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Wu et al.

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(54) **DEVICES FOR EXTRACTING AT LEAST ONE ANALYTE**

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C12Q 1/68 (2018.01)

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(Continued)

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CPC G01N 27/44739; G01N 27/44778; G01N 27/44791; G01N 27/44726; G01N 27/44721; C12Q 1/6806
See application file for complete search history.

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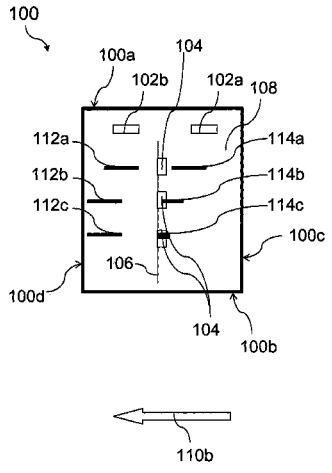
(57) **ABSTRACT**

A device for extracting at least one analyte may include: a sample reservoir configured to contain a sample comprising at least one target analyte and interfering materials; at least one extraction chamber connected to the sample reservoir; at least one porous structure lining one or more sides of the at least one extraction chamber; and a voltage source configured to provide a first voltage and a second voltage, wherein, when the first voltage is provided, the at least one target analyte and the interfering materials move towards the at least one extraction chamber or to a predetermined area from the at least one extraction chamber, wherein, when the second voltage is provided, the interfering materials pass through and exit the at least one extraction chamber, and the at least one target analyte is stopped from exiting the at least one extraction chamber by means of the at least one porous structure.

18 Claims, 13 Drawing Sheets



(52) **U.S. Cl.**
CPC . ***G01N 27/44791*** (2013.01); *G01N 27/44721* (2013.01); *G01N 27/44726* (2013.01)

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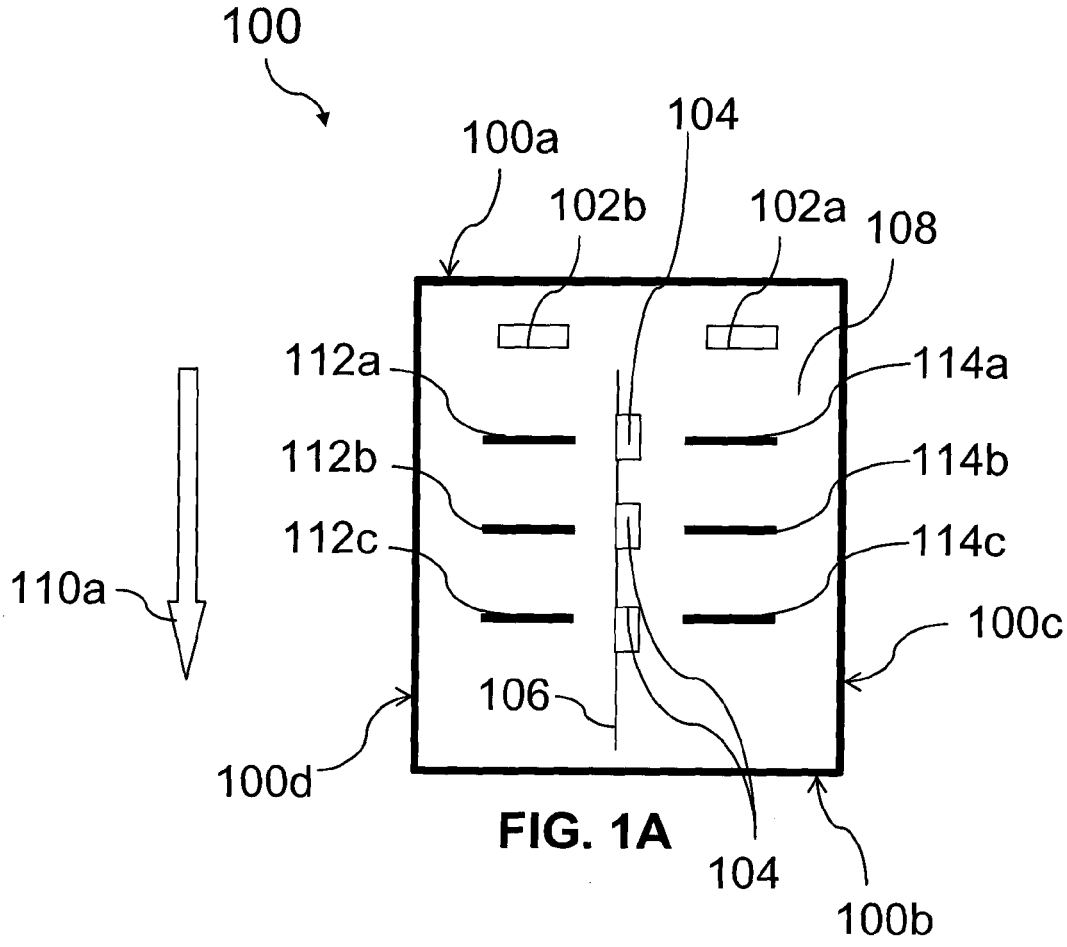

100
100a
104
102b
102a
108
112a
114a
112b
114b
112c
114c
110a
100c
106
100d
104
100b

FIG. 1A

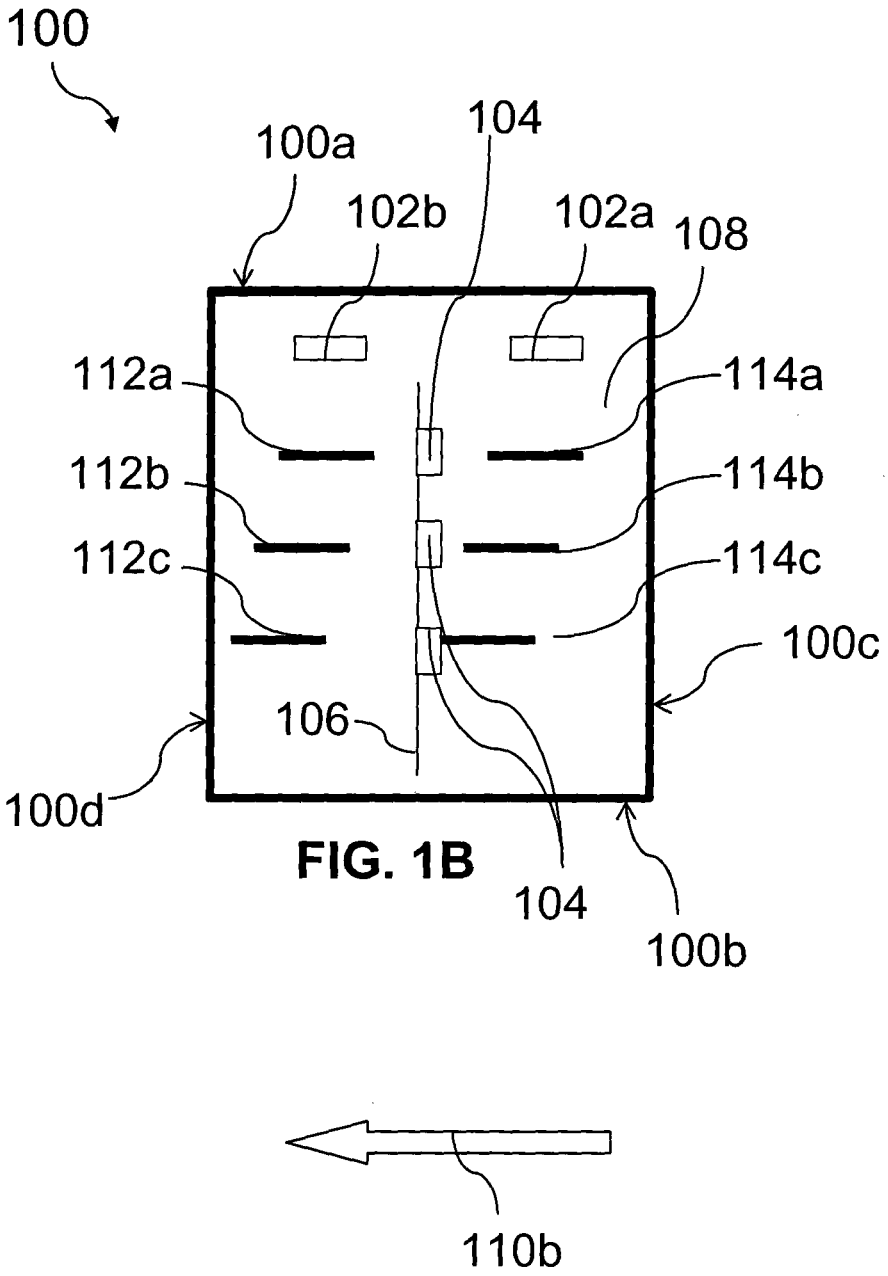

100
100a
104
102b
102a
108
112a
114a
112b
114b
112c
114c
100c
106
100d
104
100b
110b

FIG. 1B

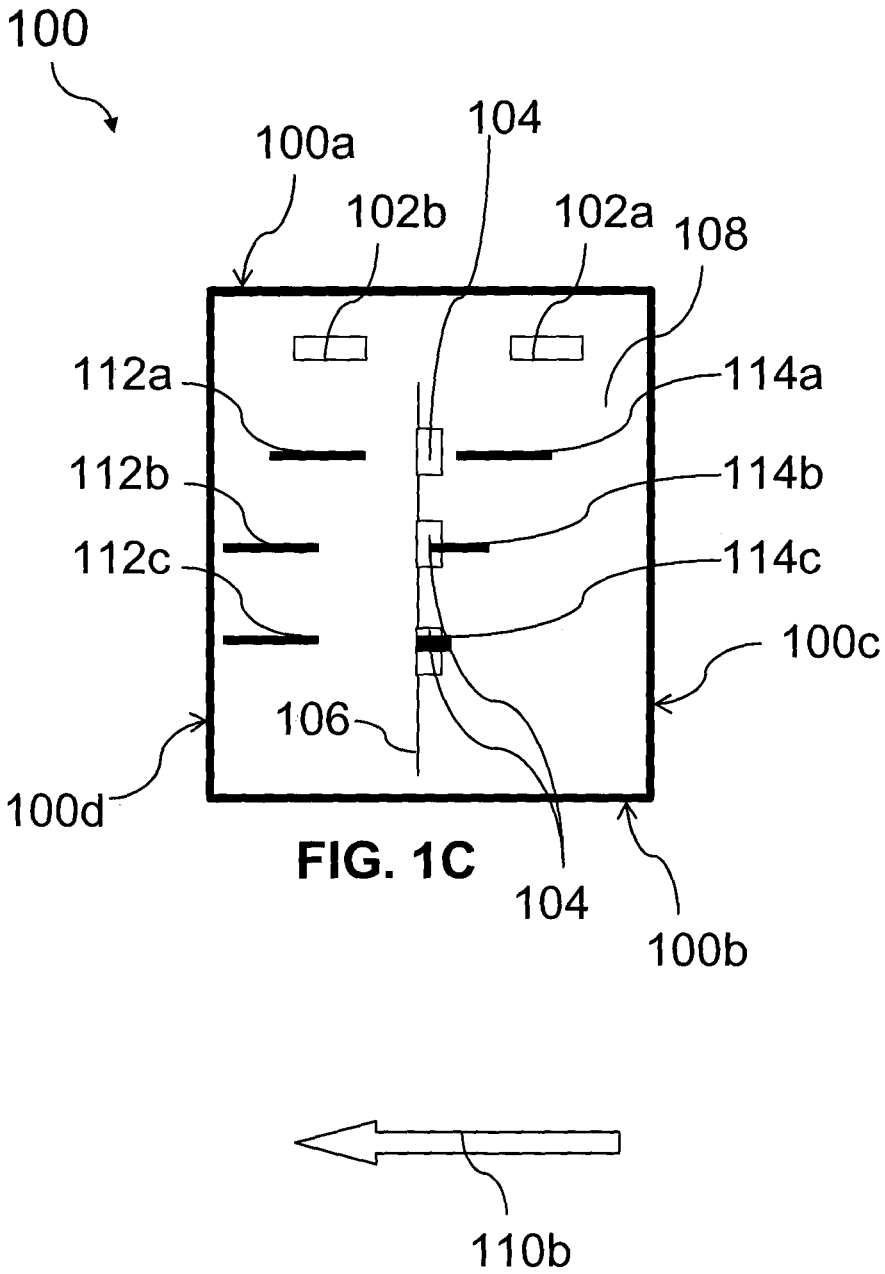
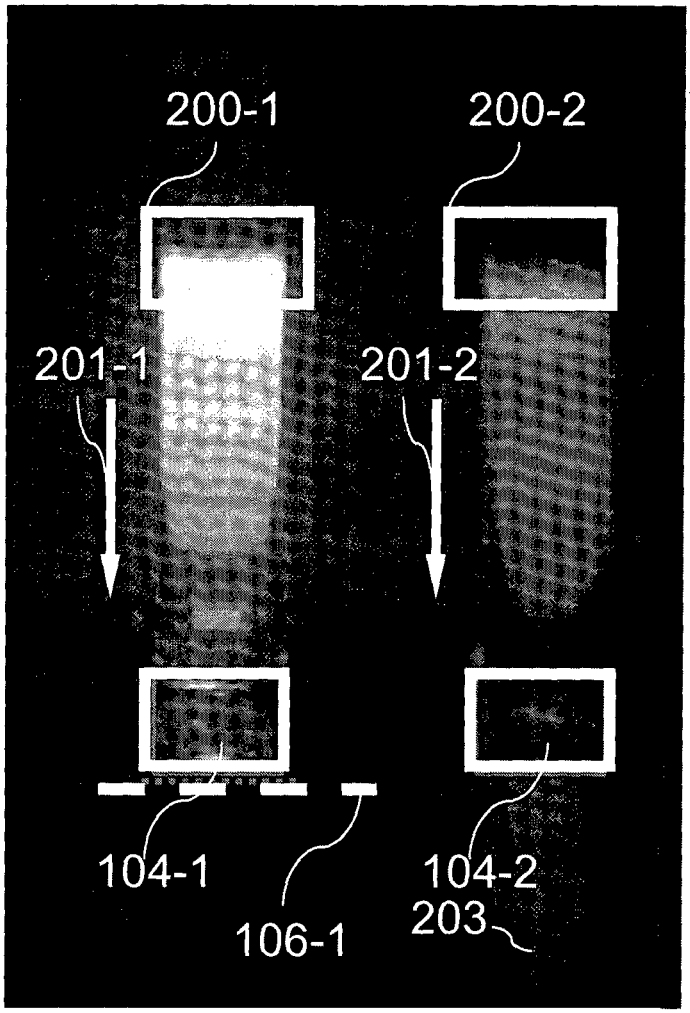

