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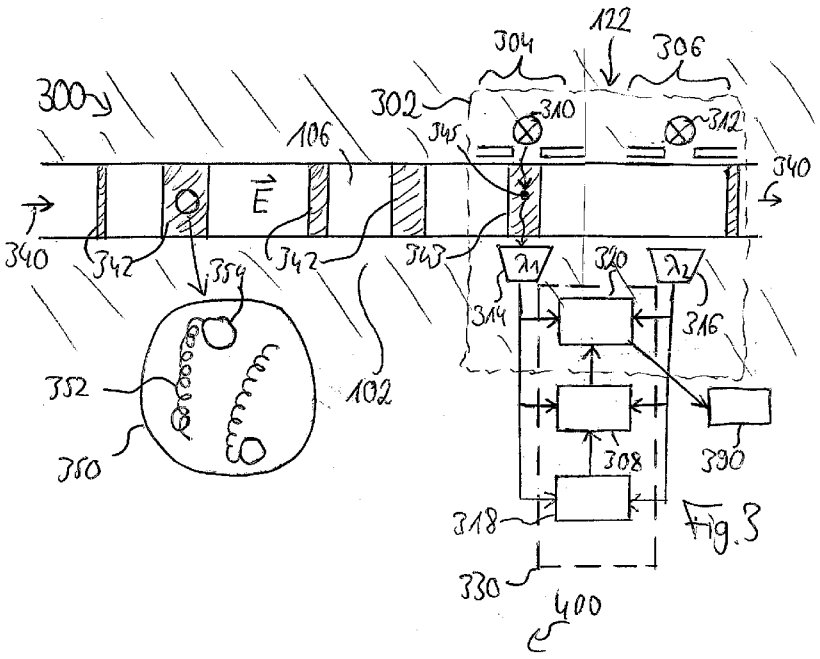


Fig. 3

(57) Abstract: A sample separation device (300) for separating different components of a sample, the sample separation device (300) comprising a detection unit (302) configured for detecting, in a first detection channel (304), components of the sample at a first wavelength (λ1), and configured for detecting, in a second detection channel (306), components of the sample at a second wavelength (λ2), and a calibration unit (308) configured for identifying a calibration marker in the first detection channel (304) and in the second detection channel (306), the calibration marker having an emission spectrum (408) covering the first wavelength (M) and the second wavelength (λ2).

IDENTIFYING CALIBRATION MARKERS IN MULTIPLE DETECTION CHANNELS

BACKGROUND ART

[0001] The present invention relates to a sample separation device.

[0002] Electrophoresis is a method to analyze complex mixtures of substances, for instance biological substances like DNA or proteins. By electrophoresis, a mixture of different substances may be separated by taking into account the intrinsic charge characteristics of the substances. In gel electrophoresis, substances of an analyt are separated into different components which are spatially separated along the extension of the gel-containing device.

[0003] Conventionally, analytes may be labeled with multiple colors within a multiplex detection scheme for a fast electrophoresis separation analysis. A separate color channel is used for a calibration using a calibration marker. However, it may still be difficult to provide a sample separation system which is reliable and simple in operation.

DISCLOSURE

[0004] It is an object of the invention to provide a sample separation system which is reliable and simple in operation. The object is solved by the independent claims. Exemplary embodiments are shown by the dependent claims.

[0005] According to an exemplary embodiment of the present invention, a sample separation device (such as an electrophoresis device) for separating different components (such as different fractions) of a sample (such as a fluidic sample, for instance a biological or chemical sample) is provided, the sample separation device comprising a detection unit (which may be based on a detection principle resulting from a characteristic interaction of components of the sample with electromagnetic radiation such as visible light) configured for detecting, in a first detection channel (which may be characterized by an operation in or a sensitivity for electromagnetic radiation of or around a first wavelength of electromagnetic radiation), components (for instance a first component) of the sample at a first wavelength, and configured for detecting, in a

second detection channel (which may be characterized by an operation in or a sensitivity for electromagnetic radiation of or around a second wavelength of electromagnetic radiation), components (for instance a second component) of the sample at a second wavelength, and a calibration unit configured for identifying (for instance for identifying one or more spectral peaks resulting from) a calibration marker (which may comprise one or more substances having preknown spectral and physical properties so that an identification of a calibration marker in a spectrum may allow to unambiguously or absolutely assign other spectral peaks to specific components of the sample) in the first detection channel and in the second detection channel (for instance in detection spectra of both detection channels which detection spectra may additionally include peaks originating from various components of the sample to be detected), the calibration marker having an emission spectrum (for instance a spectral curve indicating a wavelength dependency of an intensity of electromagnetic radiation emitted by the calibration marker or a fluorescence label attached to the calibration marker for instance in response to an excitation with primary electromagnetic radiation) covering the first wavelength and the second wavelength (in other words, the emission spectrum may include properly resolvable spectral components at both the first and the second wavelength so that the calibration marker is visible in both detection spectra which may be used for calibrating the detection spectra).

[0006] According to still another exemplary embodiment of the present invention, a method of separating different components of a sample is provided, wherein the method comprises detecting, in a first detection channel, components of the sample at a first wavelength, detecting, in a second detection channel, components of the sample at a second wavelength, and identifying a calibration marker in the first detection channel and in the second detection channel, the calibration marker having an emission spectrum covering the first wavelength and the second wavelength.

[0007] According to another exemplary embodiment of the present invention, an assay (or a kit) is provided which is configured for use with a sample separation device having the above mentioned features and/or or configured for executing a method having the above mentioned features.

[0008] According to still another exemplary embodiment of the present invention, a software program or product is provided, preferably stored on a data carrier, for controlling or executing the method having the above mentioned features, when run on a data processing system such as a computer.

[0009] Embodiments of the invention can be partly or entirely embodied or supported by one or more suitable software programs, which can be stored on or otherwise provided by any kind of data carrier, and which might be executed in or by any suitable data processing unit. Software programs or routines can be preferably applied in the context of measurement evaluation. The measurement evaluation scheme according to an embodiment of the invention can be performed or assisted by a computer program, i.e. by software, or by using one or more special electronic optimization circuits, i.e. in hardware, or in hybrid form, i.e. by means of software components and hardware components.

[0010] According to an exemplary embodiment, two or more separate detection channels of a sample separation apparatus, each channel operating at an assigned wavelength, may be used for detecting two or more components of a sample under analysis. In order to be able to quantitatively determine a position of a peak of a corresponding electropherogram (which may, more generally, be denoted as a spectrum) and to consequently identify a corresponding component of the sample unambiguously, it may be advantageous to know a position of a calibration marker having known properties, for instance known a size, charge, charge to mass ratio, mobility in a separation medium, etc. When one or more of such calibration markers is or are identified due to predetermined information, it is possible to unambiguously identify an absolute position of a sample component in the electropherogram, wherein such a position may correspond to a size or another biochemical property of the identified sample. In such a sample analysis system, it may be desired to reduce the number of used wavelengths to a minimum, in order to keep the system simple. The different detection channels can be separated by different operation wavelengths. For the calibration marker, an emission spectrum may be used which spectrally extends into the first wavelength range of the first detection channel and into the second wavelength range of the second detection channel, thereby allowing to identify the calibration

markers in the detection electropherograms of the first and the second detection channel without the need of a separate calibration channel operating at a separate wavelength. This may be obtained by selecting an emission spectrum of the calibration marker being sufficiently broad so that it results in a detectable peak in both the first and the second detection channel at a pre-known position. Therefore, the identification of a calibration peak directly in each of the first and the second detection channel may be performed using information (such as an expected spectral position and/or an expected spectral intensity of the calibration marker) regarding the calibration marker so that an absolute positioning and therefore unambiguous identification of detection peaks in the two detection channels is possible.

[0011] In the following, further exemplary embodiments of the sample separation device will be explained. However, these embodiments also apply to the assay, to the method, and to the software program or product.

[0012] The calibration unit may be configured for identifying the calibration marker based on a predetermined response factor or ratio of signal intensities (which may also be denoted as or which may be correlated to an occurrence ratio) of the calibration marker in the first detection channel and in the second detection channel. Depending on a correlation of a first wavelength assigned to the first detection channel and a second wavelength assigned to the second detection channel in comparison to a centre (for instance a third wavelength) of the emission spectrum of the calibration marker, the degree of overlap of the broad spectrum of the calibration marker into the first channel may be different from its overlap into the second channel. The corresponding ratio of the integrated intensity of the signals originating from the calibration marker in the first channel and in the second channel is defined by a relation between the three wavelengths and by a spectral shape of the emission spectrum of the calibration marker. Therefore, the occurrence ratio of the calibration marker in the two detection channels may be calculated in advance so that the identification of the calibration marker in the two detection channels may be performed using the knowledge regarding this occurrence ratio. The occurrence ratio may be indicative of a ratio of peak areas resulting from the calibration marker in both detection channels. A corresponding peak area may be calculated by integrating an area below a calibration marker peak in an

electropherogram which peak is assigned to or goes back to the calibration marker.

[0013] For a proper resolution of the calibration marker in all detection channels, it may be advantageous that the occurrence ratio is not too far away from 1. However, it is also possible that the occurrence ratio is different from 1, for instance is between about 0.5 and about 2, particularly is between about 0.75 and about 1.5, more particular is between about 0.8 and 1.2. With an occurrence ratio of 1, the intensity of the peaks originating from the calibration marker in the two detection channels should be the same. Deviations of the occurrence ratio from 1 results in different spectral contribution of the calibration marker in the two channels.

