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MICROFLUIDIC DEVICE

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29 Aug 2024
2024216439

ABSTRACT

A microfluidic device is provided that is simple and compact. The microfluidic device includes: an inlet, for receiving a fluid; one or more micromixers, operatively coupled to the inlet; and one or more substances (e.g. reagents). At least one of the one or more substances is deposited in at least one of the one or more micromixers. The at least one of the one or more micromixers are configured to mix the at least one substance with the fluid.

2024216439 29 Aug 2024

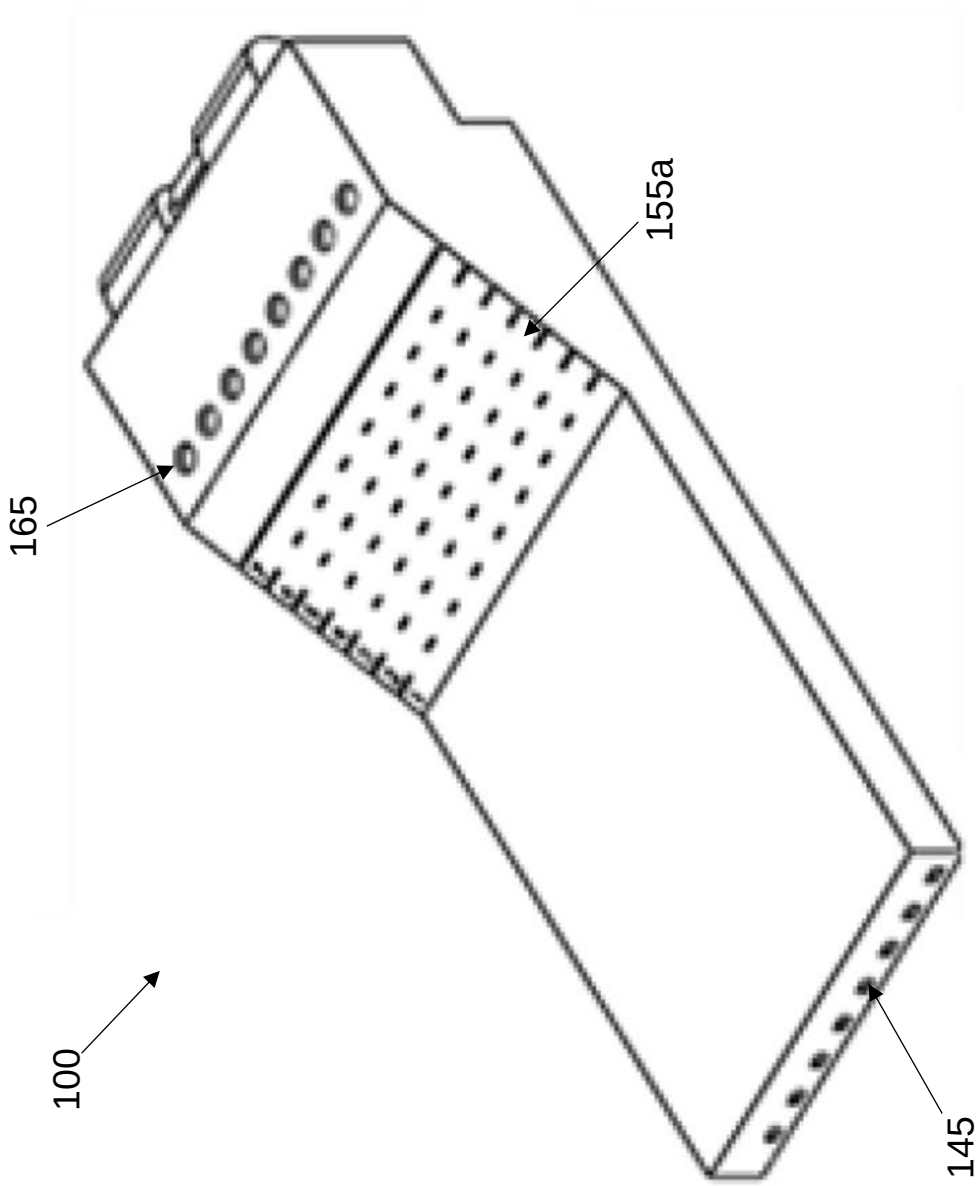

Figure 1

2024216439 29 Aug 2024

MICROFLUIDIC DEVICE

TECHNICAL FIELD

[0001] The present invention relates to microfluidic devices. In particular, although not exclusively, the present invention relates to microfluidic devices for colourimetric analysis.

BACKGROUND ART

[0002] Fertilisers are commonly used in agriculture, as the yield of crops is very much dependent on the concentration of nutrients, such as Nitrogen (N) and Phosphorus (P) in the soil. In short, if the concentration of any nutrients in the soil is low, crop yield may be significantly impacted. As such, fertilisers are used as a convenient way to amend soil to increase the concentration of nutrients in the soil.

[0003] A problem with the use of fertilisers, and in particular the overuse of fertilisers, is that fertiliser runoff can cause various environment issues and pollute waterways. Another problem with fertilisers is that they, particularly recently, have become very costly. As such, it is important that a minimum amount of fertiliser is applied to provided crop yield.

[0004] As a result, modern agricultural practices include soil analysis, so that fertiliser can be applied at rates that are needed. This soil analysis generally includes collecting samples and sending these to a laboratory for analysis, which requires specialised instrumentation, extended analysis times, and trained personnel.

[0005] A problem with such approach is that it is expensive and time consuming. In particular, such analysis equipment is very expensive, requires trained operators, and thus not readily accessible, and it is time consuming to collect and send samples for analysis. Accordingly, such testing is not performed often, and as a result, the amount of fertiliser used does not necessarily match the needs of the soil.

[0006] Certain rapid testing kits have been developed for testing soil. A problem with such test kits, however, is that they rely on the experience and judgement of the person using the kit, and are thus not accurate when used more generally. Furthermore, even when accurately interpreted, they provide only a very approximate range of nutrient concentrations rather than a precise quantitative amount.

[0007] Similar problems exist in relation to the analysis of other types of samples, including water, fluids, suspensions, solutions, and plant tissue, such as flowers and fruits, for example.

2024216439 29 Aug 2024

[0008] As such, there is clearly a need for improved ways to measure chemicals in agricultural settings.

[0009] It will be clearly understood that, if a prior art publication is referred to herein, this reference does not constitute an admission that the publication forms part of the common general knowledge in the art in Australia or in any other country.

SUMMARY OF INVENTION

[0010] The present invention relates to systems for microfluidic devices which may at least partially overcome at least one of the abovementioned disadvantages or provide the consumer with a useful or commercial choice.

[0011] With the foregoing in view, the present invention in one form, resides broadly in a microfluidic device, the microfluidic device including:

an inlet, for receiving a fluid;

one or more micromixers, operatively coupled to the inlet; and

one or more substances, wherein at least one of the one or more substances is deposited in at least one of the one or more micromixers, and wherein the at least one of the one or more micromixers are configured to mix the at least one substance with the fluid.

[0012] Advantageously, depositing the at least one substance of the one or more substances in the at least one of the one or more micromixers provides an efficient and compact means to both store the substances(s) and mix the fluid and the substance(s).

[0013] The one or more micromixers may comprise a plurality of micromixers. The at least one of the one or more substances may be deposited in the plurality of micromixers.

[0014] The plurality of micromixers may be arranged in parallel. The plurality of micromixers may be arranged in series. The plurality of micromixers may be arranged in series and parallel.

[0015] The one or more substances may include reagents. The at least one of the one or more substances may comprise at least one reagent.

[0016] The fluid may comprise a sample. The microfluidic device may be for analysing a sample.

[0017] Alternatively, the fluid may comprise a medium or other fluid. In such case, the microfluidic device may function simply as a mixer, for example.

2024216439 29 Aug 2024

[0018] The sample may comprise a liquid. The sample may comprise a solution. The sample may comprise a suspension. The sample may include organic compounds. The sample may include inorganic compounds.

[0019] In some embodiments, the sample comprises a soil sample. In other embodiments, the sample may comprise an organic material such as plant tissue.

[0020] In another embodiment, the sample may comprise a liquid sample. The liquid sample may comprise a water sample, or a liquid waste sample.

[0021] The soil sample may be generated by mixing soil with water and filtering same.

[0022] The microfluidic device may be configured to assist in determining levels of one or more analytes in the sample.

[0023] The analytes may comprise nutrients. The nutrients may include nitrogen. The nutrients may include phosphorus, magnesium, calcium, potassium, sodium, sulfur, and/or heavy metals such as copper, lead, chromium, iron, and manganese, for example.

[0024] The microfluidic device may be configured to assist in determining characteristics of the sample (e.g. soil, plant tissue, water or waste fluid sample). The characteristics of the sample may include pH, conductivity, hardness, total dissolved solids (TDS), dissolved organic carbon (DOC), biological enzyme activity and/or salinity, for example. In the case of biological enzyme activity, levels of phosphatase activity may be determined through detection of p-Nitrophenol (PNP).

[0025] The microfluidic device may be configured to measure more than one analyte, measure more than one nutrient, determine more than one characteristic, and/or a combination of one or more analytes, nutrients and characteristics. As an illustrative example, the microfluidic device may be configured to measure nitrate, ammonia and phosphate.

[0026] The at least one reagent may be deposited in the micromixer as a liquid. The at least one reagent may be stored in the micromixer as a liquid. The at least one reagent may be dried in the micromixer. The at least one reagent may be freeze dried in the micromixer.

[0027] Preferably, the one or more micromixers comprise passive mixers. Preferably, the micromixers comprise a plurality of passive micromixers.

