, in particular air, even if put under pressure (within predetermined limits). For example, the seal will remain intact up to approximately 2 bar of pressure difference between the interior and exterior sides of the slide fastener.

Optionally, at least part of the microchannel has a serpentine configuration.

Advantageously serpentine configurations can allow long fluid flow in a relatively small footprint, which means an efficient heat transfer by increasing surface to volume ratio.

In some embodiments the serpentine configuration can be useful in allowing fluid to travel over multiple different temperature zones, for example different heat plates, before curving back to repeat the process. In other embodiments the serpentine configuration can be utilised along with methods of shuttling fluid back and forth between temperature zones (i.e. shuttle flow (shuttle PCR) or

The isolatable portion may be the entire fluidic channel present on the device. In one embodiment, substantially all of the microfluidic channel present on a cassette is the isolatable portion. In this embodiment, this means that once a sample is inserted into the cassette i.e. inserted into the start of the microfluidic channel, the channel is then sealed, and the pressure is increased.

Advantageously, using positive pressure (i.e. inducing a positive pressure within the a microfluidic channel prior to significant further activity) in other microfluidic channels within a device such as a cassette, as well as in the PCR section helps to minimise fluid(liquid)-plug breakup i.e. break-up of the sample fluid plug rather than maintaining it as a single plug of fluid (the sample plug may incorporate additional reagents as it moves through the cassette). Having positive pressure acts to keep the meniscus 'tight' and prevent fluid 'creep' along the walls which can then coalesce at point up/down stream of the main plug, creating two-plugs

with an intervening air/gas bubble (this is a particular issue in the PCR section as the intervening air/gas bubble, which is not generated through de-gassing, can occur in the PCR region owing to the higher temperatures that the sample plug is subjected to.

- 5 Preferably the at least one closure means is at least one air-tight valve or sealing means actuatable to fluidically isolate the fluidically isolatable portion.

Preferably the means to increase pressure is a positive displacement pump.

- 10 Preferably the means to increase pressure may be actuated to a first position to apply a first pressure to the isolatable portion, and a second position to apply a second pressure to the isolatable portion.

Preferably the means to increase pressure is a bellows pump.

15

The bellows pump is a substantially hemispherical compressible material with an internal cavity. The bellows pump is associated with an inlet to the microfluidic channel. When pressure is applied to the external surface of the bellows pump the material is compressed such that the internal cavity is reduced in volume and any fluid in the internal cavity is pushed.

20

The bellows pump is resiliently biased to return to an expanded position.

Optionally, the means to increase pressure is valve associable with a syringe pump.

- 25 Optionally the means to increase pressure is a pneumatic pressure applicator.

Optionally, the means to increase pressure is associable with an external pressure source.

Optionally, the means to increase pressure is an on-cassette peristaltic pump.

30

An external pressure source e.g. mechanical, pneumatic or hydraulic source of pressure can be applied by an external host instrument.

Preferably there are a plurality of means to increase the pressure.

5

Preferably the plurality of means to increase the pressure comprise a plurality of positive displacement pumps, with each pump fluidically connected to the fluidically isolatable portion of the device. In a preferred embodiment these are two bellows pumps.

10 Advantageously by using multiple means to increase pressure e.g. two or more positive displacement pumps, this allows the pressure in the portion of the microchannel that includes the amplification zone to be increased without leakage.

Most preferably the plurality of means to increase the pressure are simultaneously or
15 concurrently actuatable to increase the pressure in the isolatable portion.

Advantageously by simultaneously increasing the pressure in the portion of the microchannel including the amplification zone using multiple means to increase pressure e.g. two or more positive displacement pumps, this allows the pressure in the portion of the microchannel that
20 includes the amplification zone to be increased and then the means to increase pressure can be further used to move liquid sample within the microchannel without placing a further pressure load on the system.

Preferably the means to increase the pressure are actuatable to a first compression position
25 and a second compression position.

More preferably the second compression position is further compressed (thus having less volume within it) than the first position.

30 Preferably the means to increase pressure is adapted to increase the pressure in the isolatable portion

The PCR section is heatable.

An external source of heat can be applied by an external instrument.

- 5 Preferably the means for increasing pressure in the isolatable portion induces a pressure higher than atmospheric pressure.

Preferably the means for increasing pressure in the isolatable portion induce a pressure of 1.6bar or higher.

10

Optionally the means for increasing pressure in the isolatable portion induce a pressure of 1.2bar or higher.

- Advantageously, increasing the pressure reduces the boiling point of fluids within the isolatable portion. This can be advantageous in allowing the system to be used in a range of different location, including at high altitudes.
- 15

Preferably the microfluidic device comprises on-board reagents for performing at least one diagnostic assay.

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According to another aspect of the present invention there is provided a diagnostic system comprising the microfluidic device of the first aspect, and a host instrument able to receive said microfluidic device.

- 25 Optionally, the host instrument comprises an interface enabled to convey pressure from said host instrument to said means for increasing pressure on said microfluidic device.

Optionally the host instrument comprises a pneumatic interface enabled to convey pneumatic pressure from said host instrument to said means for increasing pressure on said microfluidic device.

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Optionally, the host instrument comprises a mechanical interface enabled to convey mechanical pressure from said host instrument to said means for increasing pressure on said microfluidic device.

- 5 Preferably the host instrument is adapted to actuate an increased pressure in the isolatable portion of the cassette when it is isolated and prior to amplification of a sample by thermocycling within the PCR section.

Preferably the host instrument comprises a microprocessor.

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The microprocessor controls the interactions between the host instrument to the microfluidic device.

- 15 Optionally the host instrument comprises means for heating or applying heat to at least a portion of the PCR section of the device.

Preferably the means for heating is one or more temperature-controlling elements configured to provide at least one temperature zone.

