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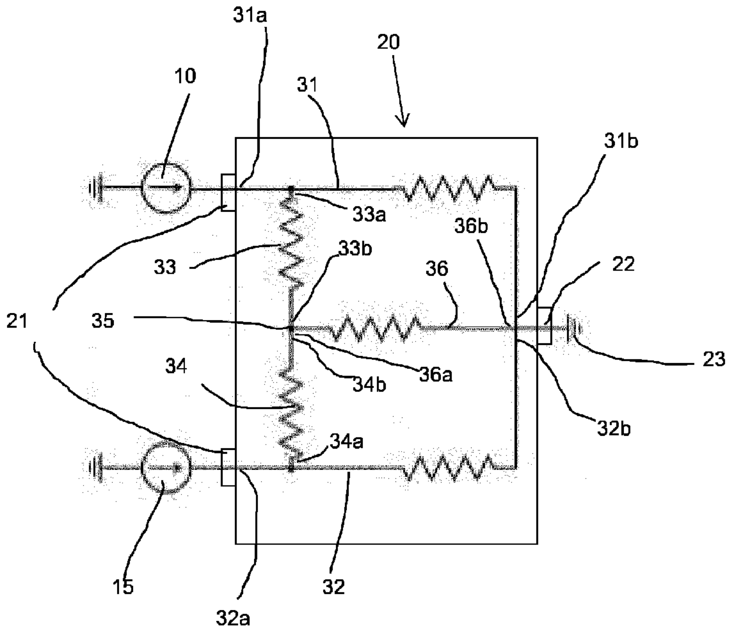

Fig. 2

(57) **Abstract:** Proposed is a nanofluidic device (20) comprising a first microchannel (31), a first branch nanochannel (33) branching off the first microchannel (31), a second microchannel (32), a second branch nanochannel (34) branching off the second microchannel (32), and a mixing nanochannel (36). The mixing nanochannel hydraulic resistance is at least one thousand times larger than each of the first and second microchannel hydraulic resistance. A junction (35) is provided which connects the first branch channel, the second branch channel and the mixing nanochannel. The junction (35): - receives a first laminar fluid flow from the first branch nanochannel (33) and a second laminar fluid flow from the second branch nanochannel (34), and - contacts the first and second laminar fluid flows with each other to establish diffusion mixing of the first and second fluids, and - discharge the mixed first and second fluids into the mixing nanochannel inlet (36a).

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Nanofluidic device, fluidic system and method for performing a test

The invention pertains to a nanofluidic device, a fluidic system and a method for performing a test. In particular, the proposed nanofluidic device, fluidic system, and method for performing a test are suitable for small scale fluidic testing.

An example of small scale fluidic testing is single-molecule detection testing. A common single-molecule detection test technique is fluorescence based detection, which includes for example confocal microscopy and imaging-based total-internal-reflection (TIRF) or wide field microscopy. These techniques are limited in their ability to properly spatially and temporally resolve fast processes or reactions, with sufficiently high resolution, in particular when this requires the parallel detection of many molecules. With these techniques it is therefore difficult to monitor chemical and enzymatic reactions in real time, in particular when such reactions happen fast, for example within milliseconds.

Another example of small scale fluidic testing involves averaged ensemble readouts as is used in experiments in life science, for example in correlation analysis which works on small ensembles of molecules.

It is the object of the invention to provide a nanofluidic device, fluidic system and method for performing a test which allow to study chemical and enzymatic reactions in real time in an improved way.

The object of the invention is achieved with a nanofluidic device which comprises:

- a first microchannel adapted to accommodate a first main fluid flow of the first fluid, which first microchannel has a fluid inlet which is in fluid communication with the first fluid source, and which first microchannel has a first microchannel hydraulic resistance,

- a first branch nanochannel which branches off the first microchannel, which first branch nanochannel has a first branch inlet and a first branch discharge, the first branch inlet being connected to the first microchannel, which first branch nanochannel is adapted to accommodate a first laminar fluid flow of the first fluid which originates from the first main fluid flow,

- a second microchannel adapted to accommodate a second main fluid flow of the second fluid, which second microchannel has a fluid inlet which is in fluid communication with the second fluid source, and which second microchannel has a second microchannel hydraulic resistance,

- a second branch nanochannel which branches off the second microchannel, which second branch nanochannel has a second branch inlet and a second branch discharge, the second branch inlet being connected to the second microchannel, which second branch nanochannel is adapted to accommodate a second laminar fluid flow of the second fluid which originates from the second main fluid flow,

- a mixing nanochannel, which has a mixing nanochannel inlet and a mixing nanochannel discharge, wherein the mixing nanochannel has a mixing nanochannel hydraulic resistance which is at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance,

- a junction, which is connected to the first branch discharge, the second branch discharge and the mixing nanochannel inlet, which junction is adapted:

  - to receive the first laminar fluid flow from the first branch nanochannel and the second laminar fluid flow from the second branch nanochannel, and

  - to contact the first and second laminar fluid flows with each other to establish diffusion mixing of the first and second fluids, and

  - to discharge the mixed first and second fluids from the junction into the mixing nanochannel inlet in the form of a combined laminar flow of the first and second fluid.

“Nanofluidic” in this context indicates that the device comprises a fluidic component, such as a channel that is adapted to accommodate a flowing fluid, which contains a functional dimension, *e.g.* the width or height of the channel that is adapted to accommodate the flowing fluid, of 500 nanometers or less.

The nanofluidic device comprises a first microchannel, a first branch nanochannel, a second microchannel, a second branch nanochannel, a mixing nanochannel and a junction. The first branch nanochannel, the second branch nanochannel and the mixing nanochannel meet at the junction. The first and second microchannels are indicated as “microchannels” to make clear that they are not the fluidic component having any functional dimension of 500 nanometers or less.

The first microchannel, the first branch nanochannel, the second microchannel, the second branch nanochannel, and/or the mixing nanochannel may be straight or may comprise one or more curves. Optionally, the first microchannel, the first branch nanochannel, the second microchannel, the second branch nanochannel, and/or the mixing nanochannel may have a meandering shape. The width and/or height of the first microchannel, the first branch nanochannel, the second microchannel, the second branch nanochannel, and the mixing nanochannel may be constant along their respective length or vary along their respective length.

The first microchannel is adapted to accommodate a first main fluid flow of a first fluid. The first microchannel has a fluid inlet which can be brought in fluid communication with a first fluid source, thereby allowing the first microchannel to receive first fluid from the first fluid source. The first microchannel has a first microchannel hydraulic resistance.

The first microchannel further has a fluid discharge.

The first branch nanochannel branches off the first microchannel. The first branch nanochannel has a first branch inlet which is connected to the first microchannel. The first branch nanochannel is adapted to accommodate a first laminar fluid flow of the first fluid. This first laminar fluid flow originates from the first main fluid flow. The first branch nanochannel further comprises a first branch discharge.

The first branch nanochannel has a first branch nanochannel hydraulic resistance. Preferably, the first branch nanochannel hydraulic resistance is at least one thousand times higher than the first microchannel hydraulic resistance.

The second microchannel is adapted to accommodate a second main fluid flow of a second fluid. The second microchannel has a fluid inlet which can be brought in fluid communication with a second fluid source, thereby allowing the second microchannel to receive second fluid from the second fluid source. The second microchannel has a second microchannel hydraulic resistance.

The second microchannel further has a fluid discharge.

The second branch nanochannel branches off the second microchannel. The second branch nanochannel has a second branch inlet which is connected to the second microchannel. The second branch nanochannel is adapted to accommodate a second laminar fluid flow of the second fluid. This second laminar fluid flow originates from the second main fluid flow. The second branch nanochannel further comprises a second branch discharge.

The second branch nanochannel has a second branch nanochannel hydraulic resistance. Preferably, the second branch nanochannel hydraulic resistance is at least one thousand times higher than the second microchannel hydraulic resistance.

The mixing nanochannel has a mixing nanochannel inlet and a mixing nanochannel discharge. The mixing nanochannel has a mixing nanochannel hydraulic resistance which is at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance.

The junction is connected to the first branch discharge, the second branch discharge and the mixing nanochannel inlet.

The junction is adapted to receive the first laminar fluid flow from the first branch nanochannel and the second laminar fluid flow from the second branch nanochannel. In addition, the junction is adapted to contact the first and second laminar fluid flows with each other to establish diffusion mixing of the first and second fluids. Furthermore, the junction is adapted to discharge the mixed first and second fluids from the junction into the mixing nanochannel inlet in the form of a combined laminar flow of the first and second fluid.

So, the first fluid flowing out of the first branch nanochannel in the form of the first laminar fluid flow and the second fluid flowing out of the second branch nanochannel in the form of the second laminar fluid flow meet each other at the junction. When they meet, diffusion mixing takes place. The first and second fluid leave the junction together via the mixing nanochannel inlet. Together they form a combined laminar flow, which then flows through the mixing nanochannel to the mixing nanochannel discharge.

The nanofluidic device according to the invention allows the parallelized detection of single molecules without the need of surface immobilisation as required in conventional imaging-based microscopy. Observations over tens of seconds of freely flowing molecules can be achieved by constraining the accessible volume such that it is smaller than the excitation/detection volume created by the conventional microscope objective, operating either in a confocal or wide field excitation arrangement.

The nanofluidic device according to the invention allows the precise synchronisation of chemical or enzymatic reactions: at the junctions, the two fluids, *e.g.* reactants, flowing from two separate fluid sources encounter each other for the very first time in a defined volume. This for example makes it possible to follow reactions, *e.g.* synthesis reactions, in real time.

The nanofluidic device when used in the fluidic system according to the invention allows various kinds of chemical reactions to be observed continuously and for a desired amount of time, as long as the two fluid sources provide the different fluids to the junction of the nanofluidic device.

The junction of the nanofluidic device works as a passive mixing device. No complex or actively controlled devices are required to obtain the desired combined laminar flow of the first and second fluid which is to be observed at the junction and/or at the mixing nanochannel.

The nanofluidic device is suitable for use in a fluidic system according to the invention.

The nanofluidic device in accordance with the invention can be any embodiment of the nanofluidic device as used in the embodiments of the fluidic system described below.

In a possible embodiment of the nanofluidic device, the mixing nanochannel hydraulic resistance is higher than each of the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance.

Optionally, in this embodiment of the nanofluidic device, the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance are both 50% or less of the mixing nanochannel hydraulic resistance, optionally between 25% and 35% of the mixing nanochannel hydraulic resistance. This embodiment provides stable mixing conditions at the junction, because the risk of parasitic flow from the first branch nanochannel into the second branch nanochannel or vice versa is reduced in case the flow rate of the first and/or second laminar fluid flow varies.

In a possible embodiment of the nanofluidic device, the mixing nanochannel has a larger cross sectional area than each of the first branch nanochannel and the second branch nanochannel. This allows to maintain the velocity of the combined laminar flow of the first and second fluid in the mixing nanochannel at a desired level. In particular, it prevents the velocity of the combined laminar flow of the first and second fluid in the mixing nanochannel to become larger than desired for certain experiments.

In a possible embodiment of the nanofluidic device, the geometries of the first and the second branch nanochannel are substantially the same. “Substantially the same” in this context means that the geometries of the first and the second branch nanochannel are designed to be the same. Inaccuracies caused by manufacturing are not taken into account.

This embodiment is in particular advantageous if the first fluid and the second fluid are to be mixed at the junction in a ratio of 1:1.

In an alternative embodiment of the nanofluidic device, the geometries of the first and the second branch nanochannel are different from each other. “Different” in this context means that the geometries of the first and the second branch nanochannel are designed to be different from each other. Inaccuracies caused by manufacturing are not taken into account.

This embodiment is in particular advantageous if the first fluid and the second fluid are to be mixed at the junction in a ratio different from 1:1.

In a possible embodiment of the nanofluidic device, at least one dimension of the first branch nanochannel, the second branch nanochannel and/or the mixing nanochannel is selected such that detection of single-molecules is possible. Optionally, this dimension is the height of the respective channel.

In a possible embodiment of the nanofluidic device, the first microchannel has a first part and a second part which are arranged in series with each other. The second part is arranged downstream of the first part and downstream of the connection of the first branch nanochannel to the first microchannel, and the cross sectional area of the second part is smaller than the cross sectional area of the first part. This helps to achieve an advantageous pressure distribution over the channels in the nanofluidic device

Alternatively or in addition, he second microchannel has a first part and a second part which are arranged in series with each other. The second part is arranged downstream of the first part and downstream of the connection of the second branch nanochannel to the second microchannel, and the cross sectional area of the second part is smaller than the cross sectional area of the first part. This helps to achieve an advantageous pressure distribution over the channels in the nanofluidic device.

In a possible embodiment of the nanofluidic device, at least one sensor is provided at the mixing nanochannel, which sensor is optionally adapted to measure at least one of electrical impedance, temperature, fluid velocity or particle velocity of the combined laminar flow of the first and second fluids through the mixing nanochannel.