[0028] The one or more micromixers may comprise lamination-based mixers, including, but not limited to, split and fold mixers, and/or chaotic advection-based micromixers, including,

2024216439 29 Aug 2024

but not limited to, intersecting channels, convergent-divergent channels, three-dimensional structures, embedded barriers, herringbone and staggered herringbone structures, twisted channels and Tesla-channels. Preferably, the one or more micromixers comprise one or more Tesla micromixers. The one or more Telsa micromixers may be configured to cause the flow of fluid in the microfluidic device to flow onto itself, and thereby mix.

[0029] The micromixers may include ridges or recesses on a side thereof. The ridges or recesses may comprise square / rectangular ridges or recesses. The ridges or recesses may increase a surface area of the mixer, e.g. to hold more reagent.

[0030] Preferably, the microfluidic device includes a flow distributor, configured to distribute the fluid into a plurality of microchannels. The number of microchannels may be any suitable number. In some embodiments, the number of microchannels is 8.

[0031] The flow distributor may comprise a plurality of bifurcations. Each bifurcation may comprise longitudinal input channel, a lateral branching channel, longitudinal output channels at ends thereof.

[0032] The bifurcations may become thinner at each bifurcation level. Such configuration may improve the precision of distribution of fluid in the flow distributor.

[0033] The bifurcations may include internal spikes positioned on outer corners of each bifurcation level. The internal spikes may extend inwardly at an angle of approximately 45º. The internal spikes may retard fluid flow, to equalize pressure.

[0034] The flow distributor may be symmetrical.

[0035] A filling zone may be provided intermediate the plurality of bifurcations and the plurality of channels. The filling zone may equalize pressure of the fluid.

[0036] The filling zone may include a plurality of pillars, to provide resistance to the flow of fluid therein. The pillars may be triangular in cross section. The pillars may be arranged in a plurality of different orientations. The pillars may include first pillars facing a first direction, and second pillars facing an opposite direction. The pillars may be arranged with uniform or non-uniform spacing. The pillars may have a first spacing in a direction parallel to the direction of flow, and a second spacing in a direction perpendicular to the flow.

[0037] Each of the plurality of channels may be associated with a fluid retardation element, intermediate the flow distributor and the plurality of channels. The fluid retardation

2024216439 29 Aug 2024

element may be configured to slow the flow of fluid when entering the channels to encourage equal distribution of fluid across the channels.

[0038] The fluid retardation element may be configured to redirect the flow of fluid back and forward. The fluid retardation element may be substantially N-shaped.

[0039] A filling segment may be provided in association with the fluid retardation element.

[0040] The plurality of micromixers may be provided in a channel of the plurality of channels.

[0041] The plurality of channels may each include a plurality of micromixers.

[0042] The plurality of channels may be substantially identical to each other in shape.

[0043] The plurality of channels may each be substantially linear, at least in part. The plurality of channels may be defined by a plurality of substantially linear portions.

[0044] The plurality of channels may be in parallel.

[0045] The plurality of channels may each include one or more reagents. At least one reagent of the one or more reagents may be deposited at a different rate or concentration in at least some of the plurality of channels. Such configuration may enable calibration through the use of reagents at different rates or concentrations. In such case, at least one of the reagents may comprise a calibrant.

[0046] The plurality of channels may extend along the microfluidic device in a first direction, and along the microfluidic device in a second direction opposite the first direction.

[0047] The plurality of channels may first extend along a length of the microfluidic device in a first direction, and then along a length of the microfluidic device in a second direction opposite the first direction.

[0048] The plurality of channels may each first extend along a length of the microfluidic device in a first direction and in a first plane, and then along a length of the microfluidic device in a second direction opposite the first direction and in a second plane, wherein the second plane is offset from the first plane.

[0049] The first and second planes may be parallel.

[0050] The second plane may be above the first plane.

2024216439 29 Aug 2024

[0051] The plurality of channels may extend substantially orthogonally between the first and second planes.

[0052] The plurality of channels may extend to an end of the microfluidic device, extending upwardly, and then back away from the end of the microfluidic device.

[0053] The channels may include micromixers in proximity to the end of the microfluidic device.

[0054] The channels may be associated with ports in proximity to the end of the microfluidic device. The ports may enable deposition of the at least one reagent of the one or more reagents in the plurality of micromixers of each of the channels. The ports may be sealable.

[0055] As such, simple access to the micromixers, e.g. for direct insertion of calibrant or reagent via a pipette tip or syringe, may be provided using the ports. This may simplify the deposition process and minimize the risk of pipette tip damage and/or contamination. This may in turn enable consistent calibrant or reagent deposition, irrespective of the orientation of the device during injection. Finally, such use of ports enables calibrants or reagents to be strategically placed at the start of the micromixers, ensuring that the calibrant is thoroughly mixed with the fluid throughout the mixing cycle.

[0056] In some embodiments the at least one reagent comprises a plurality of reagents and different reagents are deposited in at least two of the plurality of channels. Such configuration enables different channels to be used to measure different nutrients or determine different characteristics.

[0057] The microfluidic device includes a detection zone, for measurement of the sample and the one or more reagents. Each channel may include a detection portion in the detection zone. Preferably, the microfluidic device includes a detection zone for colourimetric measurement of the sample and the one or more reagents. Alternatively, the detection zone may be used for fluorometric analysis, conductimetric analysis, or electrochemical analysis.

[0058] The detection zone may include a reagent zone, for receiving the at least one reagent. The reagent zone may be defined at least in part by a barrier. The barrier may comprise a wall. The barrier may comprise a chemical barrier. The detection zone may include a plurality of reagent zones.

[0059] At least one reagent of the one or more reagents may be provided in the detection

29 Aug 2024
2024216439

zone. The reagent may comprise an indicator / colour reagent.

[0060] In some embodiments, the at least one reagent comprises an indicator for ammonia and/or ammonium ions. The at least one reagent may comprise a phenolic reagent. Specifically, the phenolic reagent may be an ammonia-salicylate reagent. Preferably, the at least one reagent comprising an indicator for ammonia and/or ammonium ions is Berthelot's reagent.

[0061] In some embodiments, the at least one reagent comprises an indicator for phosphate. The at least one reagent may comprise a molybdenum reagent. Preferably, the at least one reagent comprising an indicator for phosphate is a vanado-molybdate reagent. In other embodiments, the at least one reagent may comprise a Molybdenum Blue reagent (molybdenum oxide monohydrate).

[0062] In some embodiments, the at least one reagent comprises an indicator for potassium. The at least one reagent may comprise a dipicrylamine reagent. The at least one reagent may comprise a borate reagent. Preferably, the at least one reagent comprising an indicator for potassium is a tetraphenyl borate reagent or sodium tetraphenylboron.

[0063] Most preferably, the at least one reagent comprises a Modified Griess Reagent. The Modified Griess Reagent may be modified to omit (or reduce the amount of) phosphoric acid which enables it to be freeze dried. The Modified Griess Reagent may be deposited as liquid then solidified (e.g. frozen/dried).

[0064] The Modified Griess Reagent may be deposited as liquid then frozen/dried within the device. A barrier may isolate the Modified Griess Reagent. The barrier may comprise a physical barrier, such as a wall.

[0065] The barrier may comprise a chemical barrier. The chemical barrier may comprise a polymeric additive. Preferably, the polymeric additive is polyethylene glycol (PEG).

[0066] When used for nitrate detection, the reagent may comprise the Modified Griess Reagent. The Modified Griess Reagent requires zinc for the reaction which must be stored separately from other ingredients to avoid premature reactions. Therefore, zinc powder can be placed into the detection chamber and kept isolated from other ingredients and components by the barrier until mixing with the fluid.

[0067] The zinc powder may be deposited in the barrier. Specifically, the zinc powder may be deposited in the PEG and dried. During the drying process, the zinc powder may settle, and become protected by the barrier comprising PEG. This may also protect the zinc from

2024216439 29 Aug 2024

atmospheric moisture.

[0068] It is envisaged that the PEG may later be solubilised to release the zinc powder in the presence of a sample to allow the reaction, which is in turn used for colourimetric analysis.

[0069] The detection zone may define multi-depth pathlengths in each of the channels.

[0070] The detection zone may increase in depth compared to the depth of the channels.

[0071] The detection zone may be triangular in cross section. The detection zone may be sloped such that it increases in thickness along its length. The detection zone maybe defined at least in part by a concave or convex curve. Such configurations may allow bubbles to float to a top/rear of the detection zone, preventing interference from bubbles when performing colourimetric analysis.

[0072] The detection zone may include a transparent viewing window. The transparent viewing window may be located on an upper surface of the detection zone. The viewing window may extend across the plurality of channels.

[0073] The transparent viewing window may include indicia corresponding to different pathlength depths. Such configuration may enable an array of colour saturations to be easily visualised.

[0074] The device may include multiple channels, which may be used for self-calibration.

[0075] In particular, different concentrations of reagent may be provided in two or more of the channels, and a colour gradient may be determined according to the different concentrations. One or more characteristics of the sample may be determined according to the gradient.

[0076] Use of the gradient may be beneficial in that it uses relative differences between the channels, rather than fixed RGB measurements, and thereby may be less susceptible to the impact from environmental conditions, for example, ambient light.

[0077] Any photodetector may be used to generate RGB measurements. In some embodiments, the photodetector may comprise a photodiode array, a charge coupled device (CCD), or a light emitting diode (LED).

[0078] Preferably, the photodetector comprises a digital camera. The digital camera may be used to capture an image of the detection zone to generate RGB measurements, from which the gradient may be determined.

2024216439 29 Aug 2024

[0079] The image may be pre-processed to generate the gradient.

[0080] The image may be pre-pre-processed a general a single channel. The single channel may comprise a green channel. The single channel may comprise a combination of reg green and blue data, where green data is prioritised over red and blue data.

[0081] Any of the features described herein can be combined in any combination with any one or more of the other features described herein within the scope of the invention.

