

FLUIDIC CIRCUITS AND METHODS FOR BACTERIAL SCREENING

TECHNICAL FIELD

[0001] The present disclosure relates generally to microfluidic systems, and more particularly to capillare circuits for bacterial screening and ultra-rapid bacteria detection.

BACKGROUND

[0002] Bacterial screening provides for the diagnosis of a range of infections as is well known in the general population but also provides for other benefits including health management, food safety, and infection reduction in blood transfusions for example. Bacterial infections in blood transfusions now represent the major infection risk with the impressive reduction of transfusion transmitted virus infections. Bacterial infections such as urinary tract infections (UTIs) are extremely common. Studies indicate 50% of women have at least one UTI by age 32 and that approximately 80% of UTI infections are due to are *Escherichia coli* (*E. coli*) where the traditional clinical limit-of-detection (LOD) is 105 colony-forming units per milliliter.

[0003] Standard diagnosis is based on bacterial culture and takes several days. Whilst so-called laboratory-on-a-chip tests have been built, these often require more than an hour to complete and require complex pre-concentration and amplification steps.

[0004] In contrast to existing laboratory-on-a-chip microfluidic systems, capillary-driven microfluidics and advanced capillare circuits offer an attractive technology for rapid, user-friendly, and minimally-instrumented diagnosis. However, existing capillare circuits typically require semiconductor style cleanroom based microfabrication, using microfabrication techniques such as deep reactive ion etching (DRIE). While such microfabrication techniques afford great precision, they limit the total volumes of capillary channels formed, and thus the fluid samples tested, to about 1 μ L. While beneficial for applications in which sample volume minimization is desirable, such microfabrication manufacturing techniques for capillare circuits, which can accommodate only nanoliters of sample, precludes their use for direct bacterial detection.

[0005] Improved capillare-based microfluidic devices and methods for rapid bacteria

detection in fluid samples are therefore sought.

SUMMARY OF THE INVENTION

[0006] It is an object to mitigate limitations in the prior art relating to bacterial screening systems and more particularly to capillarie circuits for ultra-rapid bacteria detection.

[0007] There is accordingly provided, a microfluidic device adapted to perform an assay for detecting bacteria in a fluid sample, the microfluidic device comprising a body having a capillarie circuit formed therein, the capillarie circuit comprising: a main channel and a sample reservoir which are fluidically connected and separated from each other by a sample-release valve integrally formed with the capillarie circuit, the main channel having a suspension of a plurality of functionalized microbeads in a portion thereof; a capillary pump fluidly connected with the main channel and operable to drive fluid motion through the fluidic circuit by capillary action only; one or more reagent reservoirs fluidically connected with the main channel and separated therefrom by respective reagent-release valves integrally formed with the capillarie circuit; and a microbead column portion formed within the main channel, the microbead column portion receiving the plurality of functionalized microbeads therein to create a self-packing microbead column that is formed during operation of the microfluidic device by flow of the suspension of the plurality of functionalized microbeads through the main channel.

[0008] In the microfluidic device as defined above, the sample-release valve may be preprogrammed to release the fluid sample into the main channel once the self-packing microbead column has formed in the microbead column portion of the capillarie circuit.

[0009] In the microfluidic device as defined above, the one or more reagent reservoirs may include at least two reagent reservoirs, the reagent-release valves each having different preprogrammed fluid release points to sequentially deliver fluid reagents from within respective ones of the at least two reagent reservoirs into the main channel and into the self-packing microbead column, after the fluid sample is released into the microbead column portion by the first valve.

[0010] In the microfluidic device as defined above, the microbead column portion may be disposed within the main channel.

[0011] In the microfluidic device as defined above, the microbead column portion may be disposed between the first valve and the capillary pump.

[0012] In the microfluidic device as defined above, the body, and thus the capillarie circuit defined therein, may be formed using at least one of: a three-dimensional printing process; and a molding process using a mold formed using a three-dimensional printing process.

[0013] In the microfluidic device as defined above, the body may be formed of polydimethylsiloxane (PDMS).

[0014] In the microfluidic device as defined above, at least the capillarie circuit of the body may have a plasma treatment thereon for hydrophilicity.

[0015] In the microfluidic device as defined above, one or more of the sample-release valve and the reagent-release valves may be trigger valves.

[0016] In the microfluidic device as defined above, the trigger valves may be three dimensional printed resin trigger valves integrally formed with the body of capillarie circuit.

[0017] The trigger valves may also have a cross section area that is the smallest of the capillarie circuit.

[0018] The trigger valves may have at least one dimension that is greater than 100 μm .

[0019] The trigger valves may have a height-to-width aspect ratio of greater than about 1.0.

[0020] The trigger valves may have dimensional properties selected from the group consisting of: a height of about 400 μm and a height-to-width aspect ratio greater than 0.6; a height of about 600 μm and a height-to-width aspect ratio of greater than 1.3; and a height of 1000 μm and a height-to-width aspect ratio of greater than 1.5.

[0021]

[0022] The microfluidic device according to claim 9, wherein a height difference is defined between a bottom surface of the trigger valves and a bottom surface of the main channel, the height difference of the trigger valves being $\geq 300 \mu\text{m}$.

[0023] In the microfluidic device as defined above, at least one of the sample-release valve and the reagent-release valves may include a retention burst valve.

[0024] In the microfluidic device as defined above, at least the sample reservoir defines a sample-receiving volume that is greater than 10 μL .

[0025] In the microfluidic device as defined above, the sample reservoir and the reagent reservoirs define minimum volumes selected from the group consisting of: 10

μL; 20 μL; 50 μL; and 100 μL.

[0026] In the microfluidic device as defined above, the capillarie circuit may define a total volume of greater than 100 μL.

[0027] In the microfluidic device as defined above, the device may be a lab-on-a-chip.

[0028] There is also provided, a method of forming a microfluidic device adapted to perform an assay for detecting bacteria in a fluid sample, the method comprising using three-dimensional printing to create a capillarie circuit within a body, including integrally forming one or more trigger valves and a microbead column portion within fluid-carrying channels of the capillarie circuit.

[0029] The method described above may further comprise forming, by three-dimensional printing, within the capillary circuit: a main channel and a sample reservoir which are fluidically connected and separated from each other by one of said trigger valves; a capillary pump fluidly connected with the main channel for driving fluid motion through the fluidic circuit by capillary action; and one or more reagent reservoirs fluidically connected with the main channel and separated therefrom by one or more of the trigger valves.

[0030] The method described above may further comprise at least one of: forming the body directly by the three-dimensional printing process; and forming a 3D printed mold using the three-dimensional printing process and molding the body using said 3D printed mold.

[0031] The method described above may further comprise forming the body of polydimethylsiloxane (PDMS).

[0032] The method described above may further comprise forming the trigger valves having at least one dimension that is greater than 100 μm.

[0033] The method described above may further comprise applying a plasma treatment for hydrophilicity to at least the capillarie circuit of the body.

[0034] The method described above may further comprise forming the capillarie circuit with a sample reservoir, the sample reservoir having a sample-receiving volume that is greater than 10 μL.

[0035] There is further provided, a method of detecting bacteria in an assay using a microfluidic device having an integrally formed capillarie circuit, the method comprising forming a microbead column within the capillarie circuit, the microbead column having a

plurality of functionalized microbeads self-stacked therein during operation of the microfluidic device by flow of a suspension of the plurality of functionalized microbeads through a channel of the capillarie circuit.

[0036] The method described above may further comprise forming the microbead column by loading the capillarie circuit with the plurality of functionalized microbeads in a suspension.

[0037] The method described above may further comprise, once the microbead column is formed, sequentially triggering the release of a fluid sample and at least one reagent fluid into the microbead column.

[0038] In accordance with an embodiment of the present disclosure, there is thus provided a capillarie microfluidic system having a packed bead column assembled “on-the-spot” during operation of the device (i.e. the packed microbead column is formed autonomously during operation of the device and is not pre-packed). A microfluidic device formed using additive manufacturing (e.g. 3D printing technology) such that the microfluidic capillary circuit has volumes of reagents and samples exceeding 10 μL , is also described.

[0039] Optionally, the microfluidic capillary circuit may be formed having volumes of reagents and/or samples exceeding at least one of 10 μL , 20 μL , 50 μL and 100 μL .

[0040] There is also provided a method of assay for an analysis comprising forming on demand a microbead column within a capillary based fluidic circuit.

[0041] In accordance with an embodiment of the invention there is provided a method of assay for bacterial detection comprising forming, on demand, a microbead column within a capillary based fluidic circuit, wherein the microbead column is formed by loading the capillary based fluidic circuit with a microbead loaded fluid and, once formed, automatically triggers the loading of at least one of the sample and at least one other reagent fluid forming part of the assay sequence. The sample fluid and the reagent fluid may be autonomously loaded sequentially.

[0042] In accordance with an embodiment of the present disclosure there is provided a device for performing an assay for detecting bacteria comprising a fluidic capillary circuit supporting volumes of reagents and samples combined that exceed at least one of 10 μL , 20 μL , 50 μL , and 100 μL .

[0043] In accordance with an embodiment of the present disclosure there is provided

a device for performing an assay for detecting bacteria comprising a fluidic capillary circuit that is at least one of formed using a three dimensional printing process or is molded from a template formed using a three dimensional printing process.

