(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 April 2020 (23.04.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/079708 A1

(51) International Patent Classification:
B81C 1/00 (2006.01)

(21) International Application Number:
PCT/IN2019/050767

(22) International Filing Date:
16 October 2019 (16.10.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
201841039420 17 October 2018 (17.10.2018) IN

(71) Applicant: **INDIAN INSTITUTE OF TECHNOLOGY MADRAS (IIT MADRAS)** [IN/IN]; The Dean, Industrial Consultancy & Sponsored Research [ICSR], Indian Institute of Technology Madras, IIT P.O, Chennai 600036 (IN).

(72) Inventors: **S, Pushpavanam**; Department of Chemical Engineering, Indian Institute of Technology Madras, IIT P.O, Chennai 600 036 (IN). **T, Renganathan**; Department of Chemical Engineering, Indian Institute of Technology Madras, IIT P.O, Chennai 600 036 (IN). **GHOSH, Rajesh**; Department of Chemical Engineering, Indian Institute of Technology Madras, IIT P.O, Chennai 600 036 (IN). **R, Savitha**; Department of Chemical Engineering, Indian Institute of Technology Madras, IIT P.O, Chennai 600 036 (IN). **G, Saranya**; Department of Chemical Engineering, Indian Institute of Technology Madras, IIT P.O, Chennai 600 036 (IN).

(74) Agent: **SUNDAR, Swapna** et al.; Giridhar and Sai Associates, III Floor, 319, Linghi Chetty Street, George Town, Chennai 600 001 (IN).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to the identity of the inventor (Rule 4.17(i))*
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

(54) Title: A METHOD FOR FABRICATING MICROFLUIDIC DEVICES ON POROUS SUBSTRATE

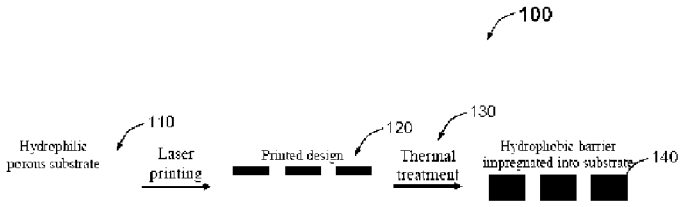


FIG. 1

(57) Abstract: A method for fabricating microfluidic devices on porous substrate. A hydrophobic toner material is deposited onto a porous substrate in a predetermined pattern wherein the porous substrate can be a compatible material for fabricating a microfluidic device. The porous substrate deposited with hydrophobic toner material is treated using a thermal treatment process for complete permeation of at least one hydrophobic polymer from the toner material across the thickness of the porous substrate resulting in at least one fluid impervious barrier patterned on the said substrate. Furthermore, at least one additional toner material layer can be deposited on the top/bottom or both surface of the patterned substrate for encapsulation of the microfluidic device with hydrophobic toner material to achieve a leak-proof device. The porous substrate deposited with additional toner material layer can be treated using a mild thermal treatment process for ensuring leak-proof settings by lateral permeation of the hydrophobic polymers.

WO 2020/079708 A1

[Continued on next page]

Published:
— *with international search report (Art. 21(3))*

DESCRIPTION

A METHOD FOR FABRICATING MICROFLUIDIC DEVICES ON POROUS SUBSTRATE

TECHNICAL FIELD

[001] This disclosure generally relates to the field of fabrication of flow control devices on porous hydrophilic substrates. Embodiments are also related to fabrication of lab-on-a-chip devices. Embodiments are further related to fabrication of lateral flow devices. Embodiments are more particularly related to methods for fabricating microfluidic devices on porous substrates by patterning using a fluid impervious hydrophobic barrier.

BACKGROUND OF THE INVENTION

[002] Fabrication of microfluidic devices by patterning porous hydrophilic substrates with hydrophobic barriers is well known in the art for at least a decade. Such patterning is generally achieved using methods such as, photolithography, wax printing, screen printing, ink-jet printing, flexographic printing and other mechanical or hand-drivel tools.

[003] Photolithography was used to pattern hydrophilic-hydrophobic regions on chromatographic paper by Whitesides and co-workers [M.G. Whitesides, T.S. Phillips, W.A. Martinez, J.M. Butte, A. Wong, W.S. Thomas, H. Sindi, J.S. Vella, E. Carrilho, A.K. Mirca, Y. Liu, Lateral flow and flow-through bioassay devices based on patterned porous media, methods of making same, methods of using same, WO 2008/049083 A2, 2007]. The method produces hydrophobic barriers having sharp boundaries but involves complex procedure with specialized equipment and expensive chemicals, which is not suitable for mass production.

[004] Wax was used as the hydrophobic material to produce permanent impervious barriers with the help of a wax printer [E. Carrilho, W.A. Martinez,

M.G. Whitesides, Methods of micro patterning paper-based microfluidics, US 20120198684A1, 2012].

[005] However, the major drawback in such methods is the lack of control over the wax deposition and wicking of the wax into the porous substrate in all the directions without restraint during thermal treatment. The hydrophobic-hydrophilic boundaries obtained using such methods are not sharp and inconsistent in fabrication. Therefore, devices produced using such method are of poor resolution. Moreover, a prior knowledge on wicking property of the wax is required, which changes with the properties of the substrate. Additionally, the printers that facilitate such methods of printing uses solid ink compositions comprising crystalline-amorphous mixtures [US 8833917B2, 2011] is not commonly available in the commercial market.

[006] Ink-jet printing is also used to fabricate microfluidic devices with demarcated hydrophilic and hydrophobic region [K. Abe, K. Kotera, K. Suzuki, D. Citterio, Inkjet-printed paper fluidic immuno-chemical sensing device, Anal. Bioanal. Chem. 398 (2010) 885–893]. But such method requires several pre-processing or post-processing steps, including several layers of printing. It also involves usage of harsh chemicals, special ink composition and requires modification of an existing printer.

[007] Several other methods were also proposed using hand driven tools, but lack reproducibility and precision [E.M. Fenton, M.R. Mascarenas, G.P. López, S.S. Sibbett, Multiplex Lateral-Flow Test Strips Fabricated by Two-Dimensional Shaping, (2009)].