100
100a
104
102b
102a
108
112a
114a
112b
114b
112c
114c
100c
106
100d
104
100b
110b

FIG. 1C



Extraction chamber

Extraction membrane

FIG. 2

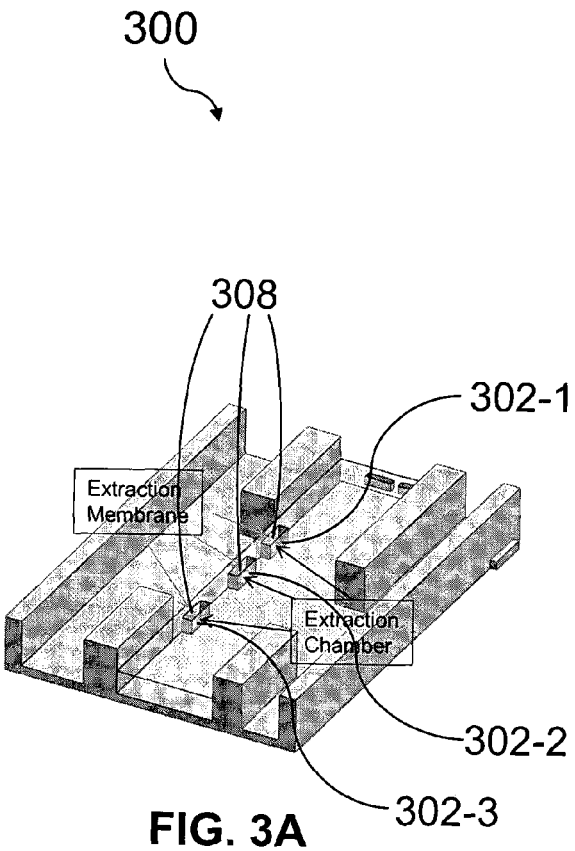

300
308
302-1
Extraction Membrane
Extraction Chamber
302-2
302-3

FIG. 3A

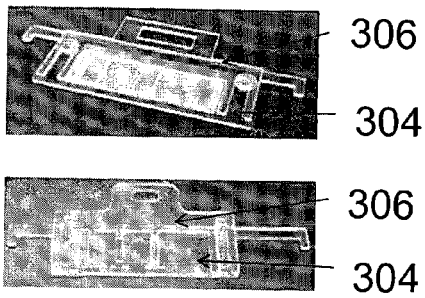

306
304
306
304

FIG. 3B

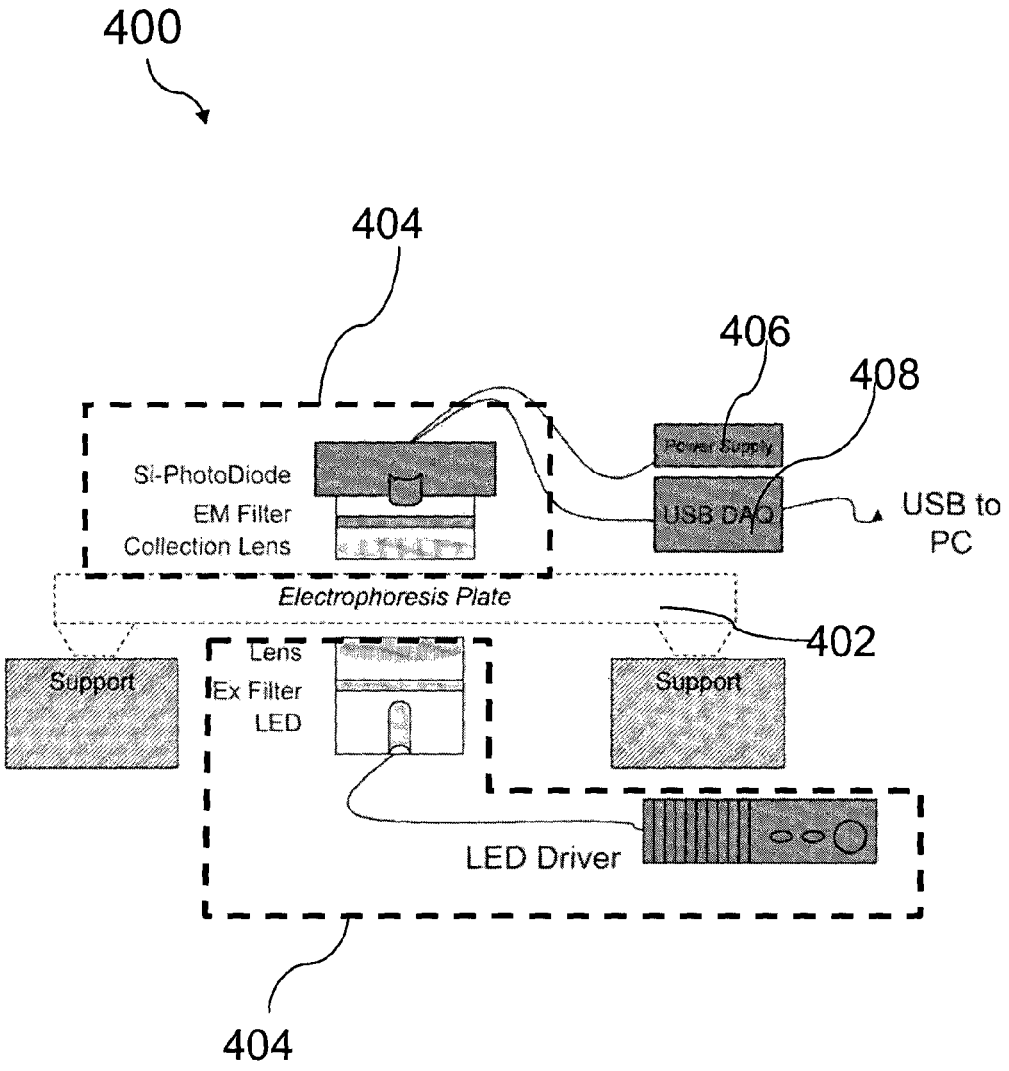

400
404
406
408
Si-PhotoDiode
EM Filter
Collection Lens
Power Supply
USB DAQ
USB to PC
Electrophoresis Plate
402
Support
Lens
Ex Filter
LED
Support
LED Driver
404

