
A multi-signal method for calibrating at least one analyte sensor for detecting at least one analyte in a sample

5

Technical Field

10 The invention relates to a method for calibrating at least one analyte sensor for detecting at least one analyte in a sample, a system configured for performing said method, a method for detecting at least one analyte in a sample by using at least one analyte sensor, a computer program, a computer-readable storage medium and non-transient computer-readable medium. The method may be used for lateral flow immunoassays. Other fields of application
15 of the present invention, however, are possible.

Background art

In Lateral Flow Immuno Assays (LFIA) an analyte to be detected and quantified is usually
20 immobilized in one or several spatially confined regions (e.g. test and control lines) on a test strip and detected with, among others, quantitative optical colorimetric means such as fluorescence. The working range achieved by LFIAs may be limited by the capacity of binding an analyte antigen to the capture and detection antibodies present in the test and control lines. With increasing analyte concentration beyond a threshold, the test line signal intensities will
25 saturate to a near constant level and with a further increase of the concentration a hook effect (also called pro-zone effect) may develop such that line signal intensities drop to a level representative of significantly lower concentrations. Line intensity saturation and hook effect inevitably lead either to highly inaccurate or outright incorrect quantitative results or even to completely false negative detections. Particularly, high concentrations of the analyte
30 being detected may be severely underestimated because the relation of concentration to measured signal (calibration curve) is no longer uniquely invertible from the signal to the concentration domain.

The analyte concentration threshold where saturation and the hook effect sets in can be changed by adjusting antibody concentrations, but accurate quantification of analytes at the higher end of the dynamic range may be nevertheless difficult. The general approach to cope with this situation is to detect the hook effect and to restrict the working range of the assay
5 to the low concentration region below the peak of the hooked calibration curve. For a single-line assays, this is actually the only possible way to derive correct concentration values.

For multi-line assays, several methodological attempts exist to remedy the non-uniqueness of hooked calibration curves and to extend the working range. Among them are US 10 942
10 179 B2, US 6 350 579 B1, US 6284472 B1, EP 2 841 918 B1, and US 10288609 B2.

In these approaches, two or more test line intensities are being used to measure multiple independent calibration curves, each of which either cover only or can be unequivocally inverted over a fraction of the envisaged working range. The subsequent common step is to
15 combine one way or another the independent individual calibration curves or segments thereof to a single calibration curve which as a whole can be used for converting signal intensities to concentrations over the full intended working range. This technique effectively creates a single univariate strictly monotonous functional dependence between signal intensity and concentration. In a two-dimensional calibration diagram the concentration appears
20 usually as the independent variable on the x-axis and the line signal as the dependent variable on the y-axis. By fitting appropriate mathematical models of the functional dependence between concentration and signal, the direction of dependence can unambiguously be inverted and eventually used to derive analyte concentration from the signal intensities. The calibration capability is thereby essentially encoded by the gradient of the functional dependence,
25 since an inversion can only be carried out in the concentration region (the “working range”) with a non-vanishing local and global calibration curve slope.

However, from an information theoretical point-of-view, it would be highly desirable to have the full information content of the individual multi-line signals combined rather than simply
30 their segmented numerical values appropriately merged to create an invertible univariate calibration curve. Moreover, from a mathematical analytical point-of-view, for the approaches described above it is strictly necessary that that the gradient be strictly one-dimensional which limits the application.

Problem to be solved

It is therefore an objective of the present invention to provide methods and devices which avoid the above-described disadvantages of known methods and devices. In particular, the method and the device shall allow improved calibration of at least one analyte sensor.

Summary

This problem is addressed by a method for calibrating at least one analyte sensor for detecting at least one analyte in a sample, a system configured for performing said method, a method for detecting at least one analyte in a sample by using at least one analyte sensor, a computer program, a computer-readable storage medium and non-transient computer-readable medium with the features of the independent claims. Advantageous embodiments which might be realized in an isolated fashion or in any arbitrary combinations are listed in the dependent claims as well as throughout the specification.

