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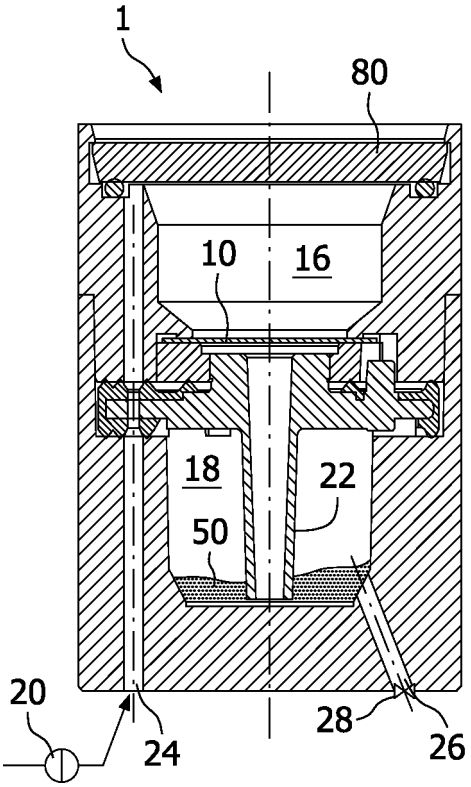
(71) Applicants:
- **Koninklijke Philips Electronics N.V.**
  5621 BA Eindhoven (NL)
- **Philips Intellectual Property & Standards GmbH**
  20099 Hamburg (DE)

(72) Inventor: **The designation of the inventor has not yet been filed**

(74) Representative: **Damen, Daniel Martijn et al**
Philips
Intellectual Property & Standards
P.O. Box 220
5600 AE Eindhoven (NL)

(54) Fluid analysis device and method

(57) An analysis device (1) for analyzing a sample fluid (50) for the presence, absence or amount of an analyte in the sample fluid (50) comprises a substrate (10) having a diameter (d) having a first surface (12) and an opposite second surface (14), and has a plurality of through going channels from the first surface (12) to the second surface (14), and is at least partially provided with a binding substance specific for the analyte. The device comprises a first volume (16) in fluid communication with the first surface (12) and a second volume (18) in fluid communication with the second surface (14). The device further comprises a means (20) for generating a pressure difference over the substrate (10) to transport the fluid from the first (16) to the second volume (18) and vice versa through the channels. The device is provided with a guide (22) placed beneath the second surface (14) of the substrate (10). The guide protrudes towards bottom of the second volume (18), wherein the guide (22) is configured for allowing flow of the sample fluid (50) from the first volume (16) to the second volume (18) and vice versa, and wherein the guide (22) has an exit diameter substantially smaller than the diameter (d) of the substrate (10).



SECTION A-A

FIG. 2b

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Description

FIELD OF THE INVENTION:

[0001] The present invention relates to fluid handling, in particular to the fluid handling in a device or to the fluid handling in a method for analysis.

BACKGROUND OF THE INVENTION:

[0002] Analysis is often performed in order to investigate qualitatively and/or quantitatively the composition of a (liquid) sample, in particular relating to the detection of the presence, absence or amount of specific DNA or RNA sequences or proteins in a sample.
Especially PCR, the Polymerase Chain Reaction has contributed enormously to the development of assays of all types for the detection of the presence or absence of DNA or RNA sequences. At present, it is possible to collect DNA containing samples from an organism and determine the presence, absence or amount therein of specific DNA sequences.

[0003] Technology is available to perform such analysis for multiple target sequences at the same time, so-called multiplex detection of target sequences to thereby increase throughput.

[0004] For example, for detection of specific bacteria in a blood sample or the like, a detection method is known which is based on a DNA multiplication process and binding of this DNA to fluorescent tracer molecules. Only specific types of DNA will bind to specific probe molecules. The presence of bound DNA is then detected by optical means, e. g. activation by a light source and detection by a camera.

[0005] The detection of the presence, absence or amount of DNA and/or RNA is indicative, for instance, for the presence, absence or amount of a gene, an allele of a gene, a genetic trait or disorder, a polymorphism, a single nucleotide polymorphism (SNP) or of the presence of exogenous DNA or RNA in an organism, i.e. the presence, absence or amount of pathogens or bacteria in organisms.