[008] These flow control devices that can be produced using one of the above techniques provide a low-cost alternate route replacing traditional laboratory sensing technology. The devices prepared are small, lightweight, portable and inexpensive. They operate without the need of any specialized tool or personnel

and are conveniently suited for diagnostic tool development in resource constrained regions and for general home healthcare requirement. But a limitation in the art has been the possibility to manufacture such devices with rapid prototyping functionality such that they can be mass produced and easily commercialized with minimum cost and processing step using tools that are easily available in any setting.

[009] Based on the foregoing, a need therefore exists for rapid prototyping of flow control devices and lab-on-a-chip devices which is prerequisite for successful commercialization.

SUMMARY OF THE INVENTION

[0010] The following summary is provided to facilitate an understanding of some of the innovative features unique to the disclosed embodiments and is not intended to be a full description or of any limiting nature and are for illustrative purposes only. A full appreciation of the various aspects of the embodiments disclosed herein can be gained by taking the entire specification, claims, drawings, and abstract as a whole.

[0011] One aspect of the disclosed embodiments is to provide a simple and robust method for the fabrication of microfluidic devices, which are also referred to as "device(s)", "flow control devices", "assay devices", "microfluidic paper-based analytical devices" or "μPADs".

[0012] Another aspect of the disclosed embodiments is to provide an improved method for making microfluidic device on porous substrates. The invention features a method for making microfluidic device on porous substrates by patterning the substrates selectively hydrophilic and hydrophobic which render one or more functionalities that can be incorporated in a microfluidic device. In some embodiments, the microfluidic device comprises a porous substrate, a composite polymeric toner material, a fluid impervious barrier permeated through the entire

thickness of the substrate, one or more hydrophilic zones, one or more assay zones, one or more sample entry zones, or one or more hydrophilic fluidic pathways or channels.

[0013] The aforementioned aspects and other objectives and advantages can now be achieved as described herein. In one or more embodiments, the method involves preparation of a channel/design/geometry using a design preparing tool depending on the end requirement. In particular embodiments, the method involves deposition of the composite toner material comprising one or more hydrophobic polymers having melting or softening point below the ignition temperature of the substrate is deposited on the suitable porous substrate.

[0014] The deposition of the composite toner material is facilitated by printing using a standard office laser printer.

[0015] The composite toner material can also be deposited on the porous substrate by other suitable means such as, manual or automated deposition for example and not limiting to screen printing or stamping. In particular embodiments, the toner material is deposited single time on a single surface of the porous substrate. In other embodiments, the toner layer is deposited one or more times on the top, bottom or both surface of the porous substrate for fabricating enclosed leak-proof microfluidic devices.

[0016] The porous substrate can be such as, for example, but is not limited to, a paper material, filter paper, nitrocellulose, cellulose acetate, nylon, cloth, tissue paper, writing paper, glass-fibre or polymer film. The porous substrate can also be polyester-cotton clean wipes or a tissue paper or a chromatography paper or filter paper. Note that the porous substrate materials discussed herein are illustrative . Any other porous substrate material can be alternatively used in the proposed invention without limiting the scope of the proposed invention. A person skilled in the art can appropriately use an appropriate porous substrate to perform the

invention. The method proposed herein is applicable to a wide range of porous substrates on which the toner material can be deposited by any suitable means, especially substrates that can be made compatible with a standard laser printer.

[0017] Similarly, the toner material described herein are particulate ink material commonly used as ink in a standard laser printer and consists of one or more hydrophobic polymers and other constituents. In certain embodiments, the toner ink is black, cyan, magenta, yellow or one or more combination of color compatible with a standard laser printer or a standard color laser printer. The combination of different colors will change the individual composition of the hydrophobic polymeric materials deposited on the paper, which in turn will change the characteristic property of the hydrophobic barrier created on a case to case basis. One skilled in the art should be able to easily decide an optimum toner combination depending on the characteristic property desired in the microfluidic device.

[0018] The method involves thermal treatment of the toner coated substrate, where the said toner coated substrate is heated to a temperature of about 50 °C to about 200 °C for a duration of about 1 to 60 minutes, above the softening or melting point of one or more constituents of the composite toner ink and below the ignition temperature of the substrate itself, which is above about 200 °C. In particular embodiments, the thermal treatment procedure includes heating at a temperature between 150 °C to about 170 °C for about 10 to 20 minutes. A person ordinarily skilled in the art can use an appropriate heating temperature and duration depending on the characteristics of the toner material and thickness of the porous substrate. The thermal treatment procedure results in the permeation of one or more hydrophobic polymeric constituent of the toner into the entire thickness of the substrate, which renders permanent hydrophobic barriers. In some embodiments, the heat required for the thermal treatment could be provided by any

standard heat source device, including and not limited to a hot plate, a heating oven or a furnace.

[0019] In one aspect, the method featured in this invention can be used to make microfluidic assay devices, which involves similar patterning of the porous substrates with hydrophilic and hydrophobic regions enabling one or more functionalities that can be incorporated in a microfluidic paper-based analytical device (μPAD). In some embodiments, the μPAD comprises a porous substrate, a composite polymeric toner material, a fluid impervious barrier permeated through the entire thickness of the substrate, one or more hydrophilic zones, one or more assay zones, one or more sample entry zones and one or more hydrophilic fluidic pathways or channels.

[0020] In certain embodiments, the hydrophilic substrates are selectively patterned with toner materials to demarcate hydrophobic and hydrophilic regions with permanent barriers created by the thermal treatment procedure. The barrier is further defined as the boundary of a hydrophilic zone which prevents flow of fluid outside the patterned hydrophilic regions of the substrate. Further, the fluid impervious barrier defines the presence of a continuous polymeric material which is permeated throughout the entire thickness of the porous substrate upon heating. These boundaries also define the peripheries of the hydrophilic regions which are also in some aspects, assay zone(s), sample loading zone(s) or channel area. In some embodiments, the method comprises construction of the fluid impervious barrier that defines a particular channel, sample loading or assay regions, which is fluidically connected by hydrophilic channels to one or more similar zones which facilitate the transport of a fluid by capillary action.