As used in the following, the terms “have”, “comprise” or “include” or any arbitrary grammatical variations thereof are used in a non-exclusive way. Thus, these terms may both refer to a situation in which, besides the feature introduced by these terms, no further features are present in the entity described in this context and to a situation in which one or more further features are present. As an example, the expressions “A has B”, “A comprises B” and “A includes B” may both refer to a situation in which, besides B, no other element is present in A (i.e. a situation in which A solely and exclusively consists of B) and to a situation in which, besides B, one or more further elements are present in entity A, such as element C, elements C and D or even further elements.

Further, it shall be noted that the terms “at least one”, “one or more” or similar expressions indicating that a feature or element may be present once or more than once typically will be used only once when introducing the respective feature or element. In the following, in most cases, when referring to the respective feature or element, the expressions “at least one” or “one or more” will not be repeated, notwithstanding the fact that the respective feature or element may be present once or more than once.

Further, as used in the following, the terms “preferably”, “more preferably”, “particularly”, “more particularly”, “specifically”, “more specifically” or similar terms are used in conjunction with optional features, without restricting alternative possibilities. Thus, features introduced by these terms are optional features and are not intended to restrict the scope of the claims in any way. The invention may, as the skilled person will recognize, be performed by

using alternative features. Similarly, features introduced by "in an embodiment of the invention" or similar expressions are intended to be optional features, without any restriction regarding alternative embodiments of the invention, without any restrictions regarding the scope of the invention and without any restriction regarding the possibility of combining the features introduced in such way with other optional or non-optional features of the invention.

In a first aspect, a method for calibrating at least one analyte sensor for detecting at least one analyte in a sample is disclosed. The analyte sensor comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals. Each of the independent sensor signals is dependent on a concentration of the analyte.

The method comprises the following steps that may be performed in the given order. However, a different order may also be possible. For example, one, more than one or even all of the method steps may be performed once or repeatedly. Further, the method steps may be performed successively or, alternatively, one or more of the method steps may be performed in a timely over-lapping fashion or even in a parallel fashion and/or in a combined fashion. The method may further comprise additional method steps that are not listed.

The method comprises the following steps:

- a) measuring at least two at least partially independent sensor signals by using the measurement unit on at least one reference sample having a known analyte concentration;
- b) determining a multidimensional calibration trajectory by combining the measured at least two at least partially independent sensor signals by using at least one processing unit.

The method may be computer-implemented, e.g. step b) may be performed by using a computer and/or computer network. The term "computer implemented method" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a method involving at least one computer and/or at least one computer network. The computer and/or computer network may comprise at least one processor which is configured for performing at least one of the steps of the method according to the present invention. Specifically, each of the method steps is performed by the computer and/or computer network. The method may be performed completely automatically, specifically without user interaction.

The term "analyte" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to an arbitrary element, component or compound which may be present in a body fluid and the concentration of which may be of interest for a user. The analyte may be or may comprise an arbitrary chemical substance or chemical compound which may take part in the metabolism of the user, such as at least one metabolite. As an example, the analyte may be selected from the group consisting of glucose, cholesterol, triglycerides, lactate, vitamin D, drugs of abuse, therapeutic drugs, hormones, cardiac markers, and metabolites in general. Additionally or alternatively, however, other types of analytes may be determined and/or any combination of analytes may be determined.

The term "sample" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to any type of composition of matter; thus, the term may refer, without limitation, to any arbitrary sample such as a biological sample. The sample may be a liquid sample. The sample may be an aqueous sample. The sample may comprise or may be suspected to comprise at least one analyte. The sample may be used directly as obtained from the respective source or may be subjected to one or more pretreatment and/or a sample preparation step(s). The sample may be pretreated by physical and/or chemical methods, in an embodiment by centrifugation, filtration, mixing, homogenization, chromatography, precipitation, dilution, concentration, contacting with a binding and/or detection reagent, and/or any other method deemed appropriate by the skilled person.