Alternatively or in addition, at least one sensor may be provided at the first branch nanochannel, which sensor is optionally adapted to measure at least one of electrical impedance, temperature, fluid velocity or particle velocity of the first laminar fluid flow through the first branch nanochannel.

Alternatively or in addition, at least one sensor may be provided at the second branch nanochannel, which sensor is optionally adapted to measure at least one of electrical impedance, temperature, fluid velocity or particle velocity of the second laminar fluid flow through the second branch nanochannel.

In a possible embodiment, the nanofluidic device is provided in a standardized microfluidic platform such as a microfluidic chip. A microfluidic platform optionally provides reusable connections to the fluid reservoirs and which allow the integration to existing fluidic and microscopy instrumentation. The microfluidic chip that can be used for the nanofluidic device according to the invention is for example made of glass or silicon. Optionally, the microfluidic chip is transparent, *e.g.* for visible light, in the vicinity of the mixing nanochannel in order to allow observation, *e.g.* optical observation, of the mixing nanochannel and its contents. An advantage of a nanofluidic device in a microfluidic chip made of glass is that glass is chemically inert for many solvents. In addition, surface modifications to prevent non-specific adsorption of reactants and molecules can follow standardized protocols using PEGylation, lipid membranes or polypeptide Poly-l-lysine.

In a possible embodiment of the nanofluidic device, at least one of the first microchannel, second microchannel, first branch nanochannel, second branch nanochannel and mixing nanochannel has an open section along at least a part of its length. Optionally, this open section is in contact with the outside atmosphere or with a controlled atmosphere when tests are being performed.

In a possible embodiment of the nanofluidic device, the first microchannel and/or the second microchannel has at least one cross sectional dimension which is between 2 and 10 micrometers, optionally between 4 and 6 micrometers, optionally 5 micrometers. For example, in an embodiment in which the nanofluidic device is arranged in a microfluidic chip, the height of the first microchannel and/or the second microchannel is between 2 and 10 micrometers, optionally between 4 and 6 micrometers, optionally 5 micrometers. The height of the channel is the dimension which extends in the direction of the thickness of the microfluidic chip, so perpendicular to the planar top- or bottom surface of the microfluidic chip.

In a possible embodiment of the nanofluidic device, the width of the first microchannel and/or the second microchannel is 500 micrometers or less.

Optionally, width of the first microchannel and/or the second microchannel varies over the length of the respective microchannel.

In a possible embodiment of the nanofluidic device, the first branch nanochannel and/or the second branch nanochannel and/or the mixing nanochannel has at least one cross sectional dimension which is between 20 and 500 nanometers, optionally between 100 and 300 nanometers, optionally between 150 and 250 nanometers.

For example, the height of the first branch nanochannel and/or the second branch nanochannel and/or the mixing nanochannel is between 20 and 500 nanometers, optionally between 100 and 300 nanometers, optionally between 150 and 250 nanometers. With such dimensions, a laminar fluid flow is ensured.

In a possible embodiment of the nanofluidic device, the mixing nanochannel has a width between 20 nanometers and 20 micrometers, optionally between 1 and 7 micrometers, optionally between 3 and 5 micrometers, optionally 4 micrometers.

In a possible embodiment of the nanofluidic device, the mixing nanochannel has a length between 5 micrometers and 1 centimeter, optionally between 160 and 260 micrometers, optionally between 100 and 120 micrometers, optionally 110 micrometers.

In a possible embodiment, the mixing nanochannel has a width between 20 nanometers and 20 micrometers and a length between 5 micrometers and 1 centimeter and a height between 20 and 500 nanometers.

In a possible embodiment of the nanofluidic device, the first branch nanochannel and/or the second branch nanochannel has a width between 20 nanometers and 20 micrometers, optionally between 1 and 5 micrometers, optionally between 2 and 4 micrometers, optionally 3 micrometers.

In a possible embodiment of the nanofluidic device, the first branch nanochannel and/or the second branch nanochannel has a length between 5 micrometers and 1 centimeter, optionally between 14 and 34 micrometers, optionally between 20 and 28 micrometers, optionally 24 micrometers.

In a possible embodiment of the nanofluidic device, the first branch nanochannel and/or the second branch nanochannel has a width between 20 nanometers and 20 micrometers and a length between 5 micrometers and 1 centimeter and a height between 20 and 500 nanometers.

In a possible embodiment of the nanofluidic device, the first branch nanochannel and the second branch nanochannel extend in line with each other.

In an alternative embodiment of the nanofluidic device, the first branch nanochannel and the second branch nanochannel extend at a relative angle to each other.

In a possible embodiment, the nanofluidic device comprises an additional microchannel and an additional branch nanochannel. The additional microchannel is adapted to accommodate an additional main fluid flow of an additional fluid, which additional fluid is optionally different from the first fluid and the second fluid. The additional microchannel has a fluid inlet which allows the additional microchannel to receive the additional fluid from an additional fluid source. The additional microchannel has an additional microchannel hydraulic resistance.

The additional microchannel further has a fluid discharge.

The additional branch nanochannel branches off the additional microchannel. The additional branch nanochannel has an additional branch inlet which is connected to the additional microchannel. The additional branch nanochannel is adapted to accommodate an additional laminar fluid flow of the additional fluid. This additional laminar fluid flow originates from the additional main fluid flow. The additional branch nanochannel further comprises an additional branch discharge.

The additional branch nanochannel has an additional branch nanochannel hydraulic resistance. Preferably, the additional branch nanochannel hydraulic resistance is at least one thousand times higher than the additional microchannel hydraulic resistance.

The additional microchannel and/or the additional branch nanochannel may be straight or may comprise one or more curves. Optionally, the additional branch nanochannel may have a meandering shape. The width and/or height of the additional branch nanochannel may be constant along its length or vary along its length.

In this embodiment, the additional branch discharge is connected to the junction. The junction is connected to the first branch discharge, the second branch discharge, the additional branch discharge and the mixing nanochannel inlet.

The junction is adapted to receive the first laminar fluid flow from the first branch nanochannel, the second laminar fluid flow from the second branch nanochannel and the additional laminar fluid flow from the additional branch nanochannel. In addition, the junction is adapted to contact the first, second and additional laminar fluid flows with each other to establish diffusion mixing of the first, second and additional fluids. Furthermore, the junction is adapted to discharge the mixed first, second and additional fluids from the junction into the mixing nanochannel inlet in the form of a combined laminar flow of the first, second and additional fluid.

So, the first fluid flowing out of the first branch nanochannel in the form of the first laminar fluid flow, the second fluid flowing out of the second branch nanochannel in the form of the second laminar fluid flow and the additional fluid flowing out of the additional branch nanochannel in the form of the additional laminar fluid flow meet each other at the junction. When they meet, diffusion mixing takes place. The first, second and additional fluids leave the junction together via the mixing nanochannel inlet. Together they form a combined laminar flow, which then flows through the mixing nanochannel to the mixing nanochannel discharge.

Optionally, in this embodiment, multiple additional microchannels and additional branch nanochannels are present. Each additional additional microchannel has its own associated branch nanochannel which is connected to the junction.

In a variant of this embodiment, instead of a single junction, a first junction and a second junction are provided. In addition, the mixing nanochannel has a first mixing nanochannel inlet and a second mixing nanochannel inlet. The first junction is connected to the first mixing nanochannel inlet and to two of the first branch discharge, the second branch discharge and the additional branch discharge. The second junction is arranged downstream (as seen in the direction of the combined fluid flow through the mixing nanochannel) of the first junction, and is connected to the second mixing nanochannel inlet and to the remaining one of the first branch discharge, the second branch discharge and the additional branch discharge. Optionally, more than two junctions may be provided.

In a possible embodiment, the nanofluidic device further comprises an additional mixing nanochannel. This additional mixing nanochannel has an additional mixing nanochannel inlet and an additional mixing nanochannel discharge. The additional mixing nanochannel has an additional mixing nanochannel hydraulic resistance which is optionally at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance.

In this embodiment, the junction is optionally connected to the first branch discharge, the second branch discharge, the mixing nanochannel inlet and the additional mixing nanochannel inlet. Optionally, a plurality of junctions is provided, and each junction is arranged to discharge a combined fluid flow in at least one of the mixing nanochannels.

This embodiment allows to spatially separate molecules of the combined fluid flow after the mixing, which makes it easier to find and monitor single entities over a longer time as they are less prone to cross their diffusion paths with other molecules. Further, this arrangement allows to arrange the individual nanochannels in such a way that various mixing ratios and/or concentration gradients of entities from the first fluid and/or the second fluid can be obtained in the mixing nanochannels.

In a possible embodiment of the nanofluidic device, a first secondary branch nanochannel is provided, which first secondary branch nanochannel branches off the first microchannel. The first secondary branch nanochannel is present in addition to the first branch nanochannel. The first secondary branch nanochannel has a first secondary branch inlet and a first secondary branch discharge. The first secondary branch inlet is connected to the first microchannel. The first secondary branch nanochannel is adapted to accommodate a first secondary laminar fluid flow of the first fluid which originates from the first main fluid flow. The first secondary branch discharge is connected to the junction, or if multiple junctions are present, to one of the junctions.

Alternatively or in addition, in the nanofluidic device, a second secondary branch nanochannel is provided, which second secondary branch nanochannel branches off the second microchannel. The second secondary branch nanochannel is present in addition to the second branch nanochannel. The second secondary branch nanochannel has a second secondary branch inlet and a second secondary branch discharge. The second secondary branch inlet is connected to the second microchannel. The second secondary branch nanochannel is adapted to accommodate a second secondary laminar fluid flow of the second fluid which originates from the second main fluid flow. The second secondary branch discharge is connected to the junction, or if multiple junctions are present, to one of the junctions.

Alternatively or in addition, in a variant of the embodiment in which an additional microchannel is present, in the nanofluidic device, an additional secondary branch nanochannel is provided, which additional secondary branch nanochannel branches off the additional microchannel. The additional secondary branch nanochannel is present in addition to the additional branch nanochannel. The additional secondary branch nanochannel has an additional secondary branch inlet and an additional secondary branch discharge. The additional secondary branch inlet is connected to the additional microchannel. The additional secondary branch nanochannel is adapted to accommodate an additional secondary laminar fluid flow of the additional fluid which originates from the additional main fluid flow. The additional secondary branch discharge is connected to the junction, or if multiple junctions are present, to one of the junctions.

In a possible embodiment, the nanofluidic device comprises a common device discharge. The first microchannel discharge, the second microchannel discharge and the mixing nanochannel discharge are connected to the common device discharge. From the common device discharge, the fluid flows from the first microchannel, second microchannel and mixing nanochannel are discharged together from the nanofluidic device. The common device discharge may for example be connected to a fluid collection container or to waste.

In a variant of this embodiment, a further microchannel, *e.g.* an additional microchannel, which comprises a further microchannel discharge, is present in the nanofluidic device. The further microchannel discharge is optionally also connected to the common device discharge.

In a variant of this embodiment, an additional mixing nanochannel, *e.g.* a second mixing nanochannel, which comprises an additional mixing nanochannel discharge is present in the nanofluidic device. The additional mixing nanochannel discharge is optionally also connected to the common device discharge.

In an alternative embodiment, the nanofluidic device comprises at least one combined device discharge. At least two of the first microchannel discharge, the second microchannel discharge and the mixing nanochannel discharge are connected to the combined device discharge. From the combined device discharge, the fluid flows from the respective channels that are connected to the combined device discharge are discharged together from the nanofluidic device. The combined device discharge may for example be connected to a fluid collection container or to waste.

In a variant of this embodiment, a further microchannel, *e.g.* an additional microchannel, which comprises a further microchannel discharge, is present in the nanofluidic device. In this variant, at least two of the first microchannel discharge, the second microchannel discharge, the further microchannel discharge and the mixing nanochannel discharge are connected to the combined device discharge.

In a variant of this embodiment, an additional mixing nanochannel, *e.g.* a second mixing nanochannel, which comprises an additional mixing nanochannel discharge is present in the nanofluidic device. In this variant, at least two of the first microchannel discharge, the second microchannel discharge, the mixing nanochannel discharge and the additional mixing nanochannel discharge are connected to the combined device discharge.

In a variant of this embodiment, an additional mixing nanochannel, *e.g.* a second mixing nanochannel, which comprises an additional mixing nanochannel discharge is present in the nanofluidic device. In addition, a further microchannel, *e.g.* an additional microchannel, which comprises a further microchannel discharge, is present in the nanofluidic device. In this variant, at least two of the first microchannel discharge, the second microchannel discharge, the further microchannel discharge, the mixing nanochannel discharge and the additional mixing nanochannel discharge are connected to the combined device discharge.

In this alternative embodiment (including its variants as described above) the discharges of all microchannels and mixing nanochannels that are present in the nanofluidic device may be connected to a combined device discharge. Alternatively, the discharge of one or more of the individual microchannels and mixing nanochannels that are present in the nanofluidic device may be connected to a fluid collection container or to waste directly.