[0021] In certain embodiments, the hydrophobic barriers are between 100 μm and about 200 μm, 200 μm and about 300 μm, 300 μm and about 400 μm, 400 μm and about 500 μm, 500 μm and about 600 μm, 600 μm and about 700 μm, 700 μm and

about 800 µm, 800 µm and about 900 µm, 900 µm and about 1000 µm. In particular embodiments, the hydrophobic barriers are created at every region of the substrate where hydrophobicity is desired and are not dimensionally limited. In another particular embodiment, the hydrophilic regions are made circular in shape having diameters ranging from about 100 µm to 5 cm. In yet another particular embodiment, the hydrophilic regions are made of any different shape or size, whether, circular, rectangle, triangle, square or any custom shape. In yet another particular embodiment, hydrophilic channel regions that can wick fluid are constructed of dimensions between about 100 µm to about 10 cm.

[0022] In certain embodiments, a plurality of hydrophilic zones is constructed in the form of an array which are marked as assay zones. Each assay zones are separated by fluid impermeable barriers which consist of the polymeric hydrophobic material from the composite toner, spread across the entire thickness of the substrate. In particular embodiments, these assay regions may be fluidically connected by a plurality of hydrophilic channels to other hydrophilic region(s) or sample loading region(s). In some embodiments, a plurality of assay regions is fluidically connected to a central hydrophilic region by means of hydrophilic channels. In all of these devices the assay zone allows for the visualization of the analytical application, the main channel zone acts as a fluidic bridge between the assay region and sample loading region and the sample loading zone is the point of introduction of one or more test fluid by the user. The construction of hydrophobic barriers in these devices prevents cross-contamination in individual assay zones and provides guided fluid flow path for the respective operation being carried out. In some embodiments, the device comprises one or more assay region(s) and one or more sample entry region(s) and each of the assay and sample entry region(s) are connected by one or more fluidic channel(s). In another aspect, the barriers can

confine a variety of liquids for example, surfactants, organic solvents and mixture of aqueous solutions.

[0023] In some embodiments, the assay regions are coated with analytical reagents which produce a visible color change upon the sensing of specific analyte(s). In particular embodiments, the reagents are confined to the respective assay regions. In yet another particular embodiment, the sample fluid is introduced at the sample loading zone and wicks to the assay regions by means of capillary action.

[0024] In such particular embodiments, the reaction between the sample and reagent could produce a signal which indicates the presence or absence of an analyte. In certain embodiments, the analytical reagent is covalently bonded to the substrate and in certain other embodiments the analytical reagent is non-covalently bonded to the substrate. In certain embodiments, the assay reagent is chosen such that it reacts with one of the reactant analytes producing a detectable signal. In certain embodiments, one or more analyte may react with one or more reagent to produce visible color outputs.

[0025] In some embodiments, one or more sample(s) and one or more reagent chemical is brought in contact with the μPAD for an application to be carried out. In certain embodiments, the sample flows to the assay region by capillary action and results in the change of a color signifying the presence or absence of an analyte. In certain embodiments, the microfluidic device is used along with an image recording device and a communication device. The color change produced at the respective assay regions are digitally recorded and the color intensity is analyzed to predict the quantitative information about the presence of an analyte. The communication device enables the captured signal to be remotely transferred to one or more other devices.

[0026] In another embodiment, the method described herein is also used for controlling the movement of fluid in a porous microfluidic device. The patterning of porous substrates as selectively hydrophobic and hydrophilic results in generation of hydrophilic pathways that can transport fluid by capillary action within the specified and fluidically connected hydrophilic regions. This allows guided pathways for fluid-fluid interaction, e.g. analytes and reagent fluids. Thus, these microfluidic devices are also labelled as flow control devices. The movement of the fluid can be from any direction e.g. sample loading zone to an assay region through a channel. In some embodiments, this disclosure features the ability to confine liquid within a specified zone, e.g. sample loading zone or assay zone, without any leakage into the hydrophobic region or outside the device. In another aspect, the barriers can confine a variety of liquids for example, surfactants, organic solvents and mixture of aqueous solutions. In another aspect, the invention features the art of making substrates hydrophobic which repel liquid and provide a self-cleaning ability to the substrate.

[0027] In another embodiment, the method involves deposition of one or more toner layers on one or more surfaces of the substrate of the fabricated final microfluidic device, enabling an enclosed feature to the microfluidic device described herein. Briefly, the method involves the preparation of a channel/design/geometry depending on the end requirement. In particular embodiments, the method involves deposition of the composite toner material on the suitable porous substrate. In some embodiments, the porous substrate is paper, filter paper, nitrocellulose, cellulose acetate, nylon, cloth, tissue paper, writing paper, polyester-cotton clean wipes, tissue paper, chromatography paper, glass fibre or polymer film. In yet another embodiment, the substrate is chromatography paper or filter paper. In certain embodiments, the toner ink deposited on the substrate is black, cyan, magenta, yellow, green, or one or more combination of

color compatible with a standard laser printer or a standard color laser printer. In yet another particular embodiment, the method involves thermal treatment of the printed or toner deposited substrate. The substrate coated with composite toner materials is heated to a temperature of about 50 °C to 200 °C for a duration of about 1 to 60 minutes, which ensured complete permeation of one or more hydrophobic polymeric constituent from the composite toner material into the entire thickness of the substrate forming permanent barriers. In some embodiments, the heat required for the thermal treatment could be provided by any standard heat source device, including and not limited to a hot plate, a heating oven or a furnace.

[0028] The finished device after toner deposition and construction of fluidic barriers by thermal treatment is subjected to coating of additional toner layers on the top and/or bottom surface of the substrate. In some embodiments, the deposition of the additional toner layers for enclosure of the microfluidic device is carried out one or more times on the top or bottom surface of the porous substrate until all the gaps or defects are sealed. Alternatively, in some embodiments, a single deposition of the toner layer on the surfaces of the microfluidic device is followed by a mild thermal treatment at 50°C to about 200 °C for 1 to about 20 minutes for lateral permeation of one or more polymeric component from the composite toner material. In particular embodiments, the enclosed substrate was heated at 140 °C to about 170 °C for about 1 minute to about 5 minutes for lateral permeation of one or more polymeric component. This results in sealing of the microfluidic device, providing a leak-proof feature. A person skilled in the art can appropriately decide the necessity for thermal treatment procedure or the duration and temperature of the thermal treatment procedure based on the thickness of the toner layers deposited. In some embodiments, the top and bottom surfaces may be selectively encapsulated with one or more regions left exposed for user interaction.