- 20 According to a further aspect of the present invention there is a method of amplifying nucleic acid from a sample comprising,
providing the device or system of the above aspects,
actuating the at least one closure means and fluidically isolating at least a portion of the device, more particularly the portion of the microfluidic channel comprising at least the PCR
25 section,
increasing the pressure within the isolated portion,
and, after the pressure has been increased within the isolated portion, carrying out polymerase chain reaction (PCR) amplification steps within the isolated portion to amplify nucleic acid within the sample.

30

Preferably the sample is inserted into the device prior to the step of fluidically isolating at least a portion of the device comprising at least the PCR section.

Optionally the entire device is fluidically isolated.

- 5 By providing a device with isolatable portions that are fluid-tight, and particularly air-tight, the cassette can be pre-pressurised prior to polymerase chain reaction (PCR) thermocycling occurring.

Alternatively, only portions of the microfluidic channel are fluidically isolated.

- 10 By isolating only portions of the microfluidic channel containing the PCR zone, other branches of the channel can be closed off, including branches to reservoirs etc, either to prevent ingress of materials detrimental to PCR reactions or to reduce the volume to which pressure is applied.
- 15 Optionally one or more fluid-tight valves are used to fluidically isolate the system.

The valves may be within the channels or form the inlet (or outlet if present).

- 20 Making the device such as a microfluidic cassette, or a portion thereof, air-tight allows pressure to then be increased.

Preferably the step of increasing the pressure comprises actuating a plurality of means to increase the pressure.

- 25 Preferably the step of increasing the pressure comprises compressing a plurality of positive displacement pumps.

- Advantageously by using multiple means to increase pressure e.g. two or more positive displacement pumps, this allows the pressure in the portion of the microchannel that includes
30 the amplification zone to be increased without leakage.

Preferably the step of increasing the pressure comprises compressing a plurality of positive displacement pumps simultaneously or concurrently to increase the pressure in the isolatable portion.

- 5 Preferably the step of increasing the pressure comprises compressing a plurality of positive displacement pumps to a first position, wherein the positive displacement pumps may still be further compressed to at least a second position.

10 Optionally the step of increasing the pressure increases the pressure in the isolated portion to greater than 1.2bar , more preferably to greater than 1.4bar yet more preferably greater than 1.6bar.

15 Optionally the step of increasing the pressure increases the pressure in the isolated portion to between 1.5bar and 2bar.

Preferably the step of increasing the pressure increases the pressure in the isolated portion to 1.6bar.

20 Throughout this document reference to “microfluidic” means with at least one dimension less than 1 millimetre and/or able to deal with microlitre or less portions of fluid.

Various further features and aspects of the invention are defined in the claims.

Brief Description of the Drawings

25 Embodiments of the present invention will now be described by way of example only with reference to the accompanying drawings in which like parts are provided with corresponding reference numerals and in which:

30 Figure 1a provides a perspective view of a microfluidics cassette according to an aspect of the present invention, the view showing an outer surface; Figure 1b provides an exploded perspective view of a similar microfluidics cassette shown from the other side, with various internal features visible.

Figure 2a, 2b, 2c is a simplified diagram showing the use of two bellows pumps to pressurise a sealed area of a cassette, with the first applying pressure and the second acting as a reservoir; and

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Figure 3a, 3b, 3c is a simplified diagram showing the use of two bellows pumps that are actuated simultaneously pressurise a sealed area of a cassette. Fig 3B shows the pressurization step while Fig. 3C shows the reciprocation mode to move the sample while keeping the pressure in a sellable portion of the cassette.

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Detailed Description

According to a first aspect of the present invention, and as generally depicted in figure 1, there is provided a microfluidic cassette **1**, more generally termed a 'device', with a micro-channel **2**, where the micro-channel 2 allows for continuous flow-through of fluid as required. As shown most clearly in Figure 1b, which shows the internal workings of a similarly configured cassette, the micro-channel 2 is formed inside the microfluidic cassette 1, in the desired length and shape to allow the passage of a sample, typically a biological sample in liquid format, and/or reagents, some of which may be incorporated on-cassette during the flow-through, along a fluid flow path and through various zones or areas which allow different activities to occur including amplification of DNA from the sample by polymerase chain reaction (PCR). A PCR section of the channel **7** is a portion of the channel that has been configured to allow thermocycling of a sample passing therethrough to occur. The PCR section 7 of the channel (and cassette) is specifically adapted to allow a liquid sample travelling therethrough to be heated and cooled in a cyclical manner to temperatures that allow for denaturing of DNA, annealing of DNA and simplification of DNA. The PCR section 7 of the channel is heatable either by an integral heat source within the channel or the wall of the device, or more typically by being brought into the proximity of an external heat source which applies heat to microfluidic device (such as a microfluidic cassette) in a manner that allows heat to transfer into the PCR section of the channel.

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Various valves and fluidically intercommunicating offshoots such as additional channels, reservoirs or chambers can be used to allow mixing, washing, removal and other actions to occur according to the needs of a diagnostic or biochemical assay to be performed therein in an automated or semi-automated manner. The assay is generally carried out by allowing a sample to interact and/or react with one or more reagents in one or more steps; typically in one or more channels or chambers of the device, for times and at temperatures effective in forming a detectable product that indicates the presence or absence of an analyte in the sample. The channel 2 is formed in a first surface of a first substrate **3**, typically a substantially planar, substantially rigid substrate which in this embodiment is polypropylene. The first substrate **3** is overlaid with a second substrate **4**, which in this embodiment is a polypropylene film. By bonding the first substrate material **3** to the film **4**, for example using laser welding, a substantially closed channel **2** is provided (inlets and outlets can be included as required). It will be understood that if the first substrate **3** is a planar element with an upper and lower surface, the majority of the microchannel **2** can be formed in the upper surface or the lower surface. It is however often desirable that the second substrate, i.e. the film **4**, forms the upper wall of the microchannel **2** in use. The use of laser welding ensured that a fluid-tight, and more specifically an air-tight seal is created around the microchannels, such that no air can escape or leak out between the first and second substrates. It will be understood that methods other than laser welding can be used and will typically be selected based on the substrate materials themselves. The seal or weld is sufficiently strong to withstand increased pressure levels within the cassette compared to outside of the cassette. Typically, it is strong enough to withstand internal gauge pressures of at least 2bar.

