

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 January 2006 (05.01.2006)

PCT

(10) International Publication Number
WO 2006/000064 A2

(51) International Patent Classification: **Not classified**

(21) International Application Number:

PCT/BE2005/000102

(22) International Filing Date: 28 June 2005 (28.06.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0414390.5 28 June 2004 (28.06.2004) GB
60/584,462 29 June 2004 (29.06.2004) US

(71) Applicant (for all designated States except US): **INTERUNIVERSITAIR MICROELEKTRONICA CENTRUM VZW** [BE/BE]; Kapeldreef 75, B-3001 Leuven (BE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BORGHS, Gustaaf** [BE/BE]; Bergstraat 70, B-3010 Leuven (BE). **BARTIC, Carmen** [RO/BE]; Bleydenberglaan 37, B-3012 Wilsele (BE). **DE GREVE, Kristiaan** [BE/BE]; Turnhoutsebaan 205, B-2970 Schilde (BE).

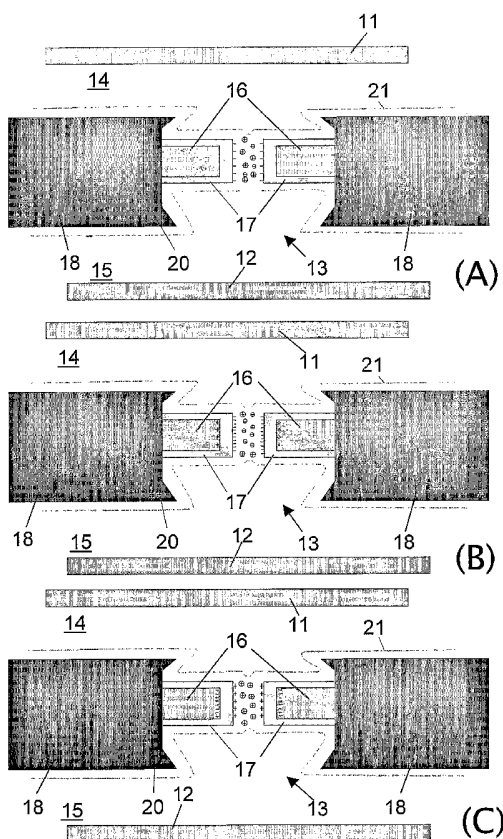
(74) Agents: **BIRD, Ariane** et al.; Bird Goën & Co, Klein Dalenstraat 42A, B-3020 Winksele (BE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: DEVICE FOR CONTROLLING THE FLOW OF CHARGED CARRIERS THROUGH A NANOPORE IN A MEMBRANE AND METHOD FOR THE FABRICATION OF SUCH A DEVICE



(57) Abstract: The present invention provides a nanofluidic device for controlling the flow of charged carriers through a nanopore extending through a membrane hereby forming a channel connecting a first side of the membrane with a second, opposite side of the membrane and a method for the manufacturing of such a device. The device comprises a first electrode positioned at the first side of the membrane, a second electrode positioned at the second side of the membrane and a third electrode integrated in the nanopore. By applying suitable voltages to the first, second and third electrode, the electrical potential distribution inside the nanopore can be modified and in that way the flow of charge carriers can be controlled.

WO 2006/000064 A2



Declaration under Rule 4.17:

— *of inventorship (Rule 4.17(iv)) for US only*

Published:

— *without international search report and to be republished
upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

DEVICE FOR CONTROLLING THE FLOW OF CHARGED CARRIERS THROUGH A NANOPORE IN A MEMBRANE AND METHOD FOR THE FABRICATION OF SUCH A DEVICE

5 **Technical field of the invention**

The present invention relates to nanofluidic devices for controlling the flow of charged carriers in a fluid through at least one nanopore in a membrane, by means of controlling the charge distribution in the at least one nanopore, to a method for the manufacturing of such devices and to a method
10 for controlling the charge distribution in at least one nanopore in a membrane by modifying the electrical potential distribution inside the nanopore.

Background of the invention

In biology, ion channels are pores through a cell membrane through
15 which ions can be transported in and/or out of the cell under the influence of an electrochemical gradient, i.e. an electrical field and/or concentration gradient. This is the so-called 'passive' transport mode, which is opposite to the active transport mode which occurs against an electrochemical gradient, and which requires dedicated, energy consuming vesicles [Alberts B., Johnson
20 A., Lewis J., Raff M., Roberts K., Walter P., "Molecular biology of the Cell", 4th ed., Garland Science, New York, 2002]. From a morphological point of view, ion channels are proteins that fold in such a way that they form natural conducting nanopores dispersed in the membrane of the cells, thereby controlling the ionic fluxes in and out of the cell. Many ion channels can
25 selectively allow or block the flow of particular ion species through the membrane. The pores are highly sensitive for particular ions and they can open and close in response to various factors such as e.g. ligand binding, changes in the electrical field or membrane tension. This process is also called gating. In this gating process the electrochemical potential profile of the
30 channel effectively changes, which subsequently alters its transport properties. Although the actual transport mechanisms and the kinetics of the gating event can differ from one pore to another (e.g. some channels are transporting only one ion at the time, while for others multiple ion transport mechanisms have

been identified [Piasecki J, Allen R. J., Hansen J-P, 'Kinetic models of ion transport through a nanopore', *Cond. Mat.*, 2004, pp.3219]; some pores are permeable to only one kind of ions, whereas others can transport a whole range of different ions [Alberts B., Johnson A., Lewis J., Raff M., Roberts K.,
5 Walter P., "Molecular biology of the Cell", 4th ed., Garland Science, New York, 2002]), the common feature is the change in current carrying capability of the channels upon gating.

This phenomenology of ion channels has been known for many years already (see e.g. [Hille, B. "Ionic channels and excitable membranes", 3rd ed.,
10 Sinauer, Sunderland MA, 2001]). Over those years, measurements of ionic currents flowing through ion channels have generated a large amount of data, which have permitted to investigate channel properties like the dependence of the ionic current through a particular channel as a function of the applied voltage and the ionic species investigated, the influence of the gradient of ion
15 concentration on channel conductivity at fixed applied voltage, change in channel conductivity under the influence of electrical or chemical/biological stimuli exerted on the channel membrane ('gating', cf. supra), etc. However, the challenge to relate these functional characteristics to the geometrical, physical and chemical structure of a pore still remains.

20 In other prior art examples, nanoporous materials have been used and/or modified in ion flow conducting experiments [Steinle E. D. et. al., 'Ion channel mimetic micropore and nanotube membrane sensors', *Anal. Chem.*, 74, 2002, pp. 2416-2422],[Jirage K.B. et al. "Nanotubule-based filtration membranes", *Science*, 278, 1997, pp. 655 - 658]. In these structures, it is a
25 chemical stimulus (i.e. interaction with the analyte species), which switches on the ion transport through the pore. As an example, an alumina membrane containing gold nanotubes has been rendered hydrophobic [Steinle E. D. et. al., 'Ion channel mimetic micropore and nanotube membrane sensors', *Anal. Chem.*, 74, 2002, pp. 2416-2422]. Therefore, initially the ion channel is in off
30 state. Once the analyte has been added in the solution, water and ions could penetrate through the membrane and a given ion current was measured.

The possibility of externally controlling the ion flow through nanochannels, preferably electrically, offers intriguing prospects in a wide

scope of fields, including (but not restricted to) battery applications, biosensors, filtration membranes, etc.

Recently, first steps have been taken towards electrical control of ionic flow through artificially fabricated micro- and nanochannels. Schasfoort et al. have reported on the modification of the electro-osmotic flow in microchannels by means of the application of an electric field, perpendicular to the ionic flow [“Field-effect flow control for microfabricated fluidic networks”, Science 286, pp. 942 – 945, 1999]. In their approach, the perpendicular field changes the charge distribution and the electrostatic profile in the so-called electrical double layer near the interface with the wall of their microchannel, which influences the electro-osmotic flow in the bulk of the channel.

However, the above-described approach does not allow full control of the electrostatic potential distribution in the whole of the channel, nor of the concentration of the ions throughout the entire pore. It is therefore impossible to essentially ‘switch off’ the conductance of the pores for particular ionic species, unlike in biological environments. This is due to the inherent screening of the electric field in a medium with essentially free, charged carriers, as a solution of ions or charged molecules, which restricts the influence of such electric field to a so-called Debye-screening-length (cf. infra).

In order to cope with this restriction, Daigiji et al. [“Ion transport in nanofluidic channels”, Nanoletters, 4, 2004, pp. 137-142] and Karnik et al. [“Electrostatic control of ions and molecules in nanofluidic transistors”, Nanoletters, 5, 2005, pp. 943 – 948] have described the use of hydrophilic nanotubes as ion channels. The dimensions of such nanotubes can be more or less tailored by changing the processing conditions, which allows for lateral dimensions in the range of the Debye-screening length, or less. In that way, full electrical control of the potential in the entire channel has been achieved [“Electrostatic control of ions and molecules in nanofluidic transistors”, Nanoletters, 5, 2005, pp. 943 – 948] and modulation of the ion current by changing the voltage applied at a third contact, also called ‘gate’ contact, independent of the voltages applied at the reservoirs at the electrodes positioned at both ends of the nanotube.