[0006] US 6886409 B2 discloses a system for controlling the flow of a sample fluid though a substrate having first and second surfaces and at least one area with a plurality of through-going capillary channels. The system comprises a housing having a chamber for receiving the substrate and a pressure differential generator capable of generating and maintaining a pressure difference over the substrate. When a small amount of sample fluid is pumped through the substrate, the fluid can form a droplet which hangs underneath the substrate. This poses potential problems for the functioning the system. The sample fluid may contain fluorescent molecules, some of which will (selectively) bind to the first surface of the substrate. The presence of molecules bounded to the substrate surface is measured using an optical detection system which illuminates the substrate surface and detects the resulting fluorescent light. The presence of a droplet with a large amount of unbound fluorescent molecules poses the risk of an increased background signal during the detection process and worsens the signal to noise ratio.

[0007] Further, WO2007/04102 provides an analysis device and method that enables repeatedly pumping a sample fluid with one or more analytes through a substrate with one or more binding materials, even when the wetted substrate has a relatively low bubble pressure. Thereto, the device comprises a first volume and a second volume, and a return channel with a valve as well as a pump. The pump can establish a pressure difference between the first volume and the second volume, to pump the sample fluid through the substrate, thereby allowing analyte to bind to the substrate. By opening the valve and establishing a reversed pressure difference, sample fluid bypasses the substrate through the return channel. This device has a number of seals which may be potential leakage points.

[0008] An object of the present invention is to provide a fluid analysis device which may not have the above-mentioned disadvantages.

SUMMARY OF THE INVENTION:

[0009] This object is achieved with an analysis device that comprises a substrate having a diameter (d) and having a first surface and an opposite second surface. The substrate has a plurality of through going channels from the first surface to the second surface, and at least partially being provided with a binding substance specific for the analyte. The device has a first volume which is in fluid communication with the first surface and a second volume which is in fluid communication with the second surface. The device is provided with a means for generating a pressure difference over the substrate to transport the fluid from the first to the second volume and vice versa through the channels. The device includes a guide placed beneath the second surface of the substrate and protruding towards the bottom of the second volume, wherein the guide has an exit diameter substantially smaller than the diameter (d) of the substrate. The guide has a small internal volume as well as an exit diameter (at the bottom end) which is significantly smaller than the diameter (d) of the substrate. This prevents the formation of large droplets and thus eliminates the risk of an increased background signal during the detection process and improves the signal to noise ratio. Furthermore, the guide protrudes down far enough towards the bottom of the volume beneath the guide. This prevents the formation of droplets due to capillary effects. The droplet formation is prevented by draining the liquid off against a surface. The sample fluid is transported from the first volume to second volume and vice versa through the guide. Therefore preferably the guide is configured for allowing flow of the sample fluid from the first volume to the second volume and vice versa. This approach of fluid

transportation increases the contact time between the sample fluid and the substrate which reduces total throughput time. As there are no extra parts for fluid transportation other than the guide, the complexity of the device is reduced. Moreover, the reliability of the device increases because of the simplification of the sample fluid path. This approach significantly reduces the number of seals which are potential leakage points. Furthermore, the device can handle more or less arbitrary amounts of sample fluid. There is no upper limit to the amount that can be handled by the device as there are no droplets hanging underneath the substrate. Functioning of the device does not depend on fluid properties such as viscosity and surface tension (which could pose a limit on the maximum amount of fluid that can be handled in case of a device in which a droplet 'hangs' underneath the substrate). Because of this independence, the device is also not influenced by thermal effects (heating / cooling) which might significantly alter the physical properties of the fluid sample. Furthermore, the distance between the lower end of the guide and the bottom of the second volume is sufficiently small to ensure that no sample fluid will remain in the second volume on application of pressure. This minimizes dead volume of the sample fluid in the second volume.

[0010] In the description, in the embodiments and in the claims, the terms "first volume" and "second volume" should be deemed interchangeable, in that they solely serve to discern the two volumes. For example by flipping the device upside down or in any other way, the two terms may be interchanged.

[0011] Moreover, the expression "in fluid communication" is intended to mean that the fluid (liquid or gas) is able to contact the surface or volume by simply flowing towards that surface or into that volume, as in communicating vessels. It is not intended to be limited to those cases that there is actually a fluid present that contacts the surface or is present in the volume.

[0012] Furthermore, it is noted that each of the first and second volume may comprise a number of sub-volumes, for example to guide sample fluid to different parallel parts of the substrate with different binding substances, or to more than one substrate. Functionally, and for the purpose of this document, these sub-volumes are considered to be one volume, either a first volume or a second volume.