Alternatively, it would be understood that although this embodiment has the second substrate as a film **4**, the second substrate can be another material and may itself have grooves or channels formed on its surface that can be aligned with the channels of the first substrate. By bonding the substrates **3**, **4** together, a closed channel **2** is provided (again inlets and outlets can be included as required).

The cartridges are typically consumables; i.e., they are used once and then discarded; and contain all or many of the reagents needed for one or more assays to be performed. Such reagents can be held on the cartridge in reservoirs or similar.

Where necessary, the first and second substrates 3, 4 can be aligned prior to bonding. The length and cross-sectional shape of the channel 2 can be any appropriate shape to allow for the desired transport and processing of a sample and or reagents. Such microfluidic systems
5 'lab-on-a-chip' type systems being well known in the art.

The cassette 1 is provided with an inlet 5 for receiving a sample into the microfluidic channel. The inlet 5 is provided with an air-tight seal in the form of an insertable cap 6. The insertable cap 6 is hinged to the surface of the cassette for ease of use. The cap 6 is sized to be received
10 into the aperture of the inlet and is provided with a resiliently deformable collar that acts to form an air-tight seal in the inlet after the sample has been inserted and when the cap 6 is closed.

In this embodiment, the other opposite end of the microchannel 2 to the inlet 5 is
15 permanently sealed as it is a single use cassette. However, a similar cap to that used on the inlet could be used to close an outlet if present. In an alternative embodiment, valves positioned within the microchannel, away from the inlet, can be used to create a smaller volume air-tight area if required (still containing the PCR amplification zone, or PCR section 7). For example, a first air-tight valve could be provided proximate and upstream of the
20 amplification zone 7 and a second air-tight valve could be provided proximate and upstream of the amplification zone 7. Once the sample is between the two valves these can be closed and the pressure in the microchannel 2, containing the amplification zone 7, which is between the two valves raised as in other embodiments.

25 The microchannel 2 is a continuous flow channel and, in this embodiment, it includes an amplification zone or PCR section 7 which includes three portions of channel that are heatable to different temperatures to allow the thermocycling of temperatures. In this embodiment there is a portion of the channel in the PCR section 7 that is heated to a temperature of 90 degrees to 98 degrees C. There is another portion of the channel in the PCR section 7 that is
30 heated to a temperature of around 45 degrees to 70 degrees C. A further portion of the channel in the PCR section 7 is heated to a temperature of around 70 degrees to 75 degrees C. In this embodiment the heating of the channel occurs when an external heater source is

brought into close proximity with the cassette 1 such that heat exchange occurs through the wall of the cassette into the relevant portions of the micro-channel. The microchannel in the amplification zone or PCR section 7 is serpentine in shape such that the sample will travel through the different temperature zones as it travels along each section of the serpentine shape. In this embodiment the heaters are provided in a separate host instrument which receives the cassette therein, but in theory the heaters could form part of the cassette, for example being positioned within the channels themselves. There are also temperature sensors positioned within or close to the relevant parts of the channel to allow the temperature in the channel to be read and if necessary controlled. Although in this embodiment a serpentine shaped microchannel 2 is used in the PCR section 7 to ensure sample moving through the channel is cyclically taken through the different temperatures required for a PCR reaction to occur, it would be understood that other layouts can be used to thermocycle a sample through different temperatures in a microfluidic cassette. For example, a shuttle flow system could be used in place of the serpentine shaping. In a shuttle flow variant, the sample is shuttled back and forward between two or more sections of the microchannel (as opposed to continuously moving forward as in the serpentine variant), each section being held at one of the required temperatures for a PCR reaction to occur.

The microchannel includes a first bellows pump **8A** in fluid communication with the microchannel 2 (via bellows inlet **10A**). The first bellows pump 8A is a positive displacement pump, comprising a hemispherical, compressible bellow which is resiliently biased to return to its expanded shape. The internal dimensions of the hemispherical bellow can be changed by applying pressure to its external surface. c. In the present embodiment, mechanical actuators found on a host instrument into which the cassette is placed act under the control of a microprocessor to actuate the bellows pumps by compression, partial compression, partial decompression or decompression of the same. The actuators could however, in theory, be incorporated onto the cassette.

The microchannel also includes a second bellows pump **8B** in fluid communication with the microchannel 2 (via bellows inlet **10B**). The second bellows pump 8B is also a positive displacement pump, in this example again comprising a hemispherical, compressible bellow

which is resiliently biased to return to its expanded shape. The second bellows 8B can be partially compressed or decompressed as well as fully compressed or decompressed.

The microchannel 2 also includes various other reservoirs, branches and chambers (such as a capture and viewing chamber for example) as are required to carry out a molecular assay. Where present, the host instrument will include elements such as actuators, heaters and optical elements.

Whilst this example describes bellows pumps with a hemispherical bellow, it would be understood by those skilled in the art that other bellows formations could be used, or indeed alternative types of positive displacement pump could be used. For example, another type of positive displacement pump is a syringe pump in which the displacement of the piston of a syringe inside a syringe cylinder causes fluid to be sucked into or pressed out of the syringe outlet.