However, in relying on the growth of nanotubes for forming ion channels, an inherent degree of 'randomness' is introduced in the processing, which seriously questions the use of this process flow for non-proof of principle type of applications. Both in growing the wires (which gives rise to a whole
5 range of nanotubes and nanowires, with an intrinsic variation in dimensionality) and in post-processing the nanotubes into nanofluidic channels (which requires either the difficult task of accurately positioning the nanotubes onto a pre-defined position or advanced 'seeking' procedures to determine the position of the nanotubes, and subsequently aligning the remainder of the
10 process steps to this nanotube), rather limited external control can be exerted.

Summary of the invention

It is an object of the present invention to provide improved nanofluidic devices.

15 The above objective is accomplished by a device and methods according to the present invention.

In a first aspect, the present invention provides a nanofluidic device for controlling the flow of charge carriers in at least one nanopore by modifying the electrical potential distribution inside the nanopore. The device comprises:

- 20 - a membrane having a thickness and having a first side and a second side,
- a first electrode being positioned at the first side of the membrane and a second electrode being positioned at the second side of the membrane, the second side being opposite to the first side.

The thickness of the membrane typically is the smallest dimension of the
25 membrane. The membrane may be laying in a plane, and then the thickness is the dimension measured perpendicularly to the plane. According to the present invention, the membrane comprises at least one nanopore extending through the membrane over the thickness of the membrane, over its thickness, so as to form a channel connecting the first and second side of the membrane. A third
30 electrode is integrated in said at least one nanopore. The nanopore may be located perpendicular to the plane of the membrane. Alternatively, the nanopore may have a longitudinal axis of which the direction is inclined with respect to a line perpendicular to the plane of the membrane.

Advantageously, the nanofluidic device according to the first aspect of the present invention allows controlling the flow of charge carriers through at least one nanopore in which the behaviour of the at least one nanopore, and thus the flow of the charge carriers, can be externally, electrically controlled.

- 5 The device according to the invention is suitable for being integrated into larger device-structures such as, for example, lab-on-chip.

According to the embodiments of the invention, the at least one nanopore may be located at pre-determined positions. This leads to accurately positioned nanopores in the membrane.

- 10 The at least one nanopore may have a smallest pore diameter located within one Debye screening length from said third electrode, wherein the smallest diameter is equal to or smaller than twice the Debye length of the nanopore.

The nanopore may be defined by an IC-compatible fabrication method.

- 15 The nanopore may for example be defined by a lithographic process forming a lithographically defined nanopore pattern and may subsequently be etched based on said lithographically defined nanopore pattern. The lithographic process may be any of optical lithography, X-ray lithography, electron-beam lithography, focused ion beam lithography or nano-imprint lithography.

- 20 The at least one nanopore may have a smallest pore diameter smaller than or equal to 100 nm. The at least one nanopore may have a smallest pore diameter of between 1 and 100 nm, preferably between 10 and 15 nm and more preferably between 0.5 and 5 nm.

- 25 The membrane may have a thickness of smaller than or equal to 100 μm . The membrane may have a thickness between 10 μm and 100 μm , preferably between 100 nm and 10 μm .

According to embodiments of the invention, the inner surface wall of the nanopore may be chemically modified with voltage-sensitive dipolar molecules.

- 30 There may be a first space between the first electrode and the first side of the membrane and a second space between the second electrode and the second side of the membrane, wherein the first space is adapted for receiving a first solution and the second space is adapted for receiving a second solution. The first solution may be different from the second solution or the first

solution may be equal to the second solution. When using the device according to the present invention, the first and/or second solutions may extend through said nanopore.

In an embodiment, the first solution comprises water or a solvent, an
5 analyte and a first electrolyte. The second solution comprises water or a solvent and a second electrolyte. The concentration of the first electrolyte in the first solution can be equal or substantially equal to the concentration of the second electrolyte in the second solution. In another embodiment, the concentration of the first electrolyte in the first solution is different from the
10 concentration of the second electrolyte in the second solution, different being understood as higher or lower. In a preferred embodiment, the first electrolyte and the second electrolyte are the same while the first solution comprises a charged analyte. The said device can then be used to transfer analyte from said first solution to said second solution. In another embodiment, a first
15 analyte is present in the first solution, and a second analyte is present in the second solution. This second analyte can be the same as the first analyte, or different. The concentration of these two said analytes can be equal or different. The said device can then be used to transport the first analyte from the first solution into the second solution. The said device can also be used to
20 transfer the second analyte from the second solution into the first solution, and for transferring both analytes from their initial solution to the solution opposite of the pore.

In an embodiment of the invention, the device according to embodiments of the present invention is used as a transistor or in a transistor
25 means. In a preferred embodiment, the device is an ionic transistor. In another embodiment of the present invention, the device according to embodiments of the present invention is a filter or is used in a filter means, filtering charged species based on their charge. In another embodiment of the present invention, the device according to embodiments of the present invention is a
30 sensor for sensing an analyte in solution or is used in a sensor. In another embodiment, the device according to embodiments of the present invention is used as part of a galvanic cell, the establishment of the equilibrium potential over the membrane of which is modified and controlled by means of the third

electrode. In all of these applications, a feature resides in the full electrostatic control over the potential and charge distribution in the channel by means of said third contact.

In a second aspect, the present invention provides a method for forming
5 a nanofluidic device for controlling the flow of charge carriers in a fluid through at least one nanopore by modifying the electrical potential distribution inside the nanopore. The method according to the second aspect of the present invention comprises:

- providing a membrane having a thickness and having a first side and a
10 second side,
- defining at least one nanopore in said membrane,
- etching said at least one nanopore through the membrane over the thickness of the membrane, such that the at least one nanopore connects the first side and the second side of the membrane by forming a channel,
- 15 - providing a first electrode at said first side of the membrane and a second electrode at said second side of the membrane, and
- providing at least one third electrode, said at least one third electrode being integrated in the at least one nanopore.

The method may comprise providing a plurality of nanopores in the
20 membrane, wherein a third electrode is integrated into at least one nanopore. A third electrode may be integrated in each of the plurality of nanopores, each third electrode being adapted for being addressed independently from the other third electrodes.

Providing at least one third electrode may comprise extending the third
25 electrode over an entire inner surface of the at least one nanopore. Alternatively, providing at least one third electrode may comprises not extending the third electrode over an entire inner surface of the at least one nanopore, but only extending it over part of the inner surface thereof.

Defining at least one nanopore in the membrane may be performed by
30 using an IC-compatible fabrication method. Defining at least one nanopore in the membrane may be performed lithographically. Defining at least one nanopore in the membrane may be performed by means of any of optical

lithography, X-ray lithography, electron-beam lithography, focused ion beam lithography or nano-imprint lithography.

Etching said at least one nanopore may be performed by wet anisotropic etching or by dry anisotropic etching.

5 Defining at least one nanopore may be performed such that the at least one nanopore has a smallest pore diameter equal to or smaller than the Debye length of the nanopore. Defining at least one nanopore may be performed such that the at least one nanopore has a smallest pore diameter equal to or smaller than 100 nm. Defining at least one nanopore may be performed such
10 that the at least one nanopore has a pore diameter of between 1 and 100 nm, preferably between 10 and 15 nm and more preferably between 0.5 and 5 nm.

In a third aspect, the present invention provides a method for controlling the flow of charge carriers in at least one nanopore in a membrane. The method comprises:

- 15 - providing a nanofluidic device comprising a membrane having a thickness and having a first side and a second side, a first electrode positioned at the first side of the membrane and a second electrode positioned at a second side of the membrane, the membrane comprising at least one nanopore, preferably at a pre-determined position, extending through the membrane over the
20 thickness of the membrane so as to form a channel connecting the first and second side of the membrane and a third electrode integrated in the at least one nanopore,
 - providing a first solution between said first side and said first electrode and a second solution between said second side and said second electrode,
25 - applying a first and a second electrical voltage to respectively the first electrode and second electrode, and
 - applying a third electrical voltage to the third electrode, the third electrical voltage being independent from the first and second electrical voltage.

 In an embodiment of the present invention, an electric field is present in
30 the pore with a component of the electric field extending transversally through the pore, transversally meaning perpendicular to the inner surface of said pore as described in the first aspect of this invention. This transversal electric field influences the creation of a double layer potential at the walls of the nanopore.

As described below in more detail, the dimensions of the pore are preferably chosen such that full control can be exerted over the potential in at least one zone of the channel, such that no connection from the first to the second solution can be found that does not intersect with this zone.