[0013] It is to be noted that the expression means for generating pressure difference may refer to a pump or such a similar device which is described in detail in WO 2007004102-A2.

[0014] Many substrates used show a bubble pressure in the order of several bars, while the fluid pumping pressure is in the order of several tens of milli bars up to several hundred milli bars, although of course other values are possible. Such pressures allow pumping the sample fluid that is collected on one side of the substrate through the substrate to the other side thereof. There, the sample fluid will come off the substrate. In other words, the substrate is contacted by a gas on the other side. Hence, if now the pressure (difference) would be reversed, only the gas would be pumped through the substrate. However, due to the requirement of increased pressure this will not happen. As a result the pressure will increase in the second volume. In other words, the substrate acts as a one-way valve, such that the sample fluid may be pumped out of the second volume.

[0015] In a special embodiment, the substrate has a bubble pressure in a wetted condition that is higher than a sample fluid pumping pressure of said substrate in a wetted condition. As described above, a substrate which, when wetted e.g. by the sample fluid, has a high bubble pressure, the substrate may function as a gas barrier, while allowing the flow of sample fluid there through. By providing a pressure (difference) between the first volume and the second volume that is between the bubble pressure and the sample fluid pumping pressure, the sample fluid will be pumped through the substrate while the flow of gas is blocked by the substrate.

[0016] Advantageously, said bubble pressure is at least 10% higher, preferably at least 50% higher, and even more preferably at least 200% higher than said sample fluid pump pressure. When the bubble pressure is at least 10% higher, it is relatively easy to establish a suitable pressure difference, between the sample fluid pumping pressure and the bubble pressure, allowing fluid flow without gas flow. Furthermore, not too critical variations of either or both of the bubble and pumping pressure, e.g. due to binding material to the substrate, do not affect the proper functioning of the device. When the bubble pressure is at least 50% higher, it is not only easy to establish a working pressure difference, but the pressure difference may be selected such that the sample fluid flow rate is in a useful range, since a higher pressure difference ensures a higher flow rate. In particular, when the bubble pressure is at least 200% higher than the sample fluid pumping pressure, a very useful sample fluid flow may be established. Note that other relative differences between bubble pressure and sample fluid pumping pressure may still lead to useful results.

[0017] In the above discussion, only relative differences have been discussed. It is alternatively also possible to select the substrate such that the absolute difference between the bubble pressure and the sample fluid pumping pressure is as high as possible, or at least higher than a desired amount. In particular, but not limiting, the bubble pressure is at least 100 mbar, preferably at least 1 bar higher than the sample fluid pumping pressure, for a wetted substrate, with similar advantages as mentioned above. Again, other differences may also lead to desirable results.

[0018] In a particular embodiment, the device comprises a wall around at least one of the first volume and the second volume which is at least partially transparent. The partially transparent wall allows detection of DNA etc. on the substrate without removing it from the device. Of course, simple visual inspection may also be allowed by

such a transparent part.

[0019] Transparent is intended to comprise at least: transparent to visible light, and / or to ultra-violet and / or infrared radiation, although transparency for other types of radiation is also contemplated. The at least partially transparent wall may be provided as the wall material itself, as a separate transparent part in a hole in the wall (i.e. a window), etc. In a special embodiment, the device further comprises a detection system.

[0020] Providing a detection system makes the device as a whole more versatile, and it is easier to match the analysis device to particular products to be detected. The detection device may itself comprise a transparent window, or be provided in an operative position with respect to a window, a hole in the wall, et cetera.

[0021] The detection device may comprise any suitable known detection system, such as an optical detection system, e.g. fluorescence detection. If desired, the analysis device, and/or the detection device, may comprise additional parts, such as a light source, a filter etc., required for their functioning, e.g. detecting the analyte bound to the binding material. These additional parts are only optional in the analysis device.

[0022] The device may detect based on label, length, mobility, nucleotide sequence, mass or a combination thereof. In certain embodiments the device can detect based on optical, electrochemical, magnetic principles. In principle any suitable detection device known from prior art may be used.

[0023] In certain embodiments, the system also comprises a data collection device to collect data obtained from the detection device.

[0024] In certain embodiments, the system also comprises a data processing device to process the data.