In use, a liquid sample 9 is introduced into the inlet 5, which leads to the micro-fluidic channel 2 and the cap 6 is closed to form an air-tight seal. The cassette 1 is placed into a host instrument (not shown). In a first embodiment, as depicted in a basic form in figure 2, at rest, air pressure on either side of the liquid sample 9 is equal (see fig 2A). The hemispherical bellow 8A is then actuated by pins present in the host instrument pressing down on its external surface and thus reducing the volume that air can fill between the bellow and the liquid sample. The host instrument pins or actuators can be provided with sensors to detect the surface of the bellows to ensure specific control of the pressure is achieved. As the cassette is now a sealed (air-tight) system the air has nowhere to go, so the same amount of air is in a smaller volume, so the pressure of the air must increase (see fig 2B where there is an increase in pressure to the left of the sample which will act to push the sample 9 to the right). As the air pressure behind the liquid sample 9 is higher than the air pressure in front of the liquid sample, the liquid sample is pushed forward until the air pressure before and after balance again (see Fig 2C – both sides have equal pressure again). In this embodiment the second bellow 8B is acting as a pressure ‘reservoir’ thus dampening some of the movement of the sample as the relative change in pressure is not as large, meaning the sample can still move, but pressure inside the channel can stay under 2 bar (but still increase

compared to air pressure outside of the sealed cassette). In this embodiment, the absolute pressure inside the channel is increased to 1.6bar prior to the sample being amplified in the amplification zone.

- 5 Notably, if only one bellow is used, the pressure would still be applied, but the movement of the sample would be much greater. This excessive movement and pressure can be avoided (i.e. dampened) even with only one bellow by using much longer channels, but this requires a greater footprint on the cassette which is typically undesirable.
- 10 Whilst the above example describes how the second bellow can be used as a reservoir, figure 3 is a preferred variation of the present invention which shows that utilising a plurality of positive displacement pumps such as bellows or diaphragm pumps has additional benefits. The preferred use of the present invention is to include at least two bellows pumps (or other positive displacement pumps) to induce an increase in pressure to at least 1.6 bar within the
- 15 microchannel 2. Again, in use, a liquid sample 9 is introduced into the inlet 5, and into the micro-fluidic channel 2; and the cap 6 is closed to form an air-tight seal. The cassette 1 is placed into a host instrument (not shown). Prior to the cassette being closed by the cap 6, both of bellows pump 8A and bellows pump 8B are fully decompressed. In the two examples above, the volume of the entire system changes whenever bellow 8A is depressed. This
- 20 requires constantly changing the pressure inside the cassette during the assay itself which makes fluid control within the microchannel more difficult. Therefore, in this preferred embodiment, in order to increase the pressure in the sealed portion of the microchannel 2 which contains the amplification zone or PCR section 7, both first bellow 8A and second bellow 8B are actuated simultaneously to keep the overall volume of the system equal, and
- 25 so keep the overall pressure of the system equal. The host instrument applies a push force to the external surfaces of both first bellow 8A and second bellow 8B such that they are compressed by a first amount (the bellows are compressed approximately half-way) to increase the pressure inside the cassette to approx. 1.6 bar. This can be seen in Fig 3B and it can be seen that the sample at this point does not move if bellows pump 8A is positioned on
- 30 the opposite side of the sample to bellows pump 8B i.e. a bellow is provided at either side of the sample, (in this case either side of the inlet 5 into which the sample was initially inserted) and in fact preferably at either side of the amplification zone. At this stage the bellows pumps

8A and 8B can continue to be used to move the liquid sample 9 within the channel as required by the assay. By including various valves, and actuating them to open and close as required, this can allow sample to be very effectively moved throughout various regions of the micro-channel such that different actions and reactions can be carried out. In order to move the liquid sample 9 to the right, bellows pump 8A can be further compressed by a second amount and Bellow B allowed to re-inflate (the resilient material of the bellows allowing it to revert back to its original hemispherical shape). Using this mechanism of concurrently or simultaneously actuating or compressing the two valves – or using two positive displacement pumps simultaneously to raise the pressure in the portion of the microchannel containing the amplification zone 7 prior to the liquid sample 9 being amplified (which requires the heating and cooling to different temperatures) means the pressure inside always stays at 1.6bar and the liquid sample 9 can also be moved around using the same positive displacement pumps without the danger of increasing the pressure within the microchannel to a point where the bonds, such as laser bonds, valves or cap will leak.

It is preferred that the pressure in the portion of the microchannel containing the amplification zone is increased to 1.6 bar prior to thermocycling occurring in the amplification zone to obtain the benefits of reducing bubble formation, as well as ensuring that the system works even at high altitude, whilst not damaging or exceeding the limits of any of the valves, caps or seals present in the cassette. However, the pressure could also be raised to between 1.5bar and 2bar and significant beneficial effects would still be seen. In some embodiments even raising the pressure to 1.1bar or 1.2bar or higher would still allow the benefits of the system working at higher altitudes and some reduction in bubble formation when compared to a standard system where no pressure increase prior to amplification would typically occur.

All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. Each feature disclosed in this specification (including any accompanying claims, abstract and drawings) may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of

equivalent or similar features. The invention is not restricted to the details of the foregoing embodiment(s). The invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity.

It will be understood by those within the art that, in general, terms used herein, and especially in the appended claims are generally intended as "open" terms (e.g., the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.). It will be further understood by those within the art that if a specific number of an introduced claim recitation is intended, such an intent will be explicitly recited in the claim, and in the absence of such recitation no such intent is present. For example, as an aid to understanding, the following appended claims may contain usage of the introductory phrases "at least one" and "one or more" to introduce claim recitations. However, the use of such phrases should not be construed to imply that the introduction of a claim recitation by the indefinite articles "a" or "an" limits any particular claim containing such introduced claim recitation to embodiments containing only one such recitation, even when the same claim includes the introductory phrases "one or more" or "at least one" and indefinite articles such as "a" or "an" (e.g., "a" and/or "an" should be interpreted to mean "at least one" or "one or more"); the same holds true for the use of definite articles used to introduce claim recitations. In addition, even if a specific number of an introduced claim recitation is explicitly recited, those skilled in the art will recognize that such recitation should be interpreted to mean at least the recited number (e.g., the bare recitation of "two recitations," without other modifiers, means at least two recitations, or two or more recitations).