[0044] Other aspects and features of the present invention will become apparent to those ordinary skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] Embodiments of the present invention will now be described, by way of example only, with reference to the attached Figures, wherein:

[0046] Figure 1 is a schematic of a microfluidic capillary circuit employed in demonstrating embodiments of the present disclosure;

[0047] Figures 2A to 2E provide a schematic flow diagram depicting steps for a process for E. coli bacteria detection using the capillary circuit of Fig. 1, according to an embodiment of the present disclosure;

[0048] Figures 3A and 3B depict deep reactive ion etching (DRIE) fabricated trigger valves of the prior art;

[0049] Figure 3C depicts a three dimensional (3D) printed trigger valve according to an embodiment of the disclosure;

[0050] Figures 4A and 4B depict fluidic capillary circuits according to an embodiment of the present disclosure;

[0051] Figure 5A depicts a 3D printed mold template according to an embodiment of the present disclosure;

[0052] Figure 5B depicts a molded trigger valve according to an embodiment of the present disclosure;

[0053] Figure 5C depicts several molded trigger valve test geometries, according to embodiments of the present disclosure;

[0054] Figures 6A to 6D depict the equivalent circuit of the schematic of a fluidic capillary circuit employed in demonstrating embodiments of the invention depicted in Figure 1 and dimensions of the circuit grown using 3D printing technology;

[0055] Figure 7A to 7I depict optical micrographs of a fluidic capillary circuit employed in demonstrating embodiments of the invention as depicted in Figure 1 at

different times as the automatic filling sequence of sample, antibody and wash are loaded;

[0056] Figure 8 depicts azlactone-activated polyacrylamide beads functionalized with anti- E.coli-O157:H7 antibodies before and after assay together with images of PMMA beads with antibodies in control and E. coli exposure scenarios; and

[0057] Figure 9 depicts the experimental results of fluorescent imaging versus E. coli bacteria concentration measured with a 3D fluidic circuit according to an embodiment of the invention.

DETAILED DESCRIPTION

[0058] The present disclosure is directed to bacterial screening systems and more particularly to capillarie circuits for ultra-rapid bacteria detection exploiting three-dimensional printed elements and packed bead columns assembled on-the-spot.

[0059] The ensuing description provides exemplary embodiment(s) only, and is not intended to limit the scope, applicability or configuration of the disclosure. Rather, the ensuing description of the exemplary embodiment(s) will provide those skilled in the art with an enabling description for implementing an exemplary embodiment. It being understood that various changes may be made in the function and arrangement of elements without departing from the spirit and scope as set forth in the appended claims.

[0060] Bacteria screening requires detecting small sample concentrations in large volumes. Although capillary-driven microfluidics enable automated and minimally-instrumented immunoassays, their fabrication process usually limits them to small sample volumes (~1 μ L). Further, microchannel based methods exploiting semiconductor style cleanroom based microfabrication techniques not only are limited to handling very small volumes but they also require liquid handling equipment etc. and are expensive, typically require circuit run times of 1.5–4 hours. In contrast, paper based or yarn/fiber based fluidic methods and devices, whilst offering significantly lower cost, suffer from non-homogenous and/or auto-fluorescent substrates for the basis of the fluidic circuits. These offer faster assay times, typically of 5-10 minutes, but may be limited by the requirement for pre-incubation or enrichment of bacteria samples prior to testing.

[0061] Capillary-driven microfluidic devices move liquids using capillary forces defined

by the geometry and surface chemistry of microchannels. This allows liquid delivery without using external pumps and valves.

[0062] The terms “capillary” and “capillare” as used herein are meant to emulate the distinction between electric and electronic whereas the former pertains to basic principles and the latter is used in the context of advanced circuits integrating multiple functionalities. In addition, the term capillary is sometimes used both with reference to physical capillaries (including artificial and natural capillaries such as blood vessels) and in reference to surface tension-driven flow either within capillaries, microfluidic conduits or porous media. The term capillare as used herein is however understood to refer to surface tension driven microfluidic circuits. The microfluidic circuits described herein will therefore be referred to as capillare circuits (CC).

[0063] The inventors have established large-volume ($\geq 100 \mu\text{L}$) capillare circuits fabricated by exploiting 3D-printing of a reference mold and polydimethylsiloxane (PDMS) replication in conjunction with capillary-driven integrated valves for flow automation and an instantly assembled micro-bead column for sensitive bacteria detection. Devices replicated in PDMS from the 3D-printed reference mold exhibited about 100 μm lateral resolution. With plasma treatment for hydrophilicity, the inventors have demonstrated capillare circuits performing the E-coli assay autonomously with detection at 10^5 colony-forming units/mL within 5 minutes, thereby demonstrating the benefit of the methodology as described herein to the provisioning of a platform for ultra-rapid and sensitive bacteria screening.

[0064] Further, trigger valves of the microfluidic circuit as described herein have been developed having geometries up to 80-fold larger than typically cleanroom-fabricated trigger valves, while still functioning reliably. Retention burst valves are also described herein that encode sequential liquid delivery using capillary pressure differences encoded by systematically varied heights and widths. Consequently, the microfluidic capillare circuit as described herein has been found to enable the autonomous delivery of a plurality of different liquids in a pre-determined sequence, and all in a relative short time (e.g. less than 7 minutes for up to 8 different liquids, for example).

[0065] Two different types of microfluidic circuit valves are described herein, trigger valves and retention burst valves. The two-level trigger valves (TVs) described herein stop liquids for over 30 minutes using an abrupt geometry change and a hydrophobic

PDMS cover, thereby enabling pre-loading of reservoirs and subsequent liquid release when flow is triggered by a connected channel. Retention burst valves (RBVs), in contrast, have a burst pressure encoded by their geometry. When integrated with other capillary fluidic elements within the microfluidic capillary circuit, RBVs allow autonomous delivery of liquids in a pre-programmed sequence according to increasing order of RBV capillary pressure.

[0066] Referring now to Figure 1, a microfluidic device 10 which is adapted to perform an assay for detecting bacteria in a fluid sample (e.g. urine or another liquid sample to be tested) is shown. The microfluidic device 10 includes a body 105 within which is formed, for example by 3D-printing, a capillarie circuit 100 that includes, inter alia, a main channel 112 and a sample reservoir 110 which are fluidically connected with each other but initially sealed by a first trigger valve 150 located therebetween. As described herein, the first trigger valve 150 is operable to release the fluid sample (e.g. urine, etc.) from the sample channel 110 into the main channel 112. At least one capillary pump 160 is fluidly connected with the main channel 112 and is operable to drive fluid motion through the microfluidic capillarie circuit 100 by capillary action only. A paper pump 170 may also be added, before the capillary pump 160.

[0067] At least one reagent reservoir 140 is fluidically connected with the main channel 112 and initially sealed therefrom by at least a second trigger valve 152. In the depicted embodiment, a second reagent reservoir 130 is also provided and is fluidically connected with the main channel 112 and initially sealed therefrom by at least a third trigger valve 154. The second and third trigger valves 152, 154 are operable to sequentially deliver the fluidic reagents within their respective reagent reservoirs 140 and 130 into the fluid sample released into the main channel 112, and thus into the microbead column formed therein as will now be described.

[0068] The capillarie circuit 100 includes a bead suspension portion 120 at one end of the main channel 112, within which a plurality of functionalized microbeads 200 (see Fig. 2) are retained in suspension. These functionalized microbeads 200 are allowed to self-pack, autonomously and “on-the-spot” during operation of the device (i.e. they are not pre-packed in place), within the opposite end of the main channel 112 to form a self-packing microbead column within a microbead column portion 156 of the main channel 112 in the capillarie circuit 100. In the depicted embodiment, the microbead column portion 156 is disposed within the main channel 112, between the first trigger valve 150

and the capillary pump 160. The microbead column portion 156 therefore receives therein a plurality of functionalized microbeads 200, which are in suspension within the main channel 111, such that the microbeads 200 form a self-packed microbead column which forms during operation of the capillarie circuit 100. Note that the microbead column 156 is therefore not pre-packed with the microbeads 200, but the microbeads 200 in suspension flow within the main channel 112 of the circuit 100 to autonomously form the microbead column 156 (as shown in Fig. 2A, for example).

[0069] The first trigger valve 150 is pre-programmed to release the fluid sample retained within the sample reservoir 110 into the main channel 112, and into the microbead column 156 formed therein, after the microbead column 156 has formed itself by self-packing operation as described above. Thus, once the microbead column 156 forms, the sample is released therein by the first trigger valve 150. Subsequently, the second trigger valve 152 and third trigger valve 154, sequentially and in turn, release the reagents retained within their respective reagent reservoirs 140 and 130 into the main channel 112. Thus, the second and third trigger valves 152, 154 are operable to sequentially deliver the one or more fluid reagents into the fluid sample, after the fluid sample is released into the microbead column 156.

[0070] The capillarie circuit 100 thus provides passive flow control using one or more integrated valves, which permit autonomous liquid release when flow is triggered by a connected channel, including but not limited to a trigger valve 156 which permits autonomous liquid release when flow is triggered by a connected channel. The reservoirs (110, 140, 130, etc.) may thus be pre-loaded, and the capillarie circuit 100 is operable autonomously to delivery liquids in a pre-programmed sequence (e.g. according to an increasing order of capillary and/or burst pressures, as determined by the “pre-programmed” trigger valves 150, 152, 154, etc.).

[0071] In operation, the capillarie circuit 100 is initially loaded with the sample and reagents required for the assay. These may include, in one particular example: urine (sample); biotinylated detection antibodies (reagent 1); and streptavidin alexa 647 conjugate (reagent 2). These liquids are held in place without leakage by a number of integrated trigger valves 150, 152, 154. Subsequently, functionalized microbeads from a bead suspension 120 located at one end of the main are introduced into an integrated bead column 156, triggering preprogrammed release of the urine sample 110 wherein the E. coli are captured on the functionalized microbeads. Next, a second

preprogrammed liquid delivery event releases biotinylated detection antibodies, followed by a third preprogrammed drainage event which releases streptavidin alexa 647 conjugate before the microbeads are exposed to a wash. This is depicted schematically in Figure 2.