In a further alternative embodiment, the discharges of all of the individual microchannels and mixing nanochannels that are present in the nanofluidic device are be connected to a fluid collection container or to waste directly, *e.g.* via an individual device discharge.

The invention further pertains to a fluidic system for testing, which comprises

- a first fluid source which is adapted to supply a first fluid, and
- a second fluid source which is adapted to supply a second fluid, and,
- a nanofluidic device, which comprises:
  - a first microchannel adapted to accommodate a first main fluid flow of the first fluid, which first microchannel has a fluid inlet which is in fluid communication with the first fluid source, and which first microchannel has a first microchannel hydraulic resistance,
  - a first branch nanochannel which branches off the first microchannel, which first branch nanochannel has a first branch inlet and a first branch discharge, the first branch inlet being connected to the first microchannel, which first branch nanochannel is adapted to accommodate a first laminar fluid flow of the first fluid which originates from the first main fluid flow,
  - a second microchannel adapted to accommodate a second main fluid flow of the second fluid, which second microchannel has a fluid inlet which is in fluid communication with the second fluid source, and which second microchannel has a second microchannel hydraulic resistance,

- a second branch nanochannel which branches off the second microchannel, which second branch nanochannel has a second branch inlet and a second branch discharge, the second branch inlet being connected to the second microchannel, which second branch nanochannel is adapted to accommodate a second laminar fluid flow of the second fluid which originates from the second main fluid flow,

- a mixing nanochannel, which has a mixing nanochannel inlet and a mixing nanochannel discharge, wherein the mixing nanochannel has a mixing nanochannel hydraulic resistance which is at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance,

- a junction, which is connected to the first branch discharge, the second branch discharge and the mixing nanochannel inlet, which junction is adapted:

- to receive the first laminar fluid flow from the first branch nanochannel and the second laminar fluid flow from the second branch nanochannel, and

- to contact the first and second laminar fluid flows with each other to establish diffusion mixing of the first and second fluids, and

- to discharge the mixed first and second fluids from the junction into the mixing nanochannel inlet in the form of a combined laminar flow of the first and second fluid,

- an observation device, which is adapted to observe at least one parameter of at least one of the first laminar fluid flow at the first branch nanochannel, the second laminar fluid flow at the second branch nanochannel and/or the combined laminar flow of the first and second fluid at the mixing nanochannel and/or the junction.

The fluidic system according to the invention comprises a first fluid source, a second fluid source, a nanofluidic device and an observation device.

The first fluid source is adapted to supply a first fluid and the second fluid source is adapted to supply a second fluid. Preferably, the fluid is a liquid. Usually, the first fluid and the second fluid are different from each other. Optionally, at least one of the first fluid and/or second fluid is a solution or a suspension. Optionally, at least one of the first fluid and/or second fluid contains biological material. Optionally, at least one of the first fluid and/or second fluid contains DNA polymerases and recessed DNA. Optionally, the other one of the first fluid and/or second fluid contains nucleoside triphosphates. Optionally, at least one of the first fluid and/or second fluid contains DNA polymerases and recessed DNA . Optionally, the other one of the first fluid and/or second fluid contains nucleoside triphosphates.

The term recessed DNA in this context refers to DNA having sticky ends. As is well known in the art, double stranded DNA fragments can have blunt ends, i.e. where the 5'-end of the first strand is located opposite the 3'-end of the second strand of the double stranded DNA (*e.g.* ….acgtgctag-3'/…..tgcacgatc-5'). Double stranded DNA fragments can also have sticky (also termed staggered and/or cohesive) ends. In the case of sticky ends, a first strand has an overhang compared to the second strand and hence the second strand is recessed compared to the first strand. In other words, the 5'-end of the first strand is not located opposite the 3'-end of the second strand of the double stranded DNA (*e.g.* ….acgtgctag-3'/…..tgcacgatcctag-5')

In a possible embodiment of the fluidic system according to the invention, the first fluid source comprises a pump, for example a syringe pump, or a gravity controlled fluid supply.

In a possible embodiment, the second fluid source comprises a pump, for example a syringe pump, or gravity controlled fluid supply.

In a possible embodiment, each of the first fluid supply source and the second fluid source comprises a pump, for example a syringe pump, or gravity controlled fluid supply.

For example, the first and/or second fluid sources deliver fluid at 1-10 microliters per hour.

The fluidic system according to the invention comprises a nanofluidic device according to the invention. "Nanofluidic" in this context indicates that the device comprises a fluidic component, such as a channel that is adapted to accommodate a flowing fluid, which contains a functional dimension, *e.g.* the width or height of the channel that is adapted to accommodate the flowing fluid, of 500 nanometers or less.

The nanofluidic device comprises a first microchannel, a first branch nanochannel, a second microchannel, a second branch nanochannel, a mixing nanochannel and a junction. The first branch nanochannel, the second branch nanochannel and the mixing nanochannel meet at the junction. The first and second microchannels are indicated as "microchannels" to make clear that they are not the fluidic component having any functional dimension of 500 nanometers or less.

The first microchannel, the first branch nanochannel, the second microchannel, the second branch nanochannel, and/or the mixing nanochannel may be straight or may comprise one or more curves. Optionally, the first microchannel, the first branch nanochannel, the second microchannel, the second branch nanochannel, and/or the mixing nanochannel may have a meandering shape. The width and/or height of the first microchannel, the first branch nanochannel, the second microchannel, the second branch nanochannel, and the mixing nanochannel may be constant along their respective length or vary along their respective length.

The first microchannel is adapted to accommodate a first main fluid flow of the first fluid. The first microchannel has a fluid inlet which is in fluid communication with the first fluid source, which allows the first microchannel to receive first fluid from the first fluid source. The first microchannel has a first microchannel hydraulic resistance.

The first microchannel further has a fluid discharge.

The first branch nanochannel branches off the first microchannel. The first branch nanochannel has a first branch inlet which is connected to the first microchannel. The first branch nanochannel is adapted to accommodate a first laminar fluid flow of the first fluid. This first laminar fluid flow originates from the first main fluid flow. The first branch nanochannel further comprises a first branch discharge.

The first branch nanochannel has a first branch nanochannel hydraulic resistance. Preferably, the first branch nanochannel hydraulic resistance is at least one thousand times higher than the first microchannel hydraulic resistance.

The second microchannel is adapted to accommodate a second main fluid flow of the second fluid. The second microchannel has a fluid inlet which is in fluid communication with the second fluid source, which allows the second microchannel to receive second fluid from the second fluid source. The second microchannel has a second microchannel hydraulic resistance.

The second microchannel further has a fluid discharge.

The second branch nanochannel branches off the second microchannel. The second branch nanochannel has a second branch inlet which is connected to the second microchannel. The second branch nanochannel is adapted to accommodate a second laminar fluid flow of the second fluid. This second laminar fluid flow originates from the second main fluid flow. The second branch nanochannel further comprises a second branch discharge.

The second branch nanochannel has a second branch nanochannel hydraulic resistance. Preferably, the second branch nanochannel hydraulic resistance is at least one thousand times higher than the second microchannel hydraulic resistance.

The mixing nanochannel has a mixing nanochannel inlet and a mixing nanochannel discharge. The mixing nanochannel has a mixing nanochannel hydraulic resistance which is at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance.

The junction is connected to the first branch discharge, the second branch discharge and the mixing nanochannel inlet.

The junction is adapted to receive the first laminar fluid flow from the first branch nanochannel and the second laminar fluid flow from the second branch nanochannel. In addition, the junction is adapted to contact the first and second laminar fluid flows with each other to establish diffusion mixing of the first and second fluids. Furthermore, the junction is adapted to discharge the mixed first and second fluids from the junction into the mixing nanochannel inlet in the form of a combined laminar flow of the first and second fluid.

So, the first fluid flowing out of the first branch nanochannel in the form of the first laminar fluid flow and the second fluid flowing out of the second branch nanochannel in the form of the second laminar fluid flow meet each other at the junction. When they meet, diffusion mixing takes place. The first and second fluid leave the junction together via the mixing nanochannel inlet. Together they form a combined laminar flow, which then flows through the mixing nanochannel to the mixing nanochannel discharge.

The observation device is adapted to observe, *e.g.* measure, detect and/or monitor, at least one parameter of at least one of the first laminar fluid flow at the first branch nanochannel, the second laminar fluid flow at the second branch nanochannel and/or the combined laminar flow of the first and second fluid at the mixing nanochannel and/or at the junction. For example, the observation device may contain a camera which has at least a part of the first branch nanochannel and/or at least a part of the second branch nanochannel and/or at least a part of the mixing nanochannel and/or the junction in its field of view, and/or a sensor to measure one or more parameters of the first laminar fluid flow and/or the second laminar fluid flow and/or the combined laminar flow of the first and second fluid.

Monitoring optionally involves detecting and/or measuring a parameter as a function of for example time or distance.

If the monitoring involves detecting and/or measuring a parameter as a function of distance, the parameter will be measured and/or detected at two or more distinct locations within a monitoring distance window or continuously within at least a part of the monitoring distance window. The monitoring distance window preferably includes at least a part of the mixing nanochannel. Optionally, the monitoring distance window includes the junction. Optionally, the monitoring distance window includes at least a part of the first branch nanochannel and/or at least a part of the second branch nanochannel. Optionally, the monitoring distance window includes at least two of at least a part of the first branch nanochannel, at least a part of the second branch nanochannel, the junction and/or at least a part of the mixing nanochannel.

If the monitoring involves detecting and/or measuring a parameter as a function of time, the parameter will be measured or detected at two or more distinct points in time within a monitoring time window or continuously within at least a part of the monitoring time window.

Examples of the parameter to be observed are fluorescence from an individual particle (*e.g.* to determine the position of an individual particle in one or two directions, *e.g.* with a precision in the tens of nanometers range, *e.g.* with 1-10 ms or larger time resolution), energy transfer between a donor and a acceptor fluorophore (*e.g.* by simultaneous detection in a green and red fluorescence detection channel on a camera), electrical impedance, temperature, flow rate, particle velocity and/or flow velocity.

The nanofluidic device as used in the fluidic system according to the invention allows the parallelized detection of single molecules without the need of surface immobilisation as required in conventional imaging-based microscopy. Observations over tens of seconds of freely flowing molecules can be achieved by constraining the accessible volume such that it is smaller than the excitation/detection volume created by the conventional microscope objective, operating either in a confocal or wide field excitation arrangement.

The nanofluidic device as used in the fluidic system according to the invention allows the precise synchronisation of chemical or enzymatic reactions: at the junctions, the two fluids, *e.g.* reactants, flowing from two separate fluid sources encounter each other for the very first time in a defined volume. This for example makes it possible to follow reactions, *e.g.* synthesis reactions, in real time.

The nanofluidic device as used in the fluidic system according to the invention allows various kinds of chemical reactions to be observed continuously and for a desired amount of time, as long as the two fluid sources provide the different fluids to the junction of the nanofluidic device.

The junction of the nanofluidic device works as a passive mixing device. No complex or actively controlled devices are required to obtain the desired combined laminar flow of the first and second fluid which is to be observed at the junction and/or at the mixing nanochannel.

In a possible embodiment of the fluidic system according to the invention, in the nanofluidic device, the mixing nanochannel hydraulic resistance is higher than each of the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance.

In this embodiment, for example the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance are both 50% or less of the mixing nanochannel hydraulic resistance. Optionally, the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance are both between 25% and 35% of the mixing nanochannel hydraulic resistance.

In a possible embodiment of the fluidic system according to the invention, in the nanofluidic device, the mixing nanochannel has a larger cross sectional area than each of the first branch nanochannel and the second branch nanochannel.

In a possible embodiment of the fluidic system according to the invention, at least one dimension of the first branch nanochannel, the second branch nanochannel and/or the mixing nanochannel is selected such that detection of single-molecules is possible. Optionally, this dimension is the height of the respective channel.

In a possible embodiment of the fluidic system according to the invention, in the nanofluidic device, the geometries of the first and the second branch nanochannel are substantially the same. “Substantially the same” in this context means that the geometries of the first and the second branch nanochannel are designed to be the same. Inaccuracies caused by manufacturing are not taken into account.

This embodiment is in particular advantageous if the first fluid and the second fluid are to be mixed at the junction in a ratio of 1:1.

In an alternative possible embodiment, in the nanofluidic device, the geometries of the first and the second branch nanochannel are different. “Different” in this context means that the geometries of the first and the second branch nanochannel are designed to be different from each other. Inaccuracies caused by manufacturing are not taken into account.

This embodiment is in particular advantageous if the first fluid and the second fluid are to be mixed at the junction in a ratio different from 1:1.

In a possible embodiment of the fluidic system according to the invention, in the nanofluidic device, the first microchannel has a first part and a second part which are arranged in series with each other. The second part is arranged downstream of the first part and downstream of the connection of the first branch nanochannel to the first microchannel. In this embodiment, the cross sectional area of the second part is smaller than the cross sectional area of the first part. This helps to achieve an advantageous pressure distribution over the channels in the nanofluidic device.