[0025] In a particular embodiment, the analysis device according to the invention further comprises a sample fluid introduction device. This sample fluid introduction device is not particularly limited. It may for example comprise simply an introduction opening and/or an introduction channel, preferably with a closing valve. After introducing the sample, said valve may be closed, and a completely closed device is provided, or at least possible. Any other embodiment of the sample fluid introduction device is also contemplated, e.g. those allowing (substantially) contamination-free introduction of a sample fluid.

[0026] According to another embodiment, the sample fluid is introduced to the second volume through the fluid introduction device.

[0027] In a special embodiment, the device of the invention is substantially closed. Of course, during introduction of the sample fluid, there is a connection with the outside world.

[0028] However, it is intended that the analysis device is at least substantially completely closeable, by means of closure means present on or in the device. This may be achieved e.g. by providing valves on all possible channels to the environment. The big advantage is that the device may provide analysis with less risk of contamination, e.g. through exogene DNA from an operator. Furthermore contamination of surrounding environment by the analyte can be prevented.

[0029] In particular, the invention provides a substantially closeable cassette comprising the detection device according to the invention. Such a cassette is preferably compact and portable, such that it may be easily employed for use in situ. It may preferably comprise any other desired device, such as for storage of fluids, one or more pumps, et cetera, such as described in this application, or otherwise known to the skilled person.

[0030] Advantageously, the cassette is disposable, in order to prevent contamination when reusing such a cassette. It is still possible however to provide a reusable cassette according to this invention.

[0031] Note that the terms "first volume" and "second volume" do not relate to specific functions, but merely as ordinal numbers to discern the two. The names may be interchanged, as may the functions.

[0032] According to a second aspect of the invention, an analysis method for analyzing a sample fluid for the presence, absence or amount of an analyte in the sample fluid, the analysis method comprises providing an analysis device; supplying a sample fluid in said second volume; performing at least one time, preferably multiple times the following steps: operating the means for generating a pressure difference for generating the pressure difference between the second volume and the first volume such that the sample fluid flows from the second volume through the guide, through the substrate to the first volume; operating the means for generating a pressure difference for generating the pressure difference between the first volume and the second volume such that the sample fluid flows from the first volume through the substrate, through the guide to the second volume. The substrate is now ready for a detection step. This method allows advantageous use of the device according to the invention, in that sample fluid may be pumped through the substrate any desired number of times. This increases the accuracy and / or sensitivity of the analysis, both by improving the amount of analyte bound to the binding material, and by improving mixing of the constituents of the sample fluid. Herein, the amount of sample fluid is substantially irrelevant, which makes the method more versatile and robust.

[0033] In particular, the method further comprises the step of equalizing the pressure between the first volume and the second volume. In this way, it is prevented that overall pressures keep increasing or that residual pressures interfere with the method. Equalizing the pressure may be performed e.g. after the sample fluid has flowed from the first volume to the second volume, or vice versa, through the substrate or through the return channel, or even only after one or more of all the pumping steps, such as just before actually optically etc. analyzing or inspecting the substrate with the fluid. Equalizing the pressure may be brought about by opening one or more

suitable valves, by operating one or more pumps and the like.

[0034] In particular, the desired number of times is two or more. Repeatedly performing the sequence of steps improves the sensitivity of the analysis. Any number, such as ten or more, is possible. Note that the desired number of times may be determined dynamically, that is, during performing the method. For example, the desired number of times may be determined depending on the strength of a measurement signal or absence thereof.

[0035] In a special embodiment of the method, a detection step is carried out on the substrate still present between the first volume and the second volume. In other words, the substrate is not moved after the pumping actions, in order to prevent possible contamination.

[0036] Thereto, it is possible to carry out the detection from the side of the substrate where there is little or no sample fluid, in order not to disturb the analysis, such as fluorescence detection.

[0037] In the method as described, this may be the second volume side. Alternatively, it is possible to carry out another step of pumping the sample fluid through the substrate, and carry out the analysis from the first volume side of the substrate. If required, the analysis device as provided may comprise a window enabling such optical (or other) detection.

[0038] In a special embodiment of the method, the analyte comprises DNA, RNA, polynucleotides, oligonucleotides, polysaccharides or proteins. Detection of such substances may require very accurate analysis in order to establish the presence or absence of e.g. pathogenic organisms or DNA etc. thereof. The present method, with its increased sensitivity through repeatedly pumping the sample fluid through the substrate, provides advantages for such analyses.