[0072] The trigger valves 150, 152, 154 of the capillary circuit 100 allow for liquids stored in the respective portions of the capillary circuit to be held without leakage prior to their preprogrammed liquid delivery events, as controlled by the trigger valves.

[0073] In the prior art, trigger valves have traditionally been fabricated using semiconductor based cleanroom manufacturing methodologies such as deep reactive ion etching (DRIE) and can only handle small volumes on the assumption that precise fabrication of features in the 1-100 μm size range is required for capillary valves to work. Such DRIE fabricated trigger valves of the prior art are depicted at 310 and in Figures 3A and 3B. In comparison, a three dimensional (3D) printed resin trigger valve 330 of the present disclosure is depicted in Figure 3C. The 3D-printed trigger valves 330 demonstrated here are produced by 3D printing or other similar additive manufacturing technique, and have larger feature sizes ($\geq 100 \mu\text{m}$) than their cleanroom-fabricated counterparts. In addition, the surface roughness of the printed resin is $\pm 2 \mu\text{m}$. Also, the layered structure of the 3D-printed trigger valve 330 is also visible in Figure 3. Such large surface roughness and layered structures were typically thought to result in creeping flows that lead to trigger valve failure. However, the inventors have recognized that 3D-printed trigger valves 330 are reliable and the 3D-printed trigger valve 330 success rate as a function of geometry is shown in Table 1 below.

[0074] The use of 3D-printing or other additive manufacturing techniques to produce microfluidic capillary circuits, such as the capillary circuit 100 described above, is not a manufacturing choice that one skilled in the art would be readily led to use. More particularly, the resolution currently available with consumer grade 3D-printers is typically $\geq 200 \mu\text{m}$ with about $1 \mu\text{m}$ surface roughness. Capillary microfluidics however have traditionally been made with channels in the 1-100 μm range because the capillary pressure is inversely proportional to the smallest dimension, and becomes very small for large microchannels. Moreover, valving and flow control depend on the surface topography and abrupt geometric changes and low surface roughness are considered necessary to prevent pre-wetting and creeping flows. Hence the prevailing perception

by those of ordinary skill in the art is that current 3D-printing technology may not be suitable for making capillary microfluidics, because the smallest dimensions may be too large to obtain adequate capillary pressure, the resolution and precision may be insufficient for making abrupt changes needed for reliable valves – notably due to the layered structure of stereolithographic printing forming steps that lend themselves to corner flow – and the high surface roughness may lead to creeping of liquid. However, the inventors have found that this is not the case, and that 3D-printing may be used to produce microfluidic devices having well functioning capillary circuits, as described herein.

[0075] By exploiting 3D printing on a stereolithography-based 3D printer, the inventors were able to design and fabricate prototype circuit molds in less than 15 minutes. The 3D printer employed provides 1024×768 resolution with a pixel size of $100\ \mu\text{m}$ and Z-slice depth $50\ \mu\text{m}$. Polydimethylsiloxane (PDMS) replicas were made after spraying the 3D-printed mold with a silicone-based release agent. The PDMS replicas were plasma-treated for hydrophilicity and sealed against untreated PDMS covers resulting in the microfluidic circuit 100, as depicted in Fig. 4.

[0076] Referring to Figures 5A-5C, an optical micrograph of a 3D printed mold 510 is shown in Fig. 5A, and the PDMS replica 520 is shown in Fig. 5B. In both the 3D printed mold 510 and the PDMS replica 520 thereof, the trigger valve 530 is visible between a reagent reservoir 540 and the main channel 505. Fig. 5C depicts trigger valves 530 for four different channel widths, namely: $100\ \mu\text{m}$; $150\ \mu\text{m}$; $200\ \mu\text{m}$; and $250\ \mu\text{m}$.

[0077] The inventors have established that the aspect ratio (height to width) of the trigger valves 150, 152, 154, 530 etc. is one possible variable which will affect the resulting effectiveness of the trigger valve. Other variables which were also found to influence the operation of the trigger valve produced as described herein include: the height difference between the trigger valve and the release channel; the width of the trigger valve; and the height of the trigger valve.

[0078] The geometry of the trigger valves (TVs) influence their success rate. Referring to Fig. 5B, the geometric parameters known to affect the performance of capillary TVs: the height H of the TV, width W of the TV, and the height difference ΔH between the TV and its release channel. To determine which geometries provide high TV success

rates, valves with widths of 96 μm , 192 μm , 288 μm , 480 μm , 672 μm , 960 μm , and 2016 μm , were tested. TV heights were fixed at either 400 μm or 1000 μm to obtain different height-to-width ratios for these experiments. It was found that 3D-printed TVs were reliable with dimensions up to 3 times larger than reported with CO₂ laser cutting and up to 80 times larger than typical cleanroom fabricated valves. It was also found that above a height difference (ΔH) of 300 μm , TVs were 100 % successful. Since the minimum z-layer thickness of the microchannels was limited to 50 μm by the 3D-printer resolution, height differences of 100 μm , 150 μm , 200 μm , 250 μm , 300 μm , 400 μm , and 500 μm between the TV and the release channel were tested. The TVs used for these height difference tests were 300 μm wide and 50 μm deep. The height difference between the TV and the release channel had a threshold effect on TV success, wherein a height difference of ≥ 300 μm , TVs were 100 % successful in the test conducted.

[0079] Table 1 below shows experimental results for trigger valves with different geometries, wherein success is holding liquid for more than 30 minutes where $n=4$ for each geometry. From the results of Table 1 below, it can be extrapolated that across the different dimensions of trigger valves tested, the trigger valves having a height-to-width aspect ratio of greater than about 1.0 should provide successful results.

h = 400 μm		h = 600 μm		h = 1000 μm	
Aspect Ratio (h/w)	% Success	Aspect Ratio (h/w)	% Success	Aspect Ratio (h/w)	% Success
0.2	75	0.3	50	0.5	75
0.4	50	0.6	25	1.0	75
0.6	100	0.9	75	1.5	100
0.8	100	1.3	100	2.1	100
1.4	100	2.0	100	3.5	100
2.1	100	3.1	100	5.2	75

4.2	50	6.3	100	10.4	100
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Table 1: 3D Printed Trigger Valve Characterization

[0080] Despite the fact that the most common application of capillary microfluidics is to automate biological assays that often require the use of surfactant-containing reagents, the effect of surfactant concentration on TV performance is not well reported in the literature. To determine the effect of surfactant on TVs, we tested aqueous solutions with different concentrations of Tween® 20, a surfactant commonly used in immunoassay wash buffers and for cell lysis. The critical micelle concentration of Tween® 20, is 0.0074% w/v. Consequently, we tested the following concentrations of Tween® 20: 0.0074, 0.0110, 0.0650, 0.1100, and 0.2750 % weight/volume. TVs were found to be 100 % reliable when Tween® 20 concentrations were $\leq$ 0.0650 % weight/volume. A suitable surfactant concentration for use in wash buffers during immunoassays that commonly use 0.05%.

[0081] The presently described capillarie circuit 100 may also include Retention Burst Valves (RBVs) which may be used to retain liquid in a conduit up to a threshold, or bursting, pressure which if exceeded leads to bursting of the valve and draining of the liquid held downstream in a reservoir. The RBVs may be used either in addition to, or in lieu of, the trigger valves. It is thus possible to drain a series of reservoirs connected to a main channel in a predetermined sequence by terminating each of them with a RBV with increasing burst pressure. The burst pressure of a RBV can be predetermined.

[0082] In accordance with an alternate embodiment, a capillarie circuit (CC) with four RBVs was provided. PDMS replicas of the 3D-printed mold were made, plasma-treated for hydrophilicity, and sealed with a hydrophobic PDMS cover. First reservoirs were filled and TVs held each liquid in place. Next, a solution was added to the release channel, connecting the reservoirs to the pump and starting the pre-programmed liquid delivery sequence. Subsequently, the RBVs burst sequentially according to increasing capillary pressure.

[0083] In the present embodiments of the CC having TVs, the TVs may be designed to have the smallest cross section in the circuit and the highest capillary pressure in the CC since they play a dual role – stopping liquids during initial filling of reservoirs, and acting as retention valves with higher capillary pressure than the capillary pump during

reservoir drainage. These retention valves ensure that the side branches are not completely emptied (with minimal dead volume), thereby allowing sequential liquid delivery without bubble trapping.

[0084] The equivalent circuit for the 3D printed capillarity circuit depicted in Figure 1 is shown in Figure 6A. The schematic layout is shown in Fig 6B. The 3D printed mold for the capillarity circuit is shown in Figure 6C and the PDMS replica of the circuit is shown in Figure 6D.

[0085] Figures 7A to 7H depicts optical images of the 3D capillarity circuit loaded with dyed water, wherein the images represent:

- Fig. 7A at 0 seconds with an empty device;
- Fig. 7B at 2 min 23 seconds wherein wash buffer, antibody, and bacteria sample are loaded;
- Fig. 7C at 2 min 58 seconds wherein bead suspension is loaded;
- Fig. 7D at 7 min 26 seconds wherein bacteria sample has drained;
- Fig. 7E at 8 min 00 seconds wherein detection antibody has drained;
- Fig. 7F at 8 min 17s seconds wherein streptavidin alexa 647 conjugate has drained;
- Fig. 7G at 8 min 40s seconds wherein all preprogrammed liquid delivery events are complete;
- Fig. 7G shows the packed beads in front of the capillary pump at the end of the assay; and
- Figs. 7H and 7I show close-up views of the packed beads within the column.