Although the top and bottom surfaces are sealed to ensure no spillage or exposure of the device to its immediate environment, the fluid flow through one or more enclosed channels remains unaffected and the operation of one or more assay zones and other analytical functionalities are carried out generally within the enclosed device. In some embodiments, the loss of fluid by evaporation is prevented by enclosing with additional toner layer on the top and bottom surface of the substrate. In other embodiments, certain hydrophilic zones are left exposed without enclosure to allow sample and reagent interaction with the device as well as visualization of certain performance of the device. In yet another embodiment, certain hydrophilic regions are enclosed with a light color toner material, e.g. yellow or orange pigment containing toner that allow a "see through" ability and enable visualization of the enclosed device operation.

[0029] In certain embodiments, a plurality of hydrophilic zones is constructed within the enclosed device in the form of an array which are marked as assay zones. Each assay zones are separated by fluid impermeable barriers which consist of the polymeric hydrophobic material from the composite toner, spread across the entire thickness of the substrate. In particular embodiments, these assay regions may be fluidically connected within the encapsulated device by a plurality of hydrophilic channels to other hydrophilic region(s) or sample loading region(s). In all of these devices the assay zone allows for the visualization of the analytical application, the main channel zone acts as a fluidic bridge between the assay region and sample loading region and the sample loading zone is the point of introduction of one or more test fluid by the user. In some embodiments, the device comprises one or more assay region(s) and one or more sample entry region(s) and each of the assay and sample entry region(s) are connected by one or more fluidic channel(s) within the enclosed device. Some or all of the parts may be enclosed by

additional toner layers for packaging and protection of the equipment from external agents.

[0030] In some embodiments, the assay regions are coated with analytical reagents which produce a visible color change upon the sensing of specific analyte(s). In particular embodiments, the reagents are confined to the respective assay regions. In yet another particular embodiment, the sample fluid is introduced at the sample loading zone and wicks to the assay regions by means of capillary action.

[0031] In such particular embodiments, the reaction between the sample and reagent could produce a signal which indicates the presence or absence of an analyte. In certain embodiments, the assay reagent is chosen such that it reacts with one of the reactant analytes producing a detectable signal. In certain embodiments, one or more analyte may react with one or more reagent to produce visible color outputs. In certain embodiments, the microfluidic device is used along with an image recording device and a communication device. The color change produced at the respective assay regions are digitally recorded and the color intensity is analyzed to predict the quantitative information about the presence of an analyte. The communication device enables the captured signal to be remotely transferred to one or more other devices.

[0032] In some embodiments, the microfluidic devices prepared by method described herein can be stacked or piled on top each other to generate a three-dimensional fluidic network with lateral flow and flow through ability as described in certain embodiments. The layers stacked herein can include one or more sample entry zones, assay regions and fluidic pathways that connect to one or more sample entry zones or assay zones. In another aspect, the invention features a platform for the detection of presence or absence of analytes from fluidic samples using the fabricated microfluidic devices described herein. The method involves contacting

the sample with the hydrophilic sample introduction zone of the device, which in some cases is followed by transport of the analyte to a respective assay zone and the production of certain colorimetric signal which indicates the presence or absence of a specific analyte.

BRIEF DESCRIPTION OF DRAWINGS

[0033] The accompanying figures, in which like reference numerals refer to identical or functionally-similar elements throughout the separate views and which are incorporated in and form a part of the specification, further illustrate the present invention and, together with the detailed description of the invention, serve to explain the principles of the present invention.

[0034] FIG. 1 illustrates a graphical representation of the logical operational steps involved in the method for fabricating microfluidic devices on porous substrates in illustrative case using a laser printing technology, in accordance with the disclosed embodiments;

[0035] FIG. 2 illustrates a graphical representation of the logical operation steps involved in the method for fabricating enclosed and leak-proof microfluidic devices on porous substrates in illustrative case using a laser printer technology, in accordance with the disclosed embodiments;

[0036] FIG. 3A illustrates a further detailed schematic representation of the logical steps in involved in making microfluidic devices on the said porous substrates, namely toner deposition on the porous substrates using a suitable deposition technique and thermal treatment of the toner deposited substrate for complete permeation of one or more hydrophobic polymer across the entire thickness of the substrate, in accordance with the disclosed embodiments;

[0037] FIG. 3B illustrates illustrative microfluidic devices, namely, a concentration gradient generator fabricated in a tissue paper and a multiplexed

microfluidic device with superimposed multiple layer dye fabricated in polyester-cellulose task wipes, in accordance with the disclosed embodiments;

[0038] FIG. 3C illustrates an illustrative microfluidic paper-based analytical device (μPAD) for the detection of various analytes. Figure depicts the front and back surface of a lateral flow multiplexed device with multiple assay and sample entry zones for the detection of one or more analytes as described in some embodiments. The assay zone is coated with one or more analytical reagents as discussed in some embodiments and show a change in color indicating the presence of analytes;

[0039] FIG. 4A illustrates the step by step procedure for the fabrication of enclosed/encapsulated microfluidic device as described in some embodiments; and

[0040] FIG. 4B illustrates an illustrative microfluidic paper-based analytical device (μPAD) for the detection of various analytes, for example, but not limiting to, glucose, nitrite and protein, in accordance with the disclosed embodiments.

DETAILED DESCRIPTION

[0041] The particular values and configurations discussed in these non-limiting examples can be varied and are cited merely to illustrate at least one embodiment and are not intended to limit the scope thereof.

[0042] A method for fabricating microfluidic devices on porous substrates is disclosed herein. The method generally involves two steps. First step is deposition of a composite toner material containing hydrophobic polymer on the porous substrates by using easily available tools such as a laser printer and second is fusion of the same by a thermal treatment to make permanent hydrophobic barriers.

[0043] The method involves preparation of a channel/design/geometry using a printable template depending on the application. Further, printing single time and single side on the porous substrate using any laser printer with toner composition containing a hydrophobic polymer/s having melting point below the ignition

temperature of the substrate. Finally, the method involves thermal treatment of the substrate at or above the melting point of hydrophobic polymer/s but below the ignition temperature of the substrate for impregnation of the hydrophobic material into the substrate.