[0039] In a particular embodiment of the method, the substrate is placed substantially horizontally. This improves the accuracy of the method, in that it is easier to ensure that each part of the substrate receives equal amounts of sample fluid.

[0040] In a special embodiment of the method, the first volume is positioned above the substrate with respect to the direction of gravity. This ensures that the sample fluid that is pumped to the second volume is always present in a layer above and in contact with the substrate. This reduces the risk of formation of bubbles which would hinder the pumping through of the sample fluid. The bubble pressure of the substrate is high, and thus the pumping action would be hindered mechanically, by a counter pressure from the bubbles.

[0041] Otherwise, in case the bubble pressure is relatively low and the bubbles would also be pumped through the substrate, the substrate would receive less sample fluid there, which would decrease the sensitivity of the device and method. Furthermore, this configuration reduces the sensitivity for variations in the amount of sample fluid to be processed.

[0042] A general remark is that the time required for pumping the sample fluid once through the substrate depends on the applied pressure difference. By controlling said pressure difference, the time may be actively controlled.

[0043] The invention may be more clearly understood after reading the description of exemplary embodiments, with reference to the appended drawings, in which:

Fig. 1a illustrates a schematic representation of a drop formation without a guide;

Fig. 1b illustrates a schematic representation of a drop formation when a guide is present beneath a surface of a substrate;

Fig. 1c illustrates a schematic representation of a drop formation against a draining surface;

Fig. 2a illustrates a top/bottom view of the device according to an embodiment of the invention;

Fig.2b illustrates a sectional view (A-A) of the device of the Fig.2a; and

Fig.2c illustrates a sectional view (B-B) of the device of the Fg.2a.

[0044] Figures 1a to 1c explain the concept of a drop formation. Fig. 1a shows a drop formation without any guide beneath the substrate. The drop is of diameter D_1. Fig. 1b shows a droplet formed when there is a guide beneath the substrate. The droplet is of diameter D_2 and it is very clear from the figure that $D_2 << D_1$. Reducing the droplet size further or preventing formation of the droplet can be achieved by draining the sample fluid against a surface. The surface could be a fluid below the guide or a solid surface.

[0045] Fig. 2a to Fig. 2c illustrate a device 1 according to the invention. Herein, 10 denotes a porous substrate with a first surface 12 and a second surface 14. 16 denotes a first volume and 18 denotes a second volume. A means for generating pressure 20 is similar to that described in WO2007004102 A2. A guide 22 is placed beneath the second surface 14 and protrudes down to the bottom of the second volume 18. A sample fluid 50 is introduced into the device 1 via an introduction device 26. The introduction device 26 may be closed by means of a valve 28. A pressure is created in the first volume 16 by connecting a first channel 24 with the means for generating pressure 20. Similarly a pressure is created in the second volume 18 by connecting a second channel 25 with the means for generating pressure 20. The device 1 is also provided with an optical window 80 for inspecting the sample with an external optical detection system (not shown).

[0046] The porous substrate 10 may be any suitable type of substrate known in the art. For example non-woven fabrics, substrates based on polished and etched

hollow fibres of glass or other materials, electroformed substrates, and so on may be used. Preferably, the substrate 10 is at least partly transparent for radiation, preferably optical radiation, such as ultraviolet, visible light or infrared. This improves the detection possibilities for the substrate.

[0047] The substrate 10 comprises through-going channels, connecting first volume 16 and second volume 18. If the substrate 10 is wetted, it may show a high bubble pressure. This means that gases may only pass the substrate 10 when a relatively high pressure is exerted. This bubble pressure may be several bars. Contrarily, liquids may pass relatively easily through the substrate 10, requiring only modest pumping pressures of e.g. only a few mbars, although of course the pumping pressure may be selected higher, such as e.g. 0.5 bar, in order to increase the flow of fluid through the substrate 10. All this depends amongst others on capillary pressure in the channels. The device 1 shown in Fig. 2b and 2c is particularly suited for substrates 10 with a high bubble pressure. It is also possible to provide a substrate 10 in which the bubble pressure and the pressure required to pass liquid through the substrate 10 do not show such a large difference, but are more or less comparable.

[0048] The above discussion of passing liquid and/or gas through the substrate 10 in particular holds for a substantially horizontal positioning of the substrate 10. When positioned horizontally, the substrate 10 may be wetted evenly, and liquid will pass more or less homogeneously through the substrate 10. This has a positive influence on detection homogeneity and accuracy. Nevertheless, the substrate 10 may be positioned tilted or even vertically, although this may influence said detection homogeneity.