[0014] The emission spectrum of the calibration marker may be distributed around a third wavelength. Such a distribution may be a Gaussian, a Lorentzian, a Voigtian, etc. The emission electropherogram may be symmetric around a center frequency, or may be asymmetric. The emission electropherogram may overlap with both the first wavelength and the second wavelength which results in the fact that the calibration marker is visible in both the first and the second channel.

[0015] It is of course possible that more than two detection channels are present, for instance three or more detection channels. In this scenario, it may be advantageous that the calibration marker provides an overlap of its broad emission spectrum in each of these further channels so that the calibration marker can then be identified in three or more different channels being operated at different wavelengths.

[0016] In an embodiment, the detection unit may comprise an electromagnetic radiation source configured for emitting primary electromagnetic radiation towards the sample and may comprise an electromagnetic radiation detector configured for detecting secondary electromagnetic radiation from the sample in response to the emission of the primary electromagnetic radiation towards the sample. The electromagnetic radiation used according to exemplary embodiments may be optical light, i.e. electromagnetic radiation in the visible range between about 400 nm and about 800 nm. However, in alternative embodiments, it is possible to use other wavelength ranges such as infrared, ultraviolet, etc. The electromagnetic radiation source may comprise one or more sources such as light emitting diodes, lasers, etc. The corresponding electromagnetic radiation

detector may comprise a photodiode, a CCD detector, etc. Further optical or spectroscopic elements such as filters, lenses, apertures or the like may be implemented as well. The electromagnetic radiation source may emit a primary beam towards the sample for interaction with the sample. The electromagnetic radiation detector may then measure in transmission or in reflection. It is possible to measure at the primary wavelength or at a different wavelength, for instance in a scenario of fluorescence. If the components and the calibration marker emit electromagnetic radiation without excitation, the electromagnetic radiation source may be omitted. In an embodiment, the electromagnetic radiation source and the electromagnetic radiation detector may be movable relative to a fluidic conduit along which the fractions of the sample are separated for example under the influence of an electric force. In an alternative embodiment, the electromagnetic radiation source and the electromagnetic radiation detector may be spatially fixed (for instance also relative to a fluidic conduit along which the fractions of the sample are separated for example under the influence of an electric force).

[0017] More specifically, the detection unit may comprise a first electromagnetic radiation source (such as a first light source) configured for emitting primary electromagnetic radiation (such as a primary light beam) towards the sample and may comprise a first electromagnetic radiation detector (such as a first light detector) for detecting secondary electromagnetic radiation (such as a secondary light beam) of the first wavelength from the sample in response to the emission of the primary electromagnetic radiation. Additionally, the detection unit may comprise a second electromagnetic radiation source (such as a second light source) configured for emitting primary electromagnetic radiation (such as a further primary light beam) towards the sample and may comprise a second electromagnetic radiation detector (such as a second light source) configured for detecting secondary electromagnetic radiation (such as a further secondary light beam) of the second wavelength from the sample in response to the emission of the primary electromagnetic radiation. Therefore, the two different detection channels may operate at different two wavelengths and may therefore be provided completely separate, i.e. may each have an electromagnetic radiation source and an electromagnetic radiation detector. In an embodiment in which a first

electromagnetic radiation detector and a second electromagnetic radiation detector are provided separately, these may be configured for detecting electromagnetic radiation of the calibration marker's emission spectrum centered around a third wavelength but having spectral contributions also at the first and the second wavelength.

[0018] Alternatively, it is also possible that one and the same electromagnetic radiation source and/or electromagnetic radiation detector is used for the two detection channels, wherein detection spectra of the detection channels may be acquired and/or analyzed sequentially or simultaneously, for instance using filter technology or multiplexing technology.

[0019] The first wavelength may be different from the second wavelength, so that the two separation channels may be distinguished by different wavelengths. In other words, an emission spectrum of a first component of the sample (or of a fluorescence label attached to the first component of the sample) distributed around the first wavelength may be free of a detectable overlap with an emission spectrum of a second component of the sample (or of a fluorescence label attached to the second component of the sample) distributed around the second wavelength. Hence, the two detection channels may be separate detection channels which may operate without spectral overlap. These two wavelengths may correspond to different components of the sample.

[0020] A spectral width of the emission spectra around the first wavelength and around the second wavelength may be narrower than a spectral width of the emission spectrum around the third wavelength. For instance, the full width half maximum (FWHM) of the emission spectra around the first wavelength and around the second wavelength may be smaller than the FWHM of the emission spectrum around the third wavelength. More particularly, the FWHM of the emission spectra around the first wavelength and around the second wavelength may be at least three times smaller than the FWHM of the emission spectrum around the third wavelength.

[0021] The third wavelength may be different from the first wavelength and may be different from the second wavelength. Particularly, it may be advantageous that the third wavelength is larger than the first wavelength and is smaller than the second wavelength. In such an embodiment, the third wavelength is sandwiched between the

first wavelength and the second wavelength, thereby ensuring that the predefined occurrence ratio is relatively close to 1 which allows to obtain a sufficiently strong signal resulting from the calibration marker in both detection channels.

[0022] The detection unit may be configured for simultaneously detecting the sample in the first detection channel and in the second detection channel. This is for instance possible using two physically different electromagnetic radiation sources/detectors, which can then be arranged pairwise for instance along a fluidic path along which a sample is separated. Such a sample separation may be performed by gel electrophoresis or by liquid chromatography, for example. A simultaneous detection of the two detection channels may allow to perform high throughput analysis in which a multi-component sample can be analyzed or separated in a short time with high precision.

[0023] The calibration unit may be configured for identifying the calibration marker in an electropherogram measured by the detection unit in the first detection channel and in an electropherogram measured by the detection unit in the second detection channel. Therefore, due to the overlap of the emission spectrum with wavelengths for which both detection channels are sensitive, it is possible that within these detection spectra, also the calibration marker or markers can be clearly identified so as to enable the system to automatically calculate an absolute position and therefore an absolute physical property of a component of a sample to be separated by a comparison of a position of a component peak with a position of a calibration marker peak. If a marker with pre-known physical properties (for instance a known relation between electric charge and mass) can be identified in an electropherogram having an abscissa plotting the time at a specific position (i.e. detection time), a simple mathematical calculation can be performed which correlates such a position of a calibration marker with an unknown position of a molecule to be detected. Therefore, the analysis of a calibration marker may refine the detection of a specific component of the sample. In this context, a position on the time axis may correspond to a specific mobility of the corresponding species, and therefore to physical properties such as mass, electric charge, etc.

[0024] The calibration unit may be configured for identifying a first (for instance a

lower) marker portion (for instance indicative of first calibration marker molecules having a first mobility in a fluidic conduit of the device which may be filled with a separation medium such as a gel) and a second (for instance an upper) marker portion (for instance indicative of second calibration marker molecules having a second mobility in the fluidic conduit of the device which may be filled with the separation medium, wherein the first mobility may be smaller than the second mobility) of the calibration marker, wherein the first marker portion may be detectable in the first detection channel and in the second detection channel prior to subsequently detecting the components of the sample. The second marker portion may be detectable in the first detection channel and in the second detection channel later in time after already having detected the components of the sample. Therefore, the first marker portion may form a lower boundary of anelectropherogram, whereas the second marker portion may form an upper boundary of the electropherogram. In such a scenario, the first and second marker portions may be configured to have a size or mass or ratio between electric charge and mass so that all components of the sample which are to be expected are arranged above the first marker portion and below the second marker portion, in order to avoid crosstalk between different peaks which might require further computational burden for mathematically separating different peaks. The presence of two different markers in such an electropherogram having a preknown position not only allows to derive more precise absolute information regarding the sample or species to be identified, but may allow to calculate a linear scale along the time axis of the detection which allows to correlate each detection peak in the electropherogram with an assigned position along such a scale.

[0025] The sample separation device may comprise a reference measurement unit configured for measuring a reference electropherogram including a predefined ladder electropherogram between the lower marker portion and the upper marker portion. For example, such a reference measurement may be performed before and/or after performing the measurements for analyzing the sample in the first and second (and optionally further) detection channels. In such a reference electropherogram, the two markers can be positioned to sandwich a ladder electropherogram of a number of known particles of specific sizes in between. This may allow, in accordance with the

corresponding peak positions, for an unambiguous scaling which also holds for the detection channels. The reference measurement electropherogram can be correlated to the detection measurement spectra by means of the upper and lower markers which can be identified in all three spectra. The ladder measurement may allow for a pre-calibration yielding a result which may subsequently simplify a main calibration to be performed by the actual calibration unit. An algorithm applied in accordance with the actual calibration may identify a calibration marker easier in the light of the result of the pre-calibration.

[0026] In an embodiment, the reference measurement unit may be configured for measuring the reference electropherogram at one or both of the first wavelength and the second wavelength. In such a scenario, it is possible that the reference measurement unit uses one of the wavelengths of the detection channels also for performing the reference measurement, without the need to introduce a further wavelength (and corresponding measurement equipment). Hence, at least one of the detection channels may be synergetically used for acquiring reference data so that a separate reference measurement channel for the reference measurement may be dispensable. This allows to keep the sample separation device simple. However, in an alternative embodiment it is also possible to perform the reference measurement at a separate reference channel which may then operate at a fourth wavelength differing from the first and the second wavelengths.