[0082] The reference to any prior art in this specification is not, and should not be taken as an acknowledgement or any form of suggestion that the prior art forms part of the common general knowledge.

BRIEF DESCRIPTION OF DRAWINGS

[0083] Various embodiments of the invention will be described with reference to the following drawings, in which:

[0084] Figure 1 illustrates an upper front perspective view of a microfluidic device, according to an embodiment of the present invention.

[0085] Figure 2 illustrates a side view of the microfluidic device of Figure 1, according to an embodiment of the present invention.

[0086] Figure 3 illustrates a top view of the microfluidic device of Figure 1, according to an embodiment of the present invention.

[0087] Figure 4 illustrates an enlarged rear perspective view of the microfluidic device of Figure 1, according to an embodiment of the present invention.

[0088] Figure 5 illustrates an enlarged cross-sectional area of a rear end of the microfluidic device of Figure 1 through D-D of Figure 3, according to an embodiment of the present invention.

[0089] Figure 6 illustrates an enlarged cross-sectional area of a front end of the microfluidic device of Figure 1 through D-D of Figure 3, according to an embodiment of the present invention.

[0090] Figure 7 illustrates an upper cross-sectional view of the microfluidic device of Figure 1 through A-A of Figure 2, according to an embodiment of the present invention.

[0091] Figure 8 illustrates an upper cross-sectional view of the microfluidic device of Figure 1 through B-B of Figure 2, according to an embodiment of the present invention.

2024216439 29 Aug 2024

[0092] Figure 9 illustrates an upper cross-sectional view of the microfluidic device through C-C of Figure 2, according to an embodiment of the present invention.

[0093] Figure 10 illustrates the microfluidic device of Figure 1, in use, showing injection of a sample.

[0094] Figure 11 illustrates the microfluidic device of Figure 1, in use, showing use of a smartphone to capture colourimetric data.

[0095] Preferred features, embodiments and variations of the invention may be discerned from the following Detailed Description which provides sufficient information for those skilled in the art to perform the invention. The Detailed Description is not to be regarded as limiting the scope of the preceding Summary of the Invention in any way.

DESCRIPTION OF EMBODIMENTS

[0096] Figure 1 illustrates an upper front perspective view of a microfluidic device 100, according to an embodiment of the present invention. Figure 2 illustrates a side view of the microfluidic device 100, Figure 3 illustrates a top view of the microfluidic device 100, and Figure 4 illustrates an enlarged rear perspective view of the microfluidic device 100, according to an embodiment of the present invention.

[0097] Advantageously, the microfluidic device 100 provides an inexpensive, compact, easy to use and accurate way of analysing soil, e.g. in the context of nutrient testing. This enables the microfluidic device 100 to be used in the field, and the results of the analysis used to influence immediate decisions.

[0098] The microfluidic device 100 is configured for, and described with reference to, soil analysis, and in particular nitrate measurements. The skilled addressee will readily appreciate that the device 100 can be readily adapted for testing for other nutrients in soil, e.g. phosphate, or other characteristics of soil, e.g. pH, or for testing of other samples, e.g. water and plant samples, e.g. through the use of different reagents. Furthermore, aspects of the device may be taken and used independently, e.g. in non-testing scenarios.

[0099] The microfluidic device 100 includes an inlet port 105, for receiving a sample, e.g. from a syringe. The inlet port 105 is coupled to a flow distributor, configured to distribute the sample to a plurality of internal channels. Each channel includes a substance in the form of a first reagent, and a plurality of Tesla micromixers, for mixing the sample and the first reagent. Finally, the microfluidic device includes a detection zone 110, including a second reagent, where

2024216439 29 Aug 2024

colourimetric analysis may be performed in relation to each of the channels based on a reaction between the first reagent, the second reagent and the sample.

[00100] The microfluidic device 105 is multi-layered, which is more compact than a single layer equivalent device. In particular, the channels extend along the length of the microfluidic device 100 from a first end to a second end, and then from the second end to the first end.

[00101] Figure 5 illustrates an enlarged cross-sectional area of a rear end of the microfluidic device 100 through D-D of Figure 3, and Figure 6 illustrates an enlarged cross-sectional area of a front end of the microfluidic device 100 through D-D of Figure 3, according to an embodiment of the present invention.

[00102] Similarly, Figure 7 illustrates an upper cross-sectional view of the microfluidic device 100 through A-A of Figure 2, Figure 8 illustrates an upper cross-sectional view of the microfluidic device 100 through B-B of Figure 2, Figure 9 illustrates an upper cross-sectional view of the microfluidic device 100 through CC of Figure 2, according to an embodiment of the present invention.

[00103] The inlet 105 is associated with a hinged cap 110, which is configured to seal the inlet 105, when not used. This may prevent air and moisture from entering the device 100, and degrading or prematurely reacting with the reagents.

[00104] In use, the hinged cap 110 is removed, and a tip of a syringe with a sample therein is inserted into the inlet 105.

[00105] As best illustrated in Figure 5, the inlet 105 includes an inner portion 105a, which is shaped to correspond to a tip of the syringe, and thus form a seal with the tip of the syringe, and an enlarged outer portion 105b, which comprises baffle spikes which engage with the tip of the syringe. The baffle spikes guide the tip of the syringe into the inlet 105, while ensuring space is provided around a portion of the tip of the syringe, to enable air to vent, as outlined below.

[00106] As best illustrated in Figure 7, the inlet 105 is coupled to a flow distributor 115, located in a lower microfluidic layer 120. The flow distributor 115 is configured to distribute the fluid into a plurality of microchannels 125.

[00107] The device 100 is illustrated to include 8 microchannels 125, however the skilled addressee will readily appreciate that any suitable number of microchannels may be used.

[00108] The flow distributor 115 comprises a plurality of bifurcations, and at each bifurcation,

2024216439 29 Aug 2024

an additional channel is added, until 8 channels are provided. Each bifurcation comprises longitudinal input channel, a lateral branching channel, and longitudinal output channels at ends thereof.

[00109] The bifurcations become thinner at each bifurcation level. Such configuration may improve the precision of distribution of fluid in the flow distributor by promoting travel of the sample across a width of the flow distributor 115 before flowing down.

[00110] The bifurcations include internal spikes 115a positioned on outer corners of each bifurcation level. The internal spikes 115a may extend inwardly at an angle of approximately 45º at a junction between the lateral branching channel and the outermost longitudinal output channels. The internal spikes 115a function to retard fluid flow, to equalize pressure across the channels.

[00111] The flow distributor 115 is symmetrical in shape, and is configured to ensure an even flow of sample across the channels 125.

[00112] A filling zone 130 be provided intermediate the plurality of bifurcations of the flow distributor 115 and the plurality of channels 125. The filling zone 130 comprises a plurality of pillars 130a, that are triangular in cross section, and are configured to provide resistance to the flow of fluid therein, and thereby equalize pressure of the fluid.

[00113] The pillars 130a are arranged in different orientations, i.e. some are pointed towards the inlet, and some are pointed away from the inlet. This disrupts and slows down the flow of fluid in the filling zone 130, and causes the fluid to move laterally, which equalises the flow of fluid across a width of the device 100.

[00114] Each of the plurality of channels 120 is associated with a fluid retardation element 135, intermediate the flow distributor 115 and the plurality of channels 125. The fluid retardation element 135 is configured to slow the flow of fluid when entering the channels 125 to encourage equal distribution of fluid across the channels 125, and particularly in the filling zone 130.

[00115] The fluid retardation element 135 is configured to redirect the flow of fluid back and forward, with abrupt changes, thereby forming an N-shape.

[00116] A filling segment 135a is provided in association with each fluid retardation element 135, the filling segment 135a including a plurality of pillars to slow and redirect the flow of fluid.

[00117] Following the fluid retardation element 135, a plurality of micromixers 140 are

2024216439 29 Aug 2024

provided in the form of Tesla micromixers (Telsa valves), in each of the channels 125.

[00118] A reagent in the form of potassium nitrate is deposited in the micromixers as a liquid, and freeze dried in the micromixer 140. The potassium nitrate is deposited in different concentrations in each of the channels 125 (e.g. 0, 10, 20, 30, 40 and 50ppm) and thus may function as a calibrant. In short, such configuration may enable calibration when testing for nitrogen by allowing different base rates of nitrogen in the calibrants to, together with any nitrogen in the sample, to interact with colour reagents in a predictable manner to produce a gradient of colours.

[00119] The channels 125 are associated with ports 145 at the end of the microfluidic device, the ports 145 to enable deposition of the potassium nitrate in the plurality of micromixers 140 of each of the channels 125. The ports 145 are sealable, to prevent fluid from exiting the device 100 after deposition of the potassium nitrate.

[00120] The ports 145 thus provide simple access to the micromixers 140, for direct calibrant (potassium nitrate) insertion via a pipette tip or syringe. This may simplify the process of depositing the calibrant, and minimise the risk of pipette tip damage. This may in turn enable consistent calibrant deposition, irrespective of the channel's orientation during injection. Finally, such use of ports 145 enables calibrants to be strategically placed at or near the start of the micromixers 140, ensuring that the calibrant is thoroughly mixed with the sample solution throughout the mixing cycle.

[00121] The Telsa micromixers are configured to cause the flow of fluid in the microfluidic device 100 to flow back onto itself, and thereby mix the potassium nitrate that has been deposited in the micromixer 140 with the sample.

[00122] The micromixers 140 include rectangular ridges 140a on sides thereof, which function to provide additional surface area / storage space for storing the potassium nitrate.

[00123] While the device 100 includes modified Tesla micromixers 140, the skilled addressee will readily appreciate that any suitable micromixers may be used, including a combination of types of micromixers. Preferably, however, the micromixers 140 comprise passive mixers.