The sample may be a sample of bodily fluid. The term "sample of bodily fluid" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to an arbitrary aliquot part or aliquant part of the bodily fluid. The sample may be selected from the group consisting of: a physiological fluid, including whole blood, serum, plasma, saliva, ocular lens fluid, lacrimal fluid, cerebrospinal fluid, sweat, interstitial fluid, urine, saliva, milk, ascites, mucus, synovial fluid, peritoneal fluid, and amniotic fluid; lavage fluid; tissue, cells, or the like.

The term "reference sample" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a sample having a known analyte concentration.

The term "measuring sample" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a sample under measurement, e.g. having an unknown analyte concentration.

The term "sensor" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to an arbitrary element configured for detecting at least one condition or for measuring at least one measurement variable. The term "analyte sensor" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a sensor configured for detecting quantitatively or qualitatively at least one analyte.

The term "measurement unit" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to at least one unit, e.g. a region or area, of the analyte sensor configured for generating at least two at least partially independent sensor signals. For example, the analyte sensor may comprise two or more lateral flow immunoassays. The analyte sensor may comprise multi-line assays having at least two test lines. The test line may comprise at least one test chemical for detecting the analyte. The test lines may comprise identical or different test chemicals.

For example, the analyte sensor may comprise at least one test strip. The term "test strip" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a strip-shaped element configured for detecting an analyte or determining the concentration of an analyte in a sample. The test strip may also be referred to as test element. As outlined above, the test strip may comprise a plurality of test lines. Each of the test lines comprise at least one component, which changes at least one detectable property when the analyte is present in the sample, e.g. an optical detectable property such as a color change. The test strip may be suited for an ex-vivo measurement, specifically an in-vitro measurement. As used herein, the term "strip-shaped" may refer to an element having an elongated shape and a thickness, wherein an extension of the element in a lateral dimension exceeds the thickness of the element, such as by at least a factor of 2, preferably by at least a factor of 5, more preferably by at least a factor of 10 and

most preferably by at least a factor of 20 or even at least a factor of 30. These test strips are generally widely in use and available.

- The term "detecting at least one analyte" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to quantitatively or qualitative detecting the analyte. In an embodiment, determining is qualitative, semiquantitative, or quantitative determination. Qualitative determination may relate to determining whether the value, e.g. of a parameter, is above a predetermined threshold value, e.g. a detection limit or a physiologically relevant threshold value. Semiquantitative determination may be assigning a measured value to a pre-established category, e.g. "low", "medium", or "high" concentration. In an embodiment, determining is quantitative, i.e. is determining a value of a quantitative measure of a parameter.
- For example, the measurement unit may be configured for an optical detection. The term "optical detection" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a detection of a reaction using an optical test chemical, such as a color change test chemical which changes in color in the presence of the analyte. The color change specifically may depend on the amount of analyte present in the sample. Techniques for determining the analyte by optical detection and e.g. analyzing color of the spot on the test field are generally known to the skilled person.
- For example, the measurement unit may be configured for an electrochemical detection. The analyte sensor may be or may comprise at least one electrochemical sensor. The term "electrochemical sensor" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a sensor based on electrochemical measurement principles, such as by using one or more of an amperometric, coulometric or a potentiometric measurement principle. Specifically, the electrochemical sensor may comprise at least one enzyme configured for performing at least one redox reaction in the presence of the analyte to be detected, wherein the redox reaction may be detected by electrical means. The term "electrochemical detection" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a detection of an electrochemically detectable property of the analyte by electrochemical means, such as an electrochemical detection reaction. Thus, for example,

the electrochemical detection reaction may be detected by comparing one or more electrode potentials, such as a potential of a working electrode with the potential of one or more further electrodes such as a counter electrode or a reference electrode.

- 5 As used herein, the term "calibrating" is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to at least one process of determining a relationship between a sensor signal and the concentration of the analyte. The relationship may be a multidimensional calibration trajectory. The relationship
10 may be used for transforming one or more measured sensor signals into one or more concentration values.