[0086] The pre-loading of the capillary circuit is valve 1: 20 seconds, valve 2: 13 seconds, valve 3: 12 seconds and beads 25 seconds for a total of 65 seconds. Reagent delivery is time as 7.3 minutes for valve 1, 40 seconds for valve 2, 16 seconds for valve 3, and 24 seconds for valve 4 resulting in total drain time of 8.6 minutes such that the total assay time is 9.7 minutes.

[0087] The assay was performed using *E. coli* O157:H7 spiked into synthetic urine and pre-activated Ultralink™ bis-acrylamide/azlactone copolymer beads (50 – 80 µm diameter), functionalized with anti-*E. coli* O157:H7 antibodies, were used to provide large surface area and small gaps in the reaction chamber to increase the likelihood of bacteria capture. Biotinylated detection antibodies, streptavidin alexa

647 conjugate, and wash buffer comprising 1x Phosphate Buffered Saline with 0.1% Tween-20 were the other reagents used in the assay. The assay was conducted in a “walk-away” format i.e. assay reagents (sample, detection antibody, and wash buffer) were pre-loaded and stopped by capillary trigger valves until the user added the microbead suspension, at which point the beads were assembled and assay reagents were released sequentially by the capillary retention burst valves without operator intervention. The chip was imaged by fluorescence microscopy and the images analyzed with *ImageJ*.

[0088] The inventors first modified the design and verified the functionality of the scaled up capillary fluidic elements to achieve the desired capillarity circuit for bacteria detection using multilayer channels to increase the total reagent volume to ~ 120 μL . As shown in Figure 7, liquid delivery was verified using dye solutions and reliable bead-column packing using physical barriers was demonstrated. Microbeads columns were reliably packed in the reaction chamber. See for example first image 810 in Figure 7G and 7H wherein a close-up image of the reaction chamber of a 3D-printed capillary circuit is depicted packed with the beads

[0089] Referring to Figure 8 there are depicted:

- First and second images 910 and 920 depicting 50 – 80 μm azlactone-activated polyacrylamide beads functionalized with anti-E.coli-O157:H7 antibodies before and after assay; and
- Third to sixth images 930 to 960 depicting E. coli detection as spots within the bead column at 0 CFU / mL; 10^4 CFU / mL; 10^5 CFU / mL; and 10^6 CFU / mL respectively.

[0090] Accordingly, in Figure 9, the graph depicts analysis of the bacteria capture assay results for E. coli employing bacteria assay via 3D printed capillary circuit wherein the number of spots detected is plotted against bacteria concentration. These results indicate that initial exemplary circuits according to an embodiment of the invention exhibit a minimum detection threshold of approximately 3×10^3 CFU / mL.

[0091] Taken together, these results show direct bacteria detection in 3D-printed capillary circuits according to an embodiment of the invention. E.coli were detected without sample pre-concentration in fully automated chips within a few minutes. Whilst the circuits described *supra* exploit a volume of about 20 μL the inventors extrapolate

that sample volumes of $\geq 100\mu\text{L}$ may be employed to increase the performance of our chip by improving the limit of detection.

[0092] It would be evident to one of skill that embodiments of the invention exploiting consumer grade rapid prototyping systems circumventing the requirements for expensive clean rooms, while reducing turnaround time from design to device to a few hours, and not requiring to make masks for photolithography.

[0093] It would be evident to one of skill that embodiments of the invention allow for capillarie circuits to process large volumes of a sample in contrast to prior art microfluidics, which helps improve the performance as analytes, such as small molecules, nucleic acids, proteins, virus or bacteria, can be sampled in sufficient numbers to obtain statistically sound data even when they are at very low concentration.

[0094] It would be evident to one of skill that embodiments of the invention allow for capillarie circuits to process other analytes in addition to bacteria as discussed *supra* including, but not limited to, small molecules, nucleic acids, proteins, virus or bacteria..

[0095] It would be evident to one of skill that embodiments of the invention that consumer grade rapid prototyping systems allow for working capillarie circuits to be optimized rapidly at low cost and then mass produced by injection molding and other common machining techniques.

[0096] It would be evident to one of skill in the art that low cost printed / molded capillarie circuits such as discussed *supra* and feasible in variants of the techniques / methods presented may form the basis for low cost consumer orientated assays wherein a fluorescent visualization may provide the user with a desired indication or alternatively these circuits may be employed in conjunction with a compact hand-held / desktop / kiosk based reader for rapid, portable, and low- cost tests.

[0097] It would be evident to one of skill in the art whilst within the embodiments of the invention described *supra* that the inventors replicated the 3D printed device into PDMS (e.g. one prints a negative copy of the circuit). However, one could also directly print the final circuit, and upon surface treatment, it would be ready for use.

[0098] It would be evident to one of skill in the art that the microbead column as described within embodiments of the invention may be omitted in other embodiments and may alternatively simply be on a flat surface (e.g. the cover).

[0099] It would be evident to one of skill in the art that the methodologies described supra may be expanded within the scope of the invention to other fluidic circuit functions and elements including, but not limited to those described and depicted within World Patent Application WO/2013/029159 entitled “Method and System for Pre-Programmed Self-Power Microfluidic Circuits”, the entire content of which is incorporated herein by reference.

[00100] It would also be evident to one of skill in the art that the ability to assembly the bead column “on the fly” offers flexibility in the deployment of the assay as the loading of the beads may define the assay and accordingly the other reagents etc.

[00101] The foregoing disclosure of the exemplary embodiments of the present invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise forms disclosed. Many variations and modifications of the embodiments described herein will be apparent to one of ordinary skill in the art in light of the above disclosure. The scope of the invention is to be defined only by the claims appended hereto, and by their equivalents.

[00102] Further, in describing representative embodiments of the present invention, the specification may have presented the method and/or process of the present invention as a particular sequence of steps. However, to the extent that the method or process does not rely on the particular order of steps set forth herein, the method or process should not be limited to the particular sequence of steps described. As one of ordinary skill in the art would appreciate, other sequences of steps may be possible. Therefore, the particular order of the steps set forth in the specification should not be construed as limitations on the claims. In addition, the claims directed to the method and/or process of the present invention should not be limited to the performance of their steps in the order written, and one skilled in the art can readily appreciate that the sequences may be varied and still remain within the spirit and scope of the present invention.

CLAIMS:

1. A microfluidic device adapted to perform an assay for detecting bacteria in a fluid sample, the microfluidic device comprising a body having a capillarie circuit formed therein, the capillarie circuit comprising: a main channel and a sample reservoir which are fluidically connected and separated from each other by a sample-release valve integrally formed with the capillarie circuit, the main channel having a suspension of a plurality of functionalized microbeads in a portion thereof; a capillary pump fluidly connected with the main channel and operable to drive fluid motion through the fluidic circuit by capillary action only; one or more reagent reservoirs fluidically connected with the main channel and separated therefrom by respective reagent-release valves integrally formed with the capillarie circuit; and a microbead column portion formed within the main channel, the microbead column portion receiving the plurality of functionalized microbeads therein to create a self-packing microbead column that is formed during operation of the microfluidic device by flow of the suspension of the plurality of functionalized microbeads through the main channel.
2. The microfluidic device according to claim 1, wherein the sample-release valve is preprogrammed to release the fluid sample into the main channel once the self-packing microbead column has formed in the microbead column portion of the capillarie circuit.
3. The microfluidic device according to claim 2, wherein the one or more reagent reservoirs includes at least two reagent reservoirs, the reagent-release valves each having different preprogrammed fluid release points to sequentially deliver fluid reagents from within respective ones of the at least two reagent reservoirs into the main channel and into the self-packing microbead column, after the fluid sample is released into the microbead column portion by the first valve.
4. The microfluidic device according to claim 1, wherein the microbead column portion is disposed within the main channel.
5. The microfluidic device according to claim 4, wherein the microbead column portion is disposed between the first valve and the capillary pump.

6. The microfluidic device according to claim 1, wherein the body, and thus the capillarie circuit defined therein, is formed using at least one of: a three-dimensional printing process; and a molding process using a mold formed using a three-dimensional printing process.
7. The microfluidic device according to claim 6, wherein the body is formed of polydimethylsiloxane (PDMS).
8. The microfluidic device according to claim 7, wherein at least the capillarie circuit of the body has a plasma treatment thereon for hydrophilicity.
9. The microfluidic device according to claim 1, wherein one or more of the sample-release valve and the reagent-release valves are trigger valves.
10. The microfluidic device according to claim 9, wherein the trigger valves are three dimensional printed resin trigger valves integrally formed with the body of capillarie circuit.
11. The microfluidic device according to claim 9, wherein the trigger valves have a cross section area that is the smallest of the capillarie circuit.
12. The microfluidic device according to claim 9, wherein the trigger valves have at least one dimension that is greater than 100 μm .
13. The microfluidic device according to claim 9, wherein the trigger valves have a height-to-width aspect ratio of greater than about 1.0.
14. The microfluidic device according to claim 9, wherein the trigger valves have dimensional properties selected from the group consisting of: a height of about 400 μm and a height-to-width aspect ratio greater than 0.6; a height of about 600 μm and a height-to-width aspect ratio of greater than 1.3; and a height of 1000 μm and a height-to-width aspect ratio of greater than 1.5.
15. The microfluidic device according to claim 9, wherein a height difference is defined between a bottom surface of the trigger valves and a bottom surface of the main channel, the height difference of the trigger valves being $\geq 300 \mu\text{m}$.
16. The microfluidic device according to claim 1, wherein at least one of the

sample-release valve and the reagent-release valves are a retention burst valve.