[0044] The invention discusses at least in one aspect a novel approach for patterning porous hydrophilic substrates such as, but not limited to paper, with hydrophobic impassable barriers to confine fluidic movement within the region of interest using a laser printer. The proposed invention is a novel method for rapid prototyping and high throughput manufacturing of microfluidic devices that can be broadly used for lab-on-a-chip applications including point-of-care diagnostic and analytical applications involving fluid confinement, flow, mixing and reactions.

[0045] FIG. 1 illustrates a graphical schematic representation of the logical operational steps involved in the method 100 for fabricating microfluidic devices on porous substrates 110 using a laser printing technology, in accordance with the disclosed embodiments. The first step describes the preparation of a printable template that can be given as an input to a laser printer (not shown). The template can be produced using any designing software or can be an input from an already existing design using the photocopy functionality of a laser printer. The designing can be flexible according to the specific applications or user requirement.

[0046] For example, when used for assay/s, it may include one or more detection region, or sample addition/loading zones. The templates may also contain inter-connecting/segregated channels or reservoirs which may serve to be utilized in any lab-on-a-chip application where a number of sequential operations are to be conducted on a single chip. It can also be used to provide one or more fluidic pathways for the purpose of fluid flow, transport and mixing. Overall, any design compatible with the laser printer and the printable media can be prepared.

[0047] The second step involves deposition of the toner material 120 on a printable porous substrate 110 by printing once on a single side using a laser printer having toner composition inclusive of at least one hydrophobic resin/polymers/constituent that has a melting point below the ignition temperature of the substrate 110. Upon printing, a layer of toner composite 120 is deposited uniformly on the surface according to the required design which demarcates the hydrophobic region of the device. The remaining areas which are desired to be hydrophilic 110 are left blank. The deposition of the toner 120 on the substrate 110 is a surface coating with no/minimal penetration of the material into the substrate 110. It requires a thermal treatment procedure 130 to form permanent hydrophobic barriers 140 that impregnate the thickness of the substrate 110 and are impassable by aqueous solutions.

[0048] The thermal treatment procedure 130 can be carried out by any heating device which can uniformly distribute heat to the substrate. In one particular aspect of the embodiment, the heating temperature should be at/above the melting point of the hydrophobic constituent of the toner and below the ignition temperature of the substrate for a few minutes. The thermal treatment results in the melting of the hydrophobic polymer/s and subsequent wicking of the same into the entire cross-section of the porous hydrophilic substrate 110, thereby producing a distinct hydrophobic barrier 140. In definite cases, the heating temperature and the exposure time of heating may be optimized based on the hydrophobic polymer/s in the toner 120 and the substrate 110.

[0049] In certain embodiments, the hydrophobic toner material may also be contacted to the hydrophilic porous substrate using any hand-driven or any other mechanical tool. An example of this may be screen printing, where a predefined mesh may be fabricated and the toner composite material may be distributed manually or by any other means on the desired region as per regions defined by the

mesh. This creates versatility in the approach proposed by this invention wherein the method can also be used in scenarios where laser printer may not be available. Further, a thermal treatment step is necessary to be followed, due to which the deposited hydrophobic material will impregnate the cross-section of the substrate creating impassable hydrophobic barriers.

[0050] Fully enclosed, leak-proof device: In another aspect of a particular embodiment, a fully enclosed, leak-proof device is also fabricated such that no sample or reagent leaks through the back side of the device. FIG. 2 illustrates a graphical representation of the logical operation steps involved in the method 200 for fabricating enclosed and leak-proof microfluidic devices on porous substrates using a laser printer technology, in accordance with the disclosed embodiments. This functionality is achieved by the following steps: First, the method involves preparation of a channel/design/geometry using a printable template depending on the end application. Second, printing single time and single side on the printable porous substrate using any laser printer with toner composition containing a hydrophobic polymer/s having melting point below the ignition temperature of the substrate. Next, the method involves thermal treatment of the printed substrate at or above the melting point of hydrophobic polymer/s but below the ignition temperature of the substrate, for sufficient time for the impregnation of hydrophobic material into the substrate. Finally, the prepared device is subjected to an additional step of printing on the top, bottom or both surface of the patterened device such that sufficient hydrophobic material is coated in all possible regions of the device, where leakage is anticipated. This creates a surface hydrophobic barrier preventing sample from leakage or exposure to the environment from the back and front side. However, this additional step does not create any hydrophobic barrier in the internal structures of the substrate and is an additional feature only in cases where leak-proof and enclosed functionality is desired.

[0051] FIG. 2 illustrates a graphical schematic representation of the logical operational steps involved in the method 200 for fabricating fully enclosed leak-proof microfluidic devices on printable porous substrates 210 using a laser printing technology, in accordance with the disclosed embodiments. The first step describes the preparation of a printable template that can be given as an input to a laser printer (not shown). The template can be produced using any designing software or can be an input from an already existing design using the photocopy functionality of a laser printer. The designing can be flexible according to the specific applications or user requirement.

[0052] The second step involves deposition of the toner material 220 on a porous substrate 210 by printing once on a single side using a laser printer having toner composition inclusive of at least one hydrophobic resin/polymers/constituent that has a melting point below the ignition temperature of the substrate 210. Upon printing, a layer of toner composite 220 is deposited uniformly on the surface according to the required design which demarcates the hydrophobic region of the device. The remaining areas which are desired to be hydrophilic 210 are left blank. The deposition of the toner 220 on the substrate 210 is a surface coating with no/minimal penetration of the material into the substrate 210. It requires a thermal treatment procedure 230 to form permanent hydrophobic barriers 240 that impregnate the thickness of the substrate 210 and are impassable by aqueous solutions. Finally, the prepared device is subjected to an additional step 250 of printing on the back side of the prepared device such that sufficient hydrophobic material 220 is coated in all possible regions of the device, where leakage is anticipated. This creates a surface hydrophobic barrier 260 preventing sample from leakage or exposure to the environment from the back side.