- 5 In a further aspect, the present invention provides a method for determining a voltage to be applied to an electrode for controlling the flow of charge carriers in at least one nanopore in a membrane, the electrode being positioned in a nanopore extending through a membrane and forming a channel connecting a first side of the membrane with a second side of the
- 10 membrane. The method comprises:
- in a first step loading pre-determined relationships between the flow of charge carriers through the nanopore and at least a voltage to be applied at the electrode, e.g. a list of such relationships,
 - in a second step determining the required flow of charge carriers,
 - 15 - selecting from a combination of the first step and the second step the voltage to be applied to the electrode.

The present invention also provides a computer program product which when executed on a processing device executes the method according to an embodiment of the present invention for determining a voltage to be applied to

20 an electrode for controlling the flow of charge carriers in at least one nanopore in a membrane. The present invention also provides a machine readable data storage device storing the computer program product of the present invention.

In a further aspect, the present invention also provides the use of a nanofluidic device according to the present invention as a biosensor or as an

25 ionic transistor.

Particular and preferred aspects of the invention are set out in the accompanying independent and dependent claims. Features from the dependent claims may be combined with features of the independent claims and with features of other dependent claims as appropriate and not merely as

30 explicitly set out in the claims.

The above and other characteristics, features and advantages of the present invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way

of example, the principles of the invention. This description is given for the sake of example only, without limiting the scope of the invention. The reference figures quoted below refer to the attached drawings.

5 **Brief description of the drawings**

Fig. 1 illustrates a model for the electrode/solution double layer region.

Fig. 2 schematically illustrates the build-up of an electrical double-layer at a liquid-solid interface (A), the modification of the double layer by means of an applied gate voltage V_g over a channel wall (B-C) and an equivalent
10 capacitor model (D).

Fig. 3 is an example of a nanofluidic device according to a first aspect of the present invention.

Fig. 4 is a schematic illustration of a cross-section (parallel to the membrane surface) of a nanopore.

15 Fig. 5 is a schematic illustration of the working principle a nanofluidic device according to an embodiment of the invention.

In the different figures, the same reference signs refer to the same or analogous elements.

20 **Description of the invention**

The present invention will be described with respect to particular embodiments and with reference to certain drawings but the invention is not limited thereto but only by the claims. The drawings described are only schematic and are non-limiting. In the drawings, the size of some of the
25 elements may be exaggerated and not drawn on scale for illustrative purposes.

Furthermore, the terms first, second, third and the like in the description and in the claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order. It is to be understood that the terms so used are interchangeable under appropriate
30 circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

Moreover, the terms top, bottom, over, under and the like in the description and the claims are used for descriptive purposes and not necessarily for describing relative positions. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other orientations than described or illustrated herein.

It is to be noticed that the term "comprising", used in the claims, should not be interpreted as being restricted to the means listed thereafter; it does not exclude other elements or steps. Thus, the scope of the expression "a device comprising means A and B" should not be limited to devices consisting only of components A and B. It means that with respect to the present invention, the only relevant components of the device are A and B.

The challenge in modifying and manipulating intrinsically nanoporous materials and/or structures lies in the external control over the behaviour of one single nanopore. Therefore, the development of a technology that can controllably define nanoscale pores on pre-defined positions for nanofluidic, ionic-flow related applications and that allows for external control, preferably through interaction with standard electronic equipment, would be beneficial in view of this challenge. Recent evolutions in IC-manufacturing and -processing have resulted in ever more stringent requirements for the lithography that lies at the heart thereof, with feature sizes smaller than 100 nm commonly reached with present-day technology. Also, evolutions in inherently sequential techniques as electron-beam lithography, focused ion beam lithography and nano-imprint lithography have allowed feature sizes in the range of below 10 nm to be reached. The strength of such lithographic methods lies in the possible combination of accurately writing and aligning small patterns, including patterns ultimately (after subsequent processing) yielding the nanopores as described below, thereby overcoming the inherent 'randomness' of the nanotube- and porous membrane based technologies.

The present invention therefore provides a nanofluidic device for controlling the flow of charge carriers, such as e.g. ions or charged biomolecules, through at least one nanopore in a membrane, a method for the

manufacturing of such a nanofluidic device and a method for controlling the flow of charge carriers through at least one nanopore in a membrane.

At the core thereof lies the precise and accurate definition of a predefined pattern which, after pattern transfer and subsequent process steps,
5 yields a nanoscale pore through a membrane-like structure (see further).

In a first aspect of the present invention a nanofluidic device for modifying the electrical potential distribution inside a nanopore is provided. For example, this can be in order to induce changes in the nanopore permeability for differently charged chemical species. The device according to the present
10 invention may, for example, be an artificial ion transistor based on nanofabrication technologies, i.e. based on artificially fabricated nanopore structures which may primarily be based on IC-based processing techniques. In an artificial ion transistor the ion flow through the nanopore is controlled by the potential distribution inside that nanopore. The potential distribution is
15 defined by the double layer potentials generated at the walls of the nanopore.

It is known that at the interface between a solid surface and a solution comprising one or more electrolytes, a so-called double-layer is formed [Hunter, R.J., "Zeta potential in colloid science", Academic Press, London, 1981]. The diffuse layer of this double layer extends over a Debye screening
20 length, which is dependent on the nature and the concentration of the one or more electrolytes in a fluid. When the solid surface is in contact with the solution, a layer of charges is created on the solid surface and a layer with a same total charge as the layer of charges on the solid surface, non-uniformly distributed over this diffuse layer, is created, extending in the fluid phase.
25 These parallel layers of charges form a so-called double layer capacitor.

Several models can be used to describe this double layer capacitor, essentially varying in the description of the first few monolayers of the fluid near the interface (the so-called Stern layer or capacitor). One of these models is illustrated in Fig. 1 [Bard A. J., Faulkner L.R., "Electrochemical methods: fundamentals and applications", John Wiley & Sons, New York, 1980]. The
30 Gouy-Chapman-Stern model in Fig. 1 assumes that the double layer capacitance consists of a series network of a Helmholtz-layer capacitance and a diffuse layer capacitance. In Fig. 1 the Helmholtz-layer is indicated by

reference number 1 and the diffuse layer is indicated by reference number 2. The latter describes the zone of essentially mobile ions in which a net charge (and electrical potential) extends into the fluid, effectively screening the charge in the Stern layer and at the interface itself. The Helmholtz layer 1, having an
5 inner Helmholtz plane (IHP) and an outer Helmholtz plane (OHP), is used to model the effect of the finite size of the hydrated ions 3 by which these ions are prevented from approaching the surface 4 of a solid 5 any closer than their ionic radius.

In Fig. 2 the build-up of an electrical double-layer at a fluid-solid
10 interface (part A of Fig. 2), the modification of the double layer by means of an applied gate voltage V_g over a channel wall (parts B and C of Fig. 2) and an equivalent capacitor model (part D of Fig. 2) are illustrated [Schasfoort R. B. M. et al. "Field-effect flow control for microfabricated fluidic networks", Science 286, pp. 942 – 945, 1999].

15 As modelled by Schasfoort et al. in the above publication, the double layer can be modified by applying an electrical field with a component perpendicular to the fluid-solid interface. Generally, the double layer potential formed at a given surface while immersed in a given ionic solution depends on the pH of the solution and the ionic strength thereof, as well as on the charge
20 on the pore inner surface.

The nanofluidic device according to the first aspect of the present invention can operate in a fluid, e.g. an aqueous environment, where different charged species, e.g. ions, are present. The charged species, e.g. ions, act as carrier species. The nanofluidic device according to the first aspect of the
25 present invention comprises a membrane, the membrane comprising at least one pore extending through the membrane. The pores have nanoscale dimensions (see further), therefore, in the further description the pores will be referred to as nanopores. When the nanopore is in contact with a fluid, e.g. a solution comprising an electrolyte, a layer of charges, also called Helmholtz-
30 layer 1, is created on the solid inner pore surface and a layer with a same total charge, non-uniformly distributed over this layer, called the diffuse layer 2 is created, extending in the liquid phase. These parallel layers 1, 2 of charges form the so-called 'double-layer capacitor', as described above.

A nanofluidic device 10 according to the first aspect of the present invention and as illustrated in Fig. 3, comprises a first electrode 11, a second electrode 12 and a membrane (not shown in the figure). The membrane lies in a plane and has a first surface and a second surface. The membrane
5 comprises at least one nanopore 13 situated at pre-determined positions and extending through the membrane such as to form a channel connecting the first and second surface of the membrane.

According to embodiments of the invention, the first electrode 11 is positioned at the side of the first surface of the membrane and the second
10 electrode 12 is positioned at the side of the second surface of the membrane. A first solution 14 may be present e.g. between the first surface of the membrane and the first electrode 11 and a second solution 15 may be present between the second surface of the membrane and the second electrode 12. The first solution 14 may be the same or may differ from the second
15 solution 15. The first solution 14 may comprise a first analyte and a first fluid. The second solution 15 may comprise a second fluid and may or may not comprise a second analyte. The first and second analyte may preferably be at least one ion or at least one charged molecule. According to embodiments of the invention, the first and the second fluid may be the same or may be
20 different from each other. The first and second analyte may be the same or may be different from each other. The first analyte may be present in the first solution 14 in a first concentration. The second analyte may be present in the second solution 15 in a second concentration. The first and second concentration may be the same or may be different from each other.