[00124] Each of the plurality of channels is defined by substantially linear portions that are parallel, and are substantially identical to each other in shape.

[00125] As shown in Figure 7, the plurality of channels 125 extend along the microfluidic device 100 in a first direction, in the lower microfluidic layer 120, until they reach an end (at the

2024216439 29 Aug 2024

ports 145). The channels then extend upwardly, and back along an upper microfluidic layer 150 in a second direction opposite the first direction, as illustrated in Figure 8.

[00126] The upper and lower microfluidic layers 120, 150 are slightly offset, and are parallel to each other.

[00127] The micromixers 140 extend back along the microfluidic device 100 towards the end with the inlet 105, where they are coupled to detection portions 155 defining a detection zone 155a.

[00128] At colour reagent in the form of a Modified Griess Reagent (further details below) is provided in each of the detection portions 155.

[00129] The detection zone 155a is for colourimetric measurement of the sample with the reagents (i.e. the potassium nitrate and the Modified Griess Reagent), and increases in depth compared to the depth of the channels 125. The change in depth is to define multi-depth pathlengths in each of the channels.

[00130] In short, the detection zone 155a has a triangular cross section, such that it slopes and increases in thickness along its length. Such configuration also encourages bubbles to float to a top/rear of the detection zone 155a, enabling colourimetric analysis to be performed without interference from bubbles.

[00131] The detection zone 155a includes a transparent viewing window, located on an upper surface of the detection zone 155a. The viewing window extends across the plurality of channels, to enable observation of the reaction in the detection portions 155, to perform colourimetric measurement.

[00132] The transparent viewing window includes indicia 155b corresponding to different pathlength depths, to enable an array of colour saturations to be easily captured.

[00133] As outlined above, the detection zone 155a, and thus the detection portions 155, include a Modified Griess Reagent. The detection portion 155 include reagent zones 160, for receiving the reagent, the reagent zone 160 defined at least in part by a wall 160a.

[00134] The Modified Griess Reagent requires zinc for the reaction, which must be separated from other ingredients to avoid premature reactions caused by air and moisture. Therefore, zinc powder can be placed into the reagent zone 160 and kept isolated from other components by the separation wall 160a until mixing with the fluid upon use.

2024216439 29 Aug 2024

[00135] A plurality of reagent ports 165 are provided in association with each channel 125 and thus detection portion 155, for enabling deposition of the reagent through the ports 165. The Modified Griess Reagent omits phosphoric acid, which would otherwise be used in a Griess Reagent, which enables it to be freeze dried. The Modified Griess Reagent may be deposited as liquid then frozen/dried.

[00136] As outlined above, different concentrations of reagent are provided in different channels, and the detection portions 155 increase in depth. The detection zone 155a is segmented into seven (7) depth sections, each with incrementally increasing depth. This unique configuration leads to darker colourimetric readings for deeper segments.

[00137] Such configuration enables a gradient may be determined according to the different concentrations and depths. Characteristics of the sample may be determined according to the gradient.

[00138] The use of a gradient is beneficial in that it uses relative differences between the channels and depths, rather than absolute fixed data (e.g. RGB data) from a camera, and is therefore less susceptible to the impact from ambient light, for example.

[00139] The image is pre-processed to extract a green component (of an RGB image), and generate the gradient.

[00140] Finally, leak and backpressure prevention and liquid containment is provided through a serpentine sump 170. The serpentine sump 170 catches and retains liquid from the detection portions 155 when full, and vents out through the inlet 105.

[00141] EXAMPLE PREPARATION OF MODIFIED GRIESS REAGENTS

[00142] The following Modified Griess Reagent enables the indirect detection of nitrates by initially detecting nitrites through a reduction process using Zinc dust or Cadmium. The modified Griess reagent relies on a diazotization reaction with either Sulfanilamide or Sulfanilic Acid, leading to the formation of a coloured azo dye through coupling with Naphthylethylenediamine Dihydrochloride (NED).

[00143] To create the Modified Griess Reagent, 0.6 ml of 85% phosphoric acid is initially diluted in 10 ml of water. To this solution, three components are added in no specific order: 0.15 g of Sulfanilic Acid, 0.022 g of N-(1-naphthyl)ethylenediamine dihydrochloride (NED), and 1.5 mL of Polyethylene Glycol. Additionally, 2.5 mg of Zinc Dust, with particle sizes ranging from 2μm to 400μm, is incorporated into the reagent mixture.

2024216439 29 Aug 2024

[00144] The loading method for the reagent onto the device 100 begins with the injection of 60 µL of the reagent into the reagent zone 160. Subsequently, the loaded chips are frozen at -20°C for one hour, followed by a freeze-drying process to solidify the reagent within the reagent zone 160. Each channel of the device 100 is then filled with 2.5 mg of Zinc Dust. To finalise the preparation, the device 100 is sealed in individual vacuum bags and stored at room temperature to ensure stability and prevent contamination.

[00145] Alternatively, the Modified Griess Reagent may be created by initially mixing 9.8 ml of Polyethylene Glycol and 0.2 ml of deionised water to make 10ml of 98 % polyethylene glycol solution. 0.5 g of Phosphoric acid, 0.02 g of naphthylethylenediamine dihydrochloride, and 1.0 g of sulfanilic acid may then be added into polyethylene glycol solution. 9.98ml of the prepared Polyethylene Glycol mixture is then combined with 0.02 ml of blue dye to make 10ml of overall reagent. Zinc powder may then be added to the Modified Griess Reagent.

[00146] EXAMPLE DEPOSITION OF MODIFIED GRIESS REAGENT

[00147] To establish a self-calibration function of the device, the Modified Griess Reagent (containing zinc powder) and varying concentrations of nitrate salt are deposited into their designated channels. Initially, the nitrate solutions in the channels and the device 100 are placed in a freezer at -20°C to allow for solidification. Following this, the Modified Griess Reagent (without zinc) is deposited into the channels, which have been kept at 4°C, over the nitrate solutions. Then the device is stored at -20°C to facilitate the solidification of the Modified Griess Reagent-nitrate mixture. Once solidified, the solutions are subjected to sublimation, resulting in a powdered form. Subsequently, small amounts of solid zinc powder are introduced into the channels and the device is stored in a dry condition at -20°C to eliminate any moisture.

[00148] As outlined above, a Griess reagent typically includes phosphoric acid, which helps maintain an acidic environment. However, because this acid cannot solidify at -20°C, it has been replaced (at least significantly) by an amount of sulfanilic acid.

[00149] Use of the device 100 is described with reference to the following example.

[00150] Initially, a soil sample taken. The soil sample is pre-sieved to a size of around 2mm, followed by air-drying.

[00151] The soil is then mixed with a solution five times its weight of a dilute concentration (0.01M) of calcium chloride ($CaCl_2$). It is then vigorously shaken.

[00152] The mixture is then filtered through a 0.45 µm film, to remove any particulate matter

2024216439 29 Aug 2024

from the extract, resulting in a clear (or near clear) filtrate that is ready for analysis

[00153] 5mL of the filtrate is injected into the device 100, as outlined in Figure 10.

[00154] As the filtrate is injected into the device, it is systematically divided across eight channels for uniform distribution.

[00155] The Tesla micromixers mix the solution with the calibrant, and extend the fluidic pathlength of the solutions.

[00156] The mixed solution then enters the Detection Zone, and reacts with the colour reagents.

[00157] A camera, e.g. on a smartphone, is then used to capture an image of the detection zone.

[00158] Rather than relying solely on an absolute specific pixel RGB value from a single channel segment for nitrate quantification, the colour gradient differences among the channels and their segments are extracted and a gradient is determined.

[00159] The gradient is then used to determine a nitrate level.

[00160] In addition to determining nitrate level, the smartphone may include location tracking functionality, and may include spatial mapping of agricultural fields. Similarly, current/real-time weather data including temperature (°C), humidity (%), and air pressure (hPa) may be associated with data. This weather data may be captured and/or retrieved, e.g. from a nearby weather station.

[00161] In some embodiments, by integrating machine learning and computer vision technologies, the application could automate the recognition of the chip and the collection of image data across the measurement area, streamlining the analysis process.

[00162] The above device 100 may be made by 3D printing. Alternatively, however, other methods may be used, including using injection moulding. Furthermore, the device 100 may be unitarily formed. Alternatively, the device 100 may be assembled from multiple components.

[00163] As outlined above, while the device 100 is configured to detect nitrogen in soil, with minor modifications, the device 100 may be modified to detect other things. As an illustrative example, the device 100 may be modified (or partly modified) to measure pH or detect phosphorus, e.g. using different reagents.

202421643 29 Aug 2024

[00164] EXAMPLE CREATION AND DEPOSITION OF PH REAGENT

[00165] Initially, 0.08 grams of phenolphthalein is dissolved in 5 millilitres of ethanol, followed by the dissolution of 0.04 grams of Methyl Red in 2 millilitres of ethanol. 0.08 grams of Bromothymol blue is then dissolved in 5 millilitres of pure water, alongside 0.01 grams of Methyl orange in 5 millilitres of hot water to facilitate complete dissolution. Furthermore, 0.007 grams of sodium hydroxide is dissolved in 5 millilitres of water, adding to the complexity of the solution. Once each component is individually dissolved, they are combined into a single mixture. To complete the preparation, 78 millilitres of water is added to the combined solution, bringing the total volume to 100 millilitres.

[00166] To deposit the pH reagent, initially 47.5 mL of the pH reagent is injected into the device through the apertures. The device is then frozen at -20°C for one hour, followed by a freeze-drying process to secure the reagent within the device. Subsequently, pH standard powder is added to three channels of the device. These powders are specifically selected for their suitability in calibrating the pH detection process. Finally, the device sealed in individual vacuum bags and stored at room temperature, ready for the pH detection process.