FIG. 4

500

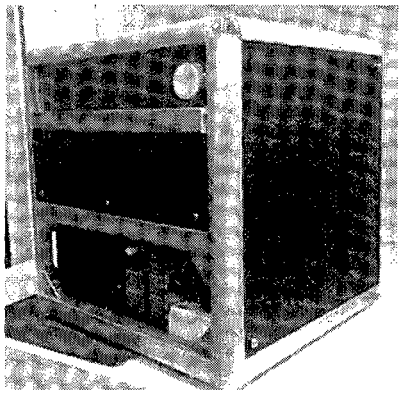


FIG. 5

600

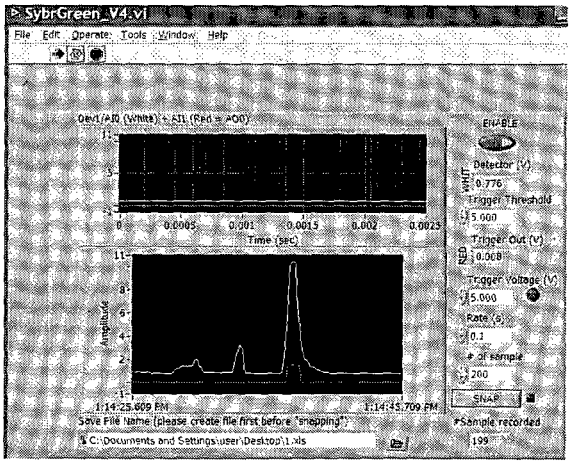


FIG. 6

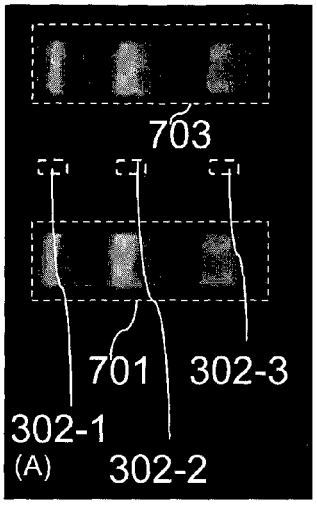


FIG. 7A

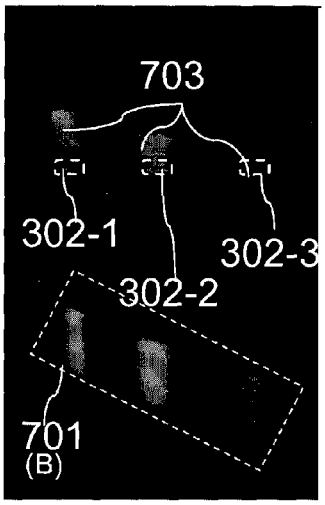


FIG. 7B

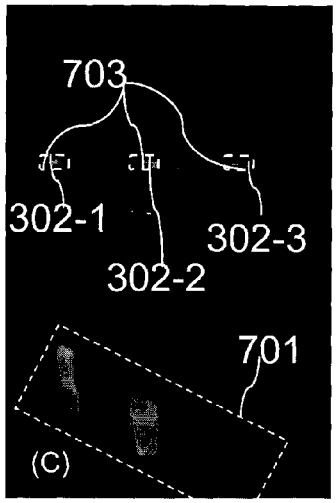


FIG. 7C

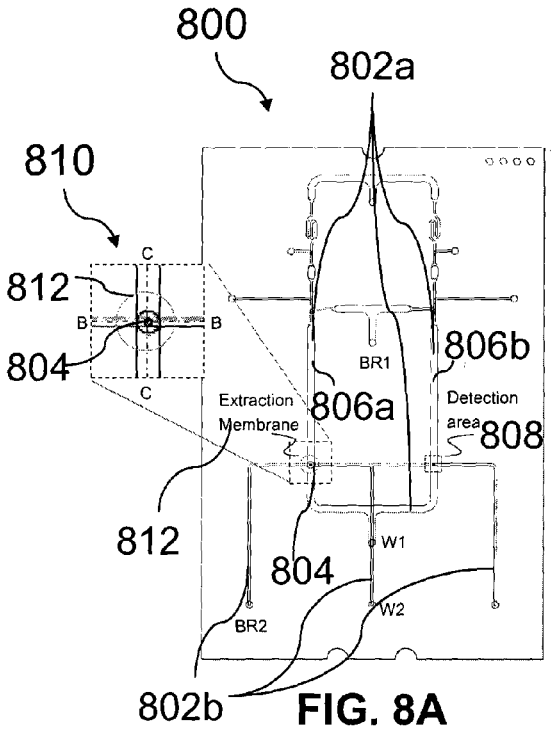


FIG. 8A

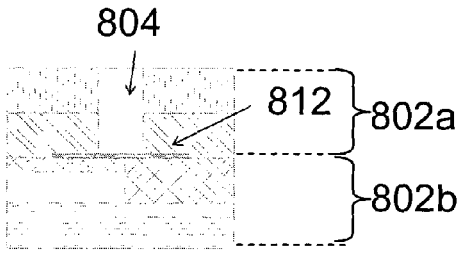


FIG. 8B

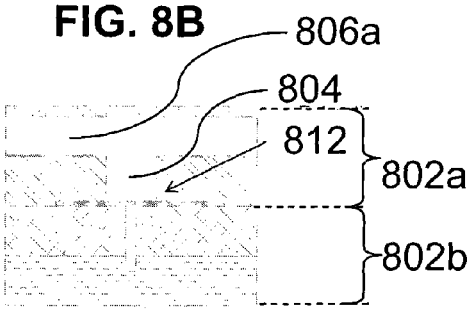


FIG. 8C

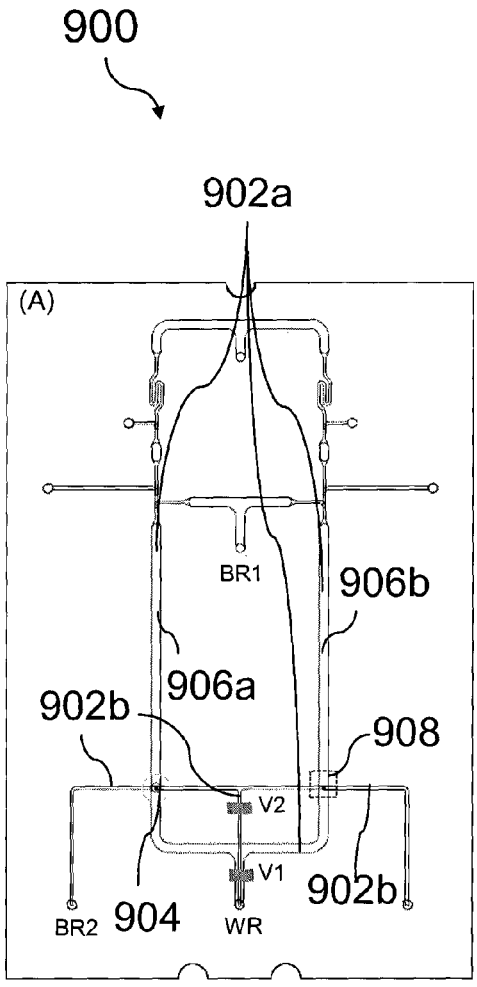

900
902a
(A)
BR1
906b
906a
902b
908
V2
V1
902b
BR2
904
WR

FIG. 9A

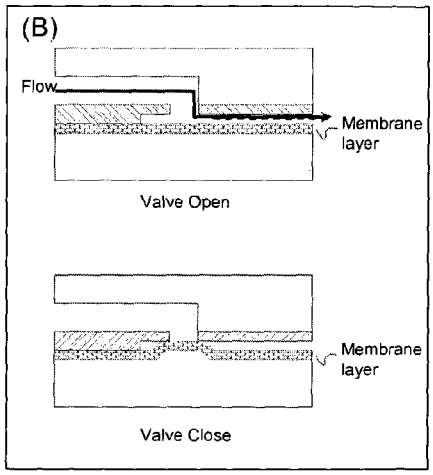

(B)
Flow
Membrane layer
Valve Open
Membrane layer
Valve Close

FIG. 9B

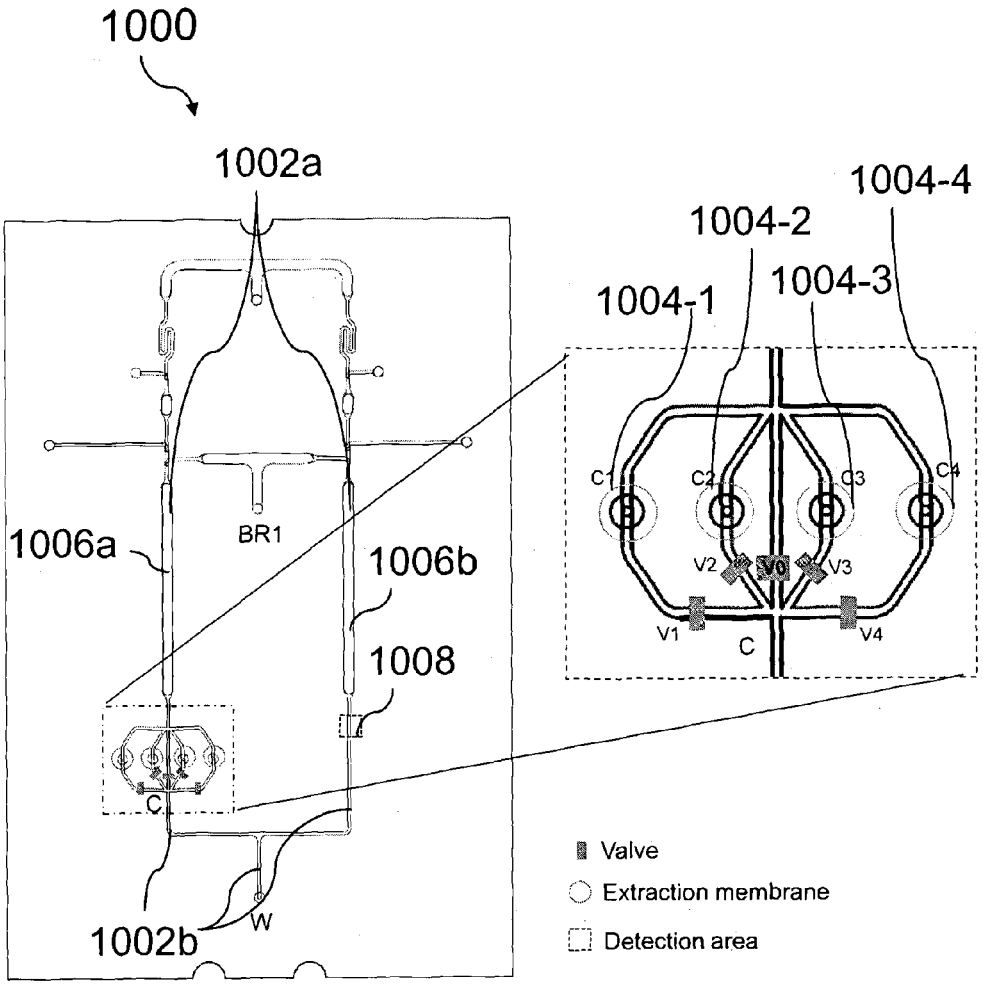

1000
1002a
1004-4
1004-2
1004-1
1004-3
C1
C2
C3
C4
V2
V0
V3
V1
C
V4
BR1
1006a
1006b
1008
C
1002b
W
Valve
Extraction membrane
Detection area