17. The microfluidic device according to claim 1, wherein at least the sample reservoir defines a sample-receiving volume that is greater than 10 μL .

18. The microfluidic device according to claim 1, wherein the sample reservoir and the reagent reservoirs define minimum volumes selected from the group consisting of: 10 μL ; 20 μL ; 50 μL ; and 100 μL .

19. The microfluidic device according to claim 1, wherein the capillarie circuit defines a total volume of greater than 100 μL .

20. The microfluidic device of claim 1, wherein the device is a lab-on-a-chip.

21. A method of forming a microfluidic device adapted to perform an assay for detecting bacteria in a fluid sample, the method comprising using three-dimensional printing to create a capillarie circuit within a body, including integrally forming one or more trigger valves and a microbead column portion within fluid-carrying channels of the capillarie circuit.

22. The method of claims 21, further comprising forming, by three-dimensional printing, within the capillary circuit: a main channel and a sample reservoir which are fluidly connected and separated from each other by one of said trigger valves; a capillary pump fluidly connected with the main channel for driving fluid motion through the fluidic circuit by capillary action; and one or more reagent reservoirs fluidly connected with the main channel and separated therefrom by one or more of the trigger valves.

23. The method of claim 21, wherein the step of using three-dimensional printing to create the capillarie circuit within a body includes at least one of: forming the body directly by the three-dimensional printing process; and forming a 3D printed mold using the three-dimensional printing process and molding the body using said 3D printed mold.

24. The method of claim 23, further comprising forming the body of polydimethylsiloxane (PDMS).

25. The method of claim 21, further comprising forming the trigger valves having at least one dimension that is greater than 100 μm .
26. The method of claim 21, further comprising applying a plasma treatment for hydrophilicity to at least the capillarie circuit of the body.
27. The method of claim 21, further comprising forming the capillarie circuit with a sample reservoir, the sample reservoir having a sample-receiving volume that is greater than 10 μL .
28. A method of detecting bacteria in an assay using a microfluidic device having an integrally formed capillarie circuit, the method comprising forming a microbead column within the capillarie circuit, the microbead column having a plurality of functionalized microbeads self-stacked therein during operation of the microfluidic device by flow of a suspension of the plurality of functionalized microbeads through a channel of the capillarie circuit.
29. The method of claim 28, further comprising forming the microbead column by loading the capillarie circuit with the plurality of functionalized microbeads in a suspension.
30. The method of claim 28, further comprising, once the microbead column is formed, sequentially triggering the release of a fluid sample and at least one reagent fluid into the microbead column.

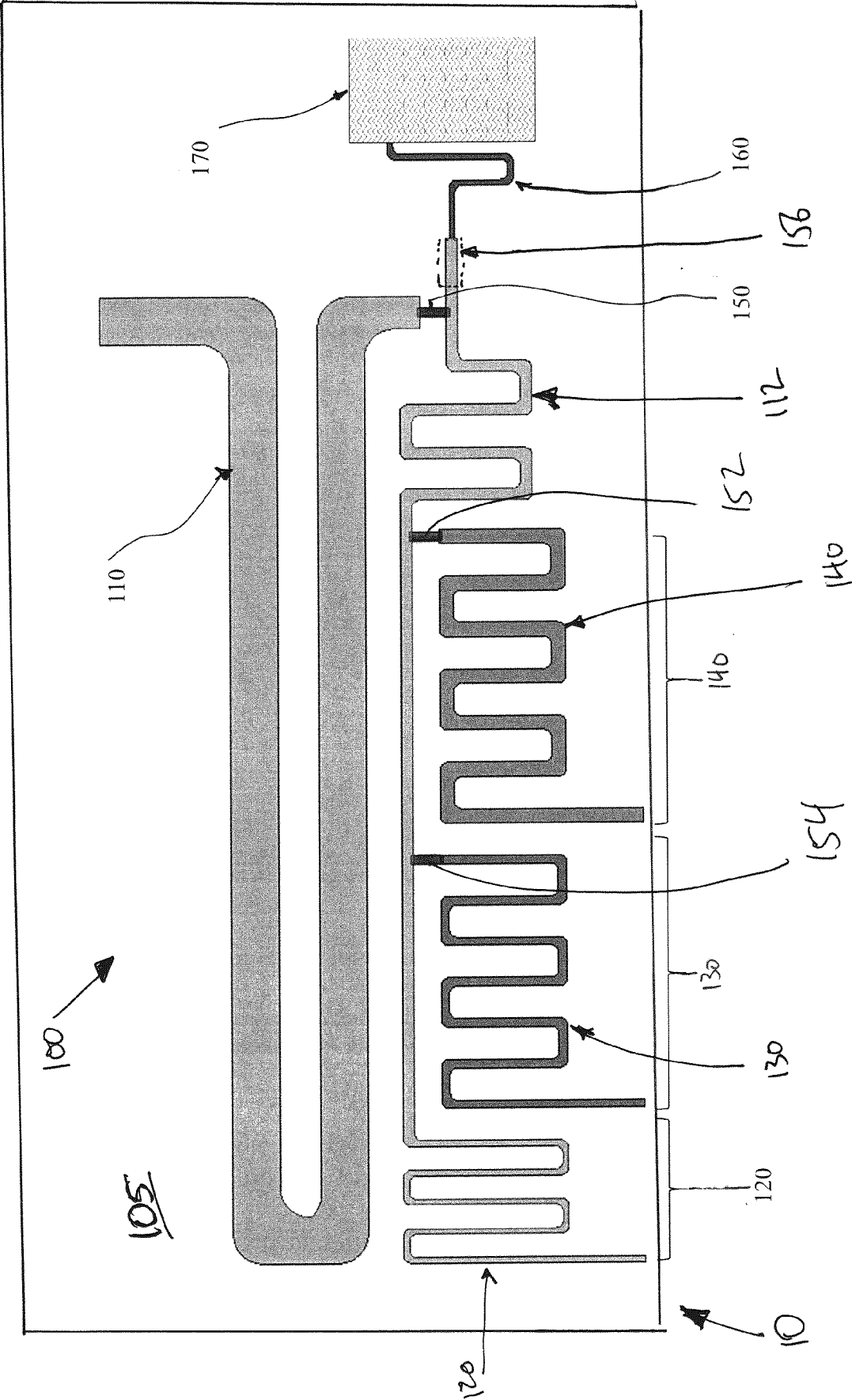
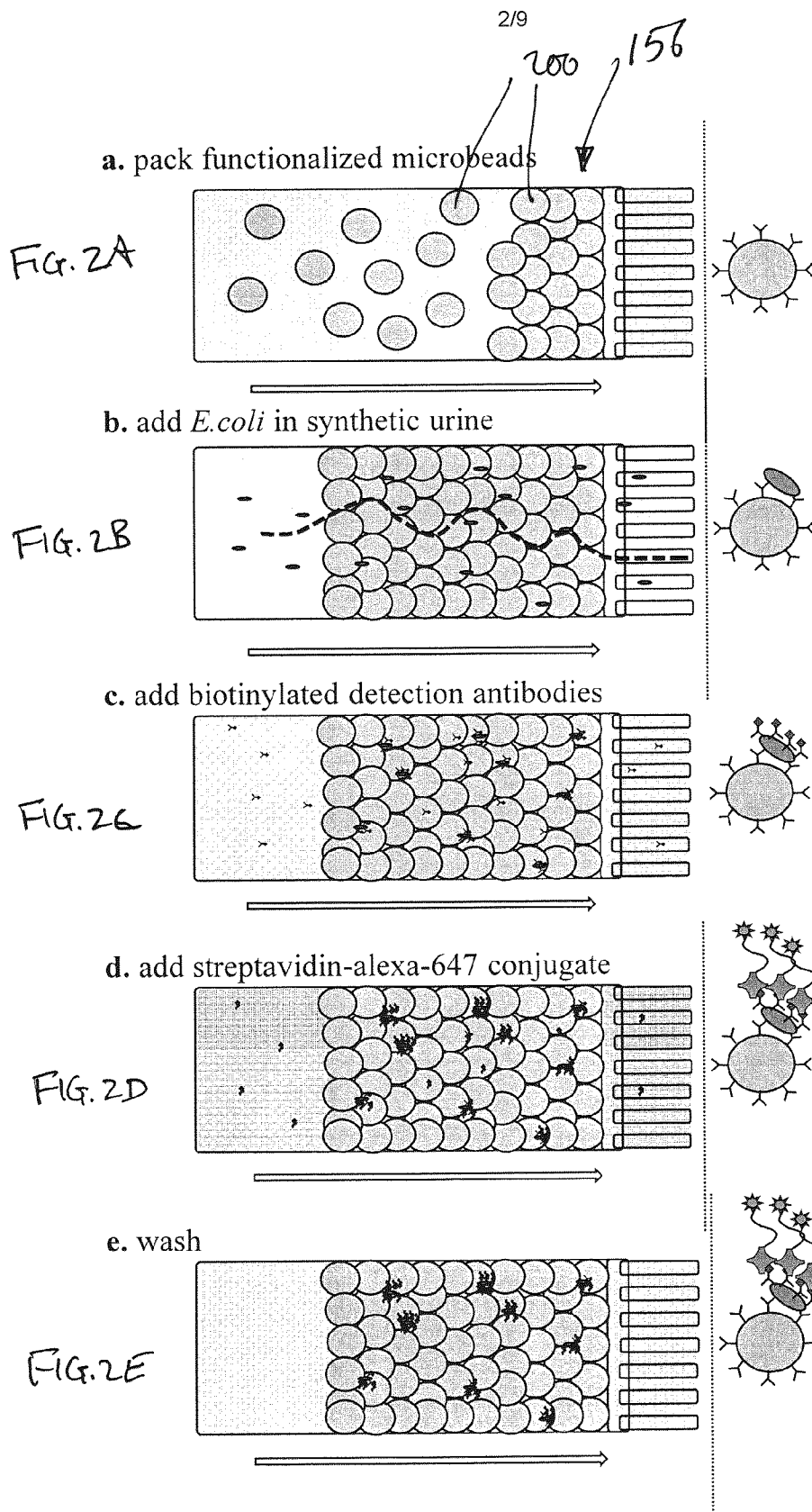
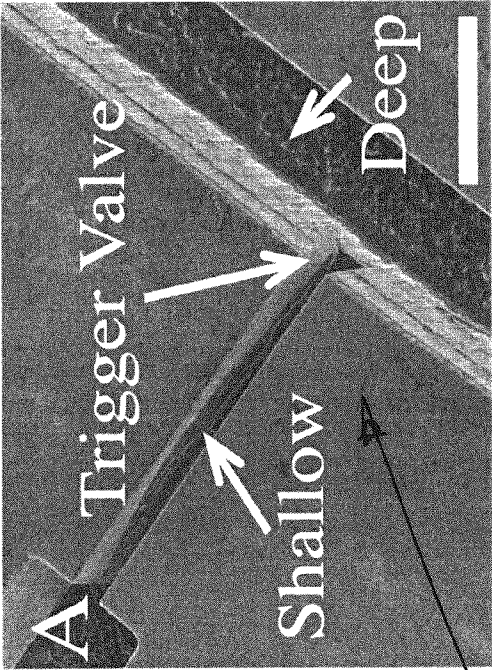