25 According to embodiments of the invention, the first and second electrodes 11, 12 may be conductive, e.g. metallic, stripes, layers, dots, etc., each physically attached on the one hand to one side of the membrane and on the other hand to a voltage source. However, in other embodiments, the first and second electrode 11, 12 may not be attached to the membrane. In the
30 latter case, standard conductive electrodes, e.g. metallic electrodes, such as Pt- or Ag/AgCl electrodes, and electrode cells may be used for biasing the respective solutions 14, 15. For biasing the electrodes 11, 12 at the appropriate electric potentials, a standard voltage source may be used. In a

preferred embodiment, a low-impedance source may be used but in other embodiments also a high-impedance source may be used.

Furthermore, the nanofluidic device 10 comprises a third electrode 16 which is integrated with the at least one nanopore 13. The third electrode 16 will be used to control the charge density at the walls of the at least one nanopore 13 for given pH and ionic strength values and therefore, obtain the desired potential distribution inside the at least one nanopore 13. The third electrode may, for example, comprise a metal (e.g. Cu, Au, Pt, Ag) or a degenerate semiconductor or may be an alloy of several metals, the electric conductivity of which is such, that the electrode surfaces may be regarded as electrical equipotential surfaces. The third electrode 16 may, according to embodiments of the invention, extend over the entire inner surface of the nanopore 13. This means that, in that case, the inner surface of the nanopore 13 is completely covered with material from the third electrode 16. In other embodiments, the third electrode 16 may not extend throughout the entire inner surface of the nanopore 13, but cover only parts thereof.

In contrast with prior art devices, according to the first aspect of the present invention, a nanofluidic device 10 is provided, the dimensions of which can be accurately predetermined and reproduced, and which can be accurately and reliably positioned on a predefined position on a substrate.

The membrane may preferably comprise a layer of IC-compatible material and may be physically attached to an IC-compatible substrate (not shown in the figure) which may be locally removed in order to expose the first and second surface of the membrane to respectively the first and second solution 14, 15 (see further). According to embodiments of the invention, the substrate may be a semiconductor substrate, such as e.g. Si, GaAs, Ge, or an insulating material, such as e.g. pyrex, silica, mica, SiC, diamond, sapphire, or any other suitable substrate.

According to embodiments of the invention, the membrane may be a "thin membrane" by which it is meant that the membrane thickness may be smaller than 10 μm . Preferably, the membrane thickness may be between 100 nm and 10 μm . In other embodiments, however, the membrane thickness may be larger, e.g. between 10 μm and 100 μm . The membrane may comprise a

thin metal sheet wherein the metal may, for example, be Au, Pt, Al, Ta, ITO, carbon or any other suitable metal, or may comprise a conducting or semiconducting polymer, such as e.g. poly(3,4-ethylenedioxythiophene) or PEDOT, a semiconductor material such as e.g. Si, or an insulating material
5 such as e.g. SiO₂, Si₃N₄, SiC, Ta₂O₅, Al₂O₃, polycarbonate, polyethylene, parylene C or any other suitable insulating material. According to further embodiments of the invention, the membrane may comprise combinations of the above-described materials, wherein with combinations is meant stacks of layers of materials or materials classes as described above, such as e.g. SiO₂
10 on top of a Si layer.

The diameter or width of the at least one nanopore 13 present in the membrane may depend on the particular application. In embodiments according to the invention, the diameter of the nanopore 13 may be equal over the entire thickness of the membrane, i.e. the nanopore 13 may have uniform
15 diameter over its entire length. In other embodiments according to the invention, the diameter of the nanopore 13 may be variable throughout the thickness of the membrane, and thus the nanopore 13 may have a non-uniform diameter over its length. In both cases, the minimum diameter of the nanopore 13 should be accurately determined. It has to be noted that this does
20 not pose any restrictions to the shape of the cross-sections through the nanopore 13, parallel to the first and second membrane surfaces. The shape of the cross-section may be either circular or non-circular (e.g. triangular, quadrangular, etc.).

According to embodiments of the invention, the dimensions of the
25 nanopore 13 are chosen such that for at least one particular zone of the nanopore 13, the potential throughout this zone can be modified. With 'zone' is meant a three-dimensional volume, enclosed within the nanopore 13, that locally fills the entire nanopore 13, i.e. no path through the nanopore 13 can be found, from the first surface to the second surface of the membrane or vice
30 versa, that does not intersect this volume. By changing the voltage applied at the third electrode 16 the surface charge on the fluid-solid interface can be changed, and subsequently the potential and charge distribution in the channel of the nanopore 13 is changed. In view of the intrinsic screening length or

Debye-length, characterising the diffuse layer 2, and in order to obtain full electrostatic control over the nanopore 13, this effectively reduces the diameter of the nanopore 13 in the vicinity of the third electrode 16 to twice the Debye length.

5 The third electrode 16 may preferably be covered with a dielectric layer 17, because, when the third electrode 16 would not be covered with a dielectric layer 17, charge transfer might occur over the interface of the electrode 16 and the solution(s) 14, 15 inside the nanopore 13, giving rise to an electrical current being drawn from the third electrode 16, and a more
10 difficult biasing scheme in which the potential drop over the dielectric capacitor would be absent. The dielectric layer 17 may comprise a single layer or may comprise a stack of layers. The dielectric layer 17 may, but should not necessarily be, morphologically and/or structurally equal to the internal surface of the entire nanopore 13. The dielectric layer 17 may, for example, be SiO₂,
15 SiO_x (non-stoichiometric silica), silicon nitride, or any other suitable dielectric material.

 If the third electrode 16 is covered with a dielectric layer 17 and extends over the entire nanopore 13, the dielectric layer 17 also extends over the entire nanopore 13 and in that case the inner surface of the nanopore 13 or the 'pore
20 inner surface' is formed by the dielectric layer 17 (or any layer on top of that, see further). If the third electrode 16 does not cover the entire nanopore 13, the pore inner surface may mainly form the interface of the nanopore 13 with the membrane (with possibly an extra layer covering it in order to change the surface charge thereof). The dielectric layer 17 and the pore inner surface (or
25 the respective covering layers of these two interfaces) may but do not have to be equal. In this case, equal may refer to the chemical composition, but also to morphology of the surface (amorphous vs. (poly/mono)crystalline, porous, ...)

 It is known that, depending on the composition and morphology of the solid-fluid interface, the pH and ionic strength of a solution, a surface charge
30 develops at the interface between a liquid and a solid, in the case of the present invention a fluid in a nanopore, which gives rise to a net charge in the diffuse part of the double layer as well, which in turn influences the permeability of the nanopore 13 to charged species of a specific nature. With

nature is meant the charge and charge distribution of the species, e.g. the valence of the ions in case of an ionic solution. For example, if a positive net charge on the walls of the nanopore is desired, e.g. to ensure anion permeability through the nanopore, and the working pH is 7, then e.g. Al_2O_3 may be used as dielectric layer. This is because Al_2O_3 has a pH_{pzc} (pH at point zero charge) of 8 which means that when immersed in pH = 7 solutions, the surface will become positively charged. Depending on the surface, the fluid and analyte used, a charge is developed on the solid material, in the case of the invention the inner pore surface, when immersed in a solution comprising a fluid and an analyte. This depends on the pH according to $-\log(\text{conc}(\text{H}_3\text{O}^+))$. At pH_{pzc}, the H_3O^+ and OH^- concentrations compensate each other, giving rise to a net zero charge. Below, the H_3O^+ concentration dominates, giving a net positive charge on the surface. This depends on many aspects such as e.g. the morphology of the surface in the case of an immersed solid (porosity, amorphous vs. crystalline etc.). In Marek Kosmulski, "The pH-dependent surface charging and the points of zero charge", Journal of colloid and Interface science, 253 (2002), p.77-78, an illustration of pH dependence of the surface charge can be found.

Optionally the dielectric layer 17 covering the third electrode 16, may itself be covered with a pore inner surface layer 19 (not shown in the Fig. 3 but illustrated in Fig. 4) which separates the dielectric layer 17 from the solution, e.g. a fluid with an analyte, inside the nanopore 13 and which in this case forms the interface between the solid surface and the fluid inside the nanopore 13. The pore inner surface layer 19 may be, for example, silica, silicon nitride, alumina, mica, or any other suitable material. The material of the pore inner surface layer 19 should be chosen such as to give an appropriate surface charge under the conditions chosen for the experiment to be conducted with a device according to the present invention.

Preferably, the layer forming the inner surface of the nanopore 13, also called the interfacial layer, which is either the third electrode 16, the dielectric layer 17 or the pore inner surface layer 19, may be chosen such that the charge which develops at the interface is minimal under the chosen conditions of pH and ionic strength, which allows for optimum modification of the surface

charge in the nanopore 13 by means of the third electrode 16 integrated in the nanopore 13.