[0053] The general steps of the method are illustrated in Figure 3A. As shown in the figure, a porous substrate 301 is coated with composite toner material 303 by a

deposition step 302. The toner deposition step 302 could be achieved by hand deposition, screen printing, automated deposition systems including printing, laser printing using standard office laser printer. The toner deposited paper is then subjected to a thermal treatment step 304 which heats the toner material coated substrate. This results in melting of one or more hydrophobic polymeric constituent of the composite toner material 303, permeating it into the thickness of the porous substrate. The substrates are heated such that hydrophobic polymeric material melts from the first hydrophobic surface at the top of the substrate and permeate to occupy the entire thickness underneath and reach the other surface on the bottom resulting in the second hydrophobic surface at the bottom. The final result is a continuous hydrophobic barrier 305 in regions where toner material is deposited. This patterning of hydrophilic and hydrophobic regions results in the formation of several functional zones such as that of a plurality of assay zones 306 and hydrophilic channels 307 and sample entry zones 308. The thermal treatment step 304 could be achieved using any heat source device, such as that of a hot plate, oven, furnace etc. The figure illustrates a cross-section view of the overall process. In the final microfluidic device 311, the substrate is patterned as selectively hydrophilic 309 and selectively hydrophobic 310 as described in embodiments. The hydrophobic barriers 305 are created at all regions where hydrophilicity is not desired.

[0054] Figure 3B illustrates different microfluidic devices fabricated using the method as described herein in various substrates. For example, a concentration gradient generator 312 fabricated using an illustrative substrate tissue paper 313, with plurality of sample entry zones such as, 314, 315 and hydrophilic fluidic pathways 316, and plurality of assay regions or outlet sections 317, 318, 319, 320, having different characteristic outputs. The fluid with dyes introduced at the inlet points such as, 314, 315 mix together at multiple levels along the hydrophilic

fluidic channels 316. The result of the multilevel mixing is the formation of output fluid with different concentration of the solute or dye at each respective outlet points such as, 317, 318, 319, 320. The size of the microfluidic device is illustrated using a 5-rupee Indian coin 322. The second illustrated device is a multiplexed microfluidic device, fabricated on porous substrate such as a polyester-cellulose task wipe 323. The hydrophilic region is marked by the presence of colored dyes which are added to central zone 326 and have wicked by capillary action along channel 327 to assay zones 325. The assay zone 325 consists of a secondary color which have been superimposed on the previous color to indicate marked colorimetric difference in a multiplexed device. The hydrophobic region 324 on the other hand do not show any presence of colored dyes as it repels liquid and does not support fluid flow.

[0055] Figure 3C illustrates an illustrative microfluidic paper-based analytical device (μPAD) 330, 350 for the detection of various analytes. Figure depicts the front 330 and back 350 surface of a lateral flow multiplexed device with multiple assay 306 and sample entry 308 zones for the detection of one or more analytes as described in some embodiments. The sample flows from sample entry port 308 through the fluidic channel 307 which connect the assay zone 306 with the sample entry port 308. The assay zone 306 is coated with one or more analytical reagents as discussed in some embodiments and show a change in color indicating the presence of analytes.

[0056] The general steps of the method to enclosed microfluidic device is illustrated in Figure 4A. As shown in figure, a porous substrate 401 is coated with composite toner material 403 by a deposition step 402. The toner deposition 402 could be achieved by hand deposition, screen printing, automated deposition systems including printing, laser printing using standard office laser printer. The toner deposited paper is then subjected to a thermal treatment step 404 which heats

the toner material coated substrate. This results in melting of one or more hydrophobic polymeric constituent of the composite toner material 403, permeating it into the thickness of the porous substrate 401. The substrates are heated such that hydrophobic polymeric material melts from the first hydrophobic surface at the top of the substrate and permeate to occupy the entire thickness underneath and reach the other surface on the bottom resulting in the second hydrophobic surface at the bottom. The final result is a continuous hydrophobic barrier 405 in regions where toner material is deposited. This patterning of hydrophilic and hydrophobic regions results in the formation of several functional zones such as that of a plurality of assay zones 406 and hydrophilic channels 407 and sample entry zones 408. The thermal treatment step 404 could achieved using any heat source device, such as that of a hot plate, oven, furnace etc. The figure illustrates a cross-section view of the overall process. In the final microfluidic device 409, the substrate is patterned as selectively hydrophilic 406 and selectively hydrophobic 405 as described in embodiments. The hydrophobic barriers 405 are created at all regions where hydrophilicity is not desired. Following this, the patterned device 409 is subjected to additional toner layers deposition step 410 for enclosure of the microfluidic device described herein. The additional toner layer 420 can be deposited on the top, bottom or both side of the microfluidic device one or more times to observe a sealing effect. This ensures leak-proof design as described in some embodiments. Similarly, the additional toner deposited device can also be subjected to mild thermal treatment as an optional measure to ensure leak-proof device 450 by lateral permeation of additional toner material 420. This results in encapsulation of the hydrophilic pathways 407 by fluid impervious barriers 430 that results in a leak-proof device 450.

[0057] FIG. 4B illustrates an illustrative microfluidic paper-based analytical device (μPAD) 432 and 472 for the detection of various analytes, for example, but

not limiting to, glucose, nitrite and protein. In a first case, figure depicts an encapsulated leak-proof device 462 wherein a distinct sample inlet port 470 is provided in the said device and the sample introduced wicks through the hydrophilic channel 468 within the enclosed device to reach the assay region 462, 464, 466 and produce analytical signal. In second case, figure depicts a dip-assay type encapsulated microfluidic device 472, wherein no distinct sample region port is provided separately. The device can be dipped into sample solution to allow contact of the enclosed hydrophilic channel within the device 470 with the sample solution and its simultaneous wicking through the said predefined channel 478 to the assay region(s).