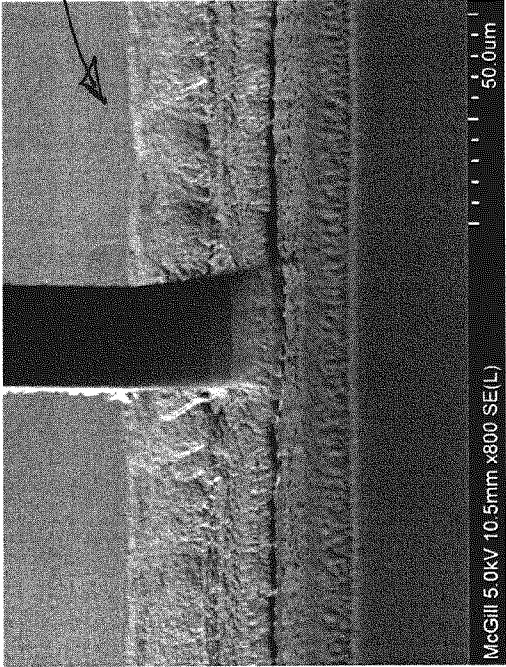
Figure 1





310

FIGURE 3A
PRIOR ART



320

FIGURE 3B
PRIOR ART

330

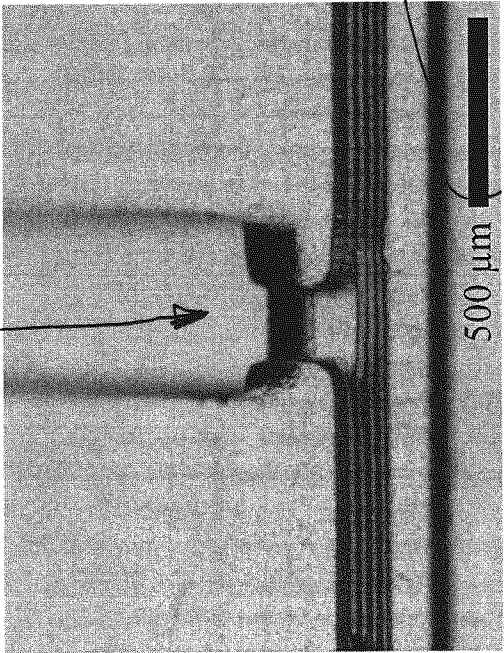


FIGURE 3C

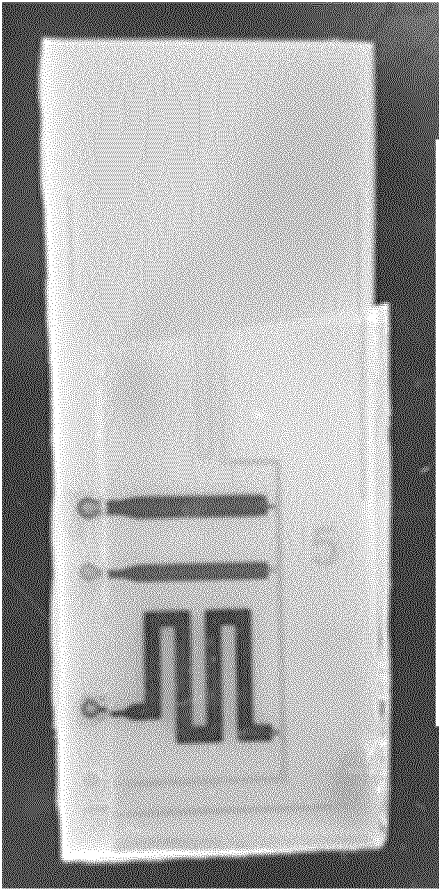


Figure 4B

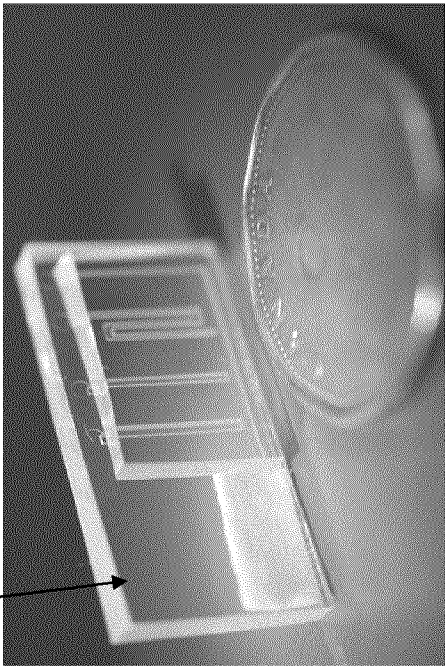


Figure 4A

100



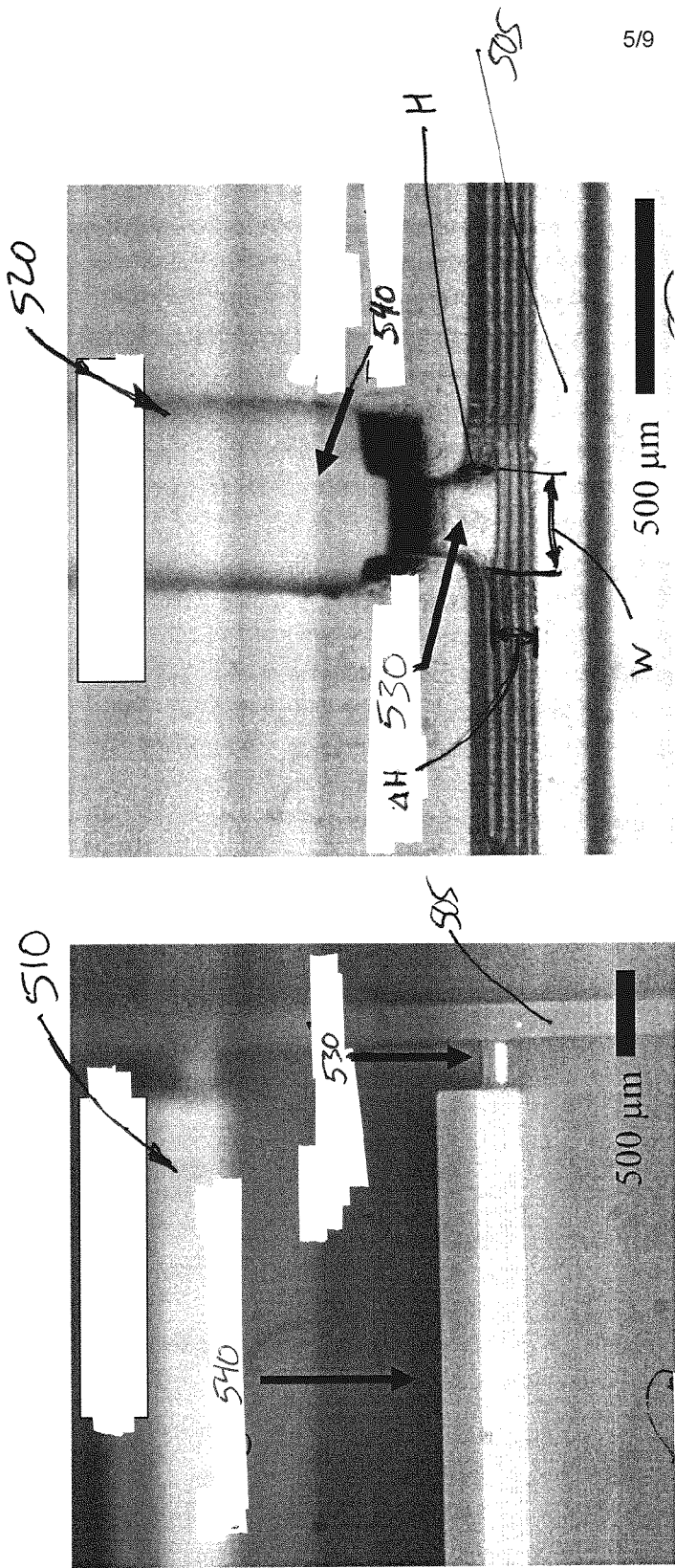


FIG. 5A

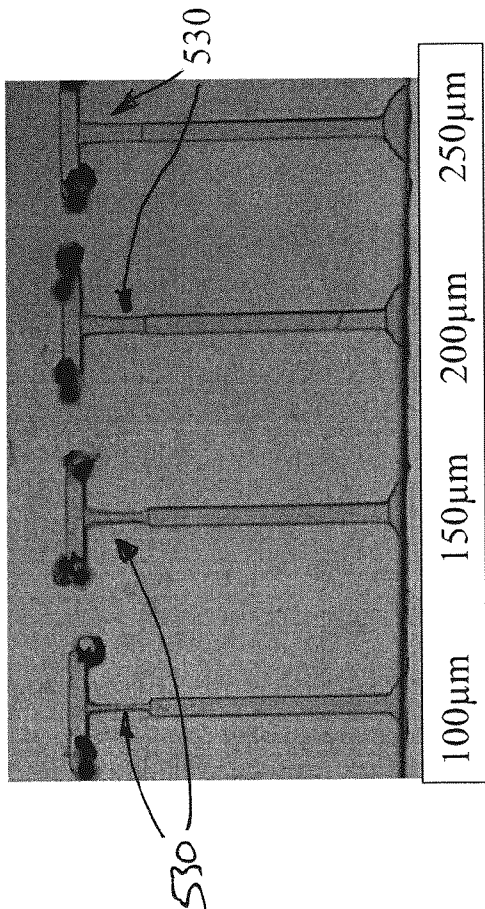


FIG. 5C

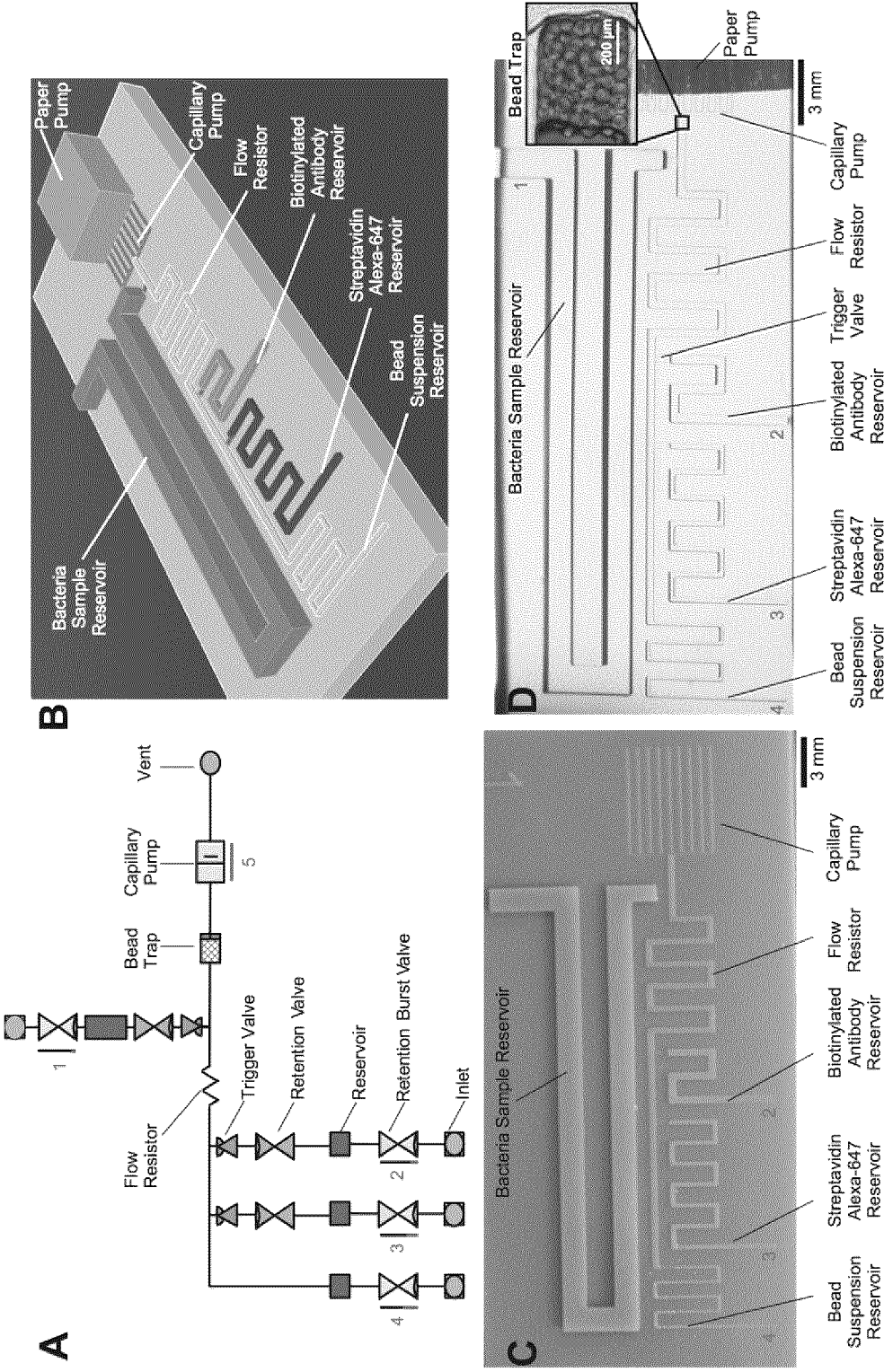


Figure 6

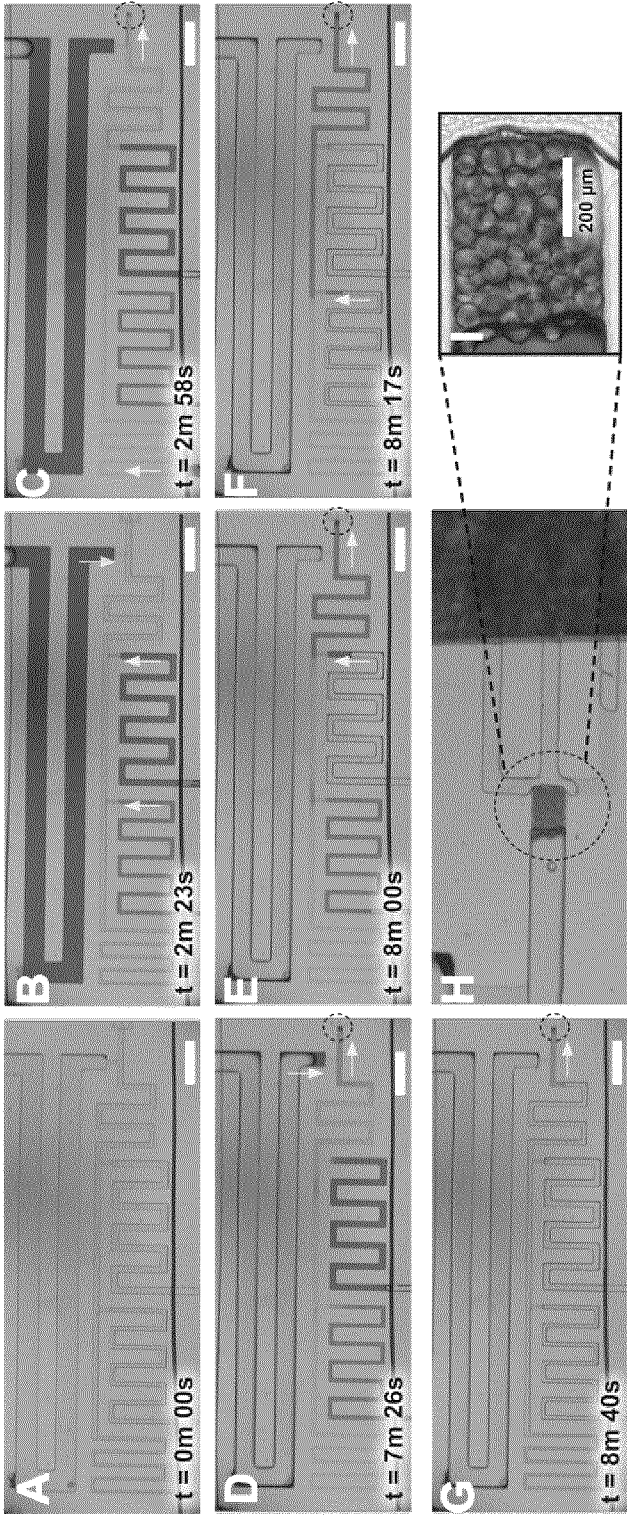


Figure 7 830 840

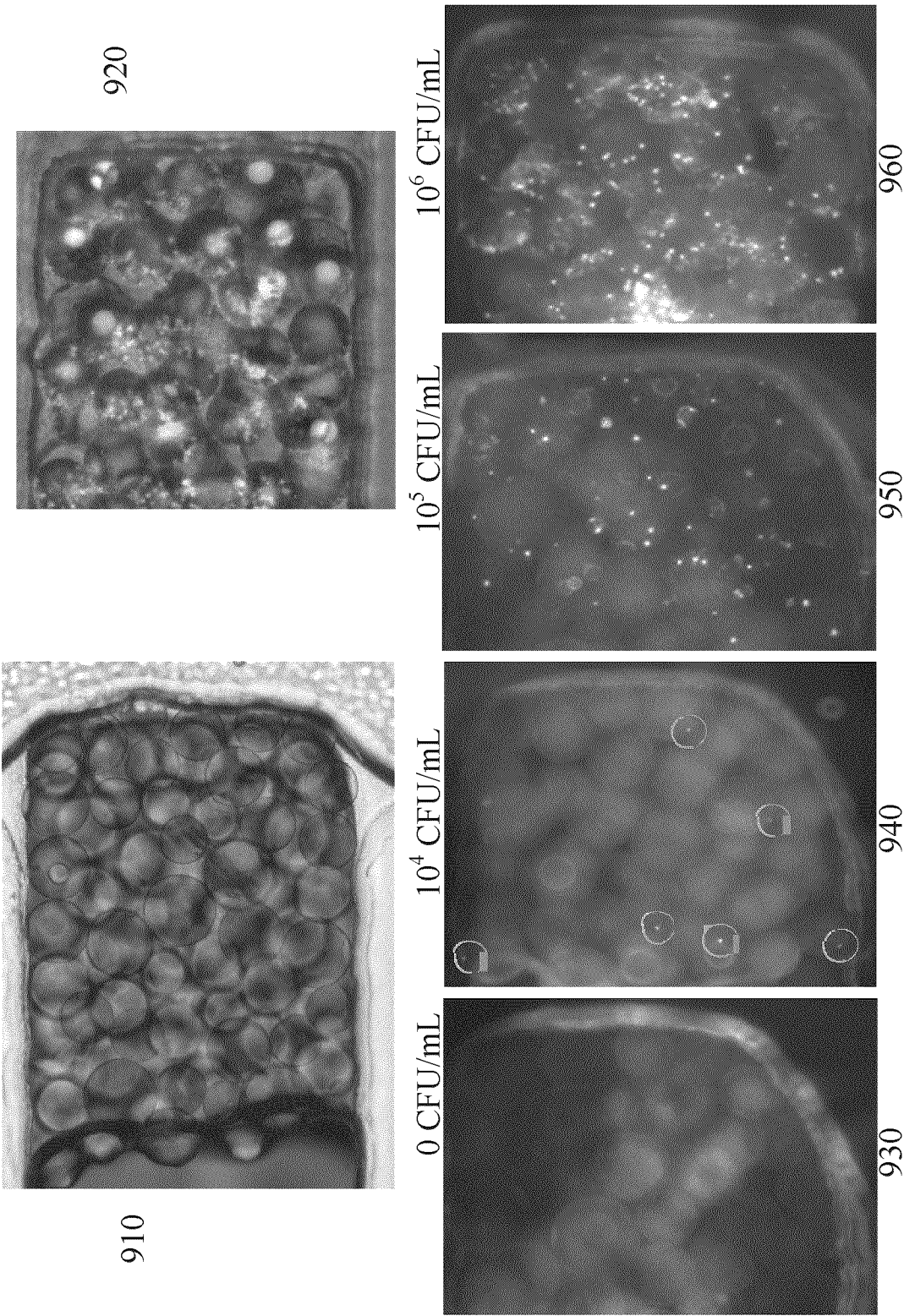


Figure 8

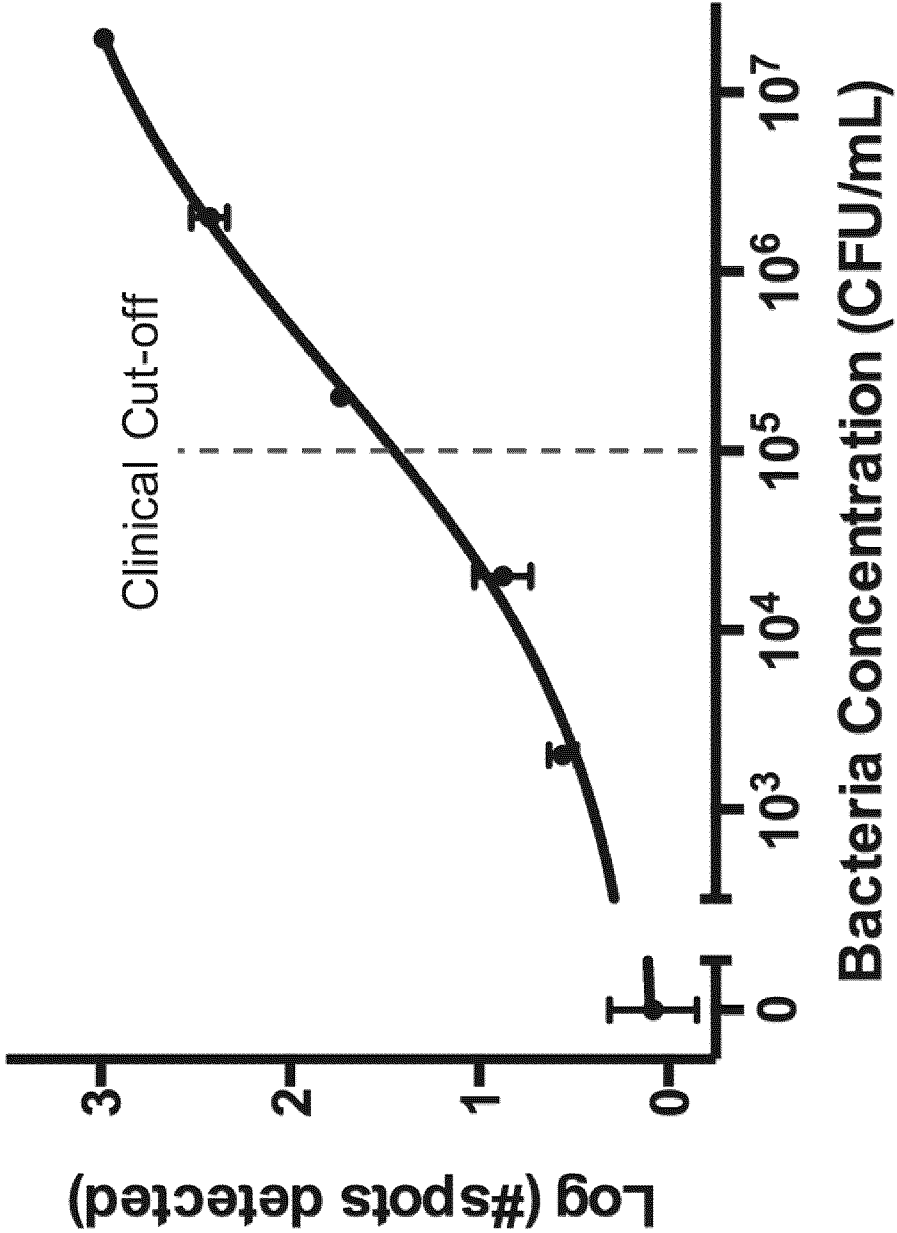


Figure 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2016/051232

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **G01N 1/00** (2006.01), **B33Y 10/00** (2015.01), **B33Y 80/00** (2015.01), **B81B 1/00** (2006.01), **B81C 1/00** (2006.01)

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)

IPC: G01N 1/00 (2006.01), B33Y 10/00 (2015.01), B33Y 80/00 (2015.01), B81B 1/00 (2006.01), B81C 1/00 (2006.01)

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

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