According to an embodiment of the invention, the longitudinal direction or length of the pore may be perpendicular or substantially perpendicular to the first and second surface of the membrane 18. In that case, the length of the nanopore 13 may be characterised by the distance between the first and second surface of the membrane 18, in other words, may be determined by the thickness of the membrane 18. In another embodiment, the longitudinal direction of the nanopore 13 may not be substantially perpendicular to the first and second surface. For example, the nanopore may be etched in a plasma non-perpendicular to the membrane 18, but still forming a channel connecting the first surface with the second surface. In this case, the length of the nanopore 13 may be different from the thickness of the membrane 18, more particularly the length of the nanopore 13 may be larger than the thickness of the membrane 18.

A schematic illustration of a possible layer stack inside a nanopore 13 is shown in Fig. 4. It has to be understood that this is only an example and that this is not limiting the invention. In the example given, a nanopore 13 extending through a membrane 18 comprises a third electrode 16 which is positioned at an inner surface of the nanopore 13. The third electrode 16 is, in the example given, covered with a dielectric layer 17. On top of the dielectric layer 17 a pore inner surface layer 19 is provided.

The dimensions of the at least one nanopore 13 are chosen such that full control can be exerted over the potential in at least one zone of the channel, such that no connection through the nanopore 13 from the first to the second solution 14, 15 can be found that does not intersect with this zone (see above).

As already described before, the interface between a solid surface, which according to the invention is the pore inner surface and/or dielectric layer 17 covering the third electrode 16 and/or the pore inner surface layer 19, and a solution 14, 15 comprising at least one electrolyte, a double layer is formed. The diffuse layer 2 of the double layer extends over a Debye screening length, which is dependent on the nature and concentration of the at

least one analyte in the solution 14, 15 in the pore channel. This Debye screening length is a measure for the ultimate extension of electric fields in the solution 14, 15. Therefore, in order to have full electrical control in at least one cross-section of the nanopore 13 near the third electrode 16, the diameter of
5 the nanopore 13 near the third electrode 16 should be chosen such that it does not exceed twice the Debye length of the nanopore 13 in the particular application.

In a preferred embodiment, the diameter or width of the at least one nanopore 13 in the vicinity of the third electrode 16, also defined as the
10 smallest diameter of the nanopore 13 in case of a non-uniform width, may be between 1 nm and 100 nm. More preferred, the width or smallest dimension of the nanopore 13 may be between 10 nm and 15 nm. Still more preferred, the width or smallest dimension of the nanopore 13 may be between 0.5 nm and 5 nm.

15 In preferred embodiments of the present invention, the inner surface of the nanopore 13 may be modified. For example, the inner surface of the nanopore 13 may be morphologically altered or may be changed by providing an accurately determined layer into the pore. This layer may, for example, comprise alumina, silica, silicon nitride, mica, or any other suitable material
20 provided that it gives rise to an appropriate surface charge under the given circumstances. In embodiments according to the present invention, the inner surface of the nanopore 13 may be coated with voltage sensitive dye molecules. Voltage sensitive dyes may be dipolar molecules that change their polarisation under influence of an electrical field. This change in polarisation
25 allows for additional, essentially electrical control of the electrical potential profile in the nanopore 13, and may or may not result in a change of the transport of an analyte through the nanopore 13.

According to embodiments of the invention, the nanofluidic device 10 as described in the first aspect of the present invention, may be used as a
30 transistor or in a transistor means. In a preferred embodiment, the nanofluidic device 10 may be an ionic transistor. In another embodiment of the present invention, the device 10 according to the first aspect of the present invention may be a filter or may be used in a filter means for filtering charged species

based on their charge. In all of these applications, one of the features resides in the full electrical control over the potential and charge distribution in the channel of the nanopore 13 by means of the third electrode 16.

The benefit of a nanofluidic device 10 according to the first aspect of the present invention compared to conventional protein ion channels is its IC-compatible fabrication method, which allows mass fabrication. Moreover, the nanofluidic device 10 according to the first aspect of the invention allows circumventing the problems of limited stability and lifetime related to the use of biological materials. This device 10 furthermore provides an additional degree of freedom in applying a potential over the third electrode 16, independent of the potential applied the first and second electrodes 11, 12 on either side of the nanopore 13. With regard to nanotube-based ion transistors as described in the prior art, the robustness and controllability of the manufacturing method, which also allows for straightforward integration into electronic circuitry, offers a clear advantage over the inherent randomness of the nanotube-approach.

Furthermore, the nanofluidic device 10 according to the first aspect of the invention can be used to transport all types of charged species across the nanopore 13, such as e.g. anions, cations, charged biomolecules, Also, the possibility of modifying the internal pore surface allows for the intrinsic surface charge (a function of the composition and morphology of the surface, the local pH and ionic strength) to be changed independently of the gate-voltage-induced charge on the pore wall, gate voltage referring to the voltage applied to the third electrode 16 integrated in the membrane 18 near the nanopore 13.

Hereinafter the operation principle of a nanofluidic device 10 according to the first aspect of the invention will be described by means of an artificial ion transistor as illustrated in Fig. 5. The solution 14, 15 penetrates inside the nanopore 13 upon application of these solutions to the membrane 18 provided with the nanopore 13, or upon immersion of the substrate 18 with nanopore 13 into the solution 14, 15. When the third electrode 16, in the example given a gate electrode, is left floating, i.e. not electrically connected to the electrodes 11, 12 in the solution 14, 15, a double layer potential drop occurs at the nanopore walls 20 and the dielectric layer 17 covering the third electrode 16, due to spontaneous charge separation. The value of this potential drop

depends on the particular experimental conditions, such as analyte concentration and pH, ion size, density of surface sites on the pore inner surface or nanopore walls 20.

In a first-order Debye-Huckel model (see e.g. Hunter) the potential $\psi(x)$ in the diffuse part 2 of the double layer at a distance x from the interface with the thin Helmholtz layer 1 attached to the wall surface 20 of the nanopore 13 (typically a few Angstrom at most) is given by:

$$\psi(x) = \psi_0 \exp(-\chi x) \quad (1)$$

wherein ψ_0 is the potential at the interface between the Helmholtz layer 1 and the diffuse part 2 of the double layer capacitor and $1/\chi$ is the Debye length. The Debye length is schematically indicated by the dashed lines in Fig. 5.

Fig. 5A illustrates a nanopore 13, comprising a third electrode 16 which does not completely cover the nanopore 13, and without a bias being applied at this third electrode 16, in the example given the gate electrode (e.g. floating gate configuration, the gate is not connected to a charge reservoir). In this part of the figure, charge separation is illustrated, as this occurs independently of the bias applied to the third or gate electrode 16 and which results in a net negative surface charge in the nanopore 13, a net positive charge in the diffuse layer 2, which extends over the entire width of the nanopore 13, at least in the vicinity of the third or gate electrode 16. The extension of the Debye-length, and hence the diffuse layer 2 of the double-layer capacitor, is indicated by means of a dashed line 21.

By modifying the potential of the third electrode 16 with regard to the potential on the first and second electrodes 11, 12, both the surface charge on the electrode-covered part of the nanopore 13, which may or may not be equal to the entire inner surface or nanopore wall 20 of the nanopore 13, and the diffuse layer charge are changed. This is illustrated in Fig. 5B and Fig. 5C. In Fig. 5B, a net positive potential is applied to the third or gate electrode 16, such that additional negative charges are attracted to the gate electrode's dielectric layer 17, partly screening the positive charge on the gate electrode 16 itself, as well as negative charges in the nanopore 13, further screening the positive charge on this gate electrode 16. Here, the permeability of the

nanopore 13 and thus of the membrane 18 is changed from preferably cationic to preferably anionic. In Fig. 5C, the applied negative potential yields a net positive surface charge (even overcoming the built-in negative surface charge), as well as a net positive charge in the diffuse layer 2. This figure
5 illustrates the opposite case than the one illustrated in Fig. 5B. A negative charge exists on the gate electrode 16, a positive charge screening this, both at the pore surface and inside the nanopore 16, cationic permeability is increased (with regard to A) and anionic permeability decreased (again compared with A).

10 The voltage to be applied to the electrodes 11, 12, 16 may be determined by means of 'voltage vs. current' experiments, changing both the electric field over the longitudinal and perpendicular directions of the nanopore 13. Effectively, this leads to a change of two independent voltages, for example the voltage applied to the gate electrode 16 (V3) and the voltage
15 applied to the second electrode 12 (V2) which may be immersed in a second solution 15 may be changed, while the voltage applied to the first electrode 11 (V1) may be left fixed. Assuming superposition may be applied, any other combination can be seen as this scheme with a superimposed fixed and uniform potential, which electrically has no meaning.

20 Three regimes may exist:

- A first one, in which the permeability of the nanopore 13 may be determined by the voltage applied to the gate electrode 16.
- A second one, in which the surface charge is dominant and the voltage applied to the third electrode 16 does not have much influence.
- 25 - And a third one in which a combination of the voltage applied to the gate electrode 16 and the voltage applied longitudinally, i.e. over the membrane 18, determines the permeability.