[0058] In one aspect of the proposed invention, a significant advantage of the method 100 is that the hydrophobic material is applied to only the regions where hydrophobicity is required which allows for the contamination free use of the device for sensitive bio assay or other analytical application. Another advantage is the utilization of solid toner powder which is evenly distributed on the substrate, thereby enabling uniform deposition and impregnation of the hydrophobic polymer into the cross-section of the substrate only in the printed region. In certain cases, where the substrate is printed multiple times, supposedly in the front and back end as well, excess hydrophobic polymer deposits on the porous substrate and may wick into the hydrophilic zones upon melting, blocking the fluidic movement in the hydrophilic channel/area. This may compromise the resolution of the fabricated devices and serve as a disadvantage. Moreover, in the similar case when printing on both side of the substrate, it increases the complexity as it is difficult to obtain the correct alignment during printing the same design overlaying the previously printed design. Thus, single printing on one side of a hydrophilic substrate as described herein enables fabrication of high-resolution devices with impassable hydrophobic barriers for fluid confinement. The method described herein can be

used for any hydrophilic substrate which is porous and can wick fluid from one region to the other through capillary action. This feature of the porous media allows for passive fluid movement in these devices without the requirement of additional pumping system. For example, paper is one such substrate which is commonly used in the field and has been used to carry out a number of analytical assays. Paper based devices are easy to handle, portable, inexpensive, flexible and biodegradable making them attractive candidates for a wide number of applications. The passive capillary fluid movement in paper and the ability to control flow in them by generation of precise hydrophobic barriers as discussed in this invention enables the use of these devices for numerous applications without any requirement electric power.

It will be appreciated that variations of the above-disclosed and other features and functions, or alternatives thereof, may be desirably combined into many other different systems or applications. Also, that various presently unforeseen or unanticipated alternatives, modifications, variations or improvements therein may be subsequently made by those skilled in the field.

CLAIMS

I/We Claim:

1. A method for fabricating microfluidic devices on porous substrate, said method comprising:

depositing a hydrophobic toner material onto a porous substrate, single time and single surface, in a predetermined pattern wherein said porous substrate can be a compatible for fabricating microfluidic devices as described in some embodiments; and

treating the porous substrate deposited with hydrophobic toner material using a thermal treatment process for complete permeation of at least one hydrophobic polymer from the toner material across the entire thickness of the porous substrate resulting in at least one fluid impervious barrier patterned on the porous substrate.

2. The method of claim 1 further comprising:

selectively depositing at least one additional toner layer on top, bottom or both surface of the patterned substrate for sealing the said substrate with hydrophobic toner material to achieve a leak-proof microfluidic device; and

treating the porous substrate deposited with additional toner layer using a mild thermal treatment process for ensuring leak-proof device by lateral permeation of the hydrophobic polymers as described in some embodiments.3. The method as claimed in claim 1 and 2 further comprising at least one hydrophilic region which is fluidically connected and defined by at least one fluid impervious hydrophobic barrier on the porous substrate.

4. The method as claimed in claim 1 and 2 wherein the hydrophobic toner material can be deposited on to the said substrate using a technique which can be a laser printing tool.

5. The method as claimed in claim 1 and 2 wherein the toner material can be deposited on to the porous substrate by using a convenient manual or automatic deposition technique.

6. The method as claimed in claim 1 and 2 wherein the thermal treatment process can be performed using one of the following thermal treatment processes: oven; hot plate; furnace; or any heat source device.

7. The method as claimed in claim 1 and 2 wherein at least one microfluidic devices can be stacked in order to fabricate a three-dimensional microfluidic device.

8. The method as claimed in claim 2 wherein the hydrophilic regions can be enclosed with light colour toner material for visualization of the enclosed device operation as well as its encapsulation.

9. The method as claimed in claim 2 wherein the fluid flow in the microfluidic network is not affected by the additional toner material deposition and thermal treatment of the additional toner material.

10. The method as claimed in claim 1 and 2 further comprising manufacturing of the microfluidic devices that can be broadly used for one or more analytical applications involving fluid confinement, flow, mixing and reactions.

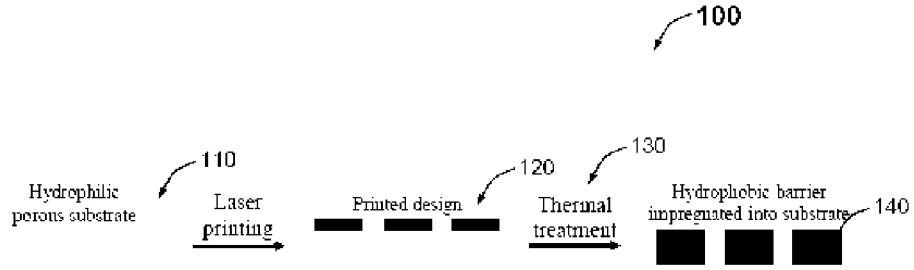

100
110
Hydrophilic
porous substrate
Laser
printing
120
Printed design
130
Thermal
treatment
Hydrophobic barrier
impregnated into substrate
140

FIG. 1

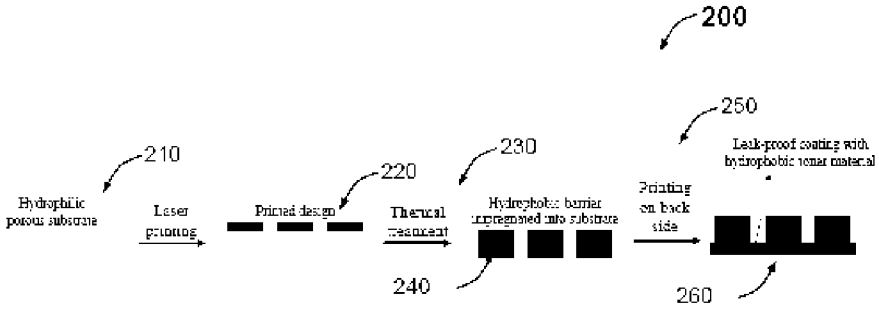

200
210
Hydrophilic
porous substrate
Laser
printing
220
Printed design
230
Thermal
treatment
Hydrophobic barrier
impregnated into substrate
240
250
Leak-proof coating with
hydrophobic toner material
Printing
on back
side
260