FIG. 10

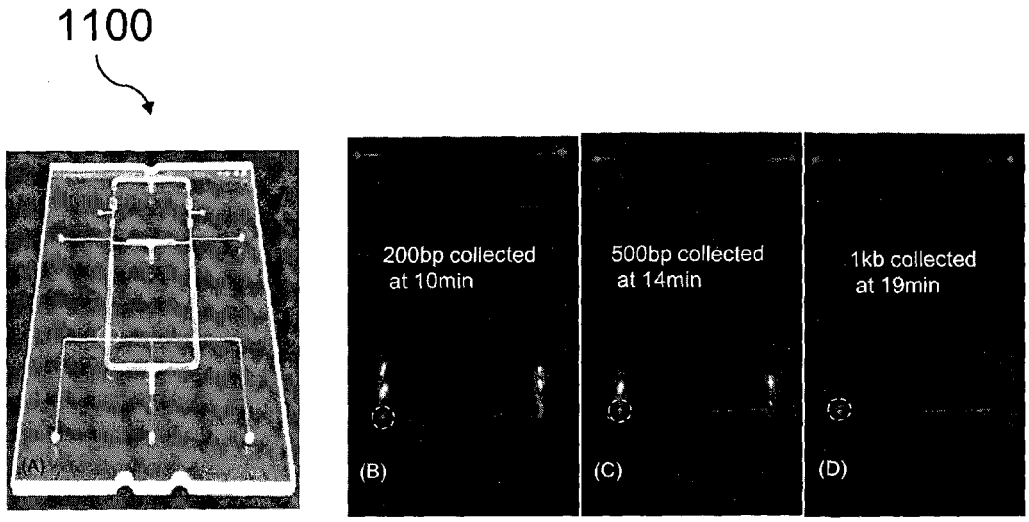


FIG. 11A FIG. 11B FIG. 11C FIG. 11D

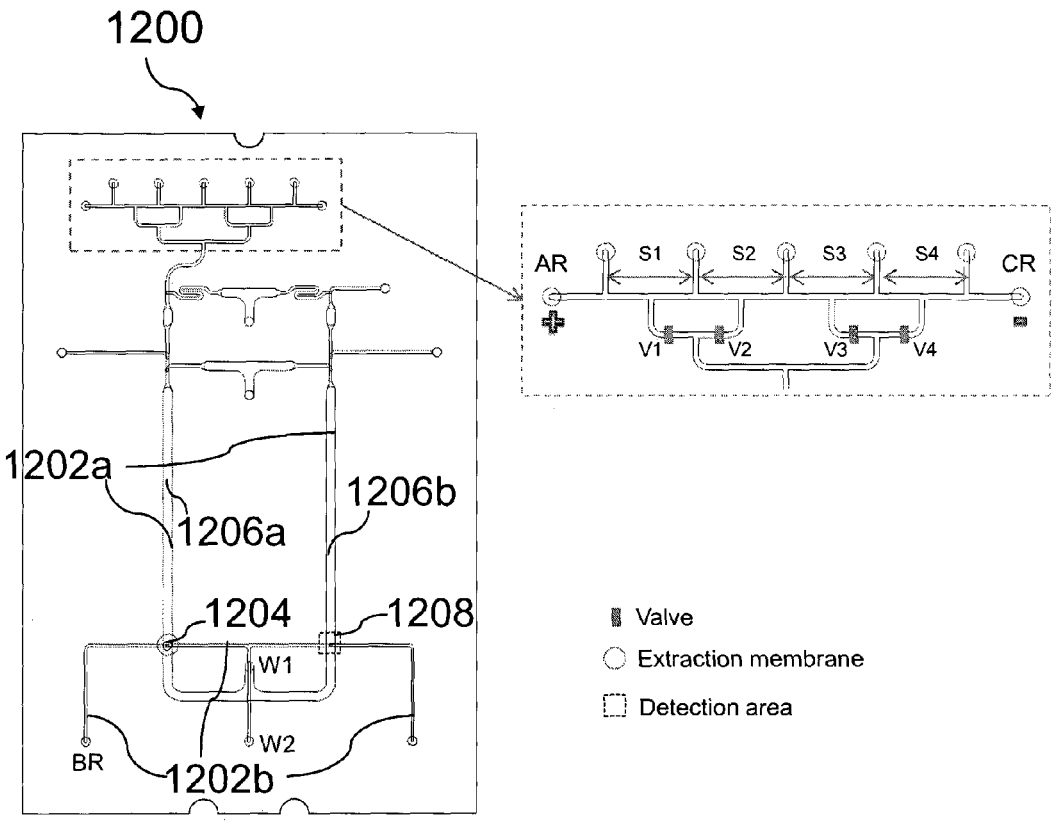

1200
AR
S1
S2
S3
S4
CR
V1
V2
V3
V4
1202a
1206a
1206b
1204
1208
W1
W2
BR
1202b
Valve
Extraction membrane
Detection area

FIG. 12

DEVICES FOR EXTRACTING AT LEAST ONE ANALYTE

TECHNICAL FIELD

Various aspects relate to devices for extracting at least one analyte.

BACKGROUND

A pure analyte (DNA, RNA or protein) fragment is important for bioanalysis. Preparation of a pure analyte fragment from a complicated sample may include separation of the target analyte fragment from interfering materials by gel electrophoresis and extraction of it from the gel matrix. To get pure target analyte fragment, the gel with all separated fragments is placed on an ultraviolet (UV) light box to visualize the location of the interested analyte fragments. A scalpel is used to cut around the interested fragment band and carefully slice the small piece of gel containing the interested band from the whole gel. After that, the sliced gel is put in a centrifuge tube with other chemicals to obtain the pure analyte fragment.

Since UV light is dangerous to the eyes and skin, protection (e.g. a protective shield and/or protective clothing) may be needed for an operator performing the separation and extraction of the target analyte fragment. Furthermore, the above-described approach to preparing and extracting the target analyte fragment (e.g. to obtain a pure analyte fragment) may be time consuming and laborious. Even further, the above-described approach cannot be done automatically and the results are operator dependent. New ways of extracting a target analyte fragment may be needed.

SUMMARY

In an embodiment, a device for extracting at least one analyte may include: a sample reservoir configured to contain a sample comprising at least one target analyte and interfering materials; at least one extraction chamber connected to the sample reservoir; at least one porous structure lining one or more sides of the at least one extraction chamber; and a voltage source configured to provide a first voltage and a second voltage, wherein, when the first voltage is provided, the at least one target analyte and the interfering materials move towards the at least one extraction chamber or to a predetermined area from the at least one extraction chamber, wherein, when the second voltage is provided, the interfering materials pass through and exit the at least one extraction chamber, and the at least one target analyte is stopped from exiting the at least one extraction chamber by means of the at least one porous structure.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings, like reference characters generally refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the invention. In the following description, various, aspects of the invention are described with reference to the following drawings, in which:

FIG. **1**A to FIG. **1**C show plan-views of a device for extracting at least one analyte.

FIG. **2** shows a result of using the device shown in FIG. **1**A to FIG. **1**C for an extraction experiment.

FIG. **3**A and FIG. **3**B show perspective view of a cartridge including a plurality of extraction chambers, a membrane frame, a chamber frame, and a sandwiched porous structure.

FIG. **4** shows a schematic of a detection system that may detect the movement of analyte fragments in electrophoresis and trigger an extraction of a target analyte fragment.

FIG. **5** shows an electrophoresis and detection system which may be used for separation and extraction of a target analyte fragment from a sample.

FIG. **6** shows a user interface of a software to detect the reference analyte fragment and trigger the switch of electrical field direction.

FIG. **7**A to FIG. **7**C show reference DNA fragments and target DNA fragments that may be separated by means of the cartridge shown in FIG. **3** and the detection system shown in FIG. **4** and FIG. **5**.

FIG. **8**A to FIG. **8**C show various views of a multi-layer microfluidic chip including at least one porous structure.

FIG. **9**A and FIG. **9**B show various views of a multi-layer microfluidic chip including a first flow controller and a second flow controller.

FIG. **10** shows a plan view of a multi-layer microfluidic chip including a plurality of flow controllers and a plurality of extraction chambers.

FIG. **11**A shows a photograph of a multi-DNA extraction chip used in an analyte extraction experiment and FIG. **11**B to FIG. **11**D show results of the experiment.

FIG. **12** shows a plan view of a two-dimensional separation-extraction microfluidic chip.

DESCRIPTION

The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and aspects in which the invention may be practised. These aspects are described in sufficient detail to enable those skilled in the art to practice the invention. Other aspects may be utilized and structural, logical, and electrical changes may be made without departing from the scope of the invention. The various aspects are not necessarily mutually exclusive, as some aspects can be combined with one or more other aspects to form new aspects. Various aspects are described for structures or devices, and various aspects are described for methods. It may be understood that one or more (e.g. all) aspects described in connection with structures or devices may be equally applicable to the methods, and vice versa.

The word "exemplary" is used herein to mean "serving as an example, instance, or illustration". Any aspect or design described herein as "exemplary" is not necessarily to be construed as preferred or advantageous over other aspects or designs.

The terms "coupled" and/or "connected" used herein to describe a feature being connected to at least one other implied feature, are not meant to mean that the feature and the at least one other implied feature must be directly coupled or connected together; intervening features may be provided between the feature and at least one other implied feature.

Directional terminology, such as e.g. "upper", "lower", "top", "bottom", "left-hand", "right-hand", etc., may be used with reference to the orientation of figure(s) being described. Because components of the figure(s) may be positioned in a number of different orientations, the directional terminology is used for purposes of illustration and is in no way limiting. It is to be understood that structural or logical changes may be made without departing from the scope of the invention.

Bioanalysis may include analysis of an analyte and/or an analyte fragment (e.g. DNA, RNA and/or protein). The description that follows provides examples of preparation, separation, and extraction of an analyte fragment (e.g. pure analyte fragment). However, the examples described may be analogously applied to preparation, separation, and extraction of an analyte (e.g. pure analyte).

Preparation of a pure analyte fragment from a sample (e.g. a sample including, or consisting of, the pure analyte fragment as well as other interfering materials) may include separating the pure analyte fragment from the interfering materials, e.g. by means of a separation process. The pure analyte fragment may also be referred to as a target analyte fragment.

The separation process may include, or may be, electrophoresis (e.g. gel electrophoresis). The separation process (e.g. electrophoresis, e.g. gel electrophoresis) may be performed in a medium. For example, in gel electrophoresis, the medium in which the separation process is performed may include, or may be, a gel matrix.

Preparation of the pure analyte fragment from the sample (e.g. a complicated sample including, or consisting of, the pure analyte fragment as well as other interfering materials) may further include extracting the target analyte fragment from the medium in which the separation process is performed (e.g. gel matrix). The extraction may be performed subsequent to the separation process (e.g. electrophoresis, e.g. gel electrophoresis).

In order to obtain a pure target analyte fragment, the medium (e.g. gel matrix) including the target analyte fragment as well as other separated analyte fragments may be placed on a surface that may be illuminated by ultraviolet (UV) light (e.g. a UV light box). This may be done in order to visualize the location of the target analyte fragment in the medium (e.g. gel matrix).

An implement (e.g. a scalpel) may be used to cut around the location of the target analyte fragment. The area around the location of the target analyte fragment may be, or may be referred to as, a band. The band having the target analyte fragment may be sliced from the medium (e.g. gel matrix) and the target analyte fragment may thereafter be extracted from the sliced band. For example, the sliced band having the target analyte fragment may be placed in a centrifuge (e.g. a centrifuge tube), e.g. with other chemicals, to obtain a pure analyte fragment.

As described above, UV light may be used to visualize the location of the target analyte fragment in the medium (e.g. gel matrix). Since UV light is dangerous to the eyes and skin, protection (e.g. a protective shield and/or protective clothing) may be needed for an operator performing the separation and extraction of the target analyte fragment. Furthermore, the above-described approach to preparing and extracting the target analyte fragment (e.g. to obtain a pure analyte fragment) may be time consuming and laborious. Even further, the above-described approach cannot be done automatically and the results are operator dependent.

Accordingly, it may be desirable to provide a method and/or device that may be simpler and safer than the current state-of-the art technology for analyte fragment extraction from a medium (e.g. gel matrix).

It may be desirable to provide a method and/or device that may select and/or extract a target analyte fragment automatically out of a medium (e.g. gel matrix) without use of UV light.

It may be desirable to provide a method and/or device that may enable an operator to only load a sample (e.g. complicated sample having the target analyte fragment and interfering materials) into a medium (e.g. gel matrix) and automatically collect the target analyte fragment (e.g. desired analyte fragment). In other words, it may be desirable to provide a method and/or device that may not be operator dependent.

FIG. **1**A to FIG. **1**C show plan-views of a device **100** for extracting at least one analyte.

The device **100** may be simpler and safer than the current state-of-the art technology for analyte fragment extraction from a medium (e.g. gel matrix).

The device **100** may be used to select and/or extract a target analyte fragment (e.g. a desired analyte fragment) automatically out of a medium (e.g. gel matrix) without use of UV light.

The device **100** may enable an operator to only load a sample (e.g. complicated sample having the target analyte fragment and interfering materials) into a medium (e.g. gel matrix) and automatically collect the target analyte fragment (e.g. desired analyte fragment).

The device **100** may be included in a detection system, that may detect the movement of analyte fragments in electrophoresis (e.g. gel electrophoresis) and trigger an extraction of a target analyte fragment when the target analyte fragment is detected (e.g. movement of the target analyte fragment is detected).

The device **100** may include a sample reservoir **102***a*, at least one extraction chamber **104**, and at least one porous structure **106** lining one or more sides of the at least one extraction chamber **104**. The device **100** may further include a reference reservoir **102***b*.

In the embodiment shown in FIG. **1**A, only three extraction chambers **104** are shown as an example. However, in another embodiment, the number of extraction chambers may be less than three (e.g. one, two) or more than three (e.g. four, five, six, etc.).

In the embodiment shown in FIG. **1**A, one porous structure **106** is shown to line one side of the at least one extraction chamber **104**. However, in another embodiment, the at least one porous structure **106** may line more than one side of the at least one extraction chamber **104**.

A sample (e.g. having a target analyte fragment and interfering materials) and a reference (e.g. having a reference analyte fragment, e.g. having molecules of the same size as the target analyte fragment of the sample) may be loaded into respective reservoirs. For example, a reference containing a reference analyte fragment (e.g. having molecules of the same size as the target analyte fragment of the sample) may be loaded in the reference reservoir **102***b*, and a sample (e.g. having a target analyte fragment and interfering materials) may be loaded in the sample reservoir **102***a*.

Accordingly, the sample reservoir **102***a* may be configured to contain a sample including at least one target analyte or target analyte fragment and interfering materials, and the reference reservoir **102***b* may be configured to contain a reference analyte or reference analyte fragment, which may have particles of the same size as the target analyte fragment of the sample.