Alternatively or in addition, the second microchannel has a first part and a second part which are arranged in series with each other. The second part is arranged downstream of the first part and downstream of the connection of the second branch nanochannel to the second microchannel. In this embodiment, the cross sectional area of the second part is smaller than the cross sectional area of the first part. This helps to achieve an advantageous pressure distribution over the channels in the nanofluidic device.

In a possible embodiment of the fluidic system according to the invention, the observation device is adapted to monitor at least one parameter of the combined laminar flow of the first and second fluids over a monitoring path which extends over at least a part of the mixing nanochannel.

Alternatively or in addition, the observation device is adapted to monitor at least one parameter of the first laminar fluid flow over a monitoring path which extends over at least a part of the first branch nanochannel.

Alternatively or in addition, the observation device is adapted to monitor at least one parameter of the second laminar fluid flow over a monitoring path which extends over at least a part of the second branch nanochannel.

In a possible embodiment of the fluidic system according to the invention, at least one sensor is provided at the mixing nanochannel, which sensor is optionally adapted to measure at least one of electrical impedance, temperature, fluid velocity or particle velocity of the combined laminar flow of the first and second fluids through the mixing nanochannel.

Alternatively or in addition, at least one sensor may be provided at the first branch nanochannel, which sensor is optionally adapted to measure at least one of electrical impedance, temperature, fluid velocity or particle velocity of the first laminar fluid flow through the first branch nanochannel.

Alternatively or in addition, at least one sensor may be provided at the second branch nanochannel, which sensor is optionally adapted to measure at least one of electrical impedance, temperature, fluid velocity or particle velocity of the second laminar fluid flow through the second branch nanochannel.

In a possible embodiment of the fluidic system according to the invention, the observation device comprises a fluorescence detector.

In a possible embodiment of the fluidic system according to the invention, the nanofluidic device is provided in a standardized microfluidic platform such as a microfluidic chip. A microfluidic platform optionally provides reusable connections to the fluid reservoirs and which allow the integration to existing fluidic and microscopy instrumentation. The microfluidic chip that can be used for the nanofluidic device according to the invention is for example made of glass or silicon. Optionally, the microfluidic chip is transparent, *e.g.* for visible light, in the vicinity of the mixing nanochannel in order to allow observation, *e.g.* optical observation, of the mixing nanochannel and its contents. An advantage of a nanofluidic device in a microfluidic chip made of glass is that glass is chemically inert for many solvents. In addition, surface modifications to prevent non-specific adsorption of reactants and molecules can follow standardized protocols using PEGylation, lipid membranes or polypeptide Poly-l-lysine.

In a possible embodiment of the fluidic system according to the invention, the observation device comprises a microscope objective, and the distance between the outside surface of the nanofluidic device on the side where the microscope objective is arranged, and the wall of the channel in which the flow is to be observed is 200 micrometers or less, optionally 175 micrometers or less. The channel in which the flow is to be observed is at least one of the first branch nanochannel (in its entirety or a part thereof), the second branch nanochannel (in its entirety or a part thereof), the junction, and/or the mixing nanochannel (in its entirety or a part thereof). This allows the use of a microscope objective with a high numerical aperture.

The microfluidic chip can be made according to known methods, for example by etching the channels in a glass plate and covering this plate by a covering layer so as to close the channels over their length.

In a possible embodiment of the fluidic system according to the invention, the nanofluidic device is provided in a microfluidic chip and the fluidic system comprises a holder for in a releasable way holding said microfluidic chip. The microfluidic chip is for example made of glass or silicon.

This embodiment allows a quick exchange of one nanofluidic device for another, for example when different flow conditions or a different mixing ratio is desired for a subsequent experiment.

In a possible embodiment of the fluidic system according to the invention, at least one of the first microchannel, second microchannel, first branch nanochannel, second branch nanochannel and mixing nanochannel has an open section along at least a part of its length. Optionally, this open section is in contact with the outside atmosphere or with a controlled atmosphere when tests are being performed.

In a possible embodiment of the fluidic system according to the invention, the first microchannel and/or the second microchannel has at least one cross sectional dimension which is between 2 and 10 micrometers, optionally between 4 and 6 micrometers, optionally 5 micrometers. For example, in an embodiment in which the nanofluidic device is arranged in a microfluidic chip, the height of the first microchannel and/or the second microchannel is between 2 and 10 micrometers, optionally between 4 and 6 micrometers, optionally 5 micrometers. The height of the channel is the dimension which extends in the direction of the thickness of the microfluidic chip, so perpendicular to the planar top- or bottom surface of the microfluidic chip.

In a possible embodiment of the fluidic system according to the invention, the width of the first microchannel and/or the second microchannel is 500 micrometers or less.

Optionally, the width of the first microchannel and/or the second microchannel varies over the length of the respective microchannels.

In a possible embodiment of the fluidic system according to the invention, the first branch nanochannel and/or the second branch nanochannel and/or the mixing nanochannel has at least one cross sectional dimension which is between 20 and 500 nanometers, optionally between 100 and 300 nanometers, optionally between 150 and 250 nanometers.

For example, the height of the first branch nanochannel and/or the second branch nanochannel and/or the mixing nanochannel is between 20 and 500 nanometers, optionally between 100 and 300 nanometers, optionally between 150 and 250 nanometers. With such dimensions, a laminar fluid flow is ensured.

In a possible embodiment of the fluidic system according to the invention, the mixing nanochannel has a width between 20 nanometers and 20 micrometers, optionally between 1 and 7 micrometers, optionally between 3 and 5 micrometers, optionally 4 micrometers.

In a possible embodiment of the fluidic system according to the invention, the mixing nanochannel has a length between 5 micrometers and 1 centimeter, optionally between 160 and 260 micrometers, optionally between 100 and 120 micrometers, optionally 110 micrometers.

In a possible embodiment of the fluidic system according to the invention, the mixing nanochannel has a width between 20 nanometers and 20 micrometers and a length between 5 micrometers and 1 centimeter and a height between 20 and 500 nanometers.

In a possible embodiment of the fluidic system according to the invention, the first branch nanochannel and/or the second branch nanochannel has a width between 20 nanometers and 20 micrometers, optionally between 1 and 5 micrometers, optionally between 2 and 4 micrometers, optionally 3 micrometers.

In a possible embodiment, the first branch nanochannel and/or the second branch nanochannel has a length between 5 micrometers and 1 centimeter, optionally between 14 and 34 micrometers, optionally between 20 and 28 micrometers, optionally 24 micrometers.

In a possible embodiment, the first branch nanochannel and/or the second branch nanochannel has a width between 20 nanometers and 20 micrometers and a length between 5 micrometers and 1 centimeter and a height between 20 and 500 nanometers.

In a possible embodiment of the fluidic system according to the invention, in the nanofluidic device, the first branch nanochannel and the second branch nanochannel extend in line with each other.

In an alternative embodiment, in the nanofluidic device, the first branch nanochannel and the second branch nanochannel extend at a relative angle to each other.

In a possible embodiment, the fluidic system according to the invention further comprises an additional fluid source which is adapted to supply an additional fluid. The additional fluid is different from the first and the second fluid. The additional fluid is for example a reactant or a dilution fluid.

In this embodiment, the fluidic system further comprises a nanofluidic device having an additional microchannel and an additional branch nanochannel. The additional microchannel is adapted to accommodate an additional main fluid flow of the additional fluid. The additional microchannel has a fluid inlet which is in fluid communication with the additional fluid source, which allows the additional microchannel to receive the additional fluid from the additional fluid source. The additional microchannel has an additional microchannel hydraulic resistance.

The additional microchannel further has a fluid discharge.

The additional branch nanochannel branches off the additional microchannel. The additional branch nanochannel has an additional branch inlet which is connected to the additional microchannel. The additional branch nanochannel is adapted to accommodate an additional laminar fluid flow of the additional fluid. This additional laminar fluid flow originates from the additional main fluid flow. The additional branch nanochannel further comprises an additional branch discharge.

The additional branch nanochannel has an additional branch nanochannel hydraulic resistance. Preferably, the additional branch nanochannel hydraulic resistance is at least one thousand times higher than the additional microchannel hydraulic resistance.

The additional microchannel and/or the additional branch nanochannel may be straight or may comprise one or more curves. Optionally, the additional branch nanochannel may have a meandering shape. The width and/or height of the additional branch nanochannel may be constant along its length or vary along its length.

In this embodiment, the additional branch discharge is connected to the junction. The junction is connected to the first branch discharge, the second branch discharge, the additional branch discharge and the mixing nanochannel inlet.

The junction is adapted to receive the first laminar fluid flow from the first branch nanochannel, the second laminar fluid flow from the second branch nanochannel and the additional laminar fluid flow from the additional branch nanochannel. In addition, the junction is adapted to contact the first, second and additional laminar fluid flows with each other to establish diffusion mixing of the first, second and additional fluids. Furthermore, the junction is adapted to discharge the mixed first, second and additional fluids from the junction into the mixing nanochannel inlet in the form of a combined laminar flow of the first, second and additional fluid.

So, the first fluid flowing out of the first branch nanochannel in the form of the first laminar fluid flow, the second fluid flowing out of the second branch nanochannel in the form of the second laminar fluid flow and the additional fluid flowing out of the additional branch nanochannel in the form of the additional laminar fluid flow meet each other at the junction. When they meet, diffusion mixing takes place. The first, second and additional fluids leave the junction together via the mixing nanochannel inlet. Together they form a combined laminar flow, which then flows through the mixing nanochannel to the mixing nanochannel discharge.

Optionally, in this embodiment, multiple additional fluid sources are present. Optionally, each additional fluid source is adapted to supply a different additional fluid. The additional fluids optionally are also different from the first and the second fluid. In this embodiment, each additional fluid source has its own associated additional microchannel, which in turn has its own associated branch nanochannel which is connected to the junction.

In a variant of this embodiment, instead of a single junction, a first junction and a second junction are provided. In addition, the mixing nanochannel has a first mixing nanochannel inlet and a second mixing nanochannel inlet. The first junction is connected to the first mixing nanochannel inlet and to two of the first branch discharge, the second branch discharge and the additional branch discharge. The second junction is arranged downstream (as seen in the direction of the combined fluid flow through the mixing nanochannel) of the first junction, and is connected to the second mixing nanochannel inlet and to the remaining one of the first branch discharge, the second branch discharge and the additional branch discharge. In case multiple additional fluid sources are present, and the nanofluidic device comprises multiple additional microchannels and associated branch nanochannels, more than two junctions may be provided.

In a possible embodiment of the fluidic system according to the invention, the nanofluidic device further comprises an additional mixing nanochannel. This additional mixing nanochannel has an additional mixing nanochannel inlet and an additional mixing nanochannel discharge. The additional mixing nanochannel has an additional mixing nanochannel hydraulic resistance which is optionally at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance.

In this embodiment, the junction is optionally connected to the first branch discharge, the second branch discharge, the mixing nanochannel inlet and the additional mixing nanochannel inlet. Optionally, a plurality of junctions is provided, and each junction is arranged to discharge a combined fluid flow in at least one of the mixing nanochannels.

This embodiment allows to spatially separate molecules of the combined fluid flow after the mixing, which makes it easier to find and monitor single entities over a longer time as they are less prone to cross their diffusion paths with other molecules. Further, this arrangement allows to arrange the individual nanochannels in such a way that various mixing ratios and/or concentration gradients of entities from the first fluid and/or the second fluid can be obtained in the mixing nanochannels.

In a possible embodiment of the fluidic system according to the invention, in the nanofluidic device, a first secondary branch nanochannel is provided, which first secondary branch nanochannel branches off the first microchannel. The first secondary branch nanochannel is present in addition to the first branch nanochannel. The first secondary branch nanochannel has a first secondary branch inlet and a first secondary branch discharge. The first secondary branch inlet is connected to the first microchannel. The first secondary branch nanochannel is adapted to accommodate a first secondary laminar fluid flow of the first fluid which originates from the first main fluid flow. The first secondary branch discharge is connected to the junction, or if multiple junctions are present, to one of the junctions.

Alternatively or in addition, in the nanofluidic device, a second secondary branch nanochannel is provided, which second secondary branch nanochannel branches off the second microchannel. The second secondary branch nanochannel is present in addition to the second branch nanochannel. The second secondary branch nanochannel has a second secondary branch inlet and a second secondary branch discharge. The second secondary branch inlet is connected to the second microchannel. The second secondary branch nanochannel is adapted to accommodate a second secondary laminar fluid flow of the second fluid which originates from the second main fluid flow. The second secondary branch discharge is connected to the junction, or if multiple junctions are present, to one of the junctions.

Alternatively or in addition, in a variant of the embodiment in which an additional microchannel is present, in the nanofluidic device, an additional secondary branch nanochannel is provided, which additional secondary branch nanochannel branches off the additional microchannel. The additional secondary branch nanochannel is present in addition to the additional branch nanochannel. The additional secondary branch nanochannel has an additional secondary branch inlet and an additional secondary branch discharge. The additional secondary branch inlet is connected to the additional microchannel. The additional secondary branch nanochannel is adapted to accommodate an additional secondary laminar fluid flow of the additional fluid which originates from the additional main fluid flow. The additional secondary branch discharge is connected to the junction, or if multiple junctions are present, to one of the junctions.