[0027] The sample separation device may comprise a signal decomposition unit configured for decomposing (analytically or numerically) a spectral component originating of a second component from a first electropherogram of the first detection channel and may be configured for decomposing a spectral component originating of a first component from a second electropherogram of the second detection channel. This may result from a small overlap of the emission spectra of the first wavelength and of the second wavelength. Consequently, a minority of molecules relating to the first component may be visible in the second detection electropherogram at a position on the time axis which basically corresponds to a time axis position of the first component in the first detection electropherogram. In a similar manner, a minority of the molecules relating to the second component may be visible in the first detection electropherogram

at a position on the time axis which basically corresponds to a time axis position of the second component in the second detection electropherogram. Using this and further criteria when analyzing the first and the second detection spectra, it may be possible to remove such signal artifacts from the spectra by a data processing analysis, to thereby compensate for or even eliminate such measurement artifacts. Using mathematical algorithms taking under account the above and other physical considerations, artificial peaks may be eliminated from the "wrong" detection electropherogram.

[0028] A component identification unit may be provided and may be configured for identifying a component of the sample in the first detection channel or the second detection channel based on a position of a corresponding peak in the first detection channel or the second detection channel in combination with a position of the calibration marker in the first detection channel or the second detection channel (and optionally in combination with information derived from a ladder electropherogram). Such a component identification unit may use information regarding the positions of the calibration marker to unambiguously identify components of the sample corresponding with detection peaks in the detection channels.

[0029] In an embodiment, the sample separation device may further comprise a substrate and at least one fluid conduit formed in and/or on the substrate and configured for conducting the sample. Such a substrate may be made of a glass material, a plastics material, a metal material and/or a ceramics material. Capillaries may be formed by bonding such a conduit comprising substrate with a further substrate. The detection unit may be configured for detecting the sample while being conducted via the at least one fluid conduit. Thus, a chip such as a glass chip may be used having capillaries formed in a surface of such a glass chip through which a fluid may be conducted. Furthermore, a sample separation medium may be filled in such a fluidic conduit, such as a gel in the case of gel electrophoresis. The substrate may comprise one or more fluidic reservoirs (or wells) in fluid communication with at least a part of the at least one fluid conduit. Therefore, fluid conduits may be interconnected to one another and may be in fluid communication with fluid reservoirs in which a sample, solvent, etc. may be injected with a pipette or the like.

[0030] It is also possible that the sample separation device has one or more electrodes for applying an electric field along the fluid conduit or fluid conduits. Therefore, an electric force may be generated acting on charged particles of the sample which may promote a motion of the charged particles along the fluid conduits so that different fractions of the sample can be separated based on a different ratio of charge and mass. These different ratios result in a different mobility and therefore a spatial separation of the different components which can be detected optically as different bands.

[0031] The sample separation device may separate a fluidic sample (such as a sample having liquid and/or gaseous components, wherein also solid particles may be optionally part of such a sample). The sample separation device may be used in the field of gel electrophoresis or capillary electrophoresis and may allow to separate the sample by an applied electric field.

[0032] Next, further exemplary embodiments of the assay will be explained. However, these embodiments also apply to the sample separation device, to the method, and to the software program or product.

[0033] In the context of this application, the term “assay” may particularly denote a kit of components used for a specific procedure by which a property (for instance the presence/absence or a concentration of a component of a sample) of an analyte is measured. Examples of assays are antigen capture assays, bioassays, competitive protein binding assays, etc.

[0034] Particularly, such an assay may comprise a plurality of substances (such as dyes, calibration marker, additives, enzymes) as well as an analysis device (such as an analysis chip) for performing a specific sample separation analysis. Thus, the components of the assay may be composed to allow to carry out a corresponding sample separation procedure, for instance in the context of a biological or chemical analysis. Optionally, also a description of such a procedure or algorithm may form part of the assay, for instance may be stored on an electronic storage device or printed as an instruction manual.

[0035] In an embodiment, the assay may comprise a first fluorescence label which may be configured to be labeled to a first component of the sample. For instance, the fluorescence label may have a linker molecule which may be specifically configured to selectively bind specifically to the first component of the sample, but not to other components of the sample. Moreover, the assay may comprise a second fluorescence label to be labeled to a second component of the sample. Also the second fluorescence label may have a specific linker molecule which selectively binds to the second component of the sample, but not to other components of the sample. A third fluorescence label may be part of the assay and may be configured for binding to the calibration marker, but not to components of the sample. For example, also the third fluorescence label may have a linker molecule for selectively binding only to the calibration marker. It is also possible that the third fluorescence label is already bound to the calibration marker in the assay.

[0036] Additionally, a plurality of ladder molecules may be provided for providing a predefined size reference electropherogram with respect to the calibration marker. Such a set of ladder molecules may comprise a number of molecules having different sizes or charge to mass ratios so that different groups of the ladder molecules can be clearly separated from one another in a reference electropherogram. Such ladder molecules may be part of the assay to enable the assay to clearly identify different species in an electropherogram. Also the ladder molecules may comprise a fluorescence label fluorescing at a proper wavelength to be visible as narrow peaks in a reference measurement. Optionally, a ladder channel or reference measurement channel may be used prior to the measurement at the first and the second detection channel, wherein it is optionally possible to run such a ladder experiment at the wavelength used in one of the detection channels. However, it is also possible to provide a separate wavelength for the ladder channel. It is however preferred to use one of the wavelengths of the first and the second detection channels also for the ladder electropherogram, since this simplifies the configuration of the system.

[0037] The assay may further comprise a substrate and at least one fluid conduit formed in and/or on the substrate and configured for conducting the sample. Therefore, an analysis chip may be part of the assay as well. Its conduits, wells, interconnections,

etc. may be adapted to the experiment to be carried out.

[0038] Next, further exemplary embodiments of the method will be explained. However, these embodiments also apply to the sample separation device, to the assay, and to the software program or product.

[0039] In this method, it is possible to label a first component with a first fluorescence label fluorescing at the first wavelength. A second component of the sample may be labeled with a second fluorescence label fluorescing at the second wavelength. Moreover, the calibration marker may be labeled or may already be labeled with a third fluorescence label fluorescing in the emission spectrum around the third wavelength. This third fluorescence label may be configured to have a relatively broad emission spectrum to be detectable in the first detection channel and in the second detection channel. This can be achieved by correspondingly selecting the emission spectrum of the third fluorescence label which should overlap with both detection channels.

[0040] During executing the method, the sample may then be separated by electrophoresis, i.e. by different charge to mass ratios of various components of the sample. Exemplary embodiments may particularly be suitable for RNA (ribonucleic acid) applications.

[0041] The two (or more) color detection scheme according to an exemplary embodiment may introduce a third color into a two (or more) color system, the third color serving for identifying a calibration marker in both (or more) detection channels. This may allow to separate differently labeled samples by electrophoresis and to detect the individual species by an optical measurement. Along a ladder electropherogram, the different ladder molecules may generate narrow peaks corresponding to different reference sizes, such as 100 bases, 120 bases, etc. Thus, these ladder molecules may be fragments for calibrating a sizing procedure for determining an absolute size of a species of a sample being separated. This may allow to determine a size of an unknown peak in one of the detection channels. Both detection channels as well as the ladder may be detected experimentally in an electropherogram. Since exemplary embodiments may use the phenomenon of fluorescence, it is possible that a detection wavelength

differs from an excitation wavelength. For example, an excitation wavelength may be 488 nm, whereas a detection wavelength may be 510 nm.

[0042] According to an exemplary embodiment, an upper calibration marker and a lower calibration marker may be labeled with a dye which is visible in both detection channels. This dye labeled to the lower marker and the upper marker may be different from a dye labeled to at least two different components of the samples. Then, the upper marker and the lower marker can be clearly visible in multiple detection channels at the same time, wherein such a response factor or occurrence ratio may be ideally 1:1 in the case of two detection channels. This may allow to unambiguously determine the upper marker and the lower marker in each of multiple detection channels. Using a known response factor (for instance 1:1 or 55:45) may allow to unambiguously detect such a marker with high precision. Of course, it is possible to use more than two detection channels, wherein a use signal of each detection channel may be captured at a different wavelength and all wavelengths should be within the emission spectrum of the calibration marker. A data processing algorithm may identify a marker at a time position of two peaks with a known ratio in the multiple detection channels. Due to this reliable calibration procedure, the method according to an exemplary embodiment is robust against failure and allows for a simple construction of the device since no separate calibration marker detection channel is necessary. Hence, a two channel measurement system may be provided in which two dyes (for instance fluorescence dyes like Alexa700 or Cy5) are specifically adjusted regarding the corresponding detection channels. Then, a third dye may be used with which an upper and lower marker may be labeled.

BRIEF DESCRIPTION OF DRAWINGS

[0043] Other objects and many of the attendant advantages of embodiments of the present invention will be readily appreciated and become better understood by reference to the following more detailed description of embodiments in connection with the accompanied drawings. Features that are substantially or functionally equal or similar will be referred to by the same reference signs.

[0044] Fig. 1 shows a gel electrophoresis chip which can be operated with a fluidic

sample separation apparatus according to an exemplary embodiment.

[0045] Fig. 2 shows an enlarged view of a well of an electrophoresis chip to be operated with a gel electrophoresis device according to an exemplary embodiment.

[0046] Fig. 3 illustrates a gel electrophoresis sample separation device according to an exemplary embodiment.

[0047] Fig. 4 illustrates narrower emission spectra relating to a sensitivity of two detection channels around a first and a second wavelength and a broader emission spectrum around a third wavelength overlapping with the first and with the second wavelength according to an exemplary embodiment.

[0048] Fig. 5 shows a ladder electropherogram and two detection spectra according to an exemplary embodiment.

[0049] Fig. 6 schematically illustrates an assay according to an exemplary embodiment.

[0050] The illustration in the drawing is schematically.

[0051] **Fig. 1** shows a biochip 100 for handling a fluidic sample such as an RNA or DNA comprising biological sample (not shown) using a fluid separation scheme according to an exemplary embodiment.