It will be appreciated that various embodiments of the present disclosure have been described herein for purposes of illustration, and that various modifications may be made without departing from the scope of the present disclosure. Accordingly, the various embodiments disclosed herein are not intended to be limiting, with the true scope being

5 indicated by the following claims.

Claims

1. A microfluidic device comprising;
a microfluidic fluidic channel having a polymerase chain reaction (PCR) section, said
5 PCR section configured to allow the temperature changes required for polymerase
chain reaction (PCR) to occur therein,
a fluidically isolatable portion of the device comprising at least the portion of the
fluidic channel having a PCR section,
at least one closure means actuatable to fluidically isolate the fluidically isolatable
10 portion from the external environment, and
at least one means to increase the pressure in said isolatable portion when it is
isolated.
2. A microfluidic device according to claim 1 wherein the at least one means to increase
15 the pressure is adapted to increase pressure prior to amplification of a sample by
thermocycling within the PCR section.
3. A microfluidic device according to any of the previous claims wherein the at least one
means to increase the pressure is a displacement pump.
20
4. A microfluidic device according to any of the previous claims wherein isolatable
portion is the entire fluidic channel present on the device.
5. A microfluidic device according to any of the previous claims wherein the device
25 comprises at least one air-tight valve or sealing means actuatable to fluidically isolate
the fluidically isolatable portion.
6. A microfluidic device according to any of the previous claims wherein the means to
increase pressure is a positive displacement pump configured to both increase
30 pressure in the fluidically isolatable portion and to move fluid within the fluidically
isolatable portion.

7. A microfluidic device according to any of the previous claims wherein the means to increase pressure is actuatable to a first position to apply a first pressure to the isolatable portion, and a second position to apply a second pressure to the isolatable portion.
- 5
8. A microfluidic device according to any of the previous claims wherein the means to increase pressure is a bellows pump.
9. A microfluidic device according to any of the previous claims wherein the means to increase pressure is associable with an external pressure source or actuator
- 10
10. A microfluidic device according to any of the previous claims wherein there are a plurality of means to increase the pressure.
11. A microfluidic device as in claim 10 wherein the plurality of means to increase the pressure comprise a plurality of positive displacement pumps.
- 15
12. A microfluidic device according to any of claims 10 or 11 wherein the plurality of means to increase the pressure are simultaneously or concurrently actuatable to increase the pressure in the isolatable portion.
- 20
13. A microfluidic device according to any of the previous claims wherein the means for increasing pressure in the isolatable portion induces a pressure higher than atmospheric pressure and, preferably 1.1bar or higher and more preferably a pressure of 1.6bar or higher.
- 25
14. A diagnostic system comprising the microfluidic device of any of the previous claims and a host instrument able to receive said microfluidic device.
15. A diagnostic system as in Claim 14 wherein the host instrument comprises an interface enabled to convey pressure from said host instrument to said means for increasing pressure on said microfluidic device.
- 30

16. A diagnostic system as in any of Claims 14 to 15 wherein the host instrument comprises a microprocessor which controls the interactions between the host instrument and the microfluidic device.

5

17. A method of amplifying nucleic acid from a sample comprising,
providing the device or system of any of Claims 1 to 16,
fluidically isolating at least a portion of the device comprising at least the PCR section,
increasing the pressure within the isolated portion,
and, after the pressure has been increased within the isolated portion, carrying out
polymerase chain reaction (PCR) amplification steps to amplify nucleic acid within the
sample.

10

18. A method of amplifying nucleic acid as in Claim 17 wherein the sample is inserted into
the device prior to the step of fluidically isolating at least a portion of the device
comprising at least the PCR section.

15

19. A method of amplifying nucleic acid as in Claim 17 or 18 wherein one or more
fluid-tight valves are closed to fluidically isolate the entire device or only a portion of
the microfluidic channel containing the PCR section is fluidically isolated.

20

20. A method of amplifying nucleic acid as in any of Claims 17 to 19 wherein the step of
increasing the pressure comprises actuating a plurality of means to increase the
pressure.

25

21. A method of amplifying nucleic acid as in any of Claims 17 to 20 wherein the step of
increasing the pressure comprises compressing a plurality of positive displacement
pumps.

30

22. A method of amplifying nucleic acid as in any of Claims 17 to 21 wherein the step of
increasing the pressure comprises compressing a plurality of positive displacement

pumps simultaneously or concurrently to increase the pressure in the isolatable portion.

5 23. A method of amplifying nucleic acid as in any of Claims 17 to 22 wherein the step of increasing the pressure comprises compressing a plurality of positive displacement pumps to a first position, wherein the positive displacement pumps may still be further compressed to at least a second position.

10 24. A method of amplifying nucleic acid as in any of Claims 17 to 23 wherein the step of increasing the pressure increases the pressure in the isolated portion to at least 1.6bar.

15 25. A method of amplifying nucleic acid as in any of Claims 17 to 23 wherein the step of increasing the pressure increases the absolute pressure in the isolated portion to between 1.2bar and 2bar.