[0049] It is noted that the terms "first volume" and "second volume" are interchangeable. This means that these terms and expressions are solely used to discern between two separate volumes 16 and 18. Their functions may be interchanged throughout this application.

[0050] The sample fluid introduction device 26 has been indicated only very diagrammatically as a kind of introduction channel. In principle, any desired introduction device known in the state of the art may be provided. The sample fluid introduction device 26 may be closeable by means of the valve 28. Note that, when the valve 28 is closed, the device 1 comprises a completely closed system. This greatly reduces the risk of contamination.

[0051] Use of the device 1 will be explained in more detail with the help of Fig. 2b and Fig. 2c. The sample fluid 50 is pumped into the second volume 18 of the device 1 via the fluid introduction device 26. Subsequent application of a pressure differential over the substrate 10 (resulting in an over pressure in the second volume 18 relative to the first volume 16 by connecting the second channel 25 to the means for generating pressure 20) will cause the sample fluid 50 to be pumped through the guide 22, through the substrate 10 into the first volume 16. In this design the distance between the lower end of the guide 22 and the bottom of the second volume 18 is sufficiently small to ensure that no sample fluid 50 will remain in the second volume 18. Similarly, the distance between the substrate 10 and the guide 22 is also sufficiently small to reduce the amount of sample fluid trapped between the second surface 14 of the substrate 10 and the fluid guide. The small amount of fluid underneath the substrate 10 eliminates the risk of an increased background signal during the detection process and improves the signal to noise ratio.

[0052] It is noted that the pressure difference between first volume 16 and second volume 18 may be established and/or released by means of a pressure generating means 20. When the sample fluid is introduced in the second volume 18, this may be pumped through the guide 22, through substrate 10 to the first volume 16 by increasing the pressure in the second volume 18. Under the influence of the increased pressure in the second volume 18, the sample will flow through the guide 22, through the substrate 10 towards the first volume 16.

[0053] In this design the substrate 10 has a certain bubble pressure. In this design the bubble pressure is lower than the applied pressure differences used for pumping the sample fluid 50. Once all the sample fluid 50 has been pumped through the substrate 10, the bubble pressure of the substrate 10 will prevent the transfer of gas from the second volume 18 into the first volume 16. This is very beneficial as the gas passing through the substrate 10 into the fluid in the first volume 16 may result in foaming. In qualitative/quantitative analysis foaming is highly undesirable.

Even if foaming were not to occur and only bubbles would pass through the fluid mass, breaking up of these bubbles could cause fluid to be 'sprayed' onto the optical window, rendering it unsuitable for the detection function.

In this application the bubble pressure of the substrate 10 in combination with depletion of the amount of fluid in the second volume 18 will cause the flow to stop automatically.

[0054] When the desired amount of fluid has been pumped through substrate 10, the pressure is released. In order to pump sample fluid back from the first volume 16 into the second volume 18, the pressure in the first volume 16 is increased. Reversal of the pressure difference will cause the fluid to be pumped down from the first volume 16, through the substrate 10 and through the guide 22 into the second volume 18. Again the bubble pressure of the substrate in combination with depletion of the amount of the sample fluid 50 in the first volume 16 will cause the flow to stop.

[0055] The sample fluid 50 will again flow from the second volume 18 through the guide 22 to the first volume 16. If the desired amount of sample fluid, for example all of the sample fluid, has been pumped to the first volume 16, the pressure difference is released. Thus the cycle can be repeated for a desired number of times.

[0056] When all fluid is beneath the substrate 10, the first surface 12 of the substrate 10 can be inspected with

an external optical detection system (not shown) through the optical window 80 in the top of the device 1.

[0057] Thus by passing the sample fluid 50 one or more times through the substrate 10, in particular in substantially equal amounts through every part of the substrate 10, it is possible to obtain very good detection results as the amount of DNA or other analytes bound to the binding materials of the substrate 10 is increased. This also increases mixing of the constituents of the sample fluid, which is beneficial for the accuracy of the detection or analysis.