In a possible embodiment of the fluidic system according to the invention, the nanofluidic device comprises a common device discharge. The first microchannel discharge, the second microchannel discharge and the mixing nanochannel discharge are connected to the common device discharge. From the common device discharge, the fluid flows from the first microchannel, second microchannel and mixing nanochannel are discharged together from the nanofluidic device. The common device discharge may for example be connected to a fluid collection container or to waste.

In a variant of this embodiment, a further microchannel, *e.g.* an additional microchannel, which comprises a further microchannel discharge, is present in the nanofluidic device. The further microchannel discharge is optionally also connected to the common device discharge.

In a variant of this embodiment, an additional mixing nanochannel, *e.g.* a second mixing nanochannel, which comprises an additional mixing nanochannel discharge is present in the nanofluidic device. The additional mixing nanochannel discharge is optionally also connected to the common device discharge.

In an alternative embodiment of the fluidic system according to the invention, the nanofluidic device comprises at least one combined device discharge. At least two of the first microchannel discharge, the second microchannel discharge and the mixing nanochannel discharge are connected to the combined device discharge. From the combined device discharge, the fluid flows from the respective channels that are connected to the combined device discharge are discharged together from the nanofluidic device. The combined device discharge may for example be connected to a fluid collection container or to waste.

In a variant of this embodiment, a further microchannel, *e.g.* an additional microchannel, which comprises a further microchannel discharge, is present in the nanofluidic device. In this variant, at least two of the first microchannel discharge, the second microchannel discharge, the further microchannel discharge and the mixing nanochannel discharge are connected to the combined device discharge.

In a variant of this embodiment, an additional mixing nanochannel, *e.g.* a second mixing nanochannel, which comprises an additional mixing nanochannel discharge is present in the nanofluidic device. In this variant, at least two of the first microchannel discharge, the second microchannel discharge, the mixing nanochannel discharge and the additional mixing nanochannel discharge are connected to the combined device discharge.

In a variant of this embodiment, an additional mixing nanochannel, *e.g.* a second mixing nanochannel, which comprises an additional mixing nanochannel discharge is present in the nanofluidic device. In addition, a further microchannel, *e.g.* an additional microchannel, which comprises a further microchannel discharge, is present in the nanofluidic device. In this variant, at least two of the first microchannel discharge, the second microchannel discharge, the further microchannel discharge, the mixing nanochannel discharge and the additional mixing nanochannel discharge are connected to the combined device discharge.

In this alternative embodiment (including its variants as described above) the discharges of all microchannels and mixing nanochannels that are present in the nanofluidic device may be connected to a combined device discharge. Alternatively, the discharge of one or more of the individual microchannels and mixing nanochannels that are present in the nanofluidic device may be connected to a fluid collection container or to waste directly.

In a further alternative embodiment of the fluidic system according to the invention, the discharges of all of the individual microchannels and mixing nanochannels that are present in the nanofluidic device are be connected to a fluid collection container or to waste directly.

The invention further pertains to a method for performing a test. In the method according to the invention, a fluidic system in accordance with the invention is used.

The method according to the invention comprises the following steps:

- in a fluidic system according to the invention, establishing a first main fluid flow of first fluid from the first fluid source to and through the first microchannel, and simultaneously, establishing a second main fluid flow of second fluid from the second fluid source to and through the second microchannel,

- establishing a first laminar fluid flow of first fluid through the first branch nanochannel, which first laminar fluid flow originates from the first main fluid flow, and simultaneously establishing a second laminar fluid flow of second fluid through the second branch nanochannel, which second laminar fluid flow originates from the second main fluid flow,

- contacting the first and second fluids at the junction of the nanofluidic device, thereby establishing diffusion mixing of the first and second fluids,

- discharging the mixed first and second fluids from the junction into the mixing nanochannel, in the form of a combined laminar flow of the first and second fluids,

- making the combined laminar flow of the first and second fluids flow through the mixing nanochannel,

- observing at least one parameter of at least one of the first laminar fluid flow at the first branch nanochannel, the second laminar fluid flow at the second branch nanochannel and/or the combined laminar flow of the first and second fluid at the mixing nanochannel and/or the junction.

In a possible embodiment of the method according to the invention, at least one parameter of the combined laminar flow of the first and second fluids through the mixing nanochannel is monitored over a monitoring path which extends over at least a part of the mixing nanochannel.

Alternatively or in addition, at least one parameter of the first laminar fluid flow through the first branch nanochannel is monitored over a monitoring path which extends over at least a part of the first branch nanochannel.

Alternatively or in addition, at least one parameter of the second laminar fluid flow through the second branch nanochannel is monitored over a monitoring path which extends over at least a part of the second branch nanochannel.

In a possible embodiment of the method according to the invention the velocity of the first laminar fluid flow in the first branch nanochannel and the velocity of the second laminar fluid flow in the second branch nanochannel are both lower than the velocity of the combined laminar flow of the first and second fluids in the mixing nanochannel.

In a possible embodiment of the method according to the invention the mean square of the diffusion length of the first laminar fluid flow is larger than the width of the first branch nanochannel, and/or the mean square of the diffusion length of the second laminar fluid flow is larger than the width of the second branch nanochannel.

The invention will be described in more detail below under reference to the drawing, in which in a non-limiting manner exemplary embodiments of the invention will be shown.

The drawing shows in:

Fig. 1: a schematic representation of a first embodiment of a fluidic system according to the invention, showing the main components of the fluidic system,

Fig. 2: a schematic representation of a possible embodiment of a fluidic system according to the invention,

Fig. 3: a schematic representation of a possible embodiment of a nanofluidic device according to the invention,

Fig. 4A, 4B, 4C: a schematic representation of further possible embodiments of a nanofluidic device according to the invention,

Fig. 5: a schematic representation of a further possible embodiment of a nanofluidic device according to the invention.

Fig. 1 shows a schematic representation of a first embodiment of a fluidic system according to the invention.

The fluidic system as shown in fig. 1 comprises a first fluid source 10, a second fluid source 15, a nanofluidic device 20 and an observation device 50.

In the embodiment shown in fig. 1, the first fluid source 10 and the second fluid source 15 are syringe pumps.

The first fluid source 10 is adapted to supply a first fluid and the second fluid source 15 is adapted to supply a second fluid. Both the first and the second fluids are liquids. For example, the first fluid is a fluid containing DNA polymerases and recessed DNA and the second fluid contains nucleoside triphosphates required for DNA synthesis.

The nanofluidic device 20 contains inlets 21 for receiving fluids form the fluid sources 10, 15, and at least outlet 22 through which fluids can be discharged.

The observation device 50 is adapted to observe at least one parameter of a fluid flow which passes through the nanofluidic device 20. The observation device 50 may contain for example an optical device 51 (*e.g.* a camera or a fluorescence detector) which has a field of view 52 in which the part of the nanofluidic device 20 that has to be observed is arranged. Alternatively or in addition, a sensor 53 and a data connection 54, which data connection may be wired or wireless, are provided. The data connection may connect the sensor 53 to the observation device 50. For example, the sensor 53 is capable of detecting current between an anode and a cathode attached to the bottom and the top, respectively, of the mixing nanochannel.

In the embodiment of fig. 1, the fluidic system further comprises a holder 25 for fixing the nanofluidic device 20 in the fluidic system.

Fig. 2 shows a schematic representation of a possible embodiment of a fluidic system according to the invention.

For the sake of clarity, the observation device is not shown in fig. 2.

Fig. 2 shows the first and second fluid sources 10,15 and the nanofluidic device 20 with the inlets 21 and the outlet 22. The outlet 22 is connected to waste 23.

The nanofluidic device 20 further comprises a first microchannel 31, a first branch nanochannel 33, a second microchannel 32, a second branch nanochannel 34, a mixing nanochannel 36 and a junction 35. The first branch nanochannel 33, the second branch nanochannel 34 and the mixing nanochannel 36 meet at the junction 35.

The first microchannel 31 is adapted to accommodate a first main fluid flow of the first fluid. The first microchannel 31 has a fluid inlet 31a which is in fluid communication with the first fluid source 10, which allows the first microchannel 31 to receive first fluid from the first fluid source 10. The first microchannel has a first microchannel hydraulic resistance.

The first microchannel 31 further has a fluid discharge 31b. Optionally, the fluid discharge is connected to waste 23, which receives the fluids from the first and second microchannels 31, 32 and the mixing nanochannel 36. Alternatively, each of these channels 31, 32, 36 is connected to each own waste, or two of these channels share a common waste.

The first branch nanochannel 33 branches off from the first microchannel 31. The first branch nanochannel 33 has a first branch inlet 33a which is connected to the first microchannel 31. The first branch nanochannel 33 is adapted to accommodate a first laminar fluid flow of the first fluid. This first laminar fluid flow originates from the first main fluid flow. The first branch nanochannel further comprises a first branch discharge 33b.

The first branch nanochannel 33 has a first branch nanochannel hydraulic resistance. Preferably, the first branch nanochannel hydraulic resistance is at least one thousand times higher than the first microchannel hydraulic resistance.

The second microchannel 32 is adapted to accommodate a second main fluid flow of the second fluid. The second microchannel 32 has a fluid inlet 32a which is in fluid communication with the second fluid source 15, which allows the second microchannel 32 to receive second fluid from the second fluid source 15. The second microchannel 32 has a second microchannel hydraulic resistance.

The second microchannel further has a fluid discharge 32b. Optionally, the fluid discharge 32b is connected to waste 23.

The second branch nanochannel 34 branches off from the second microchannel 32. The second branch nanochannel 34 has a second branch inlet 34a which is connected to the second microchannel 32. The second branch nanochannel 34 is adapted to accommodate a second laminar fluid flow of the second fluid. This second laminar fluid flow originates from the second main fluid flow. The second branch nanochannel 34 further comprises a second branch discharge 34b.

The second branch nanochannel has a second branch nanochannel hydraulic resistance. Preferably, the second branch nanochannel hydraulic resistance is at least one thousand times higher than the second microchannel hydraulic resistance.

The mixing nanochannel 36 has a mixing nanochannel inlet 36a and a mixing nanochannel discharge 36b. The mixing nanochannel 36 has a mixing nanochannel hydraulic resistance which is at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance.

The junction 35 is connected to the first branch discharge 33b, the second branch discharge 34b and the mixing nanochannel inlet 36a.

The junction 35 is adapted to receive the first laminar fluid flow from the first branch nanochannel 33 and the second laminar fluid flow from the second branch nanochannel 34. In addition, the junction 35 is adapted to contact the first and second laminar fluid flows with each other to establish diffusion mixing of the first and second fluids. Furthermore, the junction 35 is adapted to discharge the mixed first and second fluids from the junction into the mixing nanochannel inlet 36a in the form of a combined laminar flow of the first and second fluid.

So, the first fluid flowing out of the first branch nanochannel 33 in the form of the first laminar fluid flow and the second fluid flowing out of the second branch nanochannel 34 in the form of the second laminar fluid flow meet each other at the junction 35. When they meet, diffusion mixing takes place. The first and second fluid leave the junction 35 together via the mixing nanochannel inlet 36a. Together they form a combined laminar flow, which then flows through the mixing nanochannel 36 to the mixing nanochannel discharge 36b. Optionally, the mixing nanochannel discharge 36b is connected to waste 23.

Fig. 3 shows a schematic representation of a possible embodiment of a nanofluidic device according to the invention.

In the embodiment of fig.3, the nanofluidic device 20 is arranged in a microfluidic chip 26. The microfluidic chip 26 is for example made of glass. In the embodiment of fig. 3, the microfluidic chip 26 is transparent, *e.g.* for visible light, in the vicinity of the mixing nanochannel 36 in order to allow observation, *e.g.* optical observation, of the mixing nanochannel 36 and its contents.

In the embodiment of fig. 3, the nanofluidic device has two inlets 21 and two discharges 22. As can be seen in fig. 3, in this exemplary embodiment the first branch nanochannel 33 and the second branch nanochannel 34 extend in line with each other.

In the embodiment of fig. 3, the first microchannel 31 has three parts 31-1, 31-2 and 31-3 which are arranged in series with each other. The second part 31-2 is arranged downstream of the first part 31-2 and downstream of the connection of the first branch nanochannel 33 to the first microchannel 31. In this embodiment, the cross sectional area of the second part 31-2 is smaller than the cross sectional area of the first part 31-1.

The optional third part 31-3, which is arranged downstream of the second part 31-3, the cross sectional area is larger than the cross sectional area of the second part 31-2.