[00167] EXAMPLE CREATION AND DEPOSITION OF PHOSPHORUS REAGENT

[00168] The soil is prepared in a similar way to that described above, but mixed with twenty times the weight of a 0.5 M sodium bicarbonate ($NaHCO_3$) solution, which is adjusted to a pH of 8.5.

[00169] An ammonium molybdate solution is prepared, by weighing 1g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}$), which is dissolved in 40ml of pure water. The solution is stirred continuously until the ammonium molybdate is completely dissolved, ensuring a homogenous mixture. 15ml of sulfuric acid is then added to the solution while stirring.

[00170] An Antimony Potassium Tartrate Solution is then prepared, by weighing 0.05g of antimony potassium tartrate ($KSbOC_4H_4O_6$) and dissolving it in 10 ml of pure water. Similar to the ammonium molybdate solution, this mixture is stirred until the antimony potassium tartrate is fully dissolved, ensuring no solid particles remain.

[00171] The two solutions are then combined by adding the Antimony Potassium Tartrate Solution to the Ammonium Molybdate Solution. To adjust the total volume to 100 ml, an additional 35 ml of water is added to the mixture. This final step ensures that the reagent is properly diluted and ready for use.

2024216439 29 Aug 2024

[00172] For depositing the phosphorus reagent, 53 µL of the prepared reagent is injected through the apertures, together with 10 µL of phosphate (PO_4^{3-}). The device is then frozen at -20°C for one hour, which helps to solidify the reagent within the device. After freezing, a freeze-drying process is applied to the reagent inside the device, removing any moisture and thus stabilizing the reagent for storage and use. Next, 0.5mg of ascorbic acid ($C_6H_8O_6$) is weighed and added into each channel of the device. This addition of ascorbic acid is crucial for the chemical reaction that the Molybdenum Blue Reagent is intended to facilitate. Finally, to ensure the longevity and integrity of the reagent within the device, the device is sealed in individual vacuum bags and stored at room temperature. This comprehensive process ensures that the reagent is prepared, loaded, and stored in a manner that maintains its efficacy and reliability for analytical purposes.

[00173] Advantageously, the devices described above provide a simple and accurate means for testing soil. This allows growers to understand the nitrate (or other) content in soil in real time, which in turn allows them to make decisions on fertiliser amounts to apply in order to maintain crop yield while avoiding over fertilisation.

[00174] Depositing the reagents in the micromixers provides an efficient and compact means to both store the reagents and mix the fluid and the reagents.

[00175] By folding the channels of the device into multiple layers, the device is able to be more compact. Furthermore, such configuration enables easy access to be provided to a central portion of the channels, e.g. where the mixers are located, to simplify depositing reagent there.

[00176] Finally, by utilising a gradient and multiple channels with different rates of reagent, more accurate measurements may be made, including measurements that are not dependent or impacted by light levels or the like.

[00177] In the present specification and claims (if any), the word ‘comprising’ and its derivatives including ‘comprises’ and ‘comprise’ include each of the stated integers but does not exclude the inclusion of one or more further integers.

[00178] Reference throughout this specification to ‘one embodiment’ or ‘an embodiment’ means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearance of the phrases ‘in one embodiment’ or ‘in an embodiment’ in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any

29 Aug 2024 2024216439

suitable manner in one or more combinations.

[00179] In compliance with the statute, the invention has been described in language more or less specific to structural or methodical features. It is to be understood that the invention is not limited to specific features shown or described since the means herein described comprises preferred forms of putting the invention into effect. The invention is, therefore, claimed in any of its forms or modifications within the proper scope of the appended claims (if any) appropriately interpreted by those skilled in the art.

2024216439 29 Aug 2024

CLAIMS

1. A microfluidic device, the microfluidic device including:

an inlet, for receiving a fluid;

one or more micromixers, operatively coupled to the inlet; and

one or more substances, wherein at least one of the one or more substances is deposited in at least one of the one or more micromixers, and wherein the at least one of the one or more micromixers are configured to mix the at least one substance with the fluid.

2. The microfluidic device of claim 1, wherein the fluid comprises a sample, and wherein the microfluidic device is for analysing the sample.

3. The microfluidic device of claim 1, wherein the one or more substances include reagents, and wherein the at least one of the one or more substances comprises at least one reagent.

4. The microfluidic device of claim 3, wherein the at least one reagent is deposited in the at least one micromixer as a liquid, and freeze dried in the at least one micromixer.

5. The microfluidic device of claim 1, wherein the at least one micromixer comprises one or more Tesla micromixers, the Telsa micromixers configured to cause the flow of fluid in the microfluidic device to flow onto itself, and thereby mix.

6. The microfluidic device of claim 1, wherein at least a subset of the at least one micromixers includes rectangular ridges or recesses on a side thereof, to increase a surface area of the mixer.

7. The microfluidic device of claim 1, further including a flow distributor, configured to distribute the fluid into a plurality of microchannels.

8. The microfluidic device of claim 7, wherein the flow distributor comprises a plurality of bifurcations, each bifurcation comprising longitudinal input channel, a lateral branching channel, longitudinal output channels at ends thereof.

9. The microfluidic device of claim 8, wherein the bifurcations include internal spikes positioned on outer corners of each bifurcation level.

10. The microfluidic device of claim 8, wherein a filling zone is be provided intermediate the plurality of bifurcations and the plurality of channels, the filling zone configured to equalise pressure of the fluid.

2024216439 29 Aug 2024

11. The microfluidic device of claim 10, wherein the filling zone includes a plurality of pillars, to provide resistance to the flow of fluid therein, the pillars triangular in cross section and including first pillars facing a first direction, and second pillars facing an opposite direction.

12. The microfluidic device of claim 7, wherein each of the plurality of channels is associated with a fluid retardation element, intermediate the flow distributor and the plurality of channels.

13. The microfluidic device of claim 12, wherein the fluid retardation element is configured to redirect the flow of fluid back and forward, wherein the fluid retardation element is substantially N-shaped.

14. The microfluidic device of claim 7, wherein the one or more micromixers comprises a plurality of micromixers, wherein two or more of the plurality of channels include at least one micromixer.

15. The microfluidic device of claim 1, wherein the one or more micromixers comprise a plurality of micromixers, and wherein at least a subset of the plurality of micromixers are arranged in series.

16. The microfluidic device of claim 7, wherein the substances comprise reagents, and wherein at least one reagent of the one or more reagents is deposited at different concentrations in at least some of the plurality of channels.

17. The microfluidic device of claim 7, wherein the plurality of channels extend along the microfluidic device in a first direction, and along the microfluidic device in a second direction opposite the first direction.

18. The microfluidic device of claim 17, wherein the channels are associated with ports in proximity to an end of the microfluidic device, the ports to enable deposition of the at least one reagent of the one or more reagents in the plurality of micromixers of each of the channels.

19. The microfluidic device of claim 7, further including a detection zone, for colourimetric measurement of the sample and one or more reagents, wherein the detection zone defines multi-depth pathlengths in each of the channels.

20. The microfluidic device of claim 1, wherein the at least one substance comprises a Modified Griess Reagent, wherein the Modified Griess Reagent is deposited as liquid then solidified.