[0052] The biochip 100 comprises a glass substrate 102 in which a plurality of wells 104 are arranged in a matrix-like manner. In the embodiment of Fig. 1, the matrix of wells 104 comprises four rows and seven columns. The wells 104 are formed as cavities in the glass substrate 102. Each of the wells 104 is configured for receiving a fluidic sample or a solvent. Groups of wells 104 are in fluid communication with one another using separation channels 106, wherein the separation channels 106 are etched in the surface of the substrate 102. A gel as a fluid separation medium is filled in the fluid separation channels 106.

[0053] In the embodiment of Fig. 1, wells 104 are grouped into four groups 110, 112, 114, 116 which are in fluid communication to one another and which are however

separately operable so that only the wells 104 of a respective group 110, 112, 114, 116 is capable of performing a common analysis. As further shown in Fig. 1, all wells 104 assigned to a respective group 110, 112, 114, 116 are aligned along a common direction, namely the horizontal direction in Fig. 1.

[0054] The channel 106 having the largest length of a respective group 110, 112, 114, 116 is a so-called separation channel 108. Along the separation channel 108, a separating of components of a microfluidic sample filled in one of the wells 104 can be performed. The separation channel 108 is aligned in a basically horizontal direction according to Fig. 1 and connect a majority of the wells 104 of a respective group 110, 112, 114, 116. By allowing that the separation channel 108 extends basically along the entire extension of all wells 104 of a respective group 110, 112, 114, 116 in fluid communication with one another, a sufficiently long separation path is provided.

[0055] As can be further taken from Fig. 1, a so-called injection cross 118 is provided in each of the groups 110, 112, 114, 116 for guiding fluidic samples through the wells 104 and channels 106 according to a predefined sequence.

[0056] In a detection area 122, a fluidic sample, after having passed the channels 106 according to a predefined sequence, can be separated fraction-wise and can be detected using an optical detection method.

[0057] For instance, it is possible that for separating a sample such a sample is first guided from a well 124 into a well 126 and then into a well 128 before the sample can be separated starting from well 128 along the separation channel 108 using an electrophoretic force, in order to be detected in the detection area 122.

[0058] In order to generate an electrophoretic force to the sample which migrates according to Fig. 1 along the separation channel from the left-hand side to the right-hand side, one of the groups 110, 112, 114, 116 of wells 104 is first selected for an experiment. Then, an electrode arrangement 130 of an electrophoretic measurement device (for instance similar to a Bioanalyzer of the applicant Agilent Technologies) is moved in such a manner (see reference numeral 132) that the respective electrode pins are above the wells 104 of the selected group 110, 112, 114, 116. Then, the electrode

arrangement 130 is lowered in order to immerse into the liquids included in the wells 104. By applying an electric signal to the electrodes 134, an electric separation field may be applied along the separation channel 108.

[0059] **Fig. 2** shows an enlarged view of a chip 210 of a measurement device 200 according to an exemplary embodiment.

[0060] In the arrangement of Fig. 2, a first glass substrate 102 is provided in which the wells 104 are formed as through-holes. These are connected to one another by an electrophoretic separation channel 108 which is however formed in a second glass substrate 202 which is bonded to the first glass substrate 102. A plastic caddy 204 can be attached onto this arrangement. Then, a fluidic sample 206 can be injected into the well 104 in a direction as indicated with an arrow 208 using a pipette (not shown).

[0061] In the following, referring to **Fig. 3**, a sample separation device 300 for separating different components of a sample according to an exemplary embodiment will be explained.

[0062] The system according to Fig. 3 operates in accordance with capillary electrophoresis and implements a chip similar to the one shown in Fig. 1 or Fig. 2. Therefore, a fluidic channel 106 formed in a surface of a glass substrate 102 is shown. An electric field **E** is applied along a moving direction 340 of a fluidic sample in the fluidic conduit 106. The fluidic channel 106 is filled with a fluid separating material such as a gel. The different fractions of the sample can then be separated due to different ratios of electric charge and mass or size. Therefore, different bands of components 342 are schematically illustrated in Fig. 3 which are separated due to their different behavior in the presence of the electric field **E**. As can be taken from an enlarged view 350 in Fig. 3, each of the fractions may comprise DNA molecules 352 labeled with a fluorescence label 354 visible in an optical detection scheme, as will be described in the following in more detail.

[0063] The sample separation device 300 comprises a detection unit 302 configured for detecting, in a first detection channel 304, components of the sample at a first wavelength $\lambda 1$. The detection unit 302 further comprises a second detection

channel 306, in which components of the sample may be detected at a second wavelength λ2 which is larger than λ1.

[0064] Furthermore, a calibration unit 308 is provided which is configured for identifying a calibration marker in the first detection channel 304 and also in the second detection channel 306. The calibration marker which may be present in the fluidic channel 106 has an emission spectrum (compare reference numeral 408 in Fig. 4) which is so broad as to cover both the first wavelength λ1 and the second wavelength λ2. More precisely, the calibration unit 308 identifies the calibration marker based on a predetermined response factor or occurrence ratio A1/A2 (compare Fig. 4) which is slightly larger than 1 in the present embodiment. According to this spectral area ratio, the calibration marker appears in the first detection channel and in the second detection channel.

[0065] Coming back to Fig. 3, the detection unit 302 comprises a first light source 310 configured for emitting a primary light beam towards the sample travelling through the fluid conduit 106 and comprises a first light detector 314 for detecting a secondary light beam at or around the first wavelength λ1 in response to the emission of the primary light beam. Furthermore, the detection unit 302 comprises a second light source 312 for emitting a further primary light beam towards the sample travelling through another portion of the fluidic conduit 106 and comprises a second light detector 316 for detecting a further secondary light beam at or around a second wavelength λ2 from the sample in response to the emission of the further primary light beam.

[0066] In the present scenario shown in Fig. 3, a specific fraction 343 is presently located between the first light source 310 and the first light detector 314 so that a fluorescence label 345 attached to a molecule relating to this fraction 343 is presently emitting the radiation at the first wavelength λ1 to be detected by the first light detector 314.

[0067] Now, referring to **Fig. 4**, a diagram 400 is shown having an abscissa 402 along which a wavelength is plotted, wherein a detection amplitude is plotted along an ordinate 404. As can be taken from diagram 400, the first detection channel 304 is sensitive to a detection at and narrowly around the first wavelength λ1.

[0068] As can be taken from a diagram 406 a detection may be performed at and closely around the second wavelength λ2 in the second detection channel 306.

[0069] An emission spectrum of a calibration marker is shown in a diagram 410 of Fig. 4. Here it can be seen that the calibration marker has the emission spectrum 408 with a broad spectral width centered around a third wavelength λ3. The emission spectrum 408 overlaps with the wavelength sensitivity range of the first channel 304 (compare hatched area A1) and also of the second channel 306 (compare hatched area A2). Therefore, with the calibration marker having the spectral response as shown as the emission spectrum 408, the calibration marker can be seen in both of first detection channel 304 and in the second detection channel 306. Therefore, during detecting closely around the first wavelength λ1 in the first detection channel 304 or during detecting around the second wavelength λ2 in the second detection channel 306, calibration signals can be captured as well during performing a component measurement in the detection channels 304 or 306.

[0070] Characteristic detection spectra with an abscissa along which the time is plotted (and not the wavelength as in Fig. 4) can be seen in **Fig. 5** illustrating a sequence of a measurement to be performed with the device 300 shown in Fig. 3.

[0071] Fig. 5 shows a reference measurement diagram 506 having an abscissa 502 along which the time is plotted. The reference measurement diagram 506 may be acquired between t=0 and t=t1. Along an ordinate 504 of the diagram 506, the intensity of a measured signal is plotted. The reference measurement 506 can be captured prior to an actual detection of a sample, since the reference measurement 506 is for calibration purposes. It comprises a resonance 508 originating from a lower calibration marker and comprises a peak 510 originating from an upper calibration marker. The upper calibration marker and the lower calibration marker may be guided through the channel 106 prior to the conduction of a sample to be separated through the fluidic channel 106. The lower marker 508 can be detected at a very early stage during the reference measurement, for instance since it corresponds to a molecule with a very high mobility. At the very end of the reference measurement, the upper marker portion 510 can be detected, for instance since it has a very small mobility. Between the lower

marker 508 and the upper marker 510, a plurality of pre-known ladder molecules 512 can be seen generating characteristic peaks and having a predetermined size and hence a mobility between the mobility of the lower marker 508 and the mobility of the upper marker 510. Therefore, the reference electropherogram 506 allows to obtain a scaling for a subsequent determination of molecules to be detected from a sample, as can be taken from a first detection channel 516 and a second detection channel 526. In other words, the time scale 502 may be recalculated into a mobility (and hence a mass or size) scale using the reference measurement.

[0072] As can be taken from the abscissa 502 of the diagrams 516, 526, the detection in the first detection channel (see diagram 516) and in the second detection channel (see diagram 526) may be carried out simultaneously and after carrying out the reference measurement (compare diagram 506). The diagrams 516, 526 may be acquired between t=t2>t1 and t=t3. In each of the detection channels 516, 526, the lower marker 508 can be identified as well as the upper marker 510. Since the ratio A1/A2 > 1, the intensity of the markers 508, 510 in the first detection channel 516 is larger than the intensity in the second detection channel 526.