In the embodiment of figure 3, the second microchannel 32 has three parts 32-1, 32-2 and 32-3 which are arranged in series with each other. The second part 32-2 is arranged downstream of the first part 32-2 and downstream of the connection of the second branch nanochannel 34 to the second microchannel 32. In this embodiment, the cross sectional area of the second part 32-2 is smaller than the cross sectional area of the first part 32-1.

The optional third part 32-3, which is arranged downstream of the second part 32-3, the cross sectional area is larger than the cross sectional area of the second part 32-2.

In the embodiment of fig. 3, the mixing nanochannel hydraulic resistance is higher than each of the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance, for example he first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance are both between 25% and 35% of the mixing nanochannel hydraulic resistance, *e.g.* 30%.

In the embodiment of fig. 3, the mixing nanochannel 36 has a larger cross sectional area than each of the first branch nanochannel 33 and the second branch nanochannel 34. This allows to maintain the velocity of the combined laminar flow of the first and second fluid in the mixing nanochannel at a desired level.

In the embodiment of fig. 3, the geometries of the first and the second branch nanochannel 33, 34 are substantially the same. “Substantially the same” in this context means that the geometries of the first and the second branch nanochannel 33, 34 are designed to be the same. Inaccuracies caused by the manufacturing are not taken into account.

This embodiment is in particular advantageous if the first fluid and the second fluid are to be mixed at the junction 35 in a ratio of 1:1.

In the embodiment of fig. 3, by way of example, the height of the first microchannel 31 and/or the second microchannel 32 is between 2 and 10 micrometer, for example about optionally 5 micrometers. The height of the channel is the dimension which extends in the direction of the thickness of the microfluidic chip, so perpendicular to the planar top- or bottom surface of the microfluidic chip.

In the embodiment of fig. 3, by way of example, the first branch nanochannel 33 and the second branch nanochannel 34 and optionally also the mixing nanochannel 36 have a height between 150 and 250 nanometers.

In the embodiment of fig. 3, by way of example, the first branch nanochannel 33 and the second branch nanochannel 34 have a width between 2 and 4 micrometers, optionally 3 micrometers.

In the embodiment of fig. 3, by way of example, the first branch nanochannel 33 and the second branch nanochannel 34 have a length between 20 and 28 micrometers, optionally 24 micrometers.

In the embodiment of fig. 3, by way of example, the mixing nanochannel 36 has a width between 3 and 5 micrometers, optionally 4 micrometers.

In the embodiment of fig. 3, by way of example, the mixing nanochannel 36 has a length between 100 and 120 micrometers, optionally 110 micrometers.

Fig. 4A, 4B and 4C show a schematic representation of further possible embodiments of a nanofluidic device 20 according to the invention.

In the embodiment of fig. 4A, a first microchannel 31, a second microchannel 32, a first branch nanochannel 33, a second branch nanochannel 34, a junction 35 and a mixing nanochannel 36 are present.

In this embodiment, the nanofluidic device comprises a common device discharge 41. The first microchannel discharge, the second microchannel discharge and the mixing nanochannel discharge are connected to the common device discharge 41. From the common device discharge 41, the fluid flows from the first microchannel 31, second microchannel 32 and mixing nanochannel 36 are discharged together from the nanofluidic device 20. The common device discharge 41 may for example be connected to a fluid collection container 40 or to waste.

In the embodiment of fig. 4B, a first microchannel 31, a second microchannel 32, a first branch nanochannel 33, a second branch nanochannel 34, a junction 35 and a mixing nanochannel 36 are present.

In this embodiment of the nanofluidic device 20, the discharges of all of the individual microchannels 31, 32 and mixing nanochannels 36 that are present in the nanofluidic device are be connected to a fluid collection container 40a, 40b, 40c or to waste directly, *e.g.* via an individual device discharge 42.

In the embodiment of fig. 4C, a first microchannel 31, a second microchannel 32, a first branch nanochannel 33, a second branch nanochannel 34, a junction 35 and a mixing nanochannel 36 are present.

In this embodiment, the nanofluidic device 20 comprises at least one combined device discharge 43. At least two of the first microchannel discharge, the second microchannel discharge and the mixing nanochannel discharge are connected to the combined device discharge 43. From the combined device discharge 43, the fluid flows from the respective channels that are connected to the combined device discharge 43, which in the embodiment shown in fig. 4C are the first microchannel 31 and the second microchannel 32, are discharged together from the nanofluidic device 20. The combined device discharge 43 may for example be connected to a fluid collection container 40e or to waste.

In the embodiment of fig. 4C, the mixing nanochannel 36 is provided with its own individual device discharge 42, which is for example connected to a fluid collection container 40d or to waste.

Fig. 5 shows a schematic representation of a further possible embodiment of a nanofluidic device according to the invention. The arrows indicate the direction of the fluid flow.

In the embodiment of fig. 5, the nanofluidic device 20 comprises a first microchannel 31, a second microchannel 32, a first branch nanochannel 33, a second branch nanochannel 34, a junction 35 and a mixing nanochannel 36a.

In addition, the nanofluidic device 20 comprises an additional mixing nanochannel 36a. This additional mixing nanochannel 36a has an additional mixing nanochannel inlet and an additional mixing nanochannel discharge. The additional mixing nanochannel 36a has an additional mixing nanochannel hydraulic resistance which is optionally at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance.

In the embodiment of fig. 5, the junction 35 is connected to the first branch channel 33, the second branch channel 34, the mixing nanochannel 36a and the additional mixing nanochannel 36b.

Furthermore, the nanofluidic device 20 of fig. 5 comprises an additional microchannel 51 and an additional branch nanochannel 52. The additional microchannel 51 is adapted to accommodate an additional main fluid flow of an additional fluid, which additional fluid is different from the first fluid and the second fluid. The additional microchannel 51 has a fluid inlet which allows the additional microchannel 51 to receive the additional fluid from an additional fluid source. The additional microchannel 51 has an additional microchannel hydraulic resistance.

The additional microchannel further has a fluid discharge.

The additional branch nanochannel 52 branches off the additional microchannel 51. The additional branch nanochannel 52 has an additional branch inlet which is connected to the additional microchannel 51. The additional branch nanochannel 52 is adapted to accommodate an additional laminar fluid flow of the additional fluid. This additional laminar fluid flow originates from the additional main fluid flow. The additional branch nanochannel further comprises an additional branch discharge.

The additional branch nanochannel 52 has an additional branch nanochannel hydraulic resistance. Preferably, the additional branch nanochannel hydraulic resistance is at least one thousand times higher than the additional microchannel hydraulic resistance.

In this embodiment, the additional branch discharge is connected to the junction 35. The junction is connected to the first branch channel 33, the second branch channel 34, the additional branch channel 52 the mixing nanochannels 36a and the additional mixing nanochannel 36b.

Example

In an example of the method according to the invention, the first fluid is a fluid containing DNA polymerases and fluorescently labelled recessed DNA and the second fluid contains nucleoside triphosphates. They are brought into contact with each other at the junction 35 so diffusion mixing takes place at the junction and optionally also downstream thereof, in the mixing nanochannel 36. The recessed DNA includes an acceptor labelled DNA primer annealed to a DNA template that is labelled on its single stranded, downstream overhang with a donor fluorophore. Upon extension of the primer using a DNA polymerase and the provided nucleoside triphosphates, the overhang of the template alters its conformation from a random coil to the canonical structure of double stranded DNA. This conformational change increases the distance between the donor and the acceptor fluorophore and can be detected as a decrease in the Förster resonance energy transfer efficiency between both fluorophores. Therefore, the time depended extension of the DNA primer can be monitored in the mixing nanochannel 36 and can be detected by the observation device 50. The enzymatic reaction is (quasi-) irreversible and that the presented nanofluidic device allows the continuous monitoring of reactions as only at junction 35 both recessed DNA and DNA polymerases bound to it encounter the required nucleoside triphosphates. The result of the monitoring is the determination of polymerisation rates of DNA polymerases provided in the first fluid under different buffer conditions of the second fluid, for example, having varying salt content, varying concentrations of nucleoside triphosphates, different temperatures of both fluids, etc.

In this example of the method, first a first main fluid flow of the first fluid from the first fluid source 10 is established to and through the first microchannel 31. Simultaneously, a second main fluid flow of second fluid from the second fluid source 15 to and through the second microchannel 32 is established in a fluidic system according to the invention.

Because of the design of the nanofluidic device, this results in establishing a first laminar fluid flow of first fluid through the first branch nanochannel 33. This first laminar fluid flow originates from the first main fluid flow. Simultaneously a second laminar fluid flow of second fluid through the second branch nanochannel 34 is established. This second laminar fluid flow originates from the second main fluid flow.

At the junction 35, the first and second fluids meet each other. This results in diffusion mixing of the first and second fluids.

The mixed first and second fluids are discharged from the junction 35 into the mixing nanochannel 36 in the form of a combined laminar flow of the first and second fluids. This combined laminar flow of the first and second fluids flow is made to flow through the mixing nanochannel 36, *e.g.* by the action of the fluid sources 10, 15 (which are for example syringe pumps) which continue to supply fluid to the nanofluidic device, and/or by establishing and maintaining a pressure difference between the first branch nanochannel inlet 33a and second branch nanochannel inlet 34a on the one hand a the mixing nanochannel discharge 36b on the other hand during the test.

At least one parameter of the combined laminar flow of the first and second fluids in the mixing nanochannel and/or at the junction is observed, *e.g.* detected, measured and/or monitored during the test.

In this example of the method, at least one parameter of the combined laminar flow of the first and second fluids through the mixing nanochannel 36 is monitored over a monitoring path which extends over at least a part of the mixing nanochannel 36.

Alternatively or in addition, at least one parameter of the first laminar fluid flow through the first branch nanochannel 33 is monitored over a monitoring path which extends over at least a part of the first branch nanochannel 33.

Alternatively or in addition, at least one parameter of the second laminar fluid flow through the second branch nanochannel 34 is monitored over a monitoring path which extends over at least a part of the second branch nanochannel 34.

In this example of the method, the velocity of the first laminar fluid flow in the first branch nanochannel 33 and the velocity of the second laminar fluid flow in the second branch nanochannel 34 are both lower than the velocity of the combined laminar flow of the first and second fluids in the mixing nanochannel 36.

CLAIMS

1. Nanofluidic device, which comprises:

- a first microchannel (31) adapted to accommodate a first main fluid flow of a first fluid, which first microchannel (31) has a fluid inlet (31a) which is in fluid communication with a first fluid source (10), and which first microchannel (31) has a first microchannel hydraulic resistance,

- a first branch nanochannel (33) which branches off the first microchannel (31), which first branch nanochannel (33) has a first branch inlet (33a) and a first branch discharge (33b), the first branch inlet (33a) being connected to the first microchannel (31), which first branch nanochannel (33) is adapted to accommodate a first laminar fluid flow of the first fluid which originates from the first main fluid flow,

- a second microchannel (32) adapted to accommodate a second main fluid flow of a second fluid, which second microchannel (32) has a fluid inlet (32a) which is in fluid communication with a second fluid source (15), and which second microchannel (32) has a second microchannel hydraulic resistance,

- a second branch nanochannel (34) which branches off the second microchannel (32), which second branch nanochannel (34) has a second branch inlet (24a) and a second branch discharge (34b), the second branch inlet (34a) being connected to the second microchannel (32), which second branch nanochannel (34) is adapted to accommodate a second laminar fluid flow of the second fluid which originates from the second main fluid flow,

- a mixing nanochannel (36), which has a mixing nanochannel inlet (36a) and a mixing nanochannel discharge (36b), wherein the mixing nanochannel (36) has a mixing nanochannel hydraulic resistance which is at least one thousand times larger than each of the first microchannel hydraulic resistance and the second microchannel hydraulic resistance,

- a junction (35), which is connected to the first branch discharge (33b), the second branch discharge (34b) and the mixing nanochannel inlet (36a), which junction (35) is adapted:

  - to receive the first laminar fluid flow from the first branch nanochannel (33) and the second laminar fluid flow from the second branch nanochannel (34), and
  - to contact the first and second laminar fluid flows with each other to establish diffusion mixing of the first and second fluids, and

- to discharge the mixed first and second fluids from the junction (35) into the mixing nanochannel inlet (36a) in the form of a combined laminar flow of the first and second fluid.

2. Nanofluidic device according to claim 1,
wherein the mixing nanochannel hydraulic resistance is higher than each of the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance.

3. Nanofluidic device according to claim 2,
wherein the first branch nanochannel hydraulic resistance and the second branch nanochannel hydraulic resistance are both 50% or less of the mixing nanochannel hydraulic resistance, optionally between 25% and 35% of the mixing nanochannel hydraulic resistance.

4. Nanofluidic device according to any of the preceding claims,
wherein the mixing nanochannel (36) has a larger cross sectional area than each of the first branch nanochannel (33) and the second branch nanochannel (34).

5. Nanofluidic device according to any of the preceding claims,
wherein the geometries of the first and the second branch nanochannel (33, 34) are substantially the same.