2024216439 29 Aug 2024

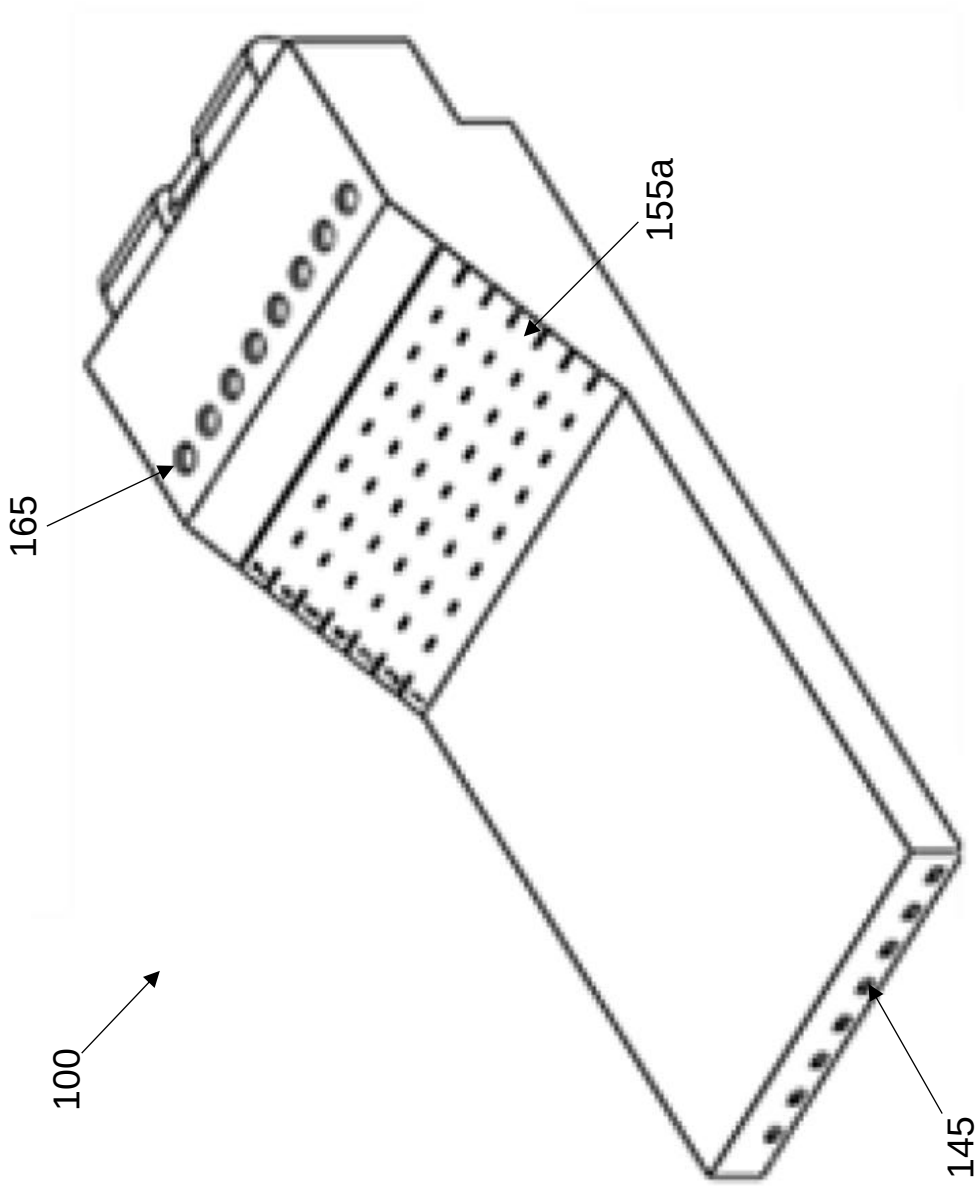

Figure 1

29 Aug 2024
2024216439

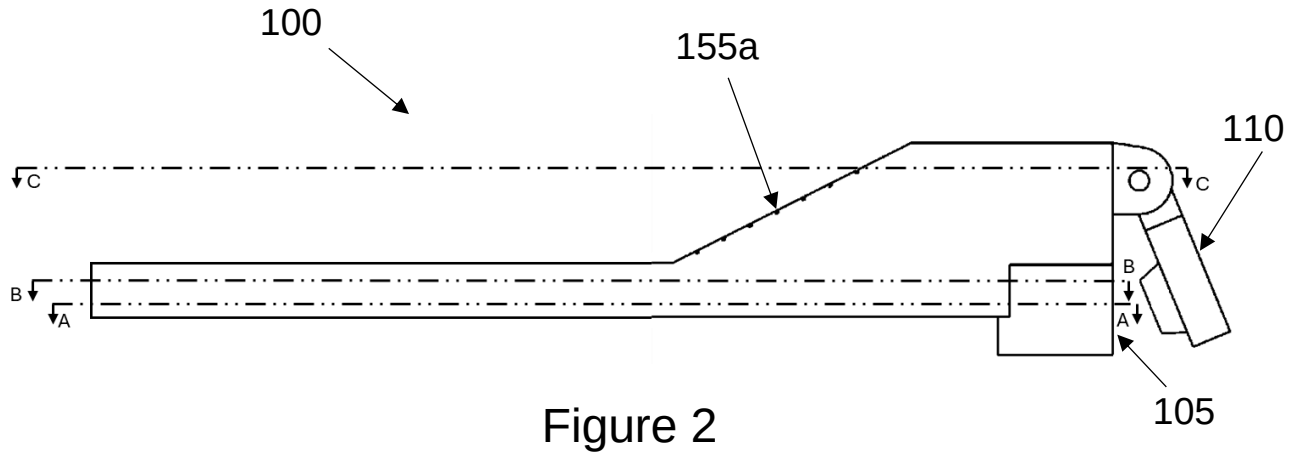

Figure 2

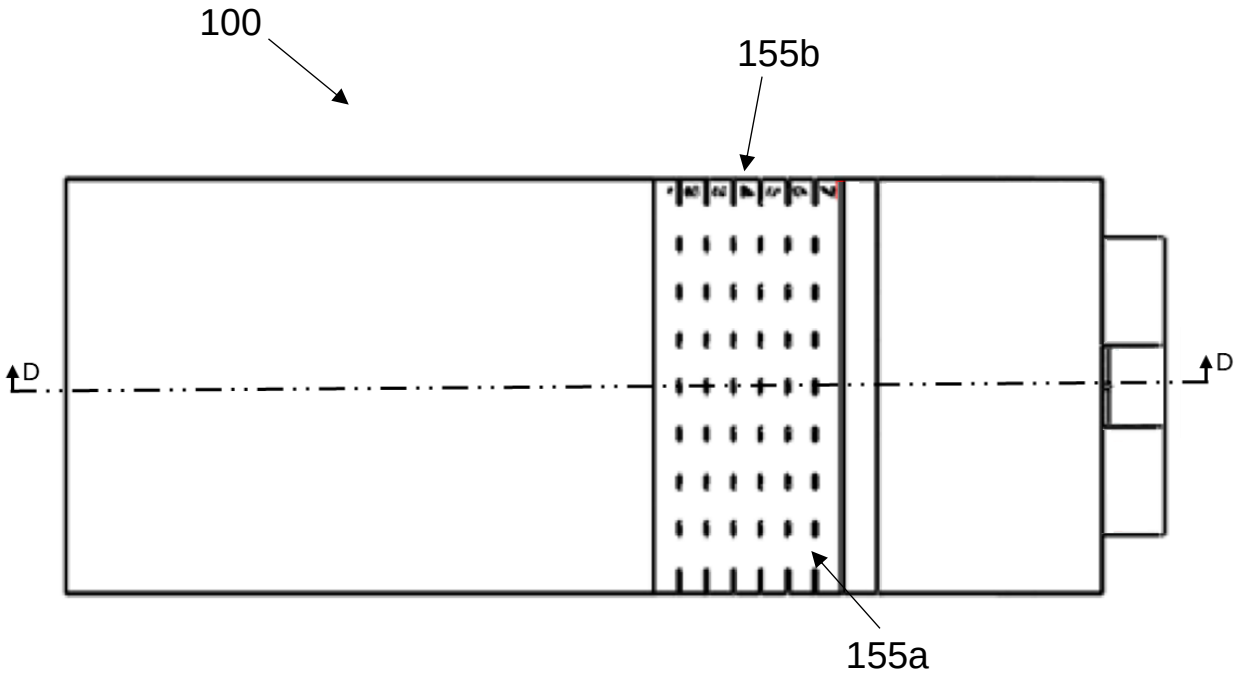

Figure 3

29 Aug 2024
2024216439