Electrodes (not shown in FIG. **1**A) may be placed in a buffer solution to form an electrical field that may drive the target analyte fragment and the reference analyte fragment to move in a medium **108** (e.g. a gel or gel matrix) based on the electrophoresis principle. For example, the target analyte fragment and the reference analyte fragment may move under the influence of the electrical field from the sample and reference reservoirs towards a predetermined area near the at least one extraction chamber **104**. The at least one

extraction chamber **104** may be connected to the sample reservoir **102***a* and the reference reservoir **102***b* by means of the medium **108** (e.g. gel matrix). Accordingly, the target analyte fragment and the reference analyte fragment may move through the medium **108** (e.g. gel matrix) towards a predetermined area near the at least one extraction chamber **104**.

For example, as shown in FIG. **1**A, an electrical field may be formed in the direction indicated by arrow **110***a* (e.g. by means of placing electrodes at sides **100***a*, **100***b* of the device **100**). After running electrophoresis (e.g. gel electrophoresis) for a period of time, analyte fragments with different size may be separated as shown in FIG. **1**A. The reference analyte fragments separated by means of electrophoresis (e.g. gel electrophoresis) are indicated in FIG. **1**A and FIG. **1**B as reference signs **112***a*, **112***b*, **112***c*; and the separated target analyte fragments of the sample are indicated in FIG. **1**A and FIG. **1**B as reference signs **114***a*, **114***b*, **114***c*.

Accordingly, the target analyte fragment and the interfering materials of the sample may move under the influence of the voltage applied to the device **100** to a predetermined area from or near the at least one extraction chamber **104**.

A detector may detect the separation of the reference analyte fragments **112***a*, **112***b*, **112***c*. For example, a detector may detect the reference analyte fragments **112***a*, **112***b*, **112***c* when the reference analyte fragments **112***a*, **112***b*, **112***c* are at least substantially aligned to the at least one extraction chamber **104**. Substantial alignment of the reference analyte fragments **112***a*, **112***b*, **112***c* to the at least one extraction chamber **104** may imply that the separated target analyte fragments (indicated by reference signs **114***a*, **114***b*, **114***c*) of the sample are also at least substantially aligned to the at least one extraction chamber **104**, as shown in FIG. **1**B.

Upon detection of the separated reference analyte fragments **112***a*, **112***b*, **112***c*, a trigger may switch the direction of the electrical field. For example, as shown in FIG. **1**B, the electrical field may be switched to the direction indicated by arrow **110***b* (e.g. by means of placing electrodes at sides **100***c*, **100***d* of the device **100**) from the direction indicated by arrow **110***a* shown in FIG. **1**A. At this time, the target analyte fragments and the interfering materials (indicated by reference signs **114***a*, **114***b*, **114***c*) may move towards the at least one extraction chamber **104** under the influence of the electrical field **110***b*. Since the direction of the electrical field may be switched when the separated target analyte fragments (indicated by reference signs **114***a*, **114***b*, **114***c*) of the sample are also at least substantially aligned to the at least one extraction chamber **104**, the at least one extraction chamber **104** may be located along the flow path of the target analyte fragments and the interfering materials (indicated by reference signs **114***a*, **114***b*, **114***c*) when the electrical field is oriented in the direction indicated by arrow **110***b*.

The at least one porous structure **106** lining one or more sides of the at least one extraction chamber **104** may have a suitable pore size which can permit small molecules like dyes and ions to pass through under electrical field, while big analyte fragments will be stopped from passing through it. Accordingly, the at least one porous structure **106** lining one or more sides of the at least one extraction chamber **104** may stop the target analyte fragment from passing through it and thus may act as a means to contain the target analyte fragment in the at least one extraction chamber **104**. The at least one porous structure **106** lining one or more sides of the at least one extraction chamber **104** may include, or may be, an extraction membrane (e.g. a porous extraction membrane).

Accordingly, the interfering materials may pass through the at least one porous structure **106** lining one- or more sides of the at least one extraction chamber **104**, and may exit the at least one extraction chamber **104** under the influence of the voltage applied to the device **100** in the direction indicated by arrow **110***b*. As shown in FIG. **1**C, the target analyte fragment may be stopped or prevented from exiting the at least one extraction chamber **104** by means of the at least one porous structure **106** lining one or more sides of the at least one extraction chamber **104**. Once all the target analyte fragments are extracted from the medium **108** (e.g. gel or gel matrix), a pipette can be used to collect the desired analyte fragments from the at least one extraction chamber **104**.

FIG. **2** shows a result of using the device **100** shown in FIG. **1**A to FIG. **1**C for an extraction experiment.

A molecular-weight size marker including a DNA ladder of at least 100 bp was used for the extraction experiment. FIG. **2** shows a first reservoir **200-1** and a second reservoir **200-2**. The first reservoir **200-1** is at least substantially aligned to a first extraction chamber **104-1** and a porous structure **106-1** lining one side of the first extraction chamber **104-1**. A target analyte fragment contained in the first reservoir **200-1** flows along a first flow path **201-1** from the first reservoir **200-1** to the first extraction chamber **104-1**, e.g. under the influence of an applied voltage.

FIG. **2** also shows that the second reservoir **200-2** is at least substantially aligned to a second extraction chamber **104-2**. The second extraction chamber **104-2** is free from a porous structure. A target analyte fragment contained in the second reservoir **200-2** flows along a second flow path **201-2** from the second reservoir **200-2** to the second extraction chamber **104-2**, e.g. under the influence of an applied voltage.

As shown in FIG. **2**, in the first flow path **201-1** having the porous structure **106-1** (e.g. extraction membrane), all DNA fragments no smaller than about 100 bp were stopped in the extraction chamber **104-1**, e.g. by means of the porous structure **106-1**. This is indicated by the containment of the sample in the extraction chamber **104-1**. However, in the second flow path **201-2** free from the porous structure, the DNA fragments passed through the extraction chamber **104-2** and moved towards the electrode (e.g. anode) under the electrical field. This is indicated in FIG. **2** as a bleeding **203** from the extraction chamber **104-2** away from the second reservoir **200-2**.

In an embodiment, the device **100** may include, or may be, a cartridge **300**.

FIG. **3**A and FIG. **3**B show perspective view of a cartridge **300** including a plurality of extraction chambers **302-1** to **302-3**, a membrane frame **304**, a chamber frame **306**, and a sandwiched porous structure **308**.

The cartridge **300** may be included in a detection system that may detect the movement of analyte fragments in electrophoresis (e.g. gel electrophoresis) and trigger an extraction of a target analyte fragment when the target analyte fragment is detected (e.g. movement of the target analyte fragment is detected). The sandwiched porous structure **308** may include or may be a sandwiched membrane.

The chamber frame **306** may be designed to assist the integration of the porous structure **308** (e.g. membrane) into the plurality of extraction chambers **302-1** to **302-3**. For example, the chamber frame **306** may be designed to line at least one side of the plurality of extraction chambers **302-1** to **302-3** with the porous structure **308** (e.g. membrane).

At first, the cartridge **300** may be used for gel casting as a gel container. At first, the porous structure **308** (e.g.

membrane) may be sandwiched between the membrane frame **304** and the chamber frame **306**, as shown in FIG. **3**B. It may then be dipped into casted gel in cartridge **300**. After the gel is solidified, the chamber frame **306** may be removed, leaving the membrane frame **304**, the porous structure **308** (e.g. membrane) and the formed plurality of extraction chambers **302-1** to **302-3** inside the gel, as shown in FIG. **3**A. A plurality of porous structures **308** (e.g. membranes) can be formed, with each extraction chamber of the plurality of extraction chambers **302-1** to **302-3** having a respective porous structure **308**. Alternatively, or in addition, as shown in FIG. **3**A and FIG. **3**B, a single porous structure **308** (e.g. membrane) can be used for all extraction chambers of the plurality of extraction chambers **302-1** to **302-3**.

As described above, the device **100** (e.g. cartridge **300**) may be included in a detection system that may detect the movement of analyte fragments in electrophoresis (e.g. gel electrophoresis) and trigger an extraction of a target analyte fragment when the target analyte fragment is detected (e.g. movement of the target analyte fragment is detected).

FIG. **4** shows a schematic of a detection system **400** that may detect the movement of analyte fragments in electrophoresis (e.g. gel electrophoresis) and trigger an extraction of a target analyte fragment when the target analyte fragment is detected (e.g. movement of the target analyte fragment is detected).

The detection system **400** may include a platform **402** where the extraction is performed, a detection system **404** to locate the reference analyte fragment to trigger the switch of electrical field direction, a power supply **406**, a data collection module **408**, and a voltage switch device (not shown in FIG. **4**).

FIG. **5** shows an electrophoresis and detection system **500** which may be used for separation and extraction of a target analyte fragment from a sample. The electrophoresis and detection system **500** may be identified with the detection system **400** shown in FIG. **4**. In other words, the detection system **400** shown in FIG. **4** may be a schematic of the detection system **500** shown in FIG. **5**.

FIG. **6** shows a user interface of a software to detect the reference analyte fragment and trigger the switch of electrical field direction.

An experiment was carried out to demonstrate multiple DNA extraction using the device **100** (e.g. cartridge **300**) and the detection system **400**. The results of the experiment are shown in FIG. **7**A to FIG. **7**C.

FIG. **7**A to FIG. **7**C show reference DNA fragments **701** and target DNA fragments **703** that may be separated by means of the cartridge **300** shown in FIG. **3** and the detection system shown in FIG. **4** and FIG. **5**.

As shown in FIG. **7**A to FIG. **7**C, extraction of three DNA fragments (200 bp, 500 bp and 1 kb), was realized with the cartridge **300** shown in FIG. **3**. After automatically switching the direction of electrical field with the trigger system, the three target DNA fragments **703** moved towards the respective extraction chamber **302-1**, **302-2**, **302-3**. To visualize and verify the extraction process, both reference DNA fragments **701** and target DNA fragments **703** were mixed with fluorescent dye SYBR green I.

DNA quantitation of extracted DNA fragment was performed using Nanodrop 2000. The A260/A280 ratio of three extracted DNA fragment were all about 1.8, which shows that the purity of collected DNA fragment is acceptable and there may not be a need for an extra purification step. Therefore, the device **100** (e.g. cartridge **300**) and the detection system **400** can help operators extract several separated target DNA fragments from a medium (e.g. gel or gel matrix) and avoid the use of hazardous UV light.

In an embodiment, extraction of one or more target analytes and/or target analyte fragments may be realized on a device configured as a multi-layer microfluidic chip.

FIG. **8**A shows a plan view of a multi-layer microfluidic chip **800** including at least one porous structure **812**.

FIG. **8**B shows a cross-sectional view of the multi-layer microfluidic chip **800** along the line B-B shown in the exploded view **810** of a part of FIG. **8**A.

FIG. **8**C shows a cross-sectional view of the multi-layer microfluidic chip **800** along the line C-C shown in the exploded view **810** of a part of FIG. **8**A

The multi-layer microfluidic chip **800** may include separation channels **802***a* disposed over supporting channels **802***b*. In the plan view of FIG. **8**A, the separation channels **802***a* and the supporting channels **802***b* may seem to be on the same level, however, they are disposed on different levels of the multi-layer microfluidic chip **800**. As more clearly shown in the cross-sectional views of FIG. **8**B and FIG. **8**C, the separation channels **802***a* may be disposed on an upper layer of the multi-layer microfluidic chip **800** and the supporting channels **802***b* may be disposed on a lower layer of the multi-layer microfluidic chip **800**. In other words, the supporting channels **802***b* may be underneath the separation channels **802***a*. The separation channels **802***a* may be filled with a medium for electrophoresis (e.g. gel) and the supporting channels **802***b* may be filled with a running buffer.

The multi-layer microfluidic chip **800** may include at least one extraction chamber **804**. Only one extraction chamber **804** is shown as an example. However the number of extraction chambers may be more than one and may, for example, be two, three, four, or more extraction chambers.