6. Nanofluidic device according to any of the preceding claims,
wherein the first microchannel (31) has a first part (31-1) and a second part (31-2) which are arranged in series with each other, which second part (31-2) is arranged downstream of the first part (31-1) and downstream of the connection of the first branch nanochannel (33) to the first microchannel (31), wherein the cross sectional area of the second part (31-2) is smaller than the cross sectional area of the first part (31-1), and/or
wherein the second microchannel (32) has a first part (32-2) and a second part (32-2) which are arranged in series with each other, which second part (32-2) is arranged downstream of the first part (32-1) and downstream of the connection of the second branch nanochannel (34) to the second microchannel (32), wherein the cross sectional area of the second part (32-2) is smaller than the cross sectional area of the first part (32-1) .

7. Fluidic system for testing, which comprises:
- a first fluid source (10) which is adapted to supply a first fluid, and
- a second fluid source (15) which is adapted to supply a second fluid, and,
- a nanofluidic device according to any of the preceding claims, and

- an observation device (50), which is adapted to observe at least one parameter of at least one of the first laminar fluid flow at the first branch nanochannel (33), the second laminar fluid flow at the second branch nanochannel (34) and/or the combined laminar flow of the first and second fluid at the mixing nanochannel (36) and/or the junction (35).

8. Fluidic system according to claim 7,
wherein the observation device (50) is adapted to monitor the at least one parameter of the combined laminar flow of the first and second fluids over a monitoring path which extends over at least a part of the mixing nanochannel (36).

9. Fluidic system according to any of the claims 7-8,
wherein at least one sensor (53) is provided at the mixing nanochannel (36), which sensor (53) is adapted to measure at least one of electrical impedance, temperature, fluid velocity or particle velocity of the combined laminar flow of the first and second fluids through the mixing nanochannel (36).

10. Fluidic system according to any of the claims 7-9,
wherein the observation device (50) comprises a fluorescence detector.

11. Fluidic system according to any of the claims 7-10,
wherein the nanofluidic device (20) is arranged in a microfluidic chip (26), and wherein the fluidic system comprises a holder (25) for in a releasable way holding said microfluidic chip (26).

12. Method for performing a test,
which method comprises the following steps:
- in a fluidic system according to claim 7, establishing a first main fluid flow of first fluid from the first fluid source (10) to and through the first microchannel (31), and simultaneously, establishing a second main fluid flow of second fluid from the second fluid source (15) to and through the second microchannel (32),
- establishing a first laminar fluid flow of first fluid through the first branch nanochannel (33), which first laminar fluid flow originates from the first main fluid flow, and simultaneously establishing a second laminar fluid flow of second fluid through the second branch nanochannel (34), which second laminar fluid flow originates from the second main fluid flow,
- contacting the first and second fluids at the junction (35) of the nanofluidic device (20), thereby establishing diffusion mixing of the first and second fluids,

- discharging the mixed first and second fluids from the junction (35) into the mixing nanochannel (36) in the form of a combined laminar flow of the first and second fluids,
- making the combined laminar flow of the first and second fluids flow through the mixing nanochannel (36),
- observing at least one parameter of at least one of the first laminar fluid flow at the first branch nanochannel (33), the second laminar fluid flow at the second branch nanochannel (34) and/or the combined laminar flow of the first and second fluid at the mixing nanochannel (36) and/or the junction (35).

13. Method according to claim 12,
wherein at least one parameter of the combined laminar flow of the first and second fluids through the mixing nanochannel (36) is monitored over a monitoring path which extends over at least a part of the mixing nanochannel (36), and/or
wherein at least one parameter of the first laminar fluid flow through the first branch nanochannel (33) is monitored over a monitoring path which extends over at least a part of the first branch nanochannel (33), and/or
wherein at least one parameter of the second laminar fluid flow through the second branch nanochannel (34) is monitored over a monitoring path which extends over at least a part of the second branch nanochannel (34).

14. Method according to any of the claims 12-13,
wherein the velocity of the first laminar fluid flow in the first branch nanochannel (33) and the velocity of the second laminar fluid flow in the second branch nanochannel (34) are both lower than the velocity of the combined laminar flow of the first and second fluids in the mixing nanochannel (36).

15. Method according to any of the claim 12-14,
wherein the mean square of the diffusion length of the first laminar fluid flow is larger than the width of the first branch nanochannel (33), and/or
wherein the mean square of the diffusion length of the second laminar fluid flow is larger than the width of the second branch nanochannel (34).

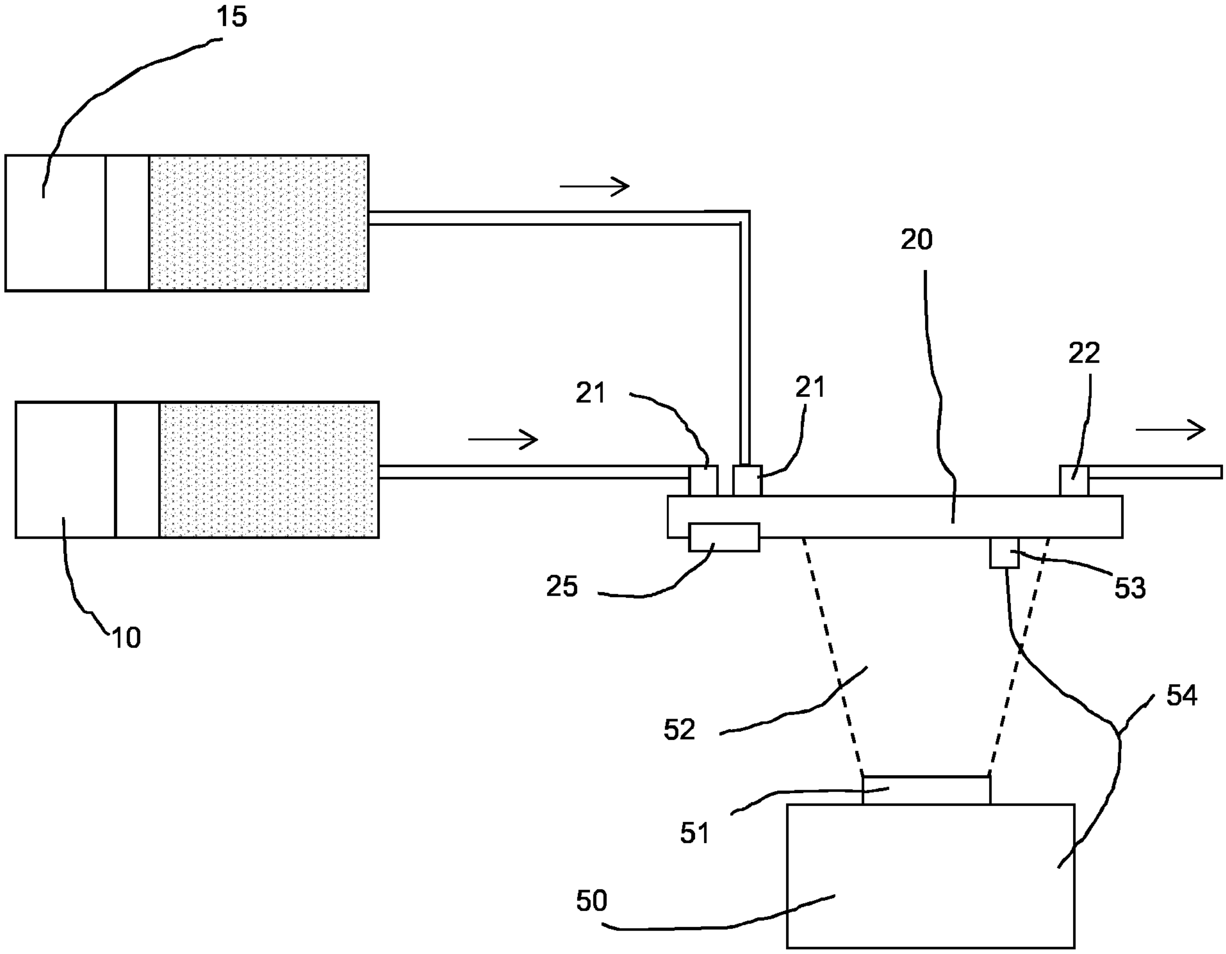

Fig. 1

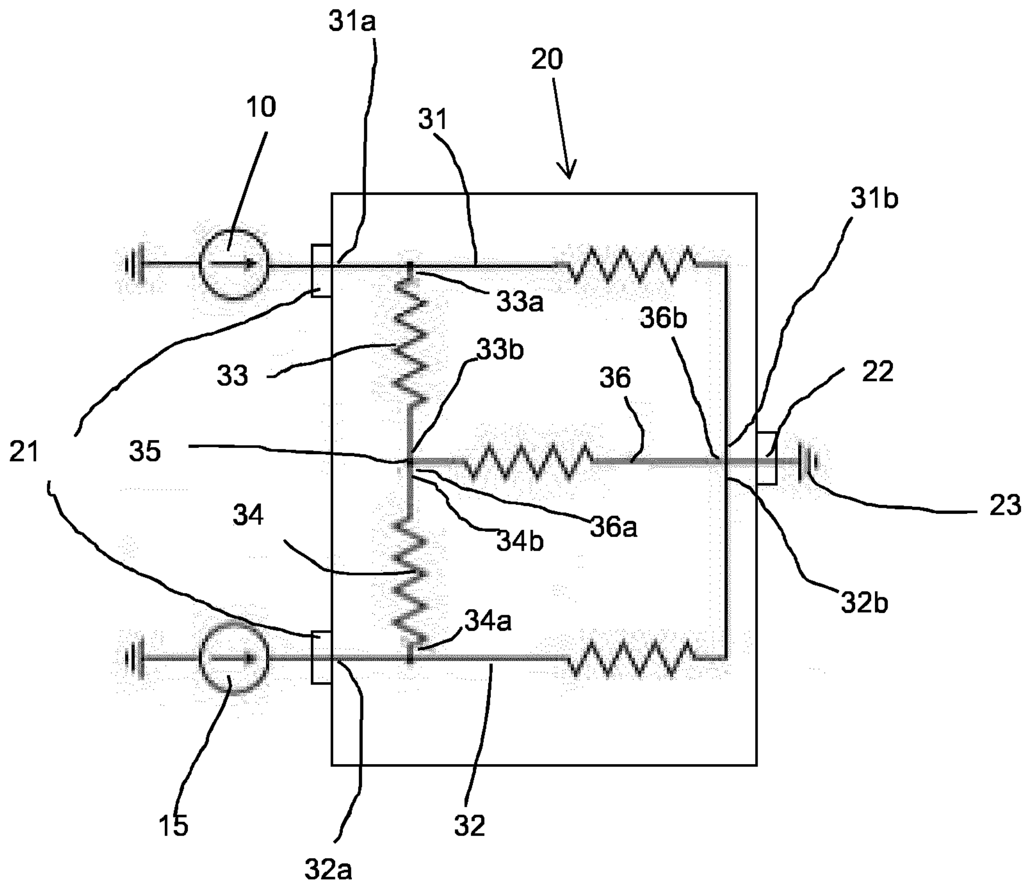
31a
20
10
31
31b
33a
36b
33
33b
36
22
21
35
23
36a
34
34b
32b
34a
15
32
32a