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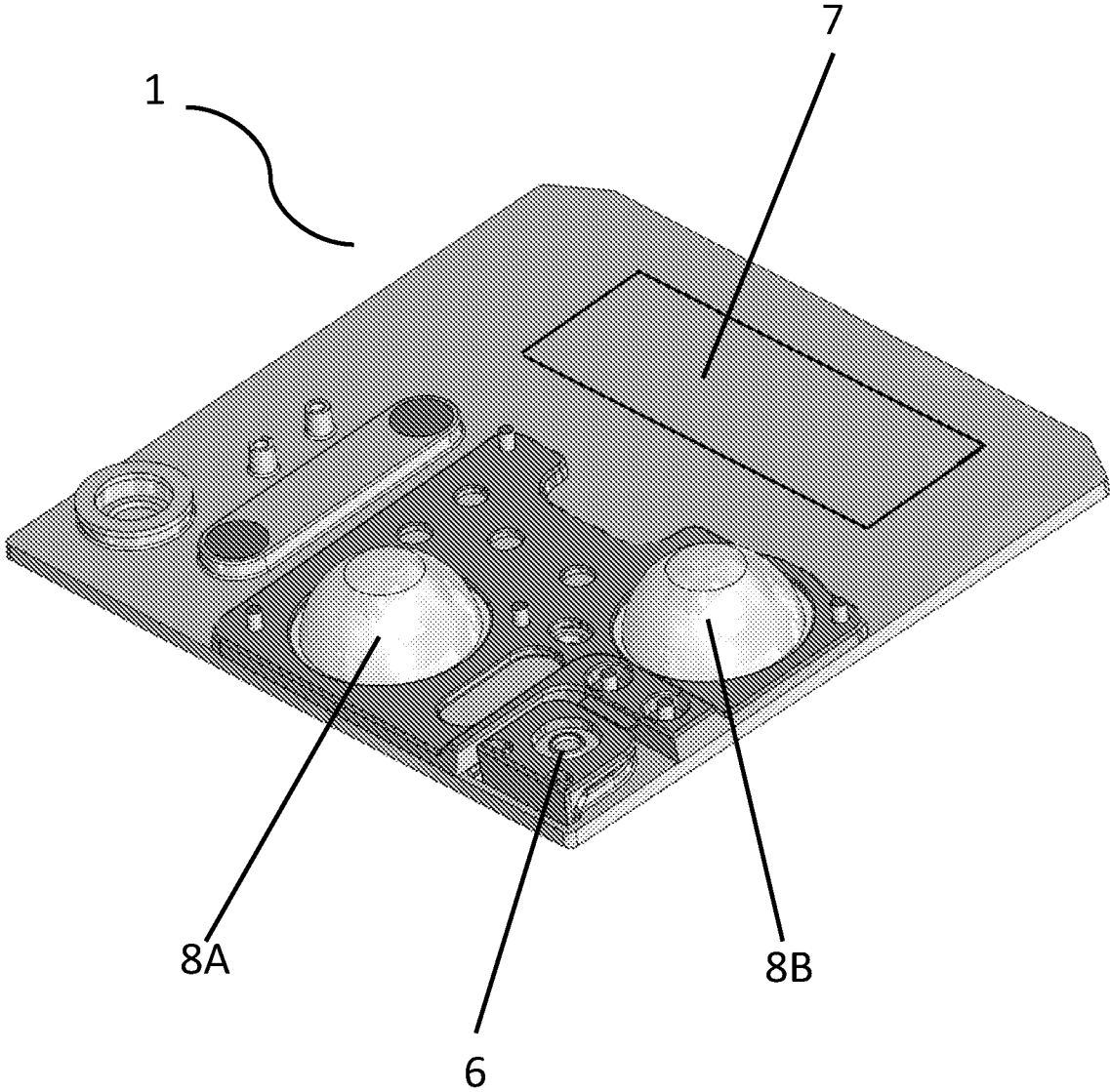


Figure 1A

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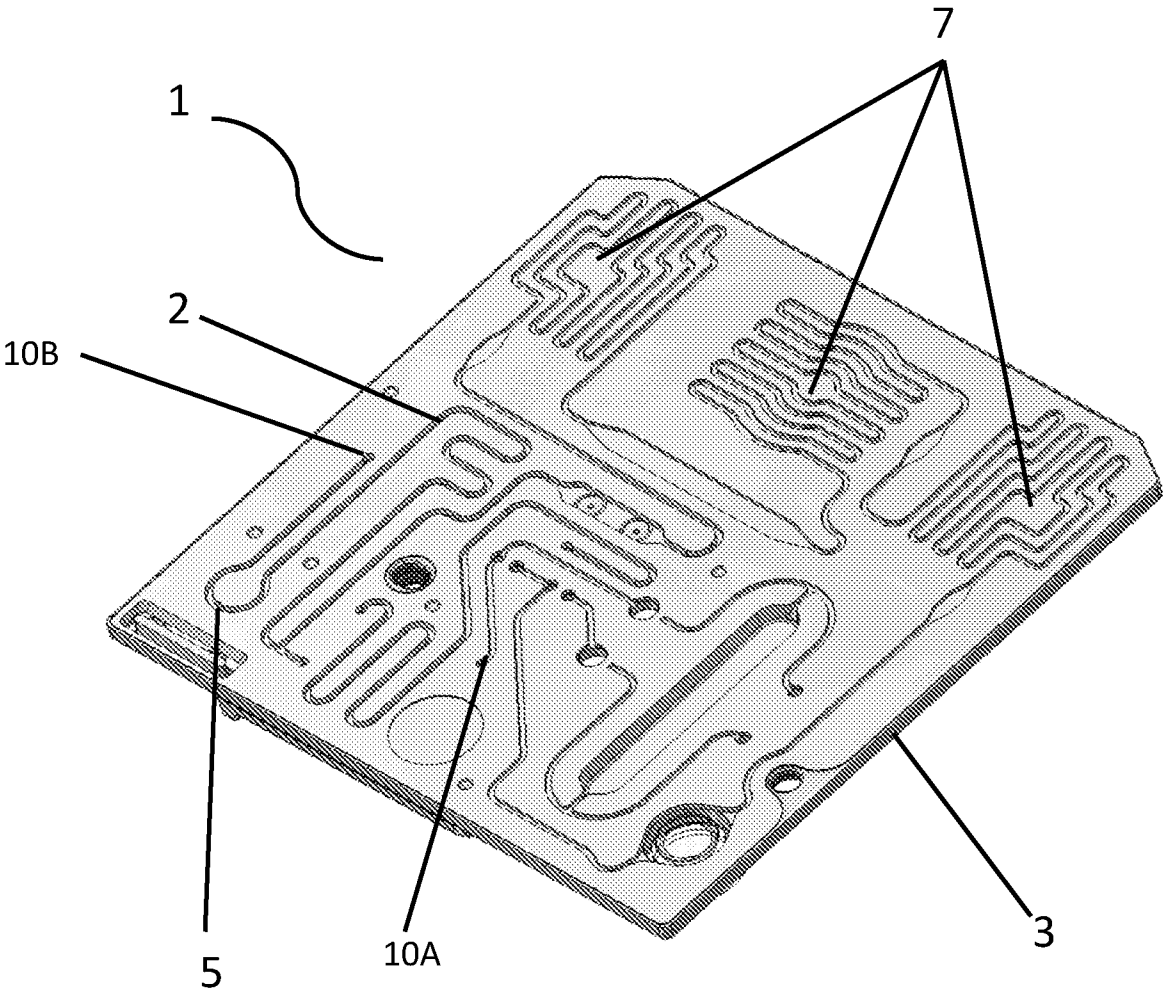