The multi-layer microfluidic chip **800** may include at least one porous structure **812** disposed between the separation channels **802***a* and the supporting channels **802***b* (e.g. as shown in FIG. **8**B and FIG. **8**C).

The separation channels **802***a* may include a plurality of parallel channels **806***a*, **806***b*, e.g. as shown in FIG. **8**A. The plurality of parallel channels **806***a*, **806***b* of the separation channels **802***a* may be designed and fabricated on the multi-layer microfluidic chip **800**. Only two parallel channels **806***a*, **806***b* are shown as an example. However the number of parallel channels may be more than two and may, for example, be three, four, or more parallel channels.

Each parallel channel of the plurality of parallel channels **806***a*, **806***b* may be connected to a respective reservoir. For example the parallel channel **806***a* may be connected to a sample reservoir and the parallel channel **806***b* may be connected to a reference reservoir. The parallel channel **806***a* connected to the sample reservoir may be additionally connected to the at least one extraction chamber **804**. For example, the parallel channel **806***a* may be disposed between the sample reservoir and the at least one extraction chamber **804**. Accordingly, the parallel channel **806***b* may be used as a reference channel and the parallel channel **806***a* connecting to the at least one extraction chamber **804** may be used for separation of target analyte fragments.

Electrodes (not shown in FIG. **8**A) may be placed at or on the multi-layer microfluidic chip **800**. For example, an electrode may be placed at each of reservoir BR**1** and first waste reservoir W**1**, which may generate an electrical field along the medium (e.g. gel) in the channel (e.g. microfluidic channel) from reservoir BR**1** to first waste reservoir W**1**. The reservoir BR**1** and the first waste reservoir W**1** may be disposed in the separation channels **802***a* of the multi-layer

microfluidic chip **800**. Accordingly, the electrical field may be generated in an upper layer of the multi-layer microfluidic chip **800**.

Multiple analyte fragments of the sample may thus separate along the parallel channel **806***a* towards the first waste reservoir W**1**, e.g. in the medium (e.g. gel) that may be pre-loaded in the parallel channel **806***a*. At the same time, a reference analyte fragment may move along the parallel channel **806***b* towards the first waste reservoir W**1**. The flow path of the reference analyte fragment between the reference reservoir and the first waste reservoir W**1** may intersect with a detection area **808** that may be disposed along the parallel channel **806***a*. The detection area **808** may be disposed in the parallel channel **806***b* of the multi-layer microfluidic chip **800**.

Once the reference analyte fragment is detected in the detection area **808**, the direction of the electrical field is switched from the first direction BR**1**-W**1** to a second direction BR**1**-W**2** towards the at least one extraction chamber **804** and the underneath supporting channel **802***b* in the bottom layer of the multi-layer microfluidic chip **800**, as shown in FIG. **8**B and FIG. **8**C. For example, an electrode may be placed at a second waste reservoir W**2**, which may be disposed in the supporting channel **802***b* of the multi-layer microfluidic chip **800**.

FIG. **8**B shows a cross-sectional view of the at least one extraction chamber **804** along the line B-B shown in the exploded view **810** of FIG. **8**A. FIG. **8**C shows a cross-sectional view of the at least one extraction chamber **804** along the line C-C shown in the exploded view **810** of FIG. **8**A.

As seen in FIG. **8**B and FIG. **8**C, at least one porous structure **812** may line one or more sides of the at least one extraction chamber **804**. As described above, the interfering materials pass through the at least one porous structure **812** and exit the at least one extraction chamber **804** under the influence of the second voltage applied in the second direction BR**1**-W**2**. The at least one target analyte may be stopped from exiting the at least one extraction chamber **804** by means of the at least one porous structure **812**. Since the target analyte fragment cannot pass through the at least one porous structure **812**, the target analyte fragment is confined within the at least one extraction chamber **804**, which may be filled with the same buffer as the running buffer of electrophoresis (e.g. gel electrophoresis).

The at least one porous structure (e.g. extraction membrane) may be embedded in the multi-layer microfluidic chip **800** by means of at least one of hot embossing, thermal bonding, laser bonding, ultrasonic bonding, although other techniques may be possible as well. After embedding, the at least one porous structure (e.g. extraction membrane) may be tightly fit in the multi-layer microfluidic chip **800** to avoid sample loss or cross contamination during the above-described extraction process.

Extraction of multiple analyte fragments can be realized sequentially or simultaneously. As shown in FIG. **8**A, once the first reference fragment is detected, the electrical field may be switched from the direction of BR**1**-W**1** to the direction of BR**1**-W**2**, and the first target analyte fragment may be collected in the at least one extraction chamber **804**.

After the first target analyte fragment is completely moved from the medium (e.g. gel) into the at least one extraction chamber **804**, the solution in the at least one extraction chamber **804** containing the first target analyte fragment can be taken out either by a pipette or a pump. Thereafter, a fresh running buffer can be re-filled for the subsequent extractions either by a pipette or a pump. To remove possible residue of the first target analyte fragment that may be left in the at least one extraction chamber **804**, rinsing of the at least one extraction chamber **804** can be done by repeating the filling-emptying process with a pipette or pump. Alternatively, it can be realized by applying the electrical field BR**2**-W**1** for a period of time. The minor quantity of first target analyte fragment residue can be completely removed from the extraction buffer toward the first waste reservoir W**1** with only the running buffer left. By repeating the separation and extraction steps, multiple target analyte fragments can be collected sequentially.

In another embodiment, extraction can be realized by using flow controllers (e.g. valve elements).

FIG. **9**A and FIG. **9**B show various views of a multi-layer microfluidic chip **900** including a first flow controller V**1** and a second flow controller V**2**.

Similar to the multi-layer microfluidic chip **800** shown in FIG. **8**A to FIG. **8**C, the multi-layer microfluidic chip **900** may include separation channels **902***a* disposed over supporting channels **902***b*. In the plan view of FIG. **9**A, the separation channels **902***a* and the supporting channels **902***b* may seem to be on the same level, however, they are disposed on different levels of the multi-layer microfluidic chip **900**. In other words, similar to the microfluidic chip **800**, the supporting channels **902***b* may be underneath the separation channels **902***a*. The separation channels **902***a* may be filled with a medium for electrophoresis (e.g. gel) and the supporting channels **902***b* may be filled with a running buffer.

The first flow controller V**1** may be disposed in the separation channels **902***a* (i.e. the upper layer) of the multi-layer microfluidic chip **900**. The second flow controller V**2** may be disposed in the supporting channels **902***b* (i.e. the lower layer) of the multi-layer microfluidic chip **900**.

Similar to the multi-layer microfluidic chip **800** shown in FIG. **8**A to FIG. **8**C, the separation channels **902***a* may include a plurality of parallel channels **906***a*, **906***b*, e.g. as shown in FIG. **9**A. The plurality of parallel channels **906***a*, **906***b* may be designed and fabricated on the multi-layer microfluidic chip **900**. Only two parallel channels **906***a*, **906***b* are shown as an example. However the number of parallel channels may be more than two and may, for example, be three, four, or more parallel channels.

Each parallel channel of the plurality of parallel channels **906***a*, **906***b* may be connected to a respective reservoir. For example the parallel channel **906***a* may be connected to a sample reservoir and the parallel channel **906***b* may be connected to a reference reservoir. The parallel channel **906***a* connected to the sample reservoir may be additionally connected to the at least one extraction chamber **904**. For example, the parallel channel **906***a* may be disposed between the sample reservoir and the at least one extraction chamber **904**. Accordingly, the parallel channel **906***b* may be used as a reference channel and the parallel channel **906***a* connecting to the at least one extraction chamber **904** may be used for separation of target analyte fragments.

Electrodes (not shown in FIG. **9**A) may be placed at or on the multi-layer microfluidic chip **900**. For example, an electrode may be placed at each of reservoir BR**1** and the waste reservoir WR, which may generate an electrical field along the medium (e.g. gel) in the channel (e.g. microfluidic channel) from reservoir BR**1** to the waste reservoir WR. The reservoir BR**1** may be disposed in the separation channels **902***a* of the multi-layer microfluidic chip **900**, while the waste reservoir WR may be disposed in the supporting channels **902***b* of the multi-layer microfluidic chip **900**. Accordingly, the electrical field may be generated from an

upper layer of the multi-layer microfluidic chip **900** to a lower layer of the multi-layer microfluidic chip **900**. For example, the plurality of parallel channels **906***a*, **906***b* and the underneath supporting channels **902***b* may be connected to the same waste reservoir WR.

When the first flow controller V**1** disposed in the separation channels **902***a* is opened, while second flow controller V**2** in the supporting channels **902***b* is closed, and when voltage is applied, an electrical field is created along the microfluidic channel from reservoir BR**1** to waste reservoir WR. At least one target analyte fragment and at least one reference analyte fragment are thus separated in the medium (e.g. gel matrix) pre-loaded in the parallel channels **906***a* and **906***b* respectively of the top layer of the multi-layer microfluidic chip **900** towards the waste reservoir WR.

Once the first reference analyte fragment is detected in the detection area **908**, the first flow controller V**1** (e.g. valve element) may subsequently be closed and second flow controller V**2** (e.g. valve element) may subsequently be opened. The electrical field is still created along the microfluidic channel from reservoir BR**1** to waste reservoir WR. However, in this case, the electrical field is created along the underneath supporting channels **902***b* from reservoir BR**1** toward waste reservoir WR. The target analyte fragment may then be extracted in the extraction chamber **904**. After the extraction is completed, a pipette or a pump is used for the first target analyte fragment collection and fresh buffer re-filling.

After this the second flow controller V**2** (e.g. valve element) may be closed and the first flow controller V**1** (e.g. valve element) may be opened and the electrical field direction will be switched back along the upper separation channels **902***a*. When the second reference analyte fragment is detected in the detection area **908**, the first flow controller V**1** (e.g. valve element) may be closed again and the second flow controller V**2** (e.g. valve element) may be opened for the extraction of the second target analyte fragment. This process may be repeated for a third reference analyte fragment and a third target analyte fragment, and so forth.

To remove possible analyte residue left in the extraction chamber **904**, rinsing of the extraction chamber **904** can be done by repeating the filling-emptying process with a pipette or pumps. Alternatively, it can also be realized by applying the electrical field from a buffer reservoir BR**2** towards the waste reservoir WR for a period of time.

In another embodiment, multiple target analyte fragments may be extracted simultaneously by using a plurality of flow controllers (e.g. valve elements) V**0** to V**4**, as shown in FIG. **10**.

FIG. **10** shows a plan view of a multi-layer microfluidic chip **1000** including a plurality of flow controllers V**0** to V**4** and a plurality of extraction chambers C**1** to C**4**.

Similar to the multi-layer microfluidic chip **800** shown in FIG. **8**A to FIG. **8**C, the multi-layer microfluidic chip **1000** may include separation channels **1002***a* disposed over supporting channels **1002***b*. In the plan view of FIG. **10**, the separation channels **1002***a* and the supporting channels **1002***b* may seem to be on the same level, however, they are disposed on different levels of the multi-layer microfluidic chip **1000**. For example, in an analogous manner to that shown in the cross-sectional view of FIG. **8**B and FIG. **8**C, the separation channels **1002***a* may be disposed on an upper layer of the multi-layer microfluidic chip **1000** and the supporting channels **1002***b* may be disposed on a lower layer of the multi-layer microfluidic chip **1000**. In other words, the supporting channels **1002***b* may be underneath the separation channels **1002***a*. The separation channels **1002***a* may be filled with a medium for electrophoresis (e.g. gel) and the supporting channels **1002***b* may be filled with a running buffer.

Similar to the multi-layer microfluidic chip **800** shown in FIG. **8**A to FIG. **8**C, the separation channels **1002***a* may include a plurality of parallel channels **1006***a*, **1006***b*, e.g. as shown in FIG. **10**. The plurality of parallel channels **1006***a*, **1006***b* may be designed and fabricated on the multi-layer microfluidic chip **1000**. Only two parallel channels **1006***a*, **1006***b* are shown as an example. However the number of parallel channels may be more than two and may, for example, be three, four, or more parallel channels.