Spontaneous separation of charge between the fluid phase and the solid interface can be caused by several mechanisms (difference in the affinity
30 of the two phases for different ions being the mechanism occurring at most solid-liquid interfaces) resulting in the creation of the double layer. Equilibrium is established when the electrochemical potential is the same for each ion that can move freely between phases. This equilibrium can be shifted by applying

an electric field perpendicular to the pore inner surface: this will result in a charge build-up at this inner surface, and a subsequent change of the charge distribution inside the diffuse layer. When the diffuse layer in at least one zone of the pore extends over the entire diameter of the pore, as described in the proposed invention, this results in an effective modulation of the permeability of the pore to charged molecules.

Besides direct field-induced actuation, an embodiment of the invention comprises an approach based on the chemical modification of the nanopore walls 20 with voltage-sensitive dipolar molecules (i.e. hyperpolarizable chromophores and proteins). In a simple approximation, a protein molecule in solution can be represented by a set of polarizable dipoles embedded into a dielectric medium of solvent molecules. Under influence of an electrical field applied onto the conductive, e.g. metal, electrode, the polarization of the molecules will change and consequently the potential distribution within the nanopore 13 will become different. In fact, the electrostatic interaction of these molecules with the ions inside the nanopore 13 can be tuned by the externally applied electric field.

In a second aspect of the invention, a method for the manufacturing of a nanofluidic device 10 according to the first aspect of the invention is provided.

In a first step, a substrate is provided. The substrate may, for example, be a semiconductor substrate, such as e.g. Si, GaAs, Ge, or an insulating material, such as e.g. pyrex, silica, mica, SiC, diamond, sapphire, or any other suitable substrate. A membrane 18 is then deposited onto the substrate. The membrane 18 may e.g. comprise a thin metal sheet wherein the metal may, for example, be Au, Pt, Al, Ta, ITO, carbon or any other suitable metal, or may e.g. comprise a conducting or semiconducting polymer, such as e.g. poly(3,4-ethylenedioxythiophene) or PEDOT, a semiconductor material such as e.g. Si, or an insulating material such as e.g. SiO₂, Si₃N₄, SiC, Ta₂O₅, Al₂O₃, polycarbonate, polyethylene, parylene C or any other suitable insulating material. According to further embodiments of the invention, the membrane 18 may comprise combinations of the above-described materials, wherein with combinations is meant stacks of layers of materials or materials classes as described above, such as e.g. SiO₂ on top of a Si layer. The membrane 18

may have a thickness of between 100 nm and 10 μ m. In other embodiments, however, the membrane 18 thickness may be between 10 μ m and 100 μ m.

For the deposition of the membrane 18, preferably standard processing techniques should be used which allow very accurate (< 10 nm) control over the thickness of the layers forming the membrane 18. With very accurate control is meant that the position of the nanopore 13 can be determined with an accuracy of less than 10 nm. Suitable methods for the deposition of the membrane 18 may be epitaxial methods (e.g. Molecular Beam Epitaxy (MBE), Metal Organic Chemical Vapour Deposition (MOCVD), Metal Organic Molecular Beam Epitaxy (MOMBE), ...) or vapour-deposition techniques, (e.g. Atomic Layer Deposition (ALD), Chemical Vapour deposition (CVD), plasma-assisted chemical vapour deposition (e.g. PECVD), vacuum evaporation, sputtering etc). In addition, wafer bonding and ion-implantation techniques may be used, such as e.g. in bonded Silicon On Insulator and Selective Implantation of oxygen SOI (SIMOX-SOI), which both yield layer stacks of silicon on oxide forming the thin membrane 18.

In order to expose the bottom membrane surface, the substrate is locally removed at those positions where a nanopore 13 is or will be extending through the membrane 18, or in other words, in the vicinity of an intersection of a nanopore 13 and the bottom surface of the membrane 18. With 'vicinity' is meant an area of the bottom surface comprising at least the zone where the nanopore 13 intersects the membrane bottom surface, and possibly but not necessarily larger. This may be done by e.g. bulk micromachining. According to embodiments of the present invention, the wafer may first be thinned by a standard IC thinning method such as e.g. grinding, plasma etching, Optionally, the wafer may also be polished, for example by Chemical Mechanical Polishing (CMP).

As part of this bulk micromachining, the substrate should be etched away locally. Suitable etching methods may, for example, be ion milling, reactive ion etching (RIE), deep reactive ion etching, sputtering or wet etching. The definition of said pore through said membrane can occur before or after the bulk micromachining.

Definition of at least one nanopore 13 through the membrane 18 may be performed before or after the bulk micromachining step, leading to a defined etch pattern. The nanopore 13 may be defined by IC-compatible fabrication methods and may preferably be defined lithographically, either
5 through optical lithography (UV, deep UV, extreme UV), electron-beam lithography, X-ray lithography, focused ion beam lithography or nano-imprint lithography, because lithographic processes allow for accurate determination with a deviation of less than 10 nm. With definition of the at least one nanopore 13 is meant the definition of the position of the nanopore 13 in the membrane
10 18. The actual conformation or formation of the nanopore 13 through the membrane 18 depends on the further processing (see below).

After, preferably lithographically, defining the nanopore position, a hole is etched through the membrane 18 based on the defined etch pattern. This may be done by wet anisotropic etching or by dry anisotropic etching. The
15 latter method may comprise etching procedures such as e.g. ion milling, powder blasting, plasma-assisted etching, focused ion beam etching, reactive ion etching, deep reactive ion etching. According to embodiments of the invention, the hole may be etched throughout the entire membrane 18 in one processing step, while in other embodiments according to the invention, the
20 hole may be etched throughout the entire membrane 18 using at least two subsequent etching steps.

The etching process for forming the holes in the membrane 18 may be combined with the deposition of conductive, e.g. metallic, insulating or semiconducting layers onto the membrane 18 and into the hole etched through
25 the membrane 18. These layers may be deposited by using methods that allow for accurate control of the thickness of the deposited layers. Preferably a self-limiting, monolayer-by-monolayer process may be used, such as e.g. Atomic Layer Deposition (ALD).

According to embodiments of the present invention, the inner surface of
30 the nanopore 13 may also be modified after pore-formation. In one embodiment, the inner surface may be morphologically altered, for example changed from amorphous to crystalline or vice versa, made porous, or having its micro-scale morphology changed, by rinsing a chemical fluid through the

nanopore, or by melting and condensing the inner surface. In another embodiment, the inner surface of the nanopore 13 may be changed by depositing a layer with accurately determined thickness into the nanopore 13. This deposition can occur either epitaxially (e.g. MBE, MOCVD, MOMBE) or
5 non-epitaxially (e.g. CVD, ...). In a preferred embodiment, a self-limiting, layer-by-layer process is used, as in Atomic layer deposition. In another embodiment, the surface of the nanopore 13 is changed by a combination of these steps.

Thereafter, the third electrode 16 is deposited onto the membrane (18),
10 extending into the nanopore 13. This may be performed by, for example, evaporation, epitaxy, sputtering or another method yielding accurate control over the layer thickness inside the nanopore 13. In a preferred embodiment, a self-limiting, monolayer-by-monolayer process may be used, as e.g. in atomic layer deposition. The third electrode 16 may comprise a metal such as e.g. Au,
15 Al, Ag, Pt, Cu, a degenerate semiconductor or an alloy. The conductivity of the third electrode 16 should be such, that the electrode forms an equipotential surface. The third electrode 16 may extend over the entire inner surface 20 of the nanopore 13. In another embodiment, this third electrode 16 does not extend throughout the entire nanopore 13.

20 In a third aspect, the present invention furthermore includes a computer program product which provides, when executed on a computing device, the functionality of a method for determining the voltage to be applied to the third electrode 16 when using the nanofluidic device 10 according to the present invention. The method comprises in a first step, loading a pre-determined
25 relationship between the applied voltage and the flow of charge carriers through at least one nanopore 13 through the membrane 18. In a second step, the flow of charge carriers required for a particular application is determined. Combination of the first step with the second step then yields the required voltage to be applied to the third electrode 16 which is integrated in the
30 nanopore 13. It has to be understood that it is the combination of the voltage applied to the third electrode 16 and the voltages applied to the first and second electrodes 11, 12, which is independent from the voltage applied to the third electrode 16, that allows for the modification of the electrical potential

distribution in the nanopore 13, and thus for the control of the flow of charge carriers through the nanopore 13.

Further, the present invention includes a data carrier such as a CD-ROM or a diskette which stores the above computer program product in a machine readable form and which executes the method for determining the voltage to be applied to the third electrode 16 when executed on a computing device. Nowadays, such software is often offered on the Internet or a company Intranet for download, hence the present invention includes transmitting the computer program product according to the present invention over a local or wide area network. The computing device may include one of a microprocessor and an FPGA.