FIG. 2

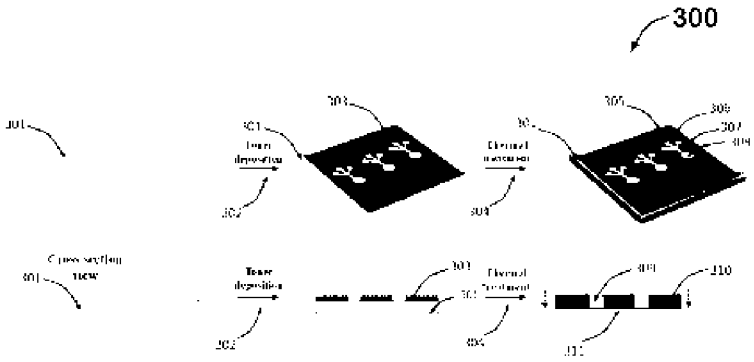


FIG. 3A

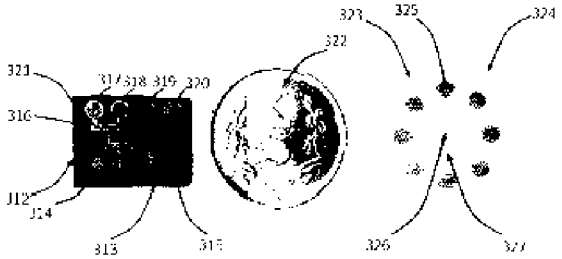


FIG. 3B

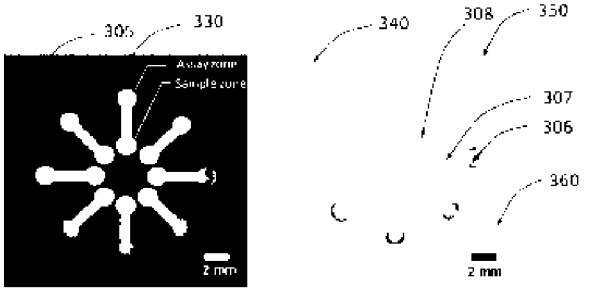


FIG. 3C

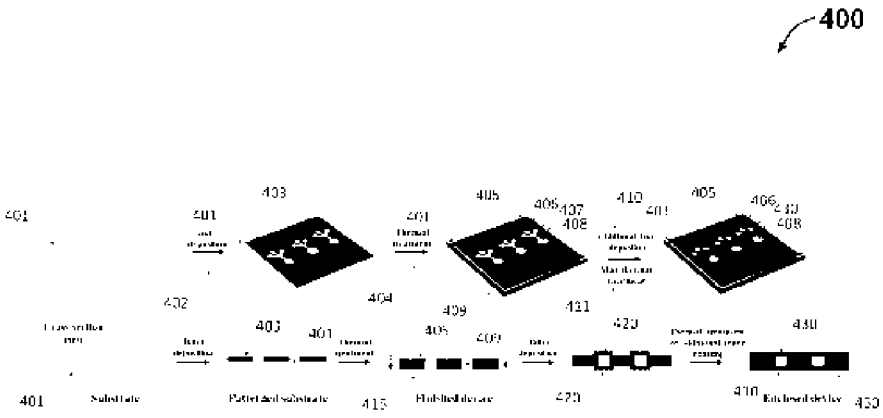

400
401
402
403
404
405
406
407
408
409
410
411
415
420
430
450

FIG. 4A

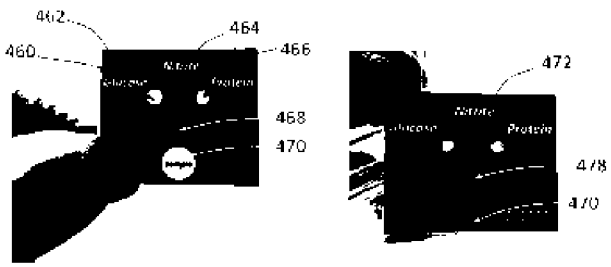

462
460
464
466
468
470
472
478
470

FIG. 4B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2019/050767

A. CLASSIFICATION OF SUBJECT MATTER
B81C1/00 Version=2020.01

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)

B81C

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

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

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2010125297A1(CENTRE NAT RECH SCIENT et.al) 04 NOV 2010 04.11.2010 figures 1A-1D; claims 1,2,14; page 6, para 002 ; page 12, para 004 of D1, English translation referred on Espacenet	1
Y	figures 1A-1D; claims 1,2,14; page 6, para 002 ; page 12, para 004 of D1, English translation referred on Espacenet	2-10
Y	US9481567B2 (TAIWAN SEMICONDUCTOR MANUFACTURING CO. LTD) 01 NOV 2016 01.11.2016 column 5, lines 19-47; figures 4,6A-6C	2-10
Y	US6074725A(CALIPER TECHNOLOGIES CORPORATION) 13 JUNE 2000 13.06.2000 Abstract; column 5, lines 22-29; column 17, lines 14-20	4-10
Y	US7259449B2(SNAPTRACK INC.) 21 AUG 2007 21.08.2007 claim 1,2 and 20; column 8, lines 48-56	8-10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" 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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27-01-2020	27-01-2020
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Mahendra Dongre Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2019/050767

Citation	Pub.Date	Family	Pub.Date
WO 2010125297 A1	04-11-2010	FR 2944713 A1	29-10-2010
US 9481567 B2	01-11-2016	US 2015360938 A1	17-12-2015
US 6074725 A	13-06-2000	AU 1534799 A	28-06-1999
		EP 1039995 A1	04-10-2000
		EP 1039995 A4	26-04-2006
		US 6509085 B1	21-01-2003
		WO 9929497 A1	17-06-1999
US 7259449 B2	21-08-2007	AU 2005203429 A1	13-04-2006
		BR PI0503940 A	09-05-2006
		CA 2516576 A1	27-03-2006
		EP 1640325 A2	29-03-2006
		EP 1640325 A3	05-12-2007
		JP 2006095673 A	13-04-2006
		JP 4331148 B2	16-09-2009
		KR 20060092891 A	23-08-2006
		MX PA05009421 A	29-03-2006
		RU 2005129864 A	10-04-2007
		SG 121061 A1	26-04-2006
		TW 200627602 A	01-08-2006
		US 2010072595 A1	25-03-2010
		US 7935555 B2	03-05-2011
		US 2007298541 A1	27-12-2007
		US 7642127 B2	05-01-2010
		US 2008038876 A1	14-02-2008
		US 7629678 B2	08-12-2009
		US 2006079098 A1	13-04-2006