[0073] As can further be taken from Fig. 5, a first species of the sample to be separated can be identified in the electropherogram 516 of the first channel 304 as a peak 530. A peak 532 corresponding to a second component can be identified in the electropherogram 526 of the second channel 306. Additionally, an artifact 530' can be seen in the electropherogram 526, whereas an artifact 532' can be seen in the electropherogram 516. A minority of molecules relating to the first component may be visible as peak 530' in the second detection channel 526 at a position on the time axis 502 which basically corresponds to a time axis position of first component peak 530 in the first detection channel 516. In a similar manner, a minority of the molecules relating to the second component may be visible as peak 532' in the first detection channel 516 at a position on the time axis 502 which basically corresponds to a time axis position of second component peak 532 in the second detection channel 526.

[0074] After having performed the measurements shown in Fig. 5, the spectra 516, 526 can be calibrated based on the reference measurement 506. In other words, the

absolute positions of the sample peak 530, 532 can be determined using electropherogram 506. A reference measurement unit 318 can use the electropherogram 506 (which may be captured at the first wavelength λ1 or at the second wavelength λ2) to supply corresponding reference measurement data to calibration unit 308. The calibration unit 308 then uses the positions of the markers 508, 510 and the scaling provided by the ladder information 512. Therefore, the spectra 516, 526 can be calibrated using the identification of the peaks 508, 510 based on the information from the reference measurement 506. Additionally, a signal decomposition unit 320 may identify and eliminate the artifacts 530' and 532' based on the above considerations so that an unambiguous identification and quantification of the sample peaks 530, 532 can be carried out.

[0075] Unit 320, 308 and 318 can be part of a processing unit 330 (or may be separate processing units) such as a microprocessor or a central processing unit or a computer. A result of the operation of units 320, 308, 318, i.e. a detection result, may be output to a user interface 390.

[0076] **Fig. 6** schematically shows an assay 700 which can be used by a user for carrying out a biochemical separation procedure in accordance with the device shown in Fig. 3 and/or the method described referring to Fig. 4 and Fig. 5.

[0077] Apart from the biochip 102 described above referring to Fig. 1 and Fig. 2, the assay 700 comprises a first vial 720 providing a substance including a first fluorescence label 702 which also has a linker molecule 703 for selective binding to a first component of the sample to be separated. A second vial 730 of the assay 700 comprises a second fluorescence label 704 to be labeled to a second component of the sample to be separated via a further linker molecule 705 for selective binding to the second component. A third vial 740 has a calibration marker 708 which is already linked to a third fluorescence label 706 with the broad emission spectrum shown in diagram 410 of Fig. 4. A fourth vial 750 comprises ladder molecules 710 of four different sizes for generating the ladder peaks 512 shown in Fig. 5.

[0078] It should be noted that the term "comprising" does not exclude other elements or steps and the "a" or "an" does not exclude a plurality. Also elements

described in association with different embodiments may be combined. It should also be noted that reference signs in the claims shall not be construed as limiting the scope of the claims.

CLAIMS

1. A sample separation device (300) for separating different components of a sample, the sample separation device (300) comprising

a detection unit (302) configured for detecting, in a first detection channel (304), components of the sample at a first wavelength (λ1), and configured for detecting, in a second detection channel (306), components of the sample at a second wavelength (λ2);

a calibration unit (308) configured for identifying a calibration marker in the first detection channel (304) and in the second detection channel (306), the calibration marker having an emission spectrum (408) covering the first wavelength (λ1) and the second wavelength (λ2).

2. The sample separation device (300) of claim 1,

wherein the calibration unit (308) is configured for identifying the calibration marker based on a predetermined occurrence ratio of the calibration marker between the first detection channel (304) and the second detection channel (306).

3. The sample separation device (300) of claim 2,

wherein the emission spectrum (408) is distributed around a third wavelength (λ3) and is configured such that the occurrence ratio of the calibration marker between the first detection channel (304) and the second detection channel (306) is in a range between 0.5 and 2, particularly is in a range between 0.75 and 1.5, more particularly is in a range between 0.8 and 1.2.

4. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the emission spectrum (408) of the calibration marker is distributed around a third wavelength (λ3) and overlaps with both the first wavelength (λ1) and the second wavelength (λ2).

5. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the detection unit (302) comprises an electromagnetic radiation source (310, 312) configured for emitting primary electromagnetic radiation towards the sample and comprises an electromagnetic radiation detector (314, 316) configured for detecting secondary electromagnetic radiation from the sample in response to the emission of the primary electromagnetic radiation towards the sample.

6. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the detection unit (302) comprises a first electromagnetic radiation source (310) configured for emitting primary electromagnetic radiation towards the sample and comprises a first electromagnetic radiation detector (314) configured for detecting secondary electromagnetic radiation of the first wavelength (λ1) from the sample in response to the emission of the primary electromagnetic radiation.

7. The sample separation device (300) of claim 6,

wherein the detection unit (302) comprises a second electromagnetic radiation source (312) configured for emitting primary electromagnetic radiation towards the sample and comprises a second electromagnetic radiation detector (316) configured for detecting secondary electromagnetic radiation of the second wavelength (λ2) from the sample in response to the emission of the primary electromagnetic radiation, wherein the first electromagnetic radiation source (310) and the first electromagnetic radiation detector (314) are decoupled from the second electromagnetic radiation source (312) and the second electromagnetic radiation detector (316).

8. The sample separation device (300) of claim 7,

wherein both the first electromagnetic radiation detector (314) and the second electromagnetic radiation detector (316) are configured for detecting electromagnetic radiation originating from the emission spectrum (408).

9. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the first wavelength (λ1) is different from the second wavelength (λ2).

10. The sample separation device (300) of claim 3 or any one of the above claims,

wherein the third wavelength (λ3) is different from the first wavelength (λ1) and is different from the second wavelength (λ2).

11. The sample separation device (300) of claim 3 or any one of the above claims,

wherein the third wavelength (λ3) is larger than the first wavelength (λ1) and smaller than the second wavelength (λ2).

12. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the detection unit (302) is configured for simultaneously detecting components of the sample in the first detection channel (304) and in the second detection channel (306).

13. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the calibration unit (308) is configured for identifying the calibration marker in an electropherogram measured by the detection unit (302) in the first detection channel (304) and in an electropherogram measured by the detection unit (302) in the second detection channel (306).

14. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the calibration unit (308) is configured for identifying a first marker portion and a second marker portion of the calibration marker, wherein the first marker portion is detectable in the first detection channel (304) and in the second detection channel (306) previously in time before detecting the components of the sample, and wherein the second marker portion is detectable in the first detection channel (304) and in the second detection channel (306) later in time after detecting the components of the sample.

15. The sample separation device (300) of claim 14,

comprising a reference measurement unit (318) configured for measuring a reference electropherogram (506) including a predefined ladder electropherogram between the first marker portion and the second marker portion.

16. The sample separation device (300) of claim 1 or any one of the above claims,

comprising a reference measurement unit (318) configured for measuring a reference electropherogram (506) including the calibration marker and a predefined ladder electropherogram.

17. The sample separation device (300) of claim 15 or any one of the above claims,

wherein the reference measurement unit (318) is configured for measuring the reference electropherogram (506) before or after detecting components of the sample in the first detection channel (304) and in the second detection channel (306).

18. The sample separation device (300) of claim 15 or any one of the above claims,

wherein the reference measurement unit (318) is configured for measuring the reference electropherogram (506) at at least one of the first wavelength (λ1) and the second wavelength (λ2).

19. The sample separation device (300) of claim 1 or any one of the above claims,

comprising a signal decomposition unit (320) configured for decomposing a spectral component originating of a first component of the sample from a second electropherogram (526) of the second detection channel (306) and configured for decomposing a spectral component originating of a second component of the sample from a first electropherogram (516) of the first detection channel (304).

20. The sample separation device (300) of claim 1 or any one of the above claims,

comprising a component identification unit (320) configured for identifying a component of the sample in the first detection channel (304) or the second detection channel (306) based on a position of a corresponding peak in the first detection channel (304) or the second detection channel (306) in combination with a position of the calibration marker in the first detection channel (304) or the second detection channel (306).

21. The sample separation device (300) of claim 1 or any one of the above claims, further comprising

a substrate (102);

at least one fluid conduit (106) formed in and/or on the substrate (102) and configured for conducting the sample;

wherein the detection unit (302) is configured for detecting the sample while being conducted via the at least one fluid conduit (106).

22. The sample separation device (300) of claim 21, comprising at least one of the following features:

the substrate (102) comprises one of the group consisting of a glass substrate, a plastics substrate, a metal substrate, and a ceramics substrate;

the substrate (102) comprises at least one fluidic reservoir (104) in fluid communication with at least a part of the at least one fluid conduit (102);

the sample separation device (300) comprises at least one electrode (134) configured for applying an electric field along the at least one fluid conduit (106).

23. The sample separation device (300) of claim 1 or any one of the above claims, comprising at least one of the following features:

the sample separation device (300) is configured for separating different components of a fluidic sample;

the sample separation device (300) is configured as a gel electrophoresis apparatus;

the sample separation device (300) is configured as a capillary electrophoresis apparatus;

the sample separation device (300) is configured for separating the sample by an applied electric field.

24. An assay (700) configured for use with a sample separation device (300) of claim 1 or any one of the above claims or configured for executing a method of claim 29 or any one of the below claims.

25. The assay (700) of claim 24, the assay (700) further comprising:

a first fluorescence label (702) to be labelled to a first component of the sample;

a second fluorescence label (704) to be labelled to a second component of the sample;

a third fluorescence label (706) to be labelled to the calibration marker (708).

26. The assay (700) of claim 25,

comprising the calibration marker (708) labelled with the third fluorescence label (706).