It is to be understood that although preferred embodiments, specific constructions and configurations, as well as materials, have been discussed herein for devices according to the present invention, various changes or modifications in form and detail may be made without departing from the scope and spirit of this invention.

CLAIMS

- 1.- Nanofluidic device (10) for controlling the flow of charge carriers in at least one nanopore (13) by modifying the electrical potential distribution inside the nanopore (13), the device comprising:
- 5 - a membrane (18) having a thickness and having a first side and a second side,
- a first electrode (11) being positioned at the first side of the membrane (18) and a second electrode (12) being positioned at the second side of the membrane (18), the second side being opposite to the first side,
- 10 wherein the membrane (18) comprises at least one nanopore (13) extending through the membrane (18) over the thickness of the membrane (18) such as to form a channel connecting the first and second side of the membrane (18) and wherein a third electrode (16) is integrated in said at least one nanopore (13).
- 15 2.- Nanofluidic device (10) according to claim 1, wherein the at least one nanopore (13) is located at a predetermined position in the membrane (18).
- 3.- Nanofluidic device (10) according to any of the previous claims, the at least one nanopore (13) having a smallest pore diameter located within
- 20 one Debye screening length from said third electrode (16), wherein the smallest pore diameter is equal to or smaller than twice the Debye length of the nanopore (13).
- 4.- Nanofluidic device (10) according to any of the previous claims, wherein the nanopore (13) is defined by an IC-compatible fabrication method.
- 25 5.- Nanofluidic device (10) according to claim 4, wherein the nanopore (13) is defined by a lithographic process forming a lithographically defined nanopore pattern and is subsequently etched based on said lithographically defined nanopore pattern.
- 6.- Nanofluidic device (10) according to claim 5, wherein the lithographic
- 30 process is any of optical lithography, X-ray lithography, electron-beam lithography, focused ion beam lithography or nano-imprint lithography.

- 7.- Nanofluidic device (10) according to any of the previous claims, wherein the at least one nanopore (13) has a smallest pore diameter smaller than or equal to 100 nm.
- 8.- Nanofluidic device (10) according to claim 7, wherein the at least one nanopore (13) has a smallest pore diameter of between 1 and 100 nm, preferably between 10 and 15 nm and more preferably between 0.5 and 5 nm.
- 9.- Nanofluidic device (10) according to any of the previous claims, there being a first space between the first electrode (11) and the first side of the membrane (18) and a second space between the second electrode (12) and the second side of the membrane (18), wherein the first space is adapted for receiving a first solution (14) and the second space is adapted for receiving a second solution (15).
- 10.- Method for forming a nanofluidic device (10) for controlling the flow of charge carriers in a fluid through at least one nanopore (13) by modifying the electrical potential distribution inside the nanopore (13), the method comprising:
- providing a membrane (18) having a thickness and having a first side and a second side,
 - defining at least one nanopore (13) in said membrane (18),
 - etching said at least one nanopore (13) through the membrane (18) over the thickness of the membrane (18), such that the at least one nanopore (13) connects the first side and the second side by forming a channel,
 - providing a first electrode (11) at said first side of the membrane (18) and a second electrode (12) at said second side of the membrane (18),
 - providing at least one third electrode (16), said at least one third electrode (16) being integrated in the at least one nanopore (13).
- 11.- Method according to claim 10, the method comprising providing a plurality of nanopores (13) in the membrane (18), wherein a third electrode (16) is integrated into at least one nanopore (13).
- 12.- Method according to claim 11, furthermore comprising integrating a third electrode (16) in each of the plurality of nanopores (13), each third

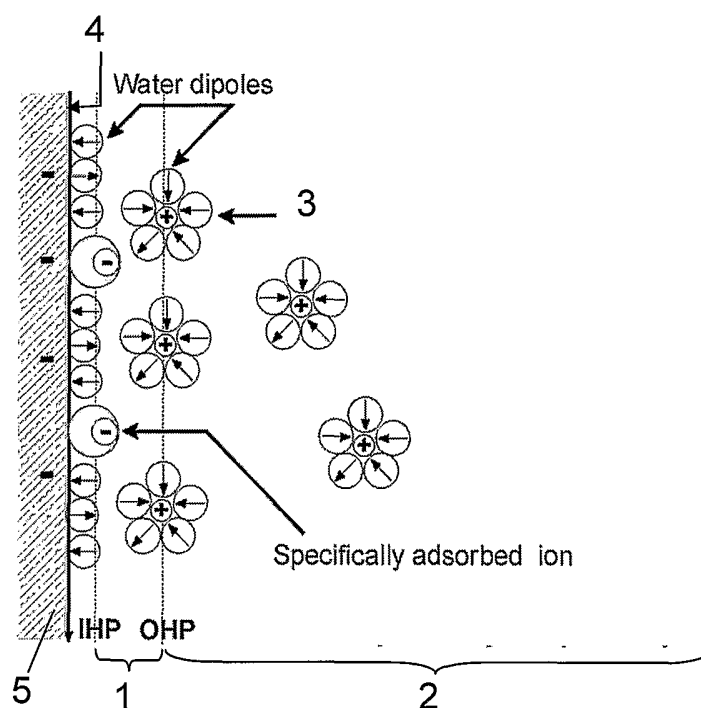
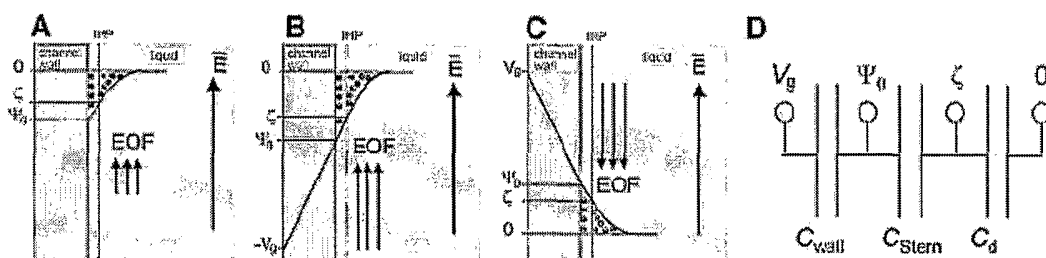
electrode (16) being adapted for being addressed independently from the other third electrodes (16).

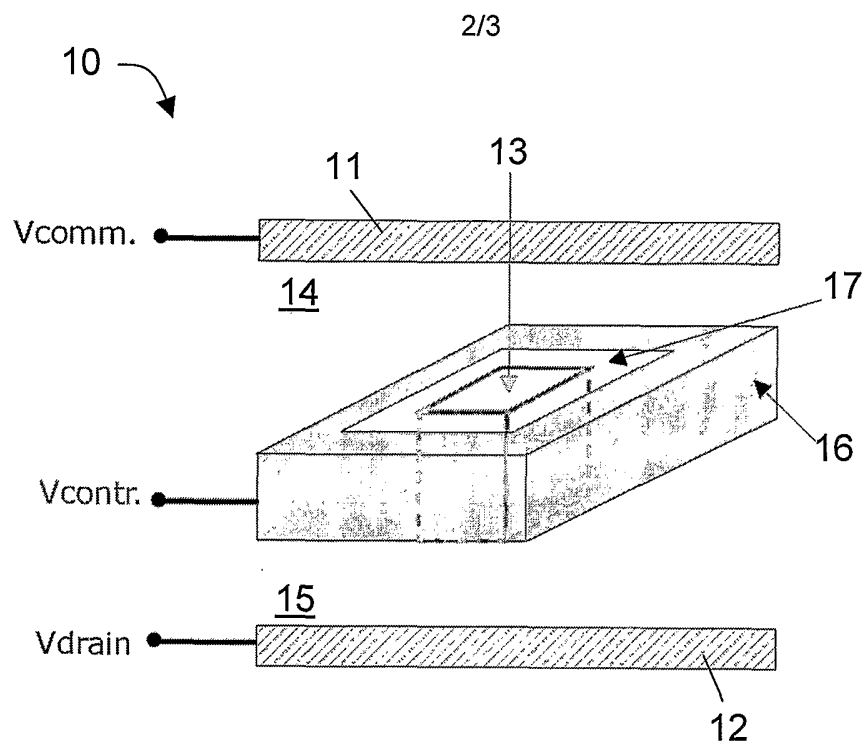
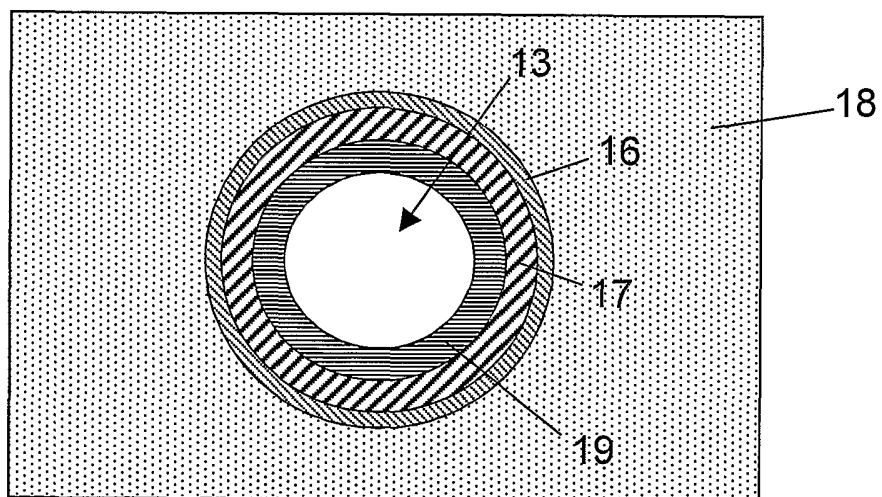
- 13.- Method according to any of claims 10 to 12, wherein providing at least one third electrode (16) comprises extending the third electrode (16) over an entire inner surface (20) of the at least one nanopore (13).
- 14.- Method according to any of claims 10 to 13, wherein providing at least one third electrode (16) comprises not extending the third electrode (16) over an entire inner surface (20) of the at least one nanopore (13).
- 15.- Method according to any of claims 10 to 14, wherein defining at least one nanopore (13) in the membrane (18) is performed by using an IC-compatible fabrication method.
- 16.- Method according to claim 15, wherein defining at least one nanopore (13) in the membrane (18) is performed lithographically.
- 17.- Method according to claim 16, wherein defining at least one nanopore (13) in the membrane (18) is performed by means of any of optical lithography, X-ray lithography, electron-beam lithography, focused ion beam lithography or nano-imprint lithography.
- 18.- Method according to any of claims 10 to 17, wherein etching said at least one nanopore (13) is performed by wet anisotropic etching or by dry anisotropic etching.
- 19.- Method according to any of claims 10 to 18, wherein defining at least one nanopore (13) is performed such that the at least one nanopore (13) has a smallest pore diameter equal to or smaller than the Debye length of the nanopore (13).
- 20.- Method according to claim 19, wherein defining at least one nanopore (13) is performed such that the at least one nanopore (13) has a smallest pore diameter equal to or smaller than 100 nm.
- 21.- Method according to claim 19 or 20, wherein defining at least one nanopore (13) is performed such that the at least one nanopore (13) has a pore diameter of between 1 and 100 nm, preferably between 10 and 15 nm and more preferably between 0.5 and 5 nm.
- 22.- Method for controlling the flow of charge carriers in at least one nanopore (13) in a membrane (18), the method comprising:

- 5 - providing a nanofluidic device (10) comprising a membrane (18) having a thickness and having a first side and a second side, a first electrode positioned (11) at the first side of the membrane (18) and a second electrode (12) positioned at a second side of the membrane (18), the membrane (18) comprising at least one nanopore (13) extending through the membrane (18) over the thickness of the membrane (18) such as to form a channel connecting the first and second side of the membrane (18) and a third electrode (16) integrated in the at least one nanopore (13),
- 10 - providing a first solution (14) between said first side and said first electrode (11) and a second solution (15) between said second side and said second electrode (12),
- applying a first and a second electrical voltage to respectively the first electrode (11) and second electrode (12), and
- 15 - applying a third electrical voltage to the third electrode (16), the third electrical voltage being independent from the first and second electrical voltage.
- 23.- Method for determining a voltage to be applied to an electrode for controlling the flow of charge carriers in at least one nanopore (13) in a membrane (18), the electrode being positioned in a nanopore (13) extending through a membrane (18) and forming a channel connecting a first side of the membrane (18) with a second side of the membrane (18), the method comprising:
- 20 - in a first step loading pre-determined relationships between the flow of charge carriers through the nanopore (13) and at least a voltage to be applied at the electrode,
- 25 - in a second step determining the required flow of charge carriers,
- selecting from a combination of the first step and the second step the voltage to be applied to the electrode.
- 30 24.- Computer program product which when executed on a processing device executes the method of claim 23.
- 25.- A machine readable data storage device storing the computer program product of claim 24.

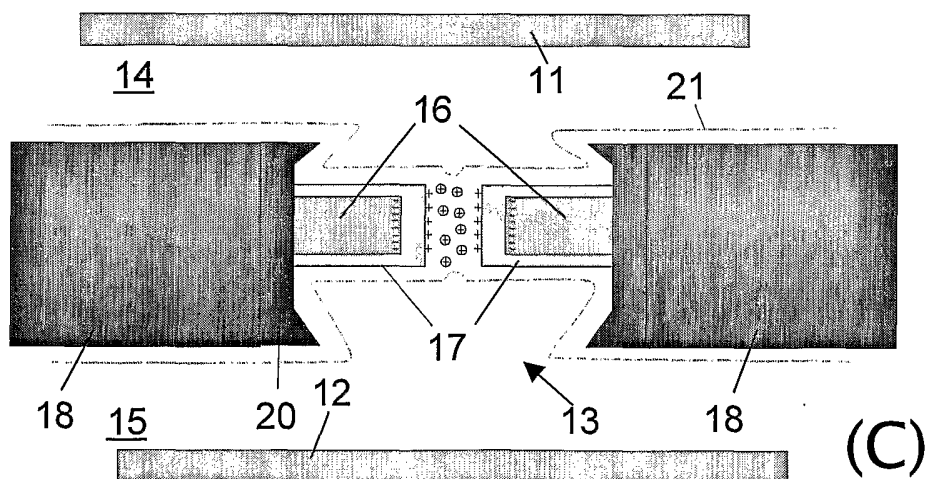
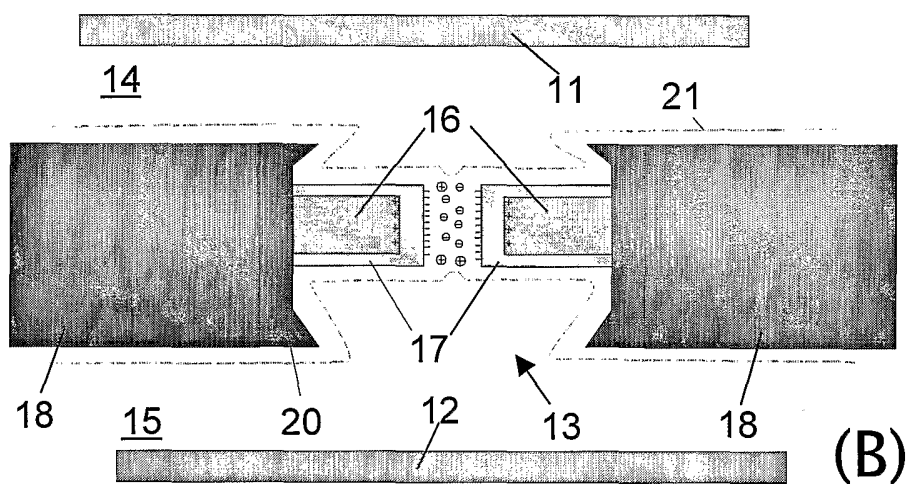
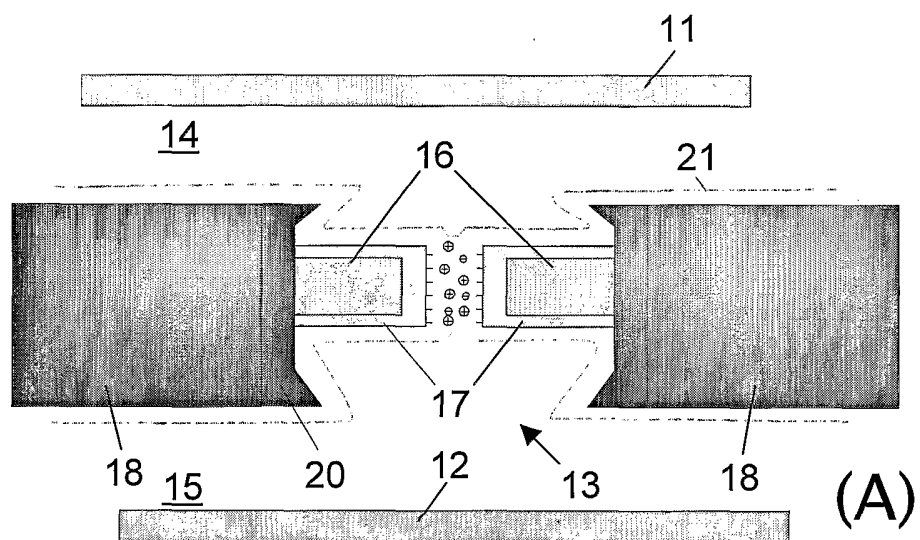
- 26.- Use of a nanofluidic device (10) according to any of claims 1 to 9 as a biosensor.
- 27.- Use of a nanofluidic device (10) according to any of claims 1 to 9 as an ionic transistor.

1/3

**FIG. 1****FIG. 2**

**FIG. 3****FIG. 4**

3/3

**FIG. 5**