[0058] A transparent window 80 couples a detection device (not shown) with the first volume 16. The window 80 is transparent for e.g. optical radiation. This allows analysis of the substrate 10, containing analyte that has been bound to binding material, for example through fluorescence lighting. Other detection methods are also possible, which may require different radiation, and thus a different transparency for the window 80. Also provided is a detection device such as a camera, a CCD or the like. Note that the detection device is optional. In other words, the analysis device according to the invention may also be provided without the detection device, but with the window 80. It is thus possible to provide the analysis device as a disposable device, without the need for a detection device, which is often very complex and expensive.

[0059] The number of cycles depends on various criteria. For example, if there is excellent binding between the analyte in the sample fluid 50 and the binding material in the substrate 10, it is possible that a single cycle (or a few) suffices for the analysis. In other cases, a higher number of cycles are required, such as 2, 3 or more. The number of cycles is in principle unlimited.

[0060] The embodiments shown in the drawings and described above are intended to be exemplary and non-limiting. The scope of the invention is defined by the appended claims, in view of the description above. Similarly, the reference numerals used in the claims solely serve to clarify the claims in view of some embodiments shown. In particular, they do not limit the claims or the parts thereof provided with such reference numerals to the specific embodiments or parts thereof as depicted in the figures. This holds especially for the first and second volumes.

Claims

1. An analysis device (1) for analyzing a sample fluid (50) for the presence, absence or amount of an analyte in the sample fluid (50), the analysis device (1) comprising:

   a. a substrate (10) having a diameter (d), the substrate having a first surface (12) and an opposite second surface (14), and having a plurality of through going channels from the first surface (12) to the second surface (14), and at least partially being provided with a binding substance specific for the analyte;
   b. a first volume (16) in fluid communication with the first surface (12);
   c. a second volume (18) in fluid communication with the second surface (14);
   d. a means (20) for generating a pressure difference over the substrate (10) to transport the fluid from the first (16) to the second volume (18) and vice versa through the channels; and
   e. a guide (22) placed beneath the second surface (14) of the substrate (10) and protruding towards the bottom of the second volume (18), wherein the guide (22) has an exit diameter substantially smaller than the diameter(d) of the substrate (10).

2. The device of claim 1, wherein the substrate (10) having a bubble pressure in a wetted condition, with respect to the sample fluid (50), that is higher than the generated pressure difference between the first volume (16) and the second volume (18).

3. The device of claim 3, wherein said bubble pressure is at least 10% higher, preferably at least 50% higher, and even more preferably at least 200% higher than said sample fluid pump pressure.

4. The device of any preceding claim, comprising a wall around at least one of the first volume (16) and the second volume (18) which is at least partially transparent.

5. The device of any preceding claim, further comprising a detection system.

6. The device of any preceding claim, further comprising a sample fluid introduction device (26).

7. The device of any preceding claim, wherein the sample fluid (50) is introduced to the second volume (18) through the fluid introduction device (26).

8. The device of any preceding claim, that is substantially closeable.

9. An analysis method for analyzing a sample fluid (50) for the presence, absence or amount of an analyte in the sample fluid (50), the analysis method comprising:

   a. providing an analysis device according to one of claims 1-8;
   b. supplying a sample fluid in said second volume (18);
   c. performing at least one time, preferably multiple times the following steps:

operating the means (20) for generating a pressure difference for generating the pressure difference between the second volume (18) and the first volume (16) such that the sample fluid (50) flows from the second volume (18) through the guide (22), through the substrate (10) to the first volume (16);
operating the means (20) for generating a pressure difference for generating the pressure difference between the first volume (16) and the second volume (18) such that the sample fluid (50) flows from the first volume (16) through the substrate (10), through the guide (22) to the second volume (18).

10. The method of claim 9, further comprising the step of equalizing the pressure between the first volume (16) and the second volume (18).

11. The method of claim 9 wherein the desired number of times is two or more.

12. The method of any of claims 9-11, wherein a detection step is carried out on the substrate (10) present between the first volume (16) and the second volume (18).

13. The method of any of claims 9-12, wherein the analyte comprises DNA, RNA, polynucleotides, oligonucleotides, polysaccharides or proteins.

14. The method of any of claims 9-13, wherein the substrate (10) is placed substantially horizontally.

15. The method of any of claims 9-13, wherein the first volume (16) is positioned above the substrate (10) with respect to the direction of gravity.