27. The assay (700) of claim 24 or any one of the above claims,

comprising a plurality of ladder molecules (710) configured for providing a predefined ladder electropherogram with respect to the calibration marker (708).

28. The assay (700) of claim 24 or any one of the above claims, further comprising

a substrate (102);

at least one fluid conduit (106) formed in and/or on the substrate (102) and configured for conducting the sample.

29. A method of separating different components of a sample, the method comprising

detecting, in a first detection channel (304), components of the sample at a first wavelength (λ1),

detecting, in a second detection channel (306), components of the sample at a second wavelength (λ2);

identifying a calibration marker in the first detection channel (304) and in the second detection channel (306), the calibration marker having an emission spectrum (408) covering the first wavelength (λ1) and the second wavelength (λ2).

30. The method of claim 29, further comprising:

labeling a first component of the sample with a first fluorescence label (702)

fluorescing at the first wavelength (λ1);

labeling a second component of the sample with a second fluorescence label (704) fluorescing at the second wavelength (λ2);

labeling the calibration marker (708) with a third fluorescence label (706) fluorescing in the emission spectrum (408) centered around a third wavelength (λ3).

31. The method of claim 30,

wherein the third fluorescence label (706) is configured to be detectable in both the first detection channel (304) and in the second detection channel (306).

32. The method of claim 29 or any one of the above claims,

comprising separating the sample by electrophoresis.

33. A software program or product, preferably stored on a data carrier, for controlling or executing the method of claim 29 or any one of the above claims, when run on a data processing system (330) such as a computer.

AMENDED CLAIMS
received by the International Bureau on 08 February 2010 (08.02.2010)

1. A sample separation device (300) for separating different components of a sample, the sample separation device (300) comprising

a detection unit (302) configured for detecting, in a first detection channel (304), components of the sample at a first wavelength ($\lambda 1$), and configured for detecting, in a second detection channel (306), components of the sample at a second wavelength ($\lambda 2$), and

a calibration unit (308) configured for identifying a calibration marker in the first detection channel (304) and in the second detection channel (306), the calibration marker having an emission spectrum (408) covering the first wavelength ($\lambda 1$) and the second wavelength ($\lambda 2$),

wherein the calibration unit (308) is configured for identifying the calibration marker based on a predetermined occurrence ratio of the calibration marker between the first detection channel (304) and the second detection channel (306).

2. The sample separation device (300) of claim 1,

wherein the emission spectrum (408) is distributed around a third wavelength ($\lambda 3$) and is configured such that the occurrence ratio of the calibration marker between the first detection channel (304) and the second detection channel (306) is in a range between 0.5 and 2, particularly is in a range between 0.75 and 1.5, more particularly is in a range between 0.8 and 1.2.

3. The sample separation device (300) of claim 1 or any one of the above claims,

wherein the emission spectrum (408) of the calibration marker is distributed around a third wavelength ($\lambda 3$) and overlaps with both the first wavelength ($\lambda 1$) and the second wavelength ($\lambda 2$).

4. The sample separation device (300) of the preceding claim, comprising at least one of:

the third wavelength ($\lambda 3$) is different from the first wavelength ($\lambda 1$) and is different from

the second wavelength ($\lambda 2$);

the third wavelength ($\lambda 3$) is larger than the first wavelength ($\lambda 1$) and smaller than the second wavelength ($\lambda 2$).

5. The sample separation device (300) of claim 1 or any one of the above claims, comprising at least one of:

the detection unit (302) comprises an electromagnetic radiation source (310, 312) configured for emitting primary electromagnetic radiation towards the sample and comprises an electromagnetic radiation detector (314, 316) configured for detecting secondary electromagnetic radiation from the sample in response to the emission of the primary electromagnetic radiation towards the sample;

wherein the detection unit (302) comprises a first electromagnetic radiation source (310) configured for emitting primary electromagnetic radiation towards the sample and comprises a first electromagnetic radiation detector (314) configured for detecting secondary electromagnetic radiation of the first wavelength ($\lambda 1$) from the sample in response to the emission of the primary electromagnetic radiation.

6. The sample separation device (300) of claim 1 or any one of the above claims, comprising at least one of:

the first wavelength ($\lambda 1$) is different from the second wavelength ($\lambda 2$);

the detection unit (302) is configured for simultaneously detecting components of the sample in the first detection channel (304) and in the second detection channel (306);

the calibration unit (308) is configured for identifying the calibration marker in an electropherogram measured by the detection unit (302) in the first detection channel (304) and in an electropherogram measured by the detection unit (302) in the second detection channel (306);

the calibration unit (308) is configured for identifying a first marker portion and a second marker portion of the calibration marker, wherein the first marker portion is detectable in the first detection channel (304) and in the second detection channel (306) previously in time before detecting the components of the sample, and wherein the second marker

portion is detectable in the first detection channel (304) and in the second detection channel (306) later in time after detecting the components of the sample.

7. The sample separation device (300) of claim 1 or any one of the above claims,

comprising a reference measurement unit (318) configured for measuring a reference electropherogram (506) including the calibration marker and a predefined ladder electropherogram.

8. The sample separation device (300) of the preceding claim, comprising at least one of:

the reference measurement unit (318) is configured for measuring the reference electropherogram (506) before or after detecting components of the sample in the first detection channel (304) and in the second detection channel (306);

the reference measurement unit (318) is configured for measuring the reference electropherogram (506) at at least one of the first wavelength ($\lambda 1$) and the second wavelength ($\lambda 2$).

9. The sample separation device (300) of claim 1 or any one of the above claims, comprising at least one of:

a signal decomposition unit (320) configured for decomposing a spectral component originating of a first component of the sample from a second electropherogram (526) of the second detection channel (306) and configured for decomposing a spectral component originating of a second component of the sample from a first electropherogram (516) of the first detection channel (304);

a component identification unit (320) configured for identifying a component of the sample in the first detection channel (304) or the second detection channel (306) based on a position of a corresponding peak in the first detection channel (304) or the second detection channel (306) in combination with a position of the calibration marker in the first detection channel (304) or the second detection channel (306).

10. The sample separation device (300) of claim 1 or any one of the above claims, further comprising at least one of:

a substrate (102), at least one fluid conduit (106) formed in and/or on the substrate (102) and configured for conducting the sample, wherein the detection unit (302) is configured for detecting the sample while being conducted via the at least one fluid conduit (106);

the sample separation device (300) is configured for separating different components of a fluidic sample;

the sample separation device (300) is configured as a gel electrophoresis apparatus;

the sample separation device (300) is configured as a capillary electrophoresis apparatus;

the sample separation device (300) is configured for separating the sample by an applied electric field.

11. An assay (700) configured for use with a sample separation device (300) of claim 1 or any one of the above claims or configured for executing a method of claim 13.

12. The assay (700) of the preceding claim, the assay (700) further comprising at least one of:

a first fluorescence label (702) to be labelled to a first component of the sample, a second fluorescence label (704) to be labelled to a second component of the sample, and a third fluorescence label (706) to be labelled to the calibration marker (708);

a plurality of ladder molecules (710) configured for providing a predefined ladder electropherogram with respect to the calibration marker (708);

a substrate (102), at least one fluid conduit (106) formed in and/or on the substrate (102) and configured for conducting the sample.

13. A method of separating different components of a sample, the method comprising

detecting, in a first detection channel (304), components of the sample at a first wavelength ($\lambda 1$),

detecting, in a second detection channel (306), components of the sample at a second wavelength ($\lambda 2$);

identifying a calibration marker in the first detection channel (304) and in the second detection channel (306) based on a predetermined occurrence ratio of the calibration marker between the first detection channel (304) and the second detection channel (306), the calibration marker having an emission spectrum (408) covering the first wavelength ($\lambda 1$) and the second wavelength ($\lambda 2$).

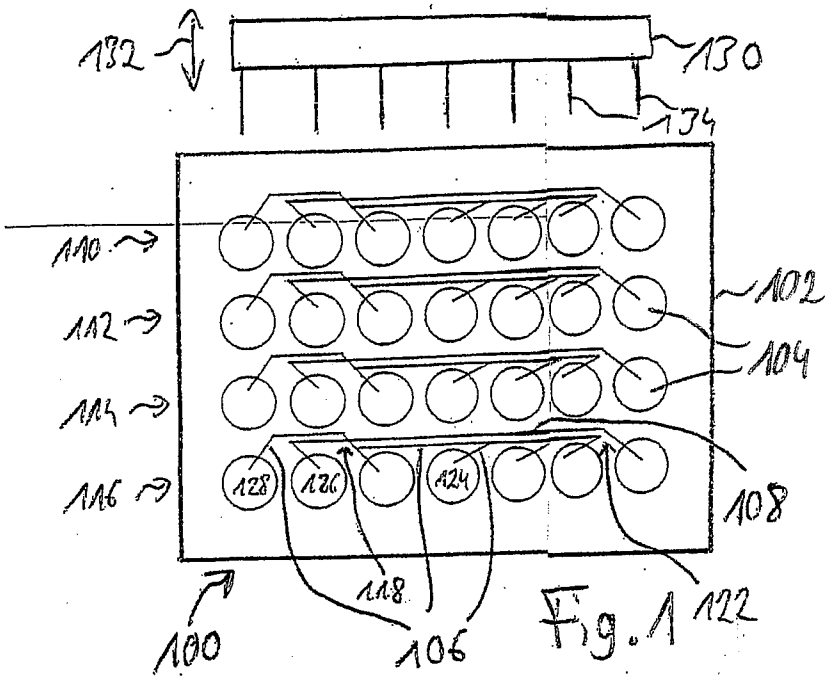
14. The method of the preceding claim, further comprising:

labeling a first component of the sample with a first fluorescence label (702) fluorescing at the first wavelength ($\lambda 1$);

labeling a second component of the sample with a second fluorescence label (704) fluorescing at the second wavelength ($\lambda 2$);

labeling the calibration marker (708) with a third fluorescence label (706) fluorescing in the emission spectrum (408) centered around a third wavelength ($\lambda 3$).