Each parallel channel of the plurality of parallel channels **1006***a*, **1006***b* may be connected to a respective reservoir. For example the parallel channel **1006***a* may be connected to a sample reservoir and the parallel channel **1006***b* may be connected to a reference reservoir. The parallel channel **1006***a* connected to the sample reservoir may be additionally connected to the plurality of extraction chambers C**1** to C**4**. For example, the parallel channel **1006***a* may be disposed between the sample reservoir and the plurality of extraction chambers C**1** to C**4**. Accordingly, the parallel channel **1006***b* may be used as a reference channel and the parallel channel **1006***a* connecting to the plurality of extraction chambers C**1** to C**4** may be used for separation of target analyte fragments.

The flow controller V**0** may be disposed in the separation channels **1002***a* of the multi layer microfluidic chip **1000**. The plurality of flow controllers V**1** to V**4** may be disposed in the supporting channels **1002***b* of the multi-layer microfluidic chip **1000**. The plurality of extraction chambers C**1** to C**4** may be disposed in the supporting channels **1002***b* of the multi-layer microfluidic chip **1000**.

A respective porous structure **1004-1** to **1004-4** may line at least one side of a respective extraction chamber of the plurality of extraction chambers C**1** to C**4**. The respective porous structures **1004-1** to **1004-4** may, for example, be disposed between the separation channels **1002***a* and the supporting channels **1002***b* of the multi-layer microfluidic chip **1000**. The respective porous structures **1004-1** to **1004-4** may, for example, be additionally disposed in the separation channels **1002***a* of the multi-layer microfluidic chip **1000** over an area spanning the underlying extraction chambers C**1** to C**4**.

An electrode may be placed in the buffer at each of reservoir BR**1** and waste reservoir W to provide an electrical field in the direction of BR**1**-W through the buffer in the gel along the microfluidics channel.

At first, all flow controllers (e.g. valve elements) V**0** to V**4** may be closed except the flow controller V**0** which may be connected with the channel branch without an extraction chamber, as shown in FIG. **10**.

When the first reference analyte fragment is detected in the detection area **1008**, the flow controller V**1** (e.g. valve element) on the supporting channel branch connecting to the extraction chamber C**1** may be opened while all the other flow controllers (e.g. valves) may be closed. The first target analyte fragment may then be transferred into the separation channel branch leading to the extraction chamber C**1**, e.g. disposed in the supporting channels **1002***b* of the multi-layer microfluidic chip **1000**.

Thereafter, the flow controller V**0** (e.g. valve element) may be opened and all other flow controllers (e.g. valve elements) may be closed. When the second reference analyte fragment is detected in the detection area **1008**, the flow controller V**2** (e.g. valve element) on the supporting channel branch connecting to the extraction chamber C**2** is opened, while all the other flow controllers (e.g. valves) may be

closed. The second target analyte fragment may then be transferred into the separation channel branch leading to the extraction chamber C2, e.g. disposed in the supporting channels **1002***b* of the multi-layer microfluidic chip **1000**. This process may be repeated for the third reference analyte fragment, the third target analyte fragment, and all subsequent reference and/or target analyte fragments.

After all target analyte fragments are transferred into respective separation channels, all flow controllers V0 to V4 may be opened to extract all target analyte fragments into the respective extraction chambers C1 to C4, e.g. disposed in the supporting channels **1002***b* of the multi-layer microfluidic chip **1000**.

Alternatively, a selective microvalve can be implemented at point C shown in FIG. **10**. In such an example, the selective microvalve may open a path without an extraction chamber for the separation of target analyte fragments. When the first reference analyte fragment is detected in the detection area **1008**, the selective microvalve at point C may open a path from extraction chamber C1 to the waste reservoir W to transfer the first target analyte fragment into the separation channel branch leading to the extraction chamber C1. When the second reference analyte fragment is detected, the selective microvalve at point C may open a path from extraction chamber C2 to waste reservoir W to transfer the second target analyte fragment into the separation channel branch leading to the extraction chamber. C2. This process may be repeated for subsequent reference analyte fragments and subsequent target analyte fragments.

An experiment was performed on the above-described multi-layer microfluidic chips **800**, **900**, **1000** to sequentially extract three DNA fragments of molecular-weights 200 bp, 500 bp and 1 kb.

FIG. **11**A shows a photograph of a multi-DNA extraction chip **1100** used in the experiment and FIG. **11**B to FIG. **11**D show results of the experiment.

As shown in FIG. **11**B to FIG. **11**D, the three DNA fragments of 200 bp, 500 bp and 1 kb were completely extracted from the medium (e.g. gel matrix) in the separation channel at 10 min, 14 min and 19 min, respectively. This is much faster compared to the conventional DNA extraction from a medium (e.g. gel matrix), which usually takes more than 3 hours from medium casting (e.g. gel casting) to DNA purification and extraction from the medium (e.g. gel). Moreover, the operation can be significantly simplified without using extra reagents or devices for DNA extraction from the medium (e.g. gel matrix). For example, an operator only needs to load the sample and reference, run the system and collect the extracted target analyte fragment (e.g. DNA fragment), e.g. with a pipette, sequentially when each individual extraction is finished.

FIG. **12** shows a plan view of a two-dimensional separation-extraction microfluidic chip **1200**.

Similar to the multi-layer microfluidic chip **800** shown in FIG. **8**A to FIG. **8**C, the multi-layer microfluidic chip **1200** may include separation channels **1202***a* disposed over supporting channels **1202***b*. In the plan view of FIG. **12**, the separation channels **1202***a* and the supporting channels **1202***b* may seem to be on the same level, however, they are disposed on different levels of the multi-layer microfluidic chip **1200**. For example, in an analogous manner to that shown in the cross-sectional views of FIG. **8**B, and FIG. **8**C, the separation channels **1202***a* may be disposed on an upper layer of the multi-layer microfluidic chip **1200** and the supporting channels **1202***b* may be disposed on a lower layer of the multi-layer microfluidic chip **1200**. In other words, the supporting channels **1202***b* may be underneath the separation channels **1202***a*. The separation channels **1202***a* may be filled with a medium for electrophoresis (e.g. gel) and the supporting channels **1202***b* may be filled with a running buffer.

In the embodiment shown in FIG. **12**, extraction of target analyte fragments like protein biomarkers from complicated samples may be done in two dimensions. The first dimension may be in the direction between an anodic reservoir AR and a cathodic reservoir CR. For example, a sample protein mixture may be focused at their respective isoelectric point in the first dimension between the anodic reservoir AR and cathodic reservoir CR by means of isoelectric focusing (IEF). The second dimension may be the above-described electrophoresis (e.g. gel electrophoresis) toward the first waste reservoir W1 to further separate the proteins of the sample according to their sizes.

The anodic reservoir AR and the cathodic reservoir CR may be connected by means of an IEF channel. The IEF channel may be divided into a plurality of segments S1, S2, . . . , Sn. In the example shown in FIG. **12**, the IEF channel may be divided into four segments S1, S2, S3, S4. Proteins with at least substantially similar isoelectric points may be focused at the same segments along the IEF channel between the anodic reservoir AR and the cathodic reservoir CR.

A target protein fragment may be extracted from proteins having at least substantially similar isoelectric points by means of its molecular weight (e.g. size), and this may be performed in the second dimension by means of electrophoresis (e.g. gel electrophoresis).

Contents of the segment S1, S2, S3, S4 of the IEF channel between the anodic reservoir AR and the cathodic reservoir CR containing the target analyte fragment may be transferred into the second dimension by opening the respective individual flow controller (e.g. control valve). For example, the contents of segment S1 may be transferred into the second dimension by means of flow controller V1; the contents of segment S2 may be transferred into the second dimension by means of flow controller V2; the contents of segment S3 may be transferred into the second dimension by means of flow controller V3; the contents of segment S4 may be transferred into the second dimension by means of flow controller V4.

Similar to the multi-layer microfluidic chips **800**, **900**, **1000** described above, the separation channels **1202***a* may include a plurality of parallel channels **1206***a*, **1206***b*, e.g. as shown in FIG. **12**. The plurality of parallel channels **1206***a*, **1206***b* may be designed and fabricated on the chip **1200**. Only two parallel channels **1206***a*, **1206***b* are shown as an example. However the number of parallel channels may be more than two and may, for example, be three, four, or more parallel channels.

Each parallel channel of the plurality of parallel channels **1206***a*, **1206***b* may be connected to a respective reservoir. For example the parallel channel **1206***a* may be connected to a sample reservoir that may include the IEF channel, the anodic reservoir AR, and the cathodic reservoir CR. The parallel channel **1206***b* may be connected to a reference reservoir.

As shown in FIG. **12**, the parallel channel **1206***a* connected to the sample reservoir may be additionally connected to the at least one extraction chamber **1204**. For example, the parallel channel **1206***a* may be disposed between the sample reservoir (that may include the IEF channel, the anodic reservoir AR, and the cathodic reservoir CR) and the at least one extraction chamber **1204**. Accordingly, the parallel channel **1206***b* may be used as a reference

channel and the parallel channel **1206***a* connecting to the at least one extraction chamber **1204** may be used for separation of target analyte fragments.

In the second dimension, the separation of the contents of the transferred segment may be done simultaneously with the reference in the parallel channel **1206***b*. When a reference analyte fragment is detected in the detection area **1208**, extraction of the target analyte fragment may be triggered to obtain pure protein fragment in the extraction chamber **1204**.

If there are more than one target protein fragment in the sample, multiple extraction can also be realized subsequently or simultaneously by repeating the above described extraction procedures in a single extraction chamber **1204** (e.g. in respect of FIG. **8** and FIG. **9**) or multiple extraction chambers (e.g. in respect of FIG. **10**).

According to various embodiments presented herein, a detection system may be used to actively locate a desired analyte fragment and switch the direction of an applied voltage to drive the desired analyte fragments to at least one extraction chamber.

According to various embodiments presented herein, a detection system may be used to actively locate a desired analyte fragment and a flow controller (e.g. valve element) may be used to control the flow and the direction of an electrical field to drive the desired analyte fragments to at least one extraction chamber.

According to various embodiments presented herein, an active extraction mechanism can be used to extract multiple analyte fragments.

According to various embodiments presented herein, a desired analyte fragment may be collected in an extraction chamber lined with a porous structure (e.g. extraction membrane) with acceptable purity.

According to various embodiments presented herein, multiple analyte extraction can be quickly realized on a microfluidic chip with parallel separation channels, at least one extraction chamber disposed above an embedded porous structure (e.g. embedded extraction membrane).

According to various embodiments presented herein, a system extracting one or more analyte fragments from a gel matrix may be provided. The system may include a power supply for running electrophoresis; an active detection system for collecting a reference signal (e.g. generated in response to detection of a reference analyte fragment); a software to trigger the start of extraction by switching the direction of an electrical field and a cartridge which includes an extraction chamber and an extraction membrane lining at least one side of the extraction chamber. The reference signal may trigger the extraction after the target analyte fragment is separated from interfering materials during electrophoresis. The analyte fragment may be driven towards the extraction chamber, being stopped by the extraction membrane, and extracted from the gel. The cartridge may include a chamber frame to produce chambers in gel, a membrane frame and an extraction membrane sandwiched between the two frames.

According to various embodiments presented herein, a system for extracting one or more analyte fragments from a gel matrix may be provided. The system may include a power supply for running electrophoresis; a detection system for detecting a reference signal (e.g. generated in response to detection of a reference analyte fragment); and a microfluidic device including an extraction chamber and an extraction membrane. The reference signal may trigger the extraction after the target analyte fragment is separated from interfering materials during electrophoresis. The extraction membrane in the microfluidic device can be embedded by thermal bonding, hot embossing, laser bonding, ultrasonic bonding or other methods to tightly attach the membrane to the microfluidic device.