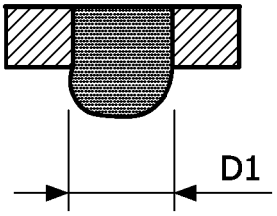


FIG. 1a

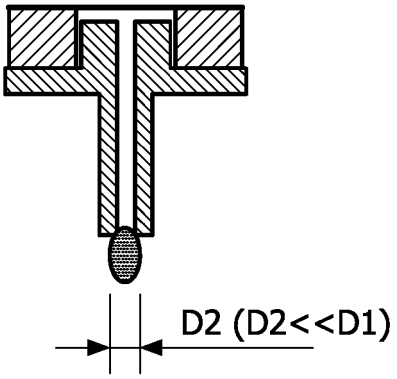


FIG. 1b

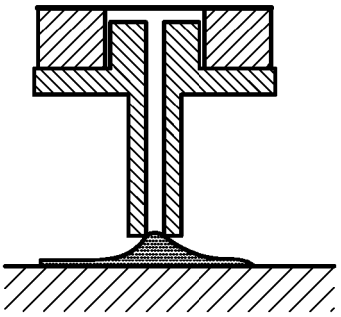


FIG. 1c

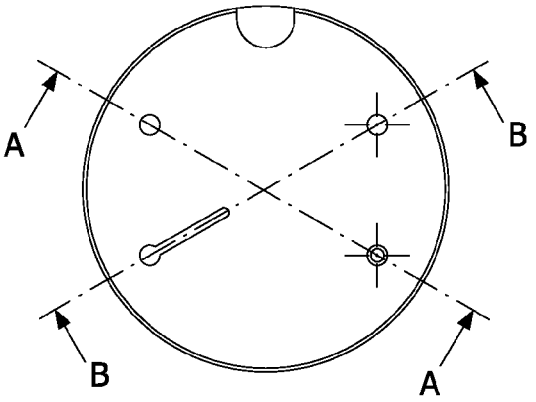
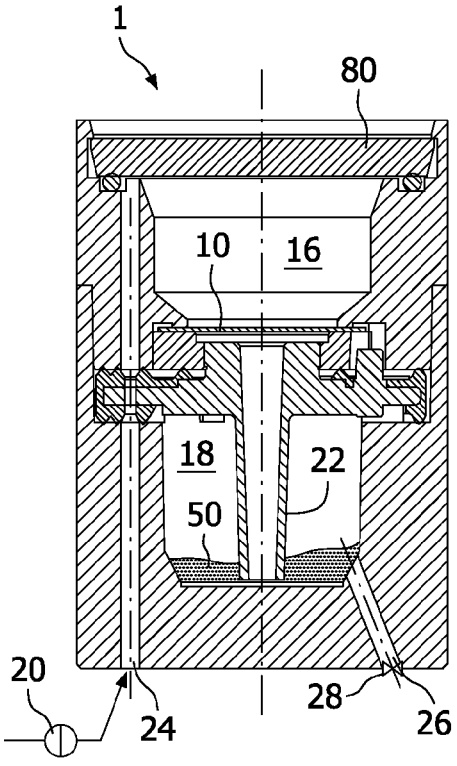
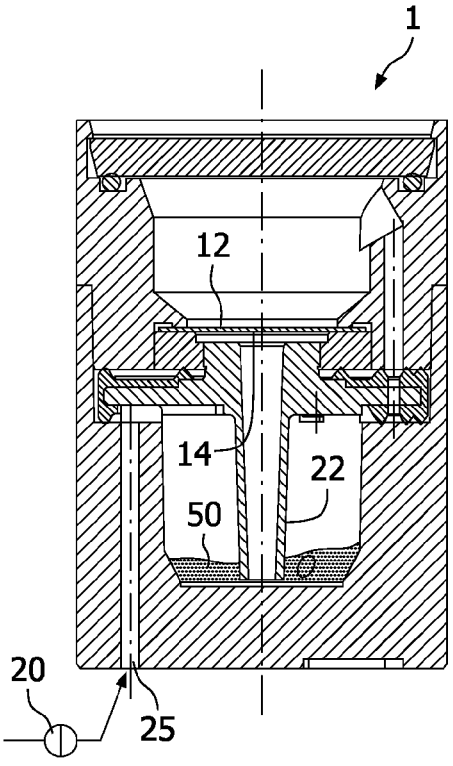


FIG. 2a



SECTION A-A

FIG. 2b



SECTION B-B

FIG. 2c

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EUROPEAN SEARCH REPORT

Application Number
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Place of search	Date of completion of the search	Examiner
Munich	9 May 2008	de Biasio, Arnaldo

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