Fig. 2

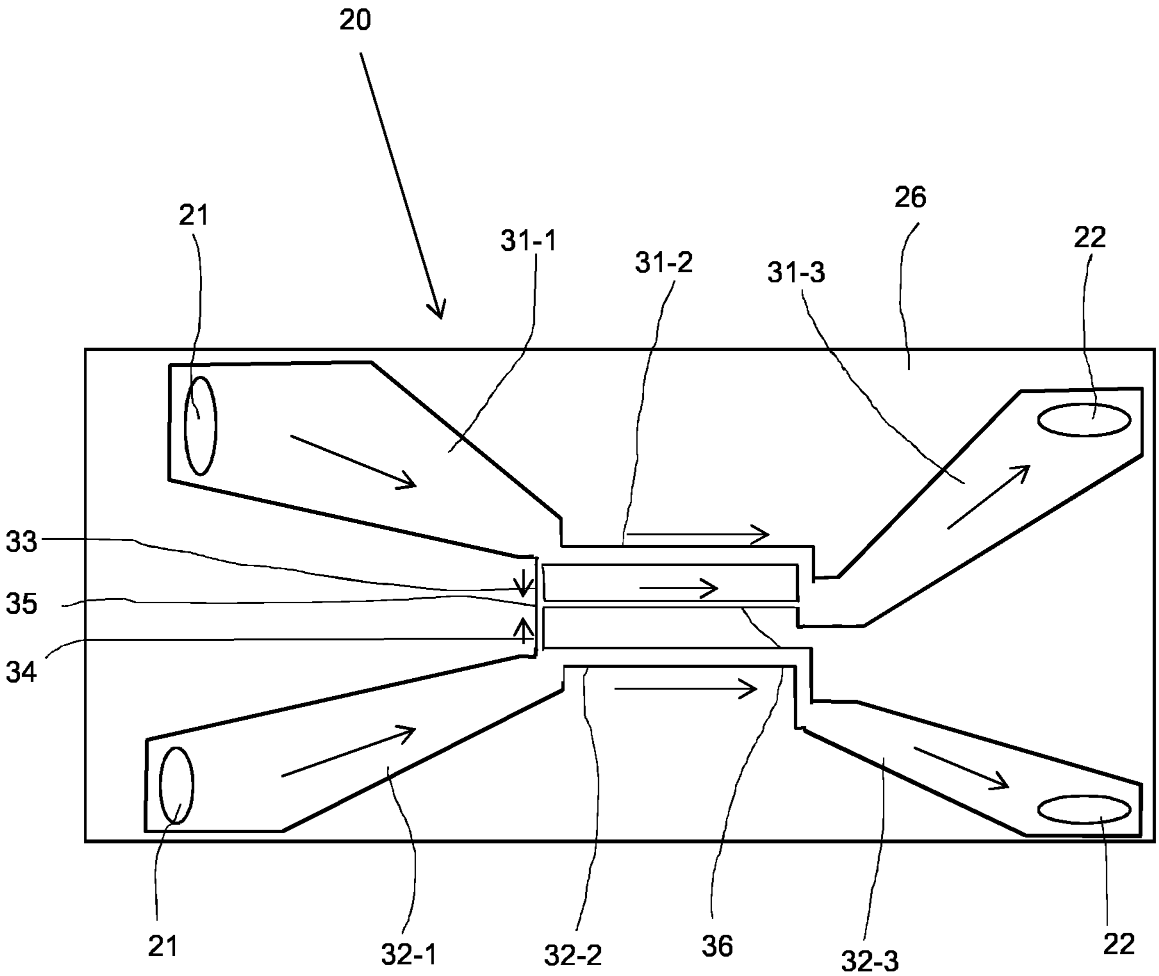

Fig. 3

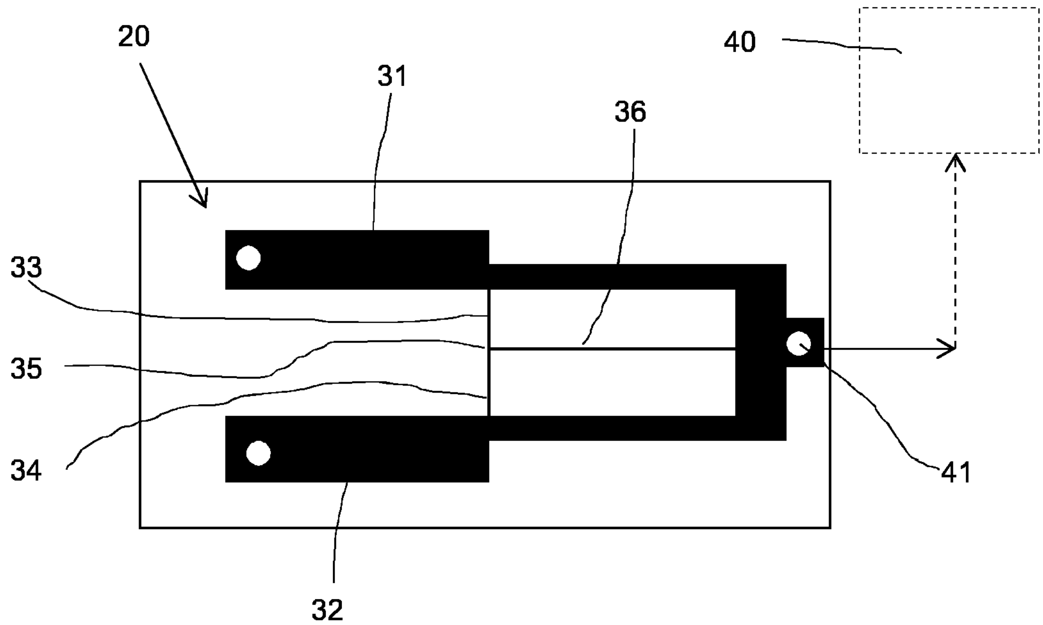

Fig. 4A

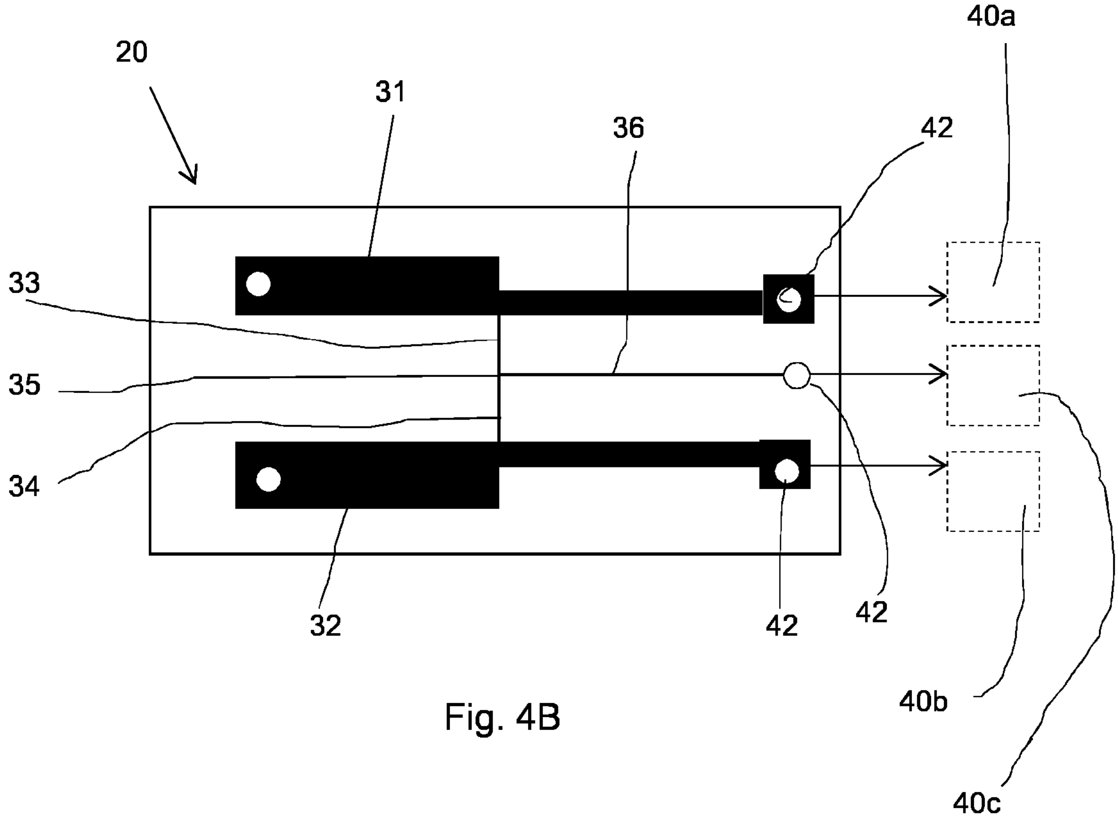

Fig. 4B

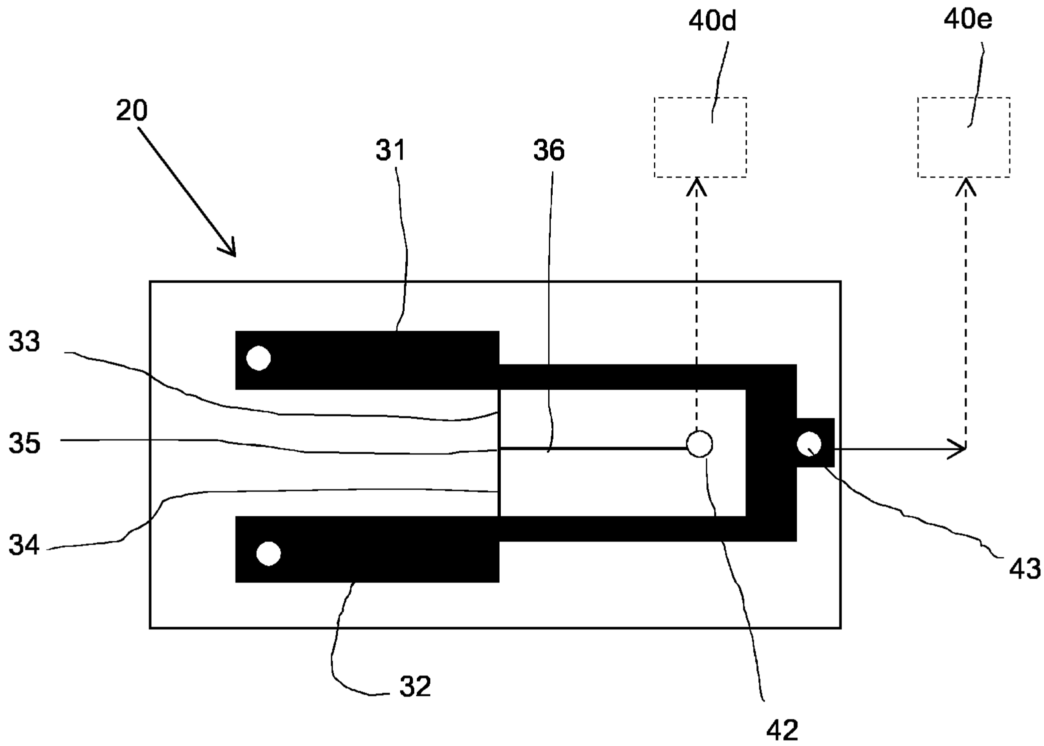

Fig. 4C

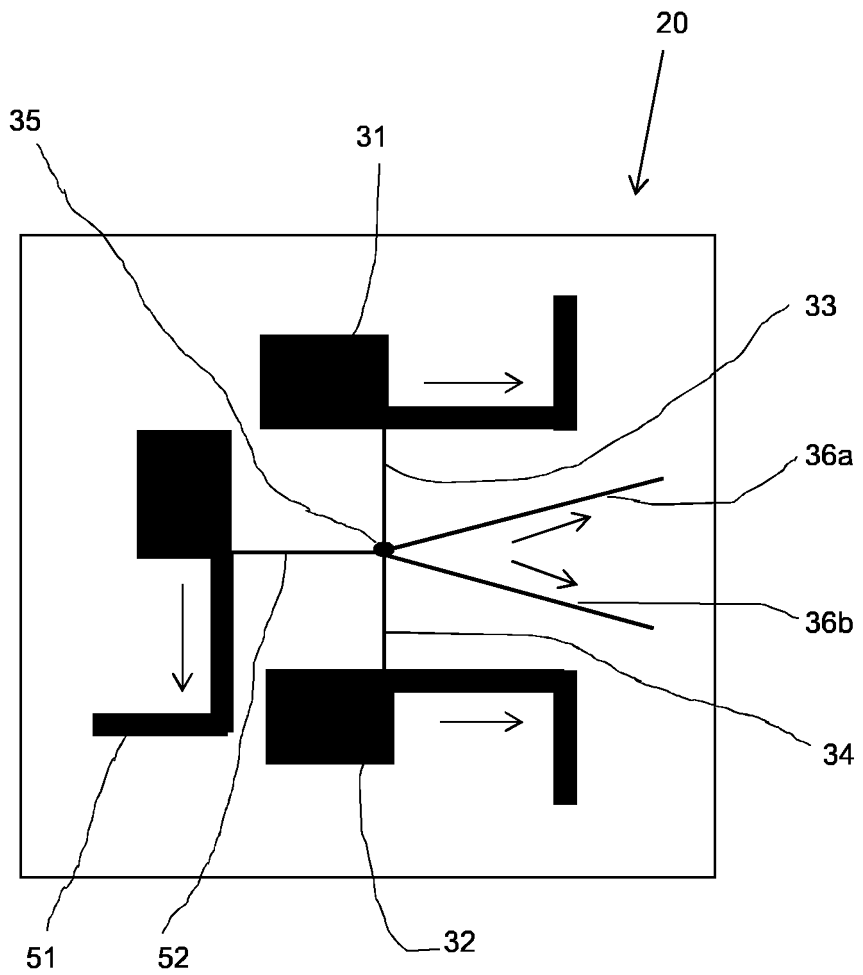

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/074521

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

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CALABRÒ F ET AL: "Modelling flow enhancement in nanochannels: Viscosity and slippage", APPLIED MATHEMATICS LETTERS, PERGAMON, AMSTERDAM, NL, vol. 26, no. 10, 20 May 2013 (2013-05-20), pages 991-994, XP028575000, ISSN: 0893-9659, DOI: 10.1016/J.AML.2013.05.004 the whole document -----	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] 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
"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
23 October 2017	03/11/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ueberfeld, Jörn

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOHAMMAD AMIN ALIBAKHSHI ET AL: "Accurate measurement of liquid transport through nanoscale conduits", SCIENTIFIC REPORTS, vol. 6, no. 1, 26 April 2016 (2016-04-26), XP055362292, DOI: 10.1038/srep24936 the whole document -----	1-15
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/074521

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