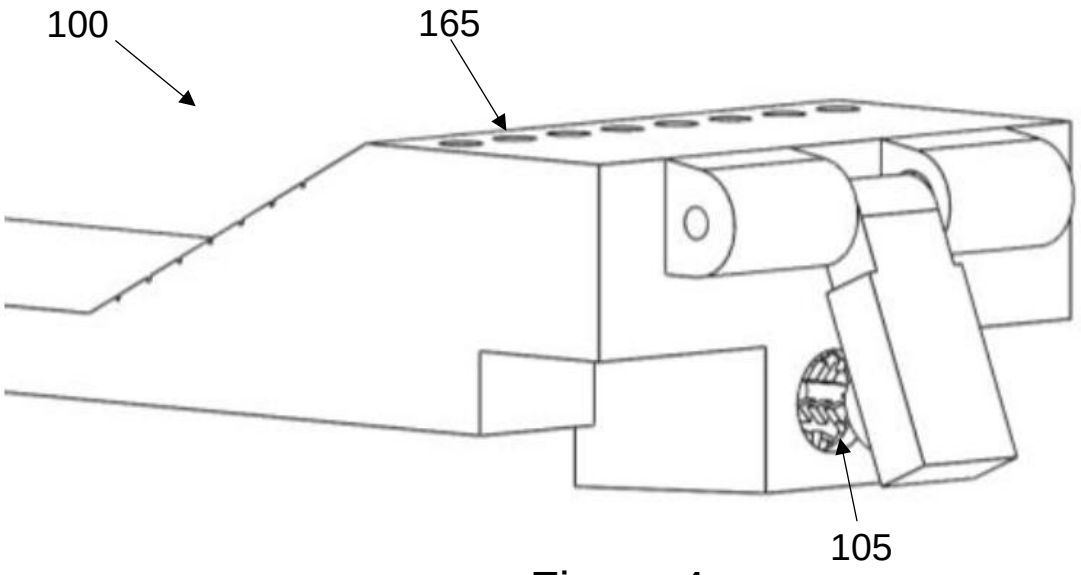

Figure 4

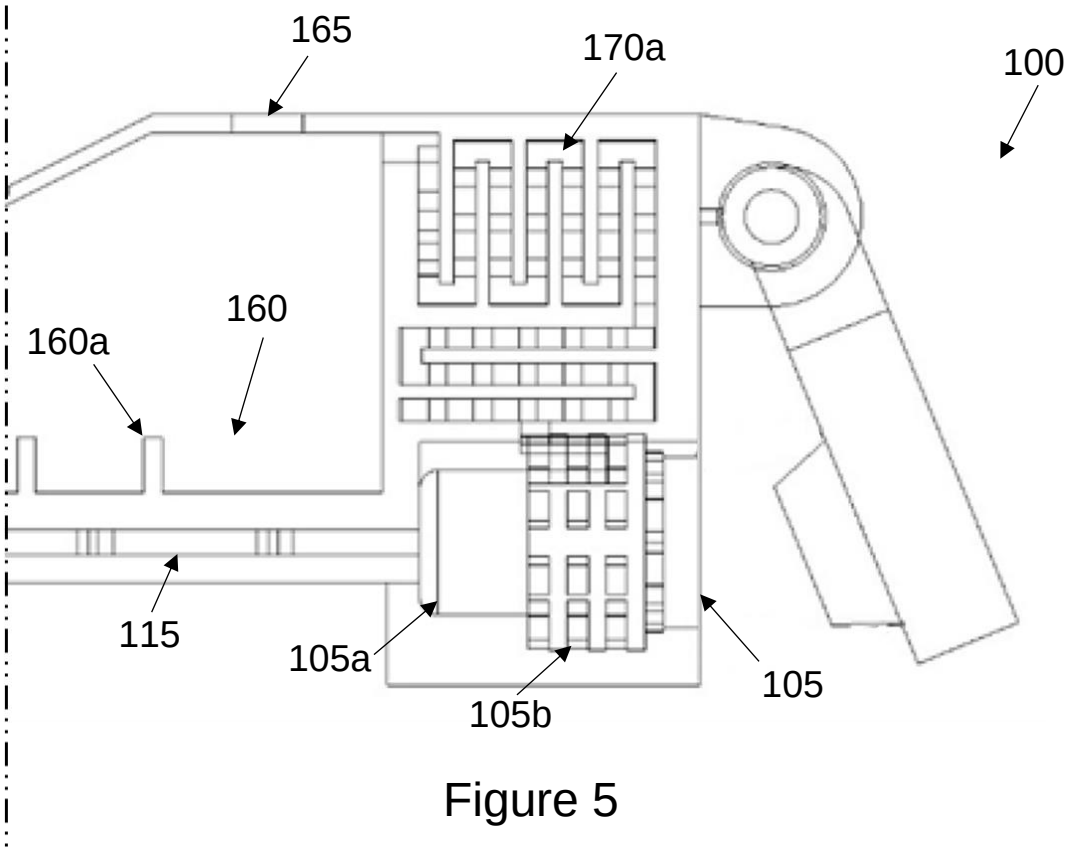

Figure 5

2024216439 29 Aug 2024

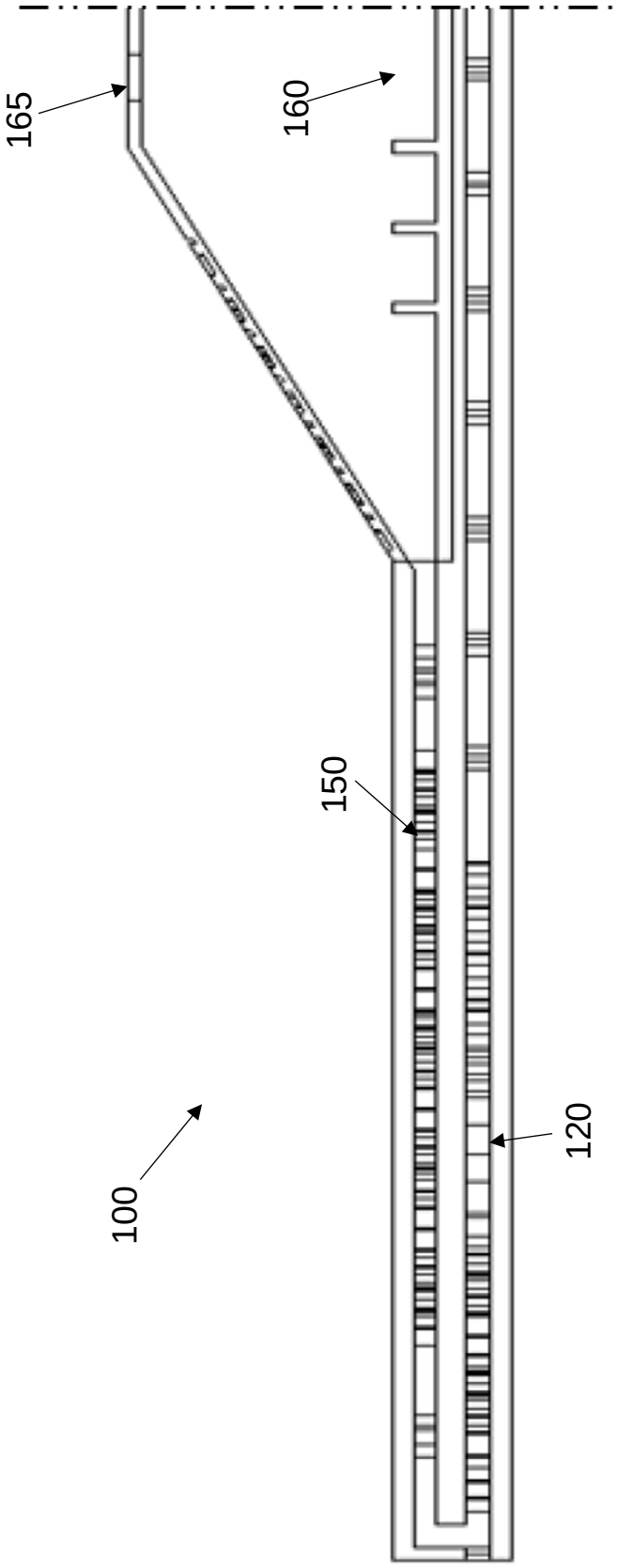

Figure 6

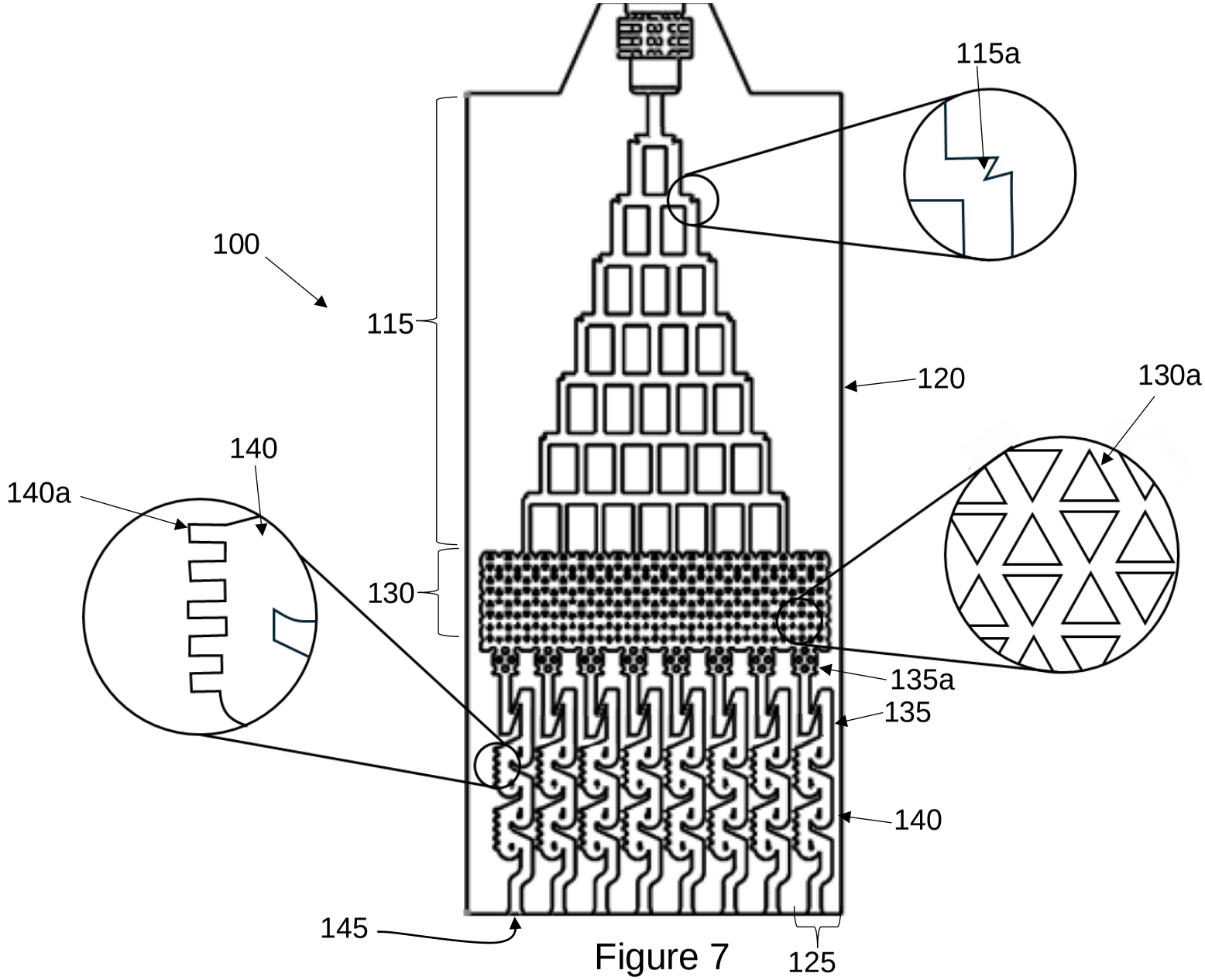