15. A software program or product, preferably stored on a data carrier, for controlling or executing the method of claim 13 or any one of the above claims, when run on a data processing system (330) such as a computer.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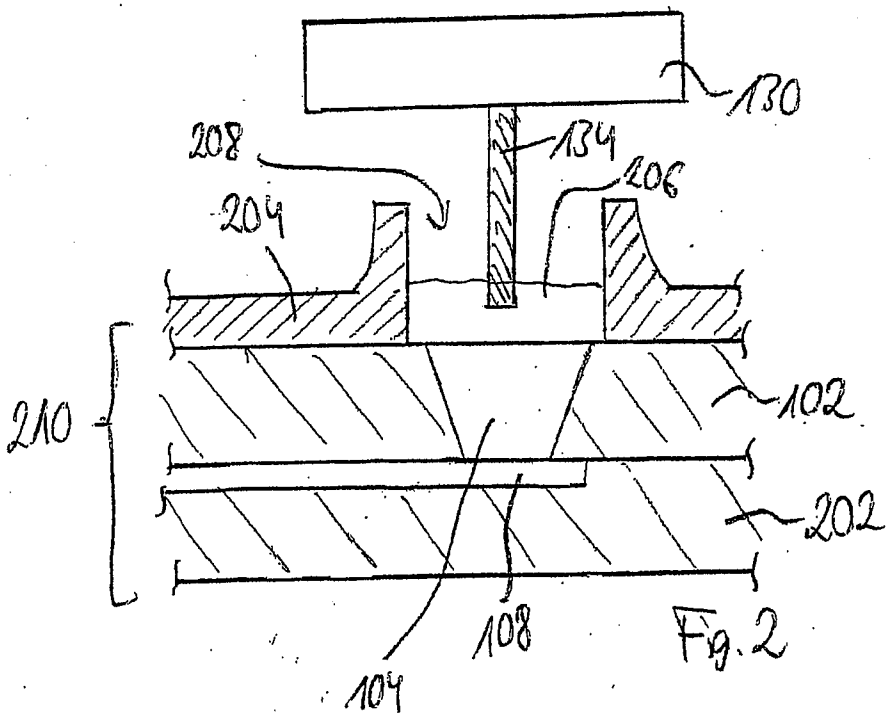
114
116
102
104
108
128
126
124
118
122
100
106

Fig. 1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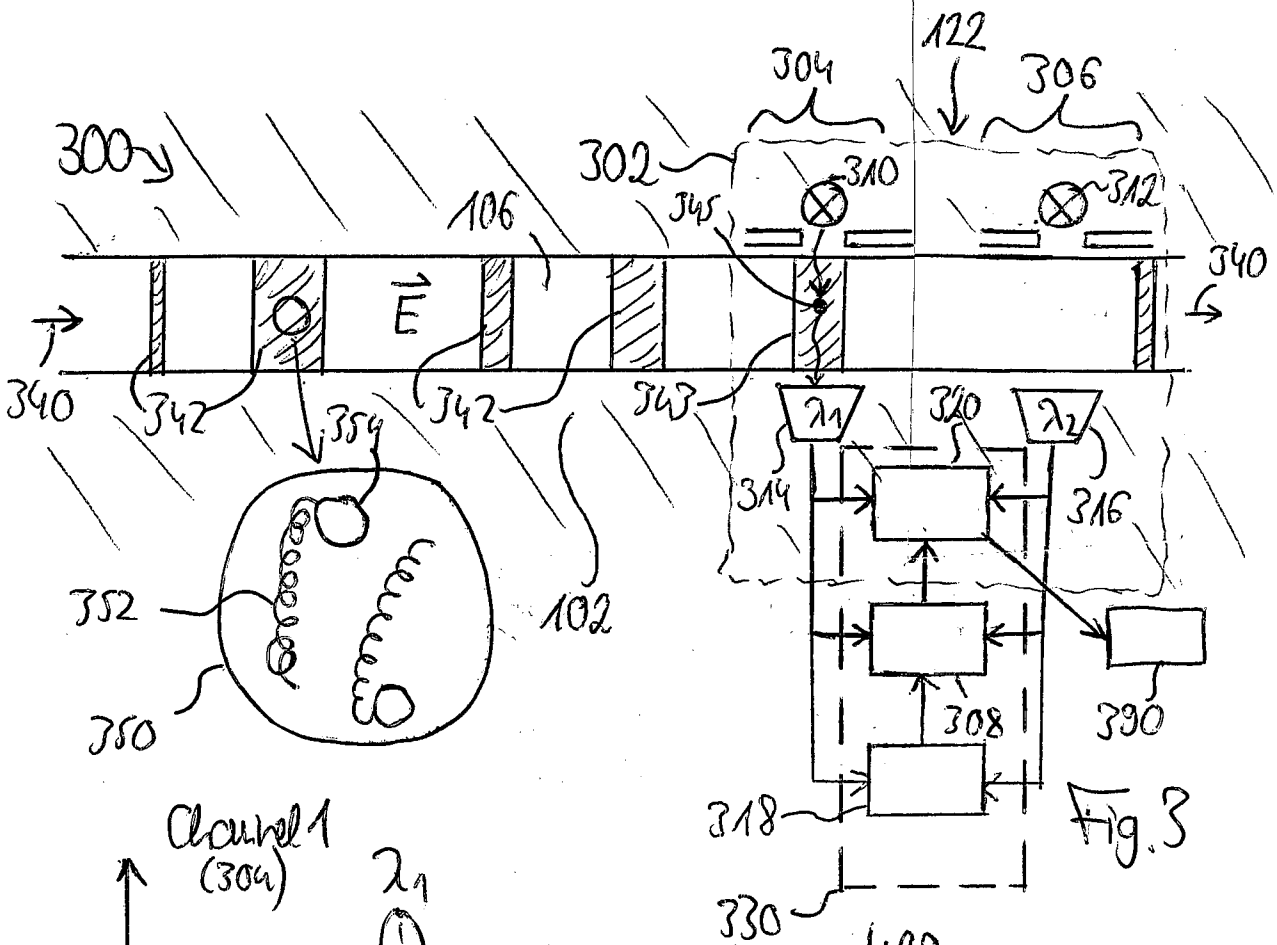
134
206
204
210
102
202
108
104

Fig. 2


300
304
122
306
302
310
312
106
345
340
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343
354
352
350
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320
314
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318
330

Fig.3

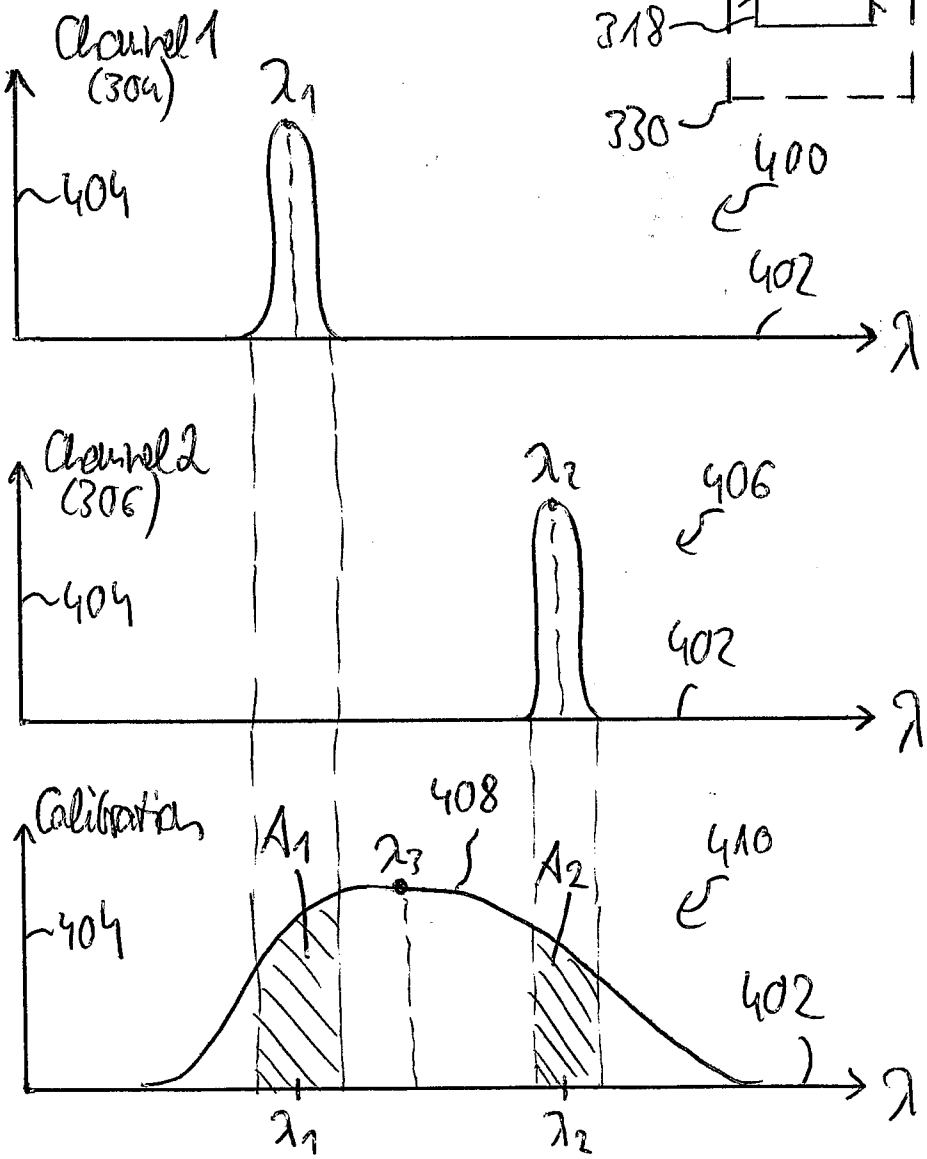

Channel 1 (304)
λ1
404
400
402
λ
Channel 2 (306)
λ2
406
404
402
λ
Calibration
A1
408
λ3
A2
410
404
402
λ
λ1
λ2