Figure 1B

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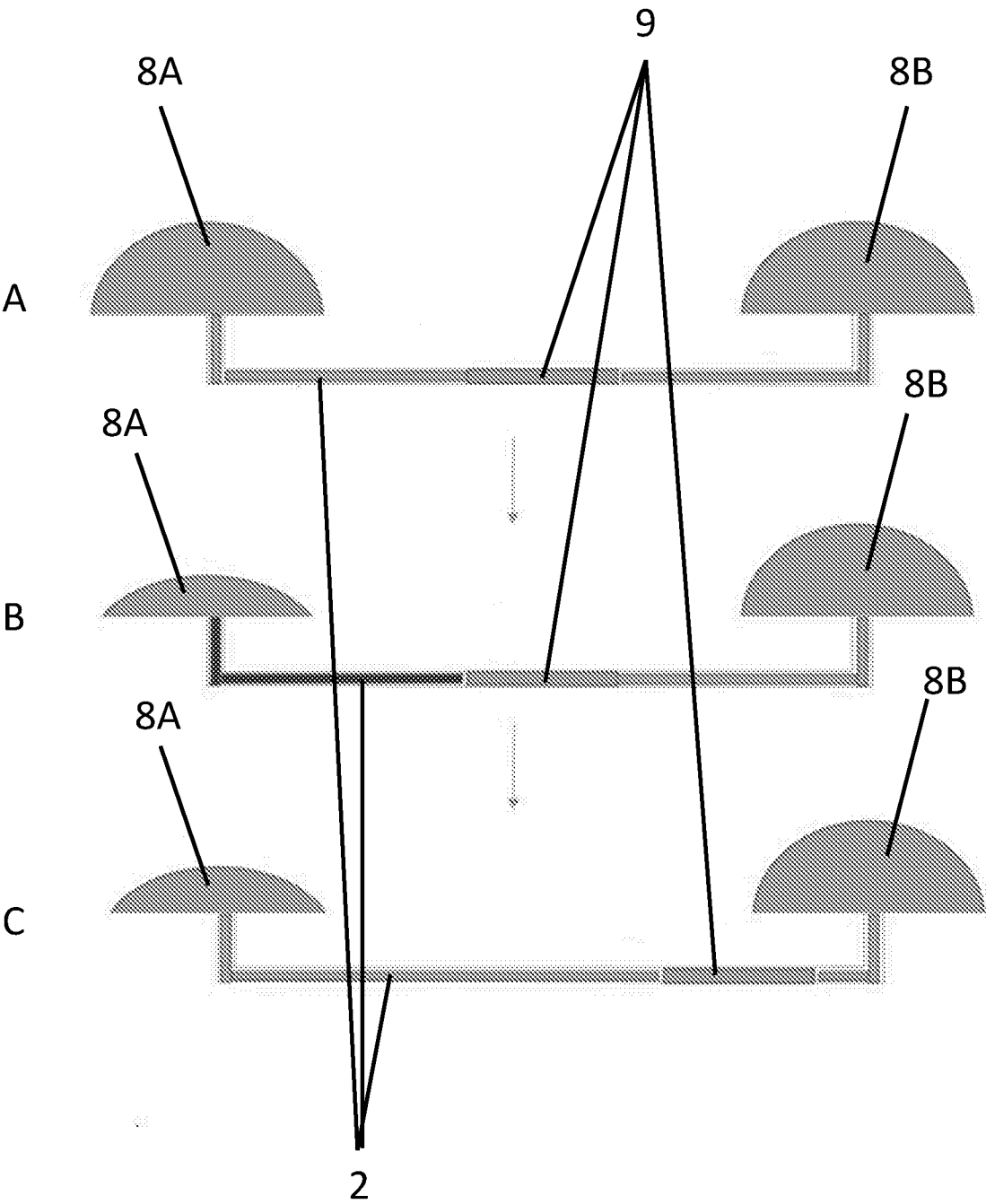