Figure 7

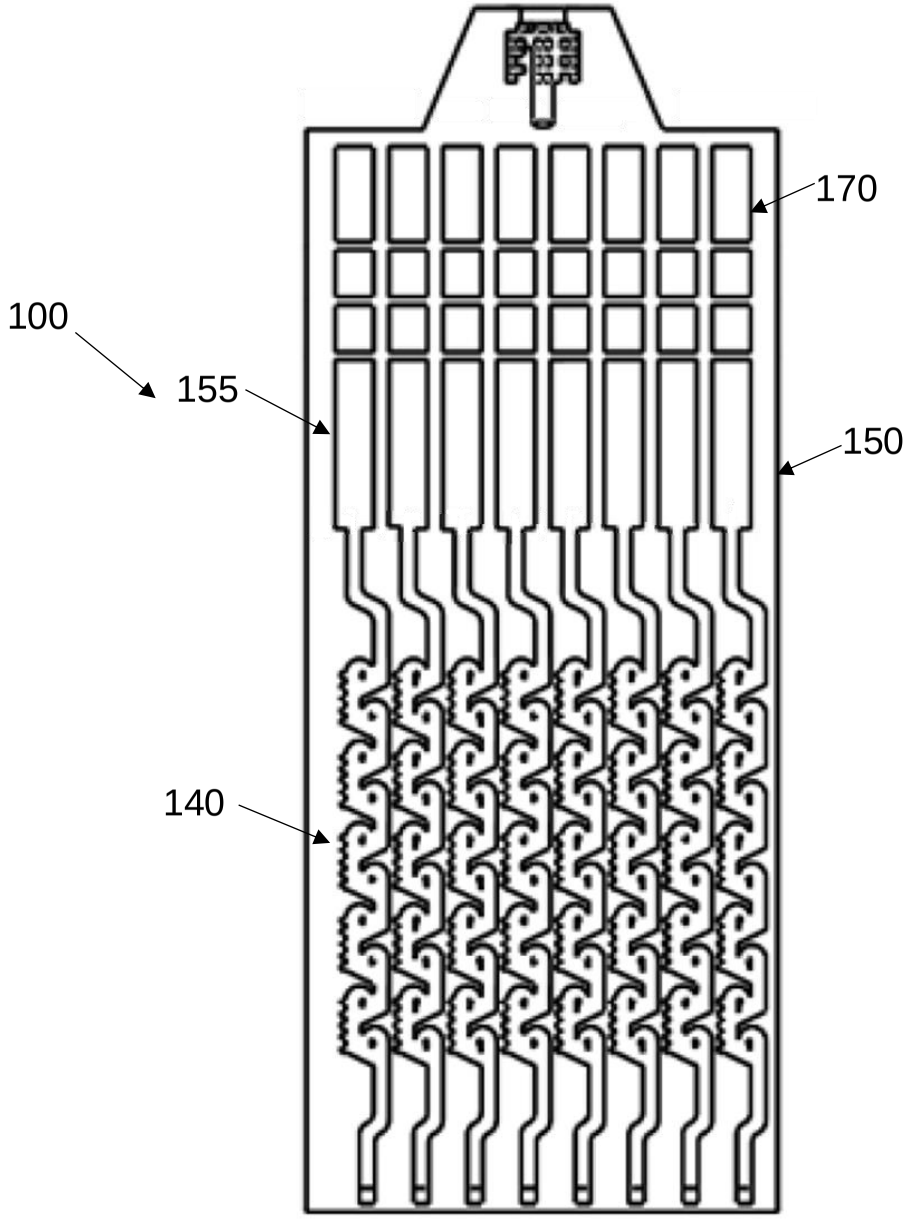

Figure 8

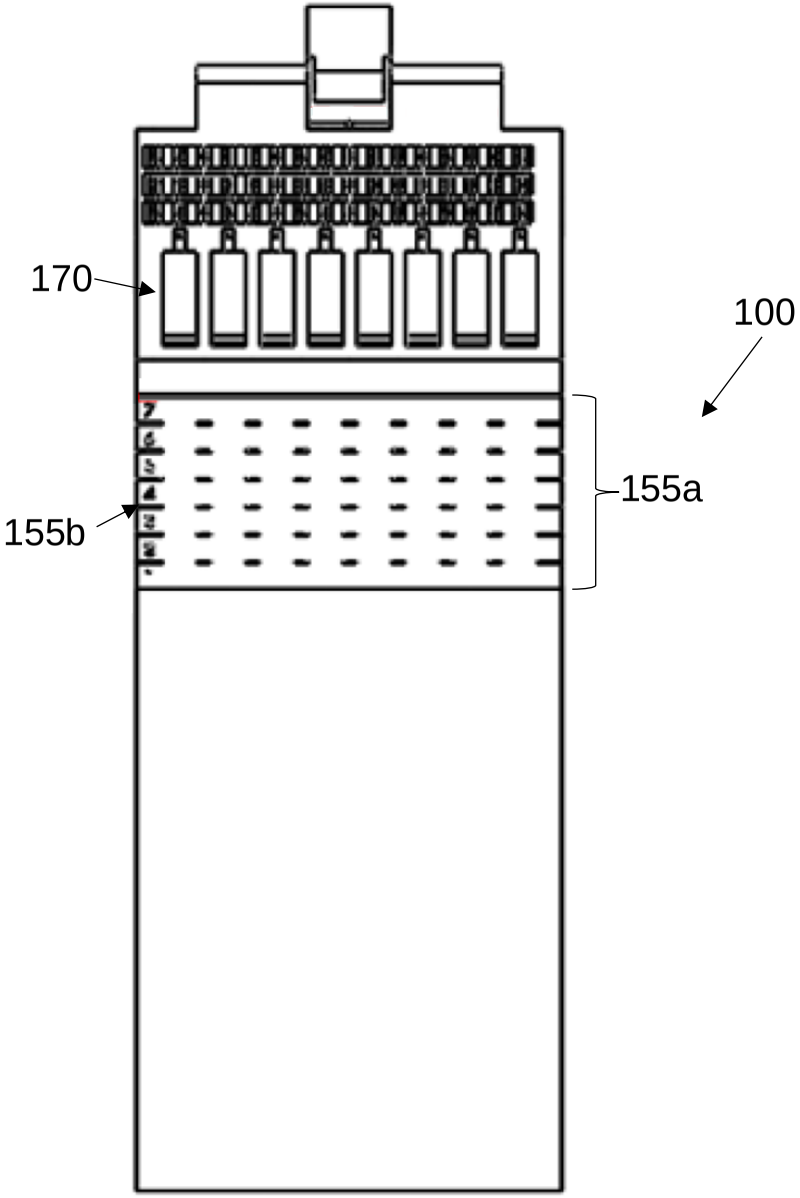

Figure 9

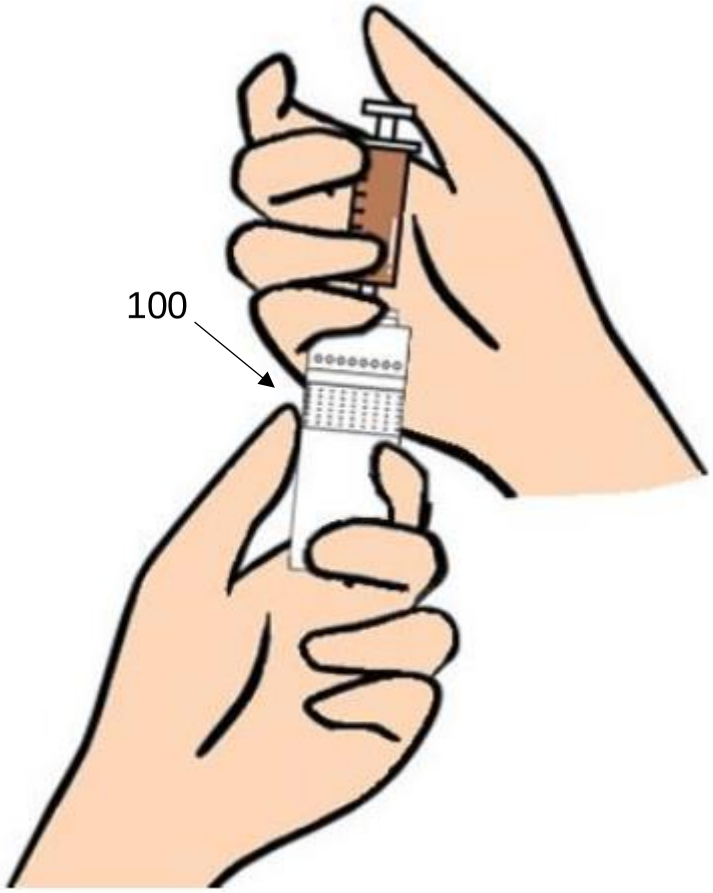

Figure 10

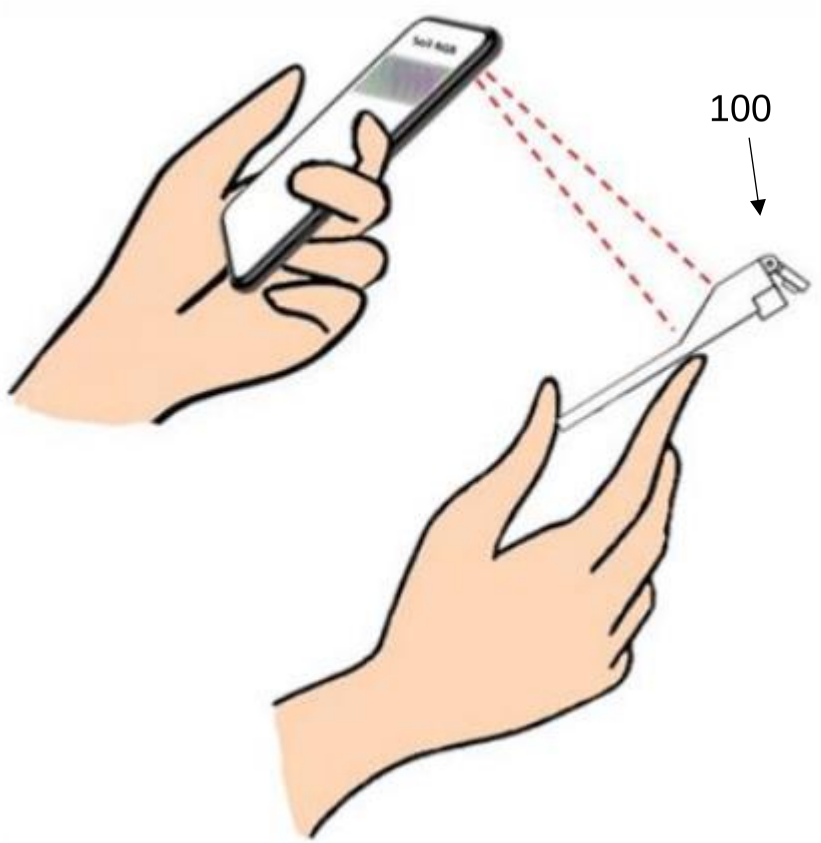

Figure 11