Database: Canadian Patent Database, Orbit Questel

Keywords: Microfluid/fluid/liquid, Detect/measure*/assay, bacteria/sample, Capillary/capillary circuit, Channel/link/connect*, Reservoir/container/tank, Valve*/stop*/flap/plug, Reagent/testing agent, Microbead*/bead*, autonomous/self*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CA 2903382 A1 (Laser et al.) 12 September 2014 (12-09-2014) Abstract; Figs. 1, 7 to 10, 16 to 18 [0035], [0091-0092], [0106], [0110], [0124], [0126-0127], [0171]	28, 29, 30 1 to 27
Y	US 2014/0332098 A1 (Juncker et al.) 13 November 2014 (13-11-2014) Fig. 3; [0092-0093]	1 to 20, 22
Y	CN104605961 A (Jia et al.) 13 May 2015 (13-05-2015) Abstract	21 to 27
A	US5885529 (Babson et al.) 23 March 1999 (23-03-1999) The whole document	1 to 30
A	CA2881841 A1 (Baxter et al.) 27 February 2014 (27-02-2014) The whole document	1 to 30

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	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 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 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
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Date of the actual completion of the international search
10 January 2017 (10-01-2017)

Date of mailing of the international search report
11 January 2017 (11-01-2017)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/051232

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2903382A1	12 September 2014 (12-09-2014)	CA2903382A1 EP2962092A1 EP2962092A4 JP2016516562A US2016008811A1 US2016010761A1 WO2014134533A1 WO2014137940A1	12 September 2014 (12-09-2014) 06 January 2016 (06-01-2016) 24 August 2016 (24-08-2016) 09 June 2016 (09-06-2016) 14 January 2016 (14-01-2016) 14 January 2016 (14-01-2016) 04 September 2014 (04-09-2014) 12 September 2014 (12-09-2014)
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CN104605961A	13 May 2015 (13-05-2015)	None	
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CA2881841A1	27 February 2014 (27-02-2014)	CA2881841A1 EP2888392A2 EP2888392A4 US2015192518A1 WO2014031902A2 WO2014031902A3	27 February 2014 (27-02-2014) 01 July 2015 (01-07-2015) 09 March 2016 (09-03-2016) 09 July 2015 (09-07-2015) 27 February 2014 (27-02-2014) 01 May 2014 (01-05-2014)