Figure 2

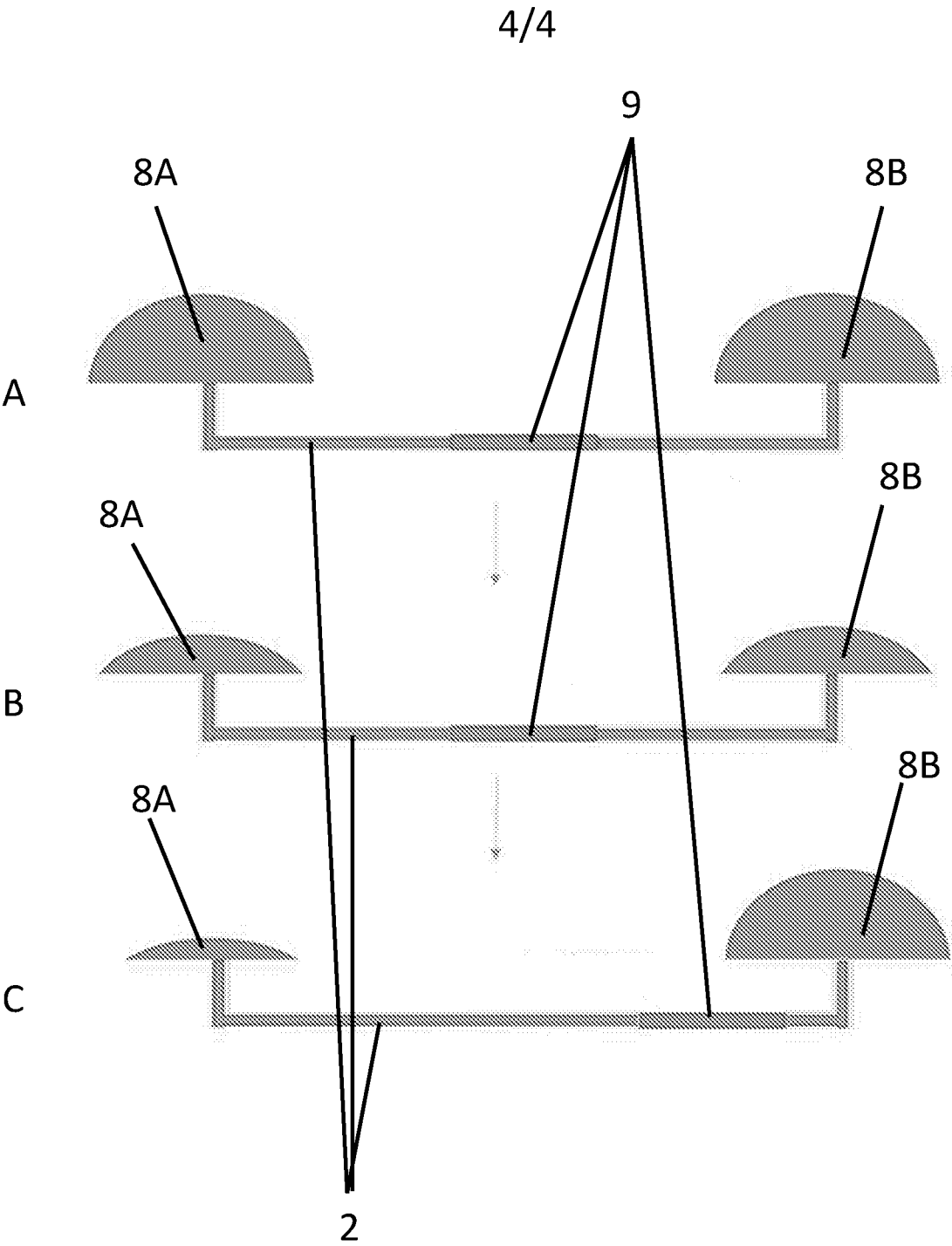


Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2019/053367

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00 B01L7/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/311673 A1 (FUKUZAWA TAKASHI [JP] ET AL) 1 November 2018 (2018-11-01) abstract; claims 5,7,11,12; figures paragraphs [0049], [0090], [0091], [0097], [0098]; figures 7,8 -----	1-25
X	US 2009/148933 A1 (BATTRELL C FREDERICK [US] ET AL) 11 June 2009 (2009-06-11) figures 15,17 -----	1-13
X	US 2011/039303 A1 (JOVANOVICH STEVAN BOGDAN [US] ET AL) 17 February 2011 (2011-02-17) paragraphs [0107], [0192], [0196]; figure 49 ----- -/-	1-16



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 January 2020

Date of mailing of the international search report

30/01/2020

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INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2019/053367

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/129872 A1 (PAIK PHILIP Y [US] ET AL) 27 May 2010 (2010-05-27) abstract; figure 4 paragraph [0062]; figure 6 -----	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2019/053367

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2018311673	A1	01-11-2018	CN 108431199 A 21-08-2018
			EP 3401386 A1 14-11-2018
			JP W02017119382 A1 08-11-2018
			SG 11201805707V A 30-07-2018
			US 2018311673 A1 01-11-2018
			WO 2017119382 A1 13-07-2017

US 2009148933	A1	11-06-2009	AU 2007225038 A1 20-09-2007
			EP 2007905 A2 31-12-2008
			ES 2393758 T3 27-12-2012
			HK 1126824 A1 24-05-2013
			JP 5254949 B2 07-08-2013
			JP 2009529883 A 27-08-2009
			US 2009148847 A1 11-06-2009
			US 2009148933 A1 11-06-2009
			US 2012329142 A1 27-12-2012
			WO 2007106579 A2 20-09-2007
			WO 2007106580 A2 20-09-2007

US 2011039303	A1	17-02-2011	CN 101715483 A 26-05-2010
			EP 2109666 A2 21-10-2009
			KR 20100028526 A 12-03-2010
			US 2011039303 A1 17-02-2011
			US 2012115189 A1 10-05-2012
			US 2014045704 A1 13-02-2014
			WO 2008115626 A2 25-09-2008

US 2010129872	A1	27-05-2010	NONE