Fig.4

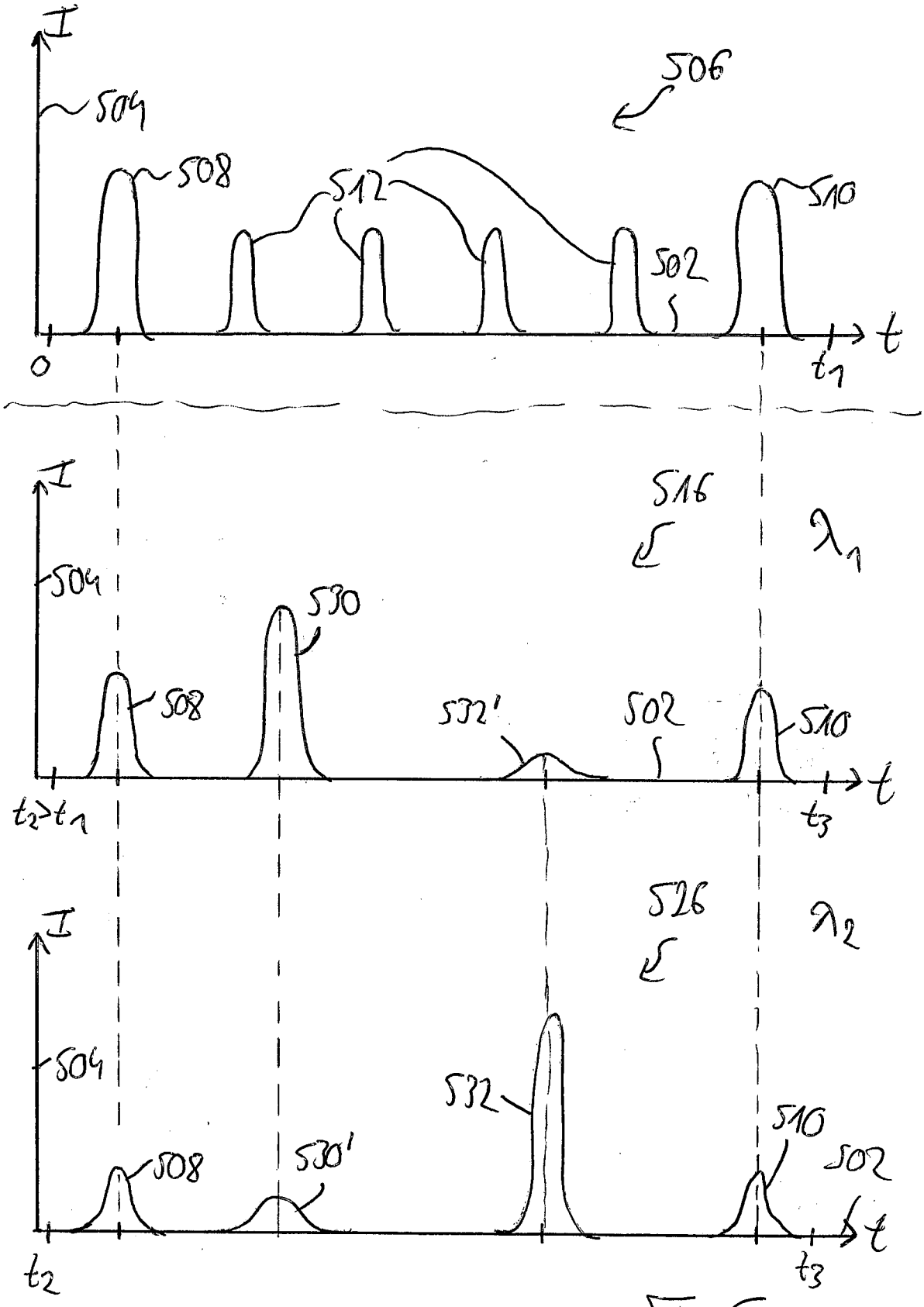
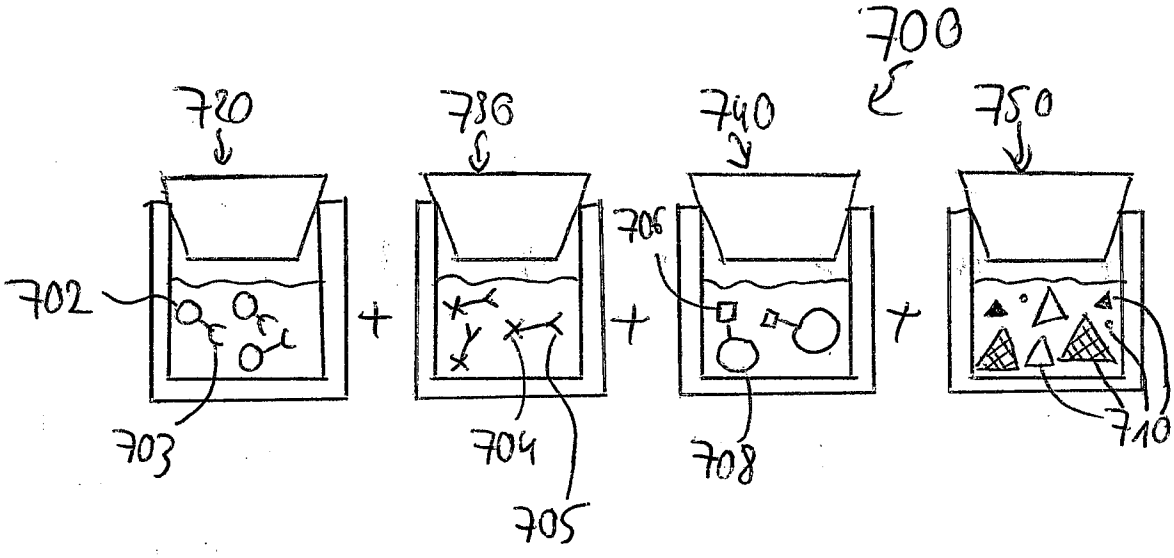

I
504
506
508
512
510
502
t
0
t_1
I
516
λ_1
504
530
508
532'
502
510
t
$t_2 > t_1$
t_3
I
526
λ_2
504
532
510
508
530'
502
t
t_2
t_3

Fig. 5


700
720
730
740
750
702
703
704
705
706
708
710

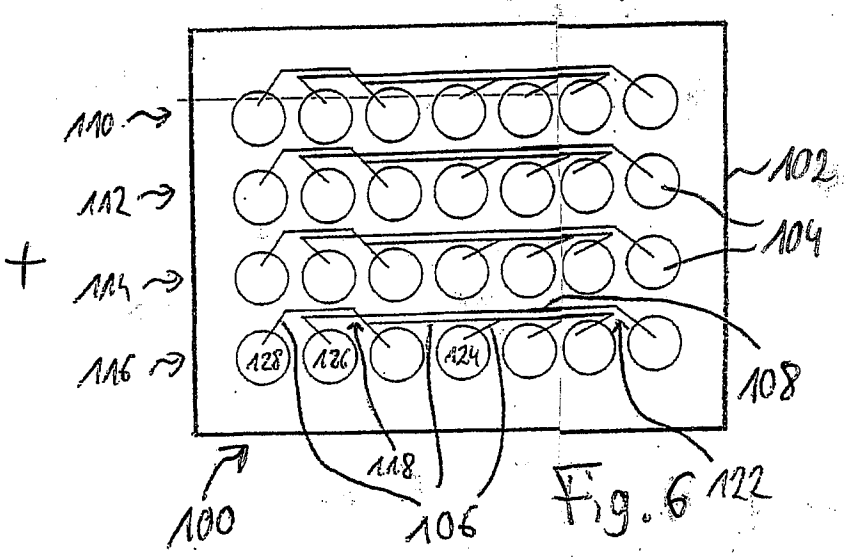

110
112
114
116
102
104
108
128
126
124
118
106
122
100

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/054666

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/64 G01N27/447

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	claims 26,28,32 paragraphs [0027], [0046]	1-33
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 2009	21/12/2009

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/054666

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 2006/014680 A (CELL BIOSCIENCES INC [US]; O'NEILL ROGER A [US]; GLAZER MARC [US]; YAN) 9 February 2006 (2006-02-09)	1,4-13, 16,17, 19-26, 28-33
A	claims 1,5-7,26 paragraph [0119] paragraphs [0118], [0121]; figure 14 paragraph [0068] paragraph [0119] paragraph [0051] paragraph [0126] paragraphs [0047], [0056] paragraphs [0051], [0055], [0058], [0098]; figures 12a,12b claims 14,24	2,3,14, 15,18,27
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A	paragraph [0005]; claim 1 claim 6	2,3,14, 15,18,27
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