According to various embodiments presented herein, a microfluidic device may be provided. The microfluidic device may include a reference channel with gel for reference; a separation channel with gel for separation of analyte sample; an extraction chamber with extraction membrane connected to supporting channel; and a first waste reservoir connecting to the separation channel and a waste reservoir connecting to the supporting channel. The target analyte fragment may move to the extraction chamber, being stopped by the extraction membrane, and extracted from the gel after the electrical field is applied from the separation channel to the extraction chamber. The electrical field may be applied by switching the positive electrode from the first waste reservoir to the second waste reservoir.

According to various embodiments presented herein, a microfluidic device may be provided. The microfluidic device may include a reference channel with gel for reference; a separation channel with gel for separation of analyte sample; an extraction chamber with extraction membrane connected to supporting channel; a first valve on the separation channel and a second valve on the supporting channel; and a waste reservoir connecting to the separation channel when the first valve opens and the second valve closes; the waste reservoir connecting to the supporting channel when the first valve closes and the second valve opens. The target analyte fragment moves to the extraction chamber, being stopped by the extraction membrane, and extracted from the gel after the electrical field is applied from the separation channel to the extraction chamber. The electrical field is applied by closing the first valve and opening the second valve. The extraction chamber can be re-used for multiple analyte fragments extraction, with the rinsing procedure to remove the minor quantity of analyte residue. The rinsing procedure can be realized by repeating filling and emptying running buffer in the extraction chamber for several times with pipette or pumps. The rinsing procedure can also be realized by switching the electrical field to rinse the extraction chamber with clean running buffer for some time.

According to various embodiments presented herein, a system for extracting multiple analyte fragments from a gel matrix may be provided. The system may include a power supply for running electrophoresis; a detection system for detecting reference signal; and a microfluidic device including multiple extraction chambers and extraction membranes. The reference signal triggers the extraction after the target analyte fragment is separated from interfering materials during electrophoresis. The extraction membrane in the microfluidic device can be embedded by thermal bonding, hot embossing, laser bonding, ultrasonic bonding or other methods to tightly attach the membrane to the microfluidic device.

According to various embodiments presented herein, a microfluidic device may be provided. The microfluidic device may include a reference channel with gel for loading reference fragments; a separation channel with gel for loading analyte sample; multiple separation channel branches at the end of the separation channel; each the separation channel branch leading to an extraction chamber and one the branch leading to a waste reservoir with a valve to control the fluid flow in the branch; each of the extraction chambers with extraction membrane connecting to a supporting channel branch; the supporting channel branches connecting to the waste reservoir. One valve on each the

supporting channel branch to open or close the fluid flow in the channel branch. The waste reservoir connecting to the separation channel branch when the valve opens and the rest of the valves close. The target analyte fragment moves to a the extraction chamber, being stopped by the extraction membrane, and extracted from the gel after the electrical field is applied from the separation channel to the extraction chamber. The electrical field is applied by closing all the valves except the one on the supporting channel branch connecting to the extraction chamber. The multiple analyte fragments extraction can be realized by replacing the multiple valves with a selective valve before the waste reservoir. The analyte fragment separation can be accomplished in one dimension or in multi-dimensions according to different separation mechanism. The detection system may be a optical system. The gel can be agrose, polyacrylamide or any solidified gel matrix. The analyte fragments can be DNAs, RNAs, proteins or any large molecules which can be separated by gel electrophoresis and extracted by the appropriate the extraction membrane from the gel.

According to various examples presented herein, a device for extracting at least one analyte is provided. The device includes a sample reservoir configured to contain a sample comprising at least one target analyte and interfering materials; at least one extraction chamber connected to the sample reservoir; at least one porous structure lining one or more sides of the at least one extraction chamber; and a voltage source configured to provide a first voltage and a second voltage, wherein, when the first voltage is provided, the at least one target analyte and the interfering materials move into the at least one extraction chamber or to a predetermined area from the at least one extraction chamber, wherein, when the second voltage is provided, the interfering materials pass through and exit the at least one extraction chamber, and the at least one target analyte is stopped from exiting the at least one extraction chamber by means of the at least one porous structure.

The first voltage is provided in a first direction and the second voltage is provided in a second direction different from the first direction.

The voltage source is configured to switch from the first voltage to the second voltage upon detection of a reference analyte by a detector.

The device further comprises the detector.

The detector is configured to provide a trigger for switching from the first voltage to the second voltage to the voltage source upon detection of the reference analyte.

The device may be configured as a microfluidic chip.

The device may further include a first waste reservoir; a second waste reservoir; wherein the first voltage is applied between the sample reservoir and the first waste reservoir, and wherein is applied between the sample reservoir and the second waste reservoir.

The device may further include a separation channel disposed at a first level; and a supporting channel disposed at a second level disposed below the first level, wherein the first waste reservoir is disposed at the first level and the second waste reservoir is disposed at the second level.

The at least one extraction chamber is disposed at the first level, and wherein the at least one porous structure is disposed between the first level and the second level and lines at least one side of the at least one extraction chamber.

According to various examples presented herein, a device for extracting at least one analyte is provided. The device may include a sample reservoir configured to contain a sample comprising at least one target analyte and interfering materials; at least one extraction chamber connected to the sample reservoir; at least one porous structure lining one or more sides of the at least one extraction chamber; a first flow controller disposed along a first channel extending from the at least one extraction chamber; and a second flow controller disposed along a second channel extending from the at least one extraction chamber, wherein, when the first flow controller is open and the second flow controller is closed, the at least one target analyte and the interfering materials move into the at least one extraction chamber under the influence of a voltage, and wherein, when the first flow controller is closed and the second flow controller is open, the interfering materials pass through the at least one porous structure and exit the at least one extraction chamber under the influence of the voltage, the at least one target analyte being stopped from exiting the at least one extraction chamber by means of the at least one porous structure.

The first channel is disposed at a first level and the second channel is disposed at a second level below the first level.

The device may further include a voltage source configured to provide the voltage.

The device may further include a waste reservoir connected to each of the first channel and the second channel, wherein the voltage is applied from the sample reservoir to the waste reservoir.

The device is further configured to close the first flow controller and open the second flow controller upon detection of a reference analyte by a detector.

The device may further include the detector.

The detector is configured to close the first flow controller and open the second flow controller upon detection of the reference analyte.

The device may be configured as a microfluidic chip.

Each of the first flow controller and the second flow controller comprises a valve.

According to various examples presented herein, a device for extracting a plurality of analytes is provided. The device may include: a sample reservoir configured to contain a sample comprising at least one target analyte and interfering materials; a plurality of extraction chambers connected to the sample reservoir, wherein each extraction chamber is connected to the sample reservoir by means of a respective separation channel branch; a respective porous structure lining one or more sides of a respective extraction chamber; a respective flow controller disposed along a respective separation channel branch and configured to control flow along the respective separation channel branch; and a voltage source configured to provide a voltage.

The device may further include a waste reservoir connected to each extraction chamber of the plurality of extraction chambers, wherein the voltage is applied from the sample reservoir to the waste reservoir.

While various aspects have been particularly shown and described with reference to these aspects of this disclosure, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from the spirit and scope of the disclosure as defined by the appended claims. The scope of the disclosure is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

What is claimed is:

1. A device for extracting at least one analyte, the device comprising:

a sample reservoir configured to contain a sample comprising at least one target analyte and interfering materials;

a reference reservoir configured to contain a reference comprising at least one reference analyte, wherein the at least one reference analyte is sized substantially similar to the at least one target analyte;

at least one extraction chamber connected to the sample reservoir;

at least one porous structure lining one or more sides of the at least one extraction chamber; and

a voltage source configured to provide a first voltage and a second voltage,

wherein, when the first voltage is provided, the at least one target analyte, the interfering materials and the at least one reference analyte move towards the at least one extraction chamber or to a predetermined area near the at least one extraction chamber,

wherein, when the second voltage is provided, the interfering materials pass through and exit the at least one extraction chamber, and the at least one target analyte is stopped from exiting the at least one extraction chamber by means of the at least one porous structure;

and wherein the voltage source is configured to switch from the first voltage to the second voltage upon detection of the at least one reference analyte by a detector.

2. The device of claim **1**, wherein the first voltage is provided in a first direction and the second voltage is provided in a second direction different from the first direction.

3. The device of claim **1**, further comprising the detector.

4. The device of claim **3**, wherein the detector is configured to provide a trigger for switching from the first voltage to the second voltage to the voltage source upon detection of the reference analyte.

5. The device of claim **1**, configured as a microfluidic chip.

6. The device of claim **1**, further comprising:

a first waste reservoir;

a second waste reservoir;

wherein the first voltage is applied between the sample reservoir and the first waste reservoir, and wherein the second voltage is applied between the sample reservoir and the second waste reservoir.

7. The device of claim **6**, further comprising:

a separation channel disposed at a first level; and

a supporting channel disposed at a second level disposed below the first level,

wherein the first waste reservoir is disposed at the first level and the second waste reservoir is disposed at the second level.

8. The device of claim **7**, wherein the at least one extraction chamber is disposed at the first level, and wherein the at least one porous structure is disposed between the first level and the second level and lines at least one side of the at least one extraction chamber.

9. A device for extracting at least one analyte, the device comprising:

a sample reservoir configured to contain a sample comprising at least one target analyte and interfering materials;

a reference reservoir configured to contain a reference comprising at least one reference analyte, wherein the at least one reference analyte is sized substantially similar to the at least one target analyte; at least one extraction chamber connected to the sample reservoir;

at least one porous structure lining one or more sides of the al least one extraction chamber;

a first flow controller disposed along a first channel extending from the at least one extraction chamber; and

a second flow controller disposed along a second channel extending from the at least one extraction chamber,

wherein, when the first flow controller is open and the second flow controller is closed, the at least one target analyte, the interfering materials and the at least one reference analyte move towards the at least one extraction chamber under the influence of a voltage, and

wherein, when the first flow controller is closed and the second flow controller is open, the interfering materials pass through the at least one porous structure and exit the at least one extraction chamber under the influence of the voltage, the at least one target analyte being stopped from exiting the at least one extraction chamber by means of the at least one porous structure,

wherein, the first flow controller is configured to close and the second flow controller is configured to open when at least one reference analyte is detected by a detector.

10. The device of claim **9**, wherein the first channel is disposed at a first level and the second channel is disposed at a second level below the first level.

11. The device of claim **9**, further comprising:

a voltage source configured to provide the voltage.

12. The device of claim **11** further comprising:

a waste reservoir connected to each of the first channel and the second channel,

wherein the voltage is applied from the sample reservoir to the waste reservoir.

13. The device of claim **9**, further comprising the detector.

14. The device of claim **13**, wherein the detector is configured to close the first flow controller and open the second flow controller upon detection of the reference analyte.

15. The device of claim **9**, configured as a microfluidic chip.

16. The device of claim **9**, wherein each of the first flow controller and the second flow controller comprises a valve.

17. A device for extracting a plurality of analytes, the device comprising:

a sample reservoir configured to contain a sample comprising at least one target analytes and interfering materials;

a reference reservoir configured to contain a reference comprising a plurality of reference analytes, wherein each reference analyte of the plurality of reference analytes is sized substantially similar to a respective target analyte of the plurality of target analytes;

a plurality of extraction chambers connected to the sample reservoir, wherein each extraction chamber is connected to the sample reservoir by means of a respective separation channel branch;

a respective porous structure lining one or more sides of a respective extraction chamber,

a respective flow controller disposed along a respective separation channel branch and configured to control flow along the respective separation channel branch; and

a voltage source configured to provide a voltage,

wherein, the respective flow controller is configured to open when a respective reference analyte of the plurality of reference analytes is detected by a detector.

18. The device of claim **17**, further comprising a waste reservoir connected to each extraction chamber of the plu-

rality of extraction chambers, wherein the voltage is applied from the sample reservoir to the waste reservoir.

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