- As used herein, the term "sensor signals" is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to an observable change in at least one physical quantity. The sensor signal may be or comprise a sign or a function conveying information about the at least one physical quantity. The sensor signal may specifically be or comprise at least one of an electronic signal, an optical signal or an optoelectronic signal. The sensor signal may be or comprise at least one of a voltage,
15 a current, a charge, an electromagnetic wave. Further options are feasible and generally known to the skilled person. The sensor signal is dependent on a concentration of the analyte. The sensor signal may comprise a signal curve. The signal curve may refer to a dependence of a detector response changing with a varying stimulus, e.g. under control of the experimenter. The signal curve may be one or more of concentration vs signal amplitude or phase,
20 angle, time lag, or otherwise appropriately derived or transformed values. The signal curve may comprise a hooked (peaked) and/or saturated (asymptotic) curve. For example, the signal curve may comprise at least one ambiguous region. The ambiguous region may comprise signal values occurring several times (denoted as "Hook" or „Hook-peak“) at different analyte concentrations. The ambiguous region may exhibit ambiguities due to changes in signal
25 height which are that low such that these cannot be distinguished within the scope of the measurement accuracy (saturation). The proposed method may allow for using even hooked and/or saturated signal curves and, thus, distinguishes from techniques trying to establish one-dimensional univariate uniquely invertible calibration curves from combinations of partial (piecewise) sensor signals.

- 35 As used herein, the term "independent sensor signals" is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be

- limited to a special or customized meaning. The term specifically may refer, without limitation, to sensor signals which do not affect each other. As used herein, the term "at least partially independent sensor signals" is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to independent in
- 5 at least one dimension of a multidimensional signal space, wherein each of the sensor signals represents an axis of the multidimensional signal space. None of the axis of the multidimensional signal space may represent the values of the analyte concentrations directly.
- 10 Step a) may comprise measuring the at least two at least partially independent sensor signals on a plurality of reference samples. The plurality of reference samples may be a limited discrete set of dedicated prepared samples having predefined and known analyte concentrations.
- 15 The at least two at least partially independent sensor signals may be generated by using at least a single or a plurality of identical or different diagnostic methods. For example, the measurement unit may be configured for generating the at least two at least partially independent sensor signals by using at least two different diagnostic methods, e.g. by using at least one optical detection and at least one electrochemical detection.
- 20 For example, the measurement unit may be configured for generating the at least two at least partially independent sensor signals by performing at least two measurements using the same diagnostic method.
- 25 For example, the measurement unit may be configured for generating the at least two at least partially independent sensor signals by using lateral flow immunoassays, e.g. at least two successively arranged lateral flow immunoassays or an assay with at least two test lines.
- 30 As used herein, the term "multidimensional calibration trajectory" is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a trajectory within the multidimensional signal space which can be used for transforming one or more measured sensor signals into one or more concentration values. A trajectory can be 1D, 2D, or nD (with $n > 2$) depending on the number of independent
- 35 variables required for its parametrization. The parametrization may be the independent variable(s) varying along the trajectory. The multidimensional calibration trajectory may be at least one-dimensional. A trajectory may be distinguished from a point (zero dimensional) in

a higher dimensional space. Each sensor signal may define a single dimension of the multi-dimensional signal space. For example, in case of two sensor signals, the multidimensional signal space may have two dimensions, one from each sensor signal. Dimensionality of a trajectory may be given by the number of independently controlled parametric variables.

- 5 Dimensionality of a trajectory may be to be distinguished from the dimensionality of the signal space, into which it will be embedded. The dimensionality of the signal space may be at least two or more, depending on the number of sensor signals being measured. A trajectory with only one parametric variable such as e.g. the concentration may be a curve with dimensionality “one”, i.e. one variable is sufficient to describe a point on the curve. A trajectory
- 10 with two independently controlled parametric variables, such as e.g. the concentration and temperature, is a surface with dimensionality “two”. Higher dimensional trajectories (hyper-surfaces) may exist for an increasing number of parametric variables. The embedding signal space may be used to have a dimensionality of at least that of a trajectory, otherwise embedding and mapping of sensor signals to parametric variables may not be possible.

- 15 The term “processing unit” as generally used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to an arbitrary logic circuitry configured for performing basic operations of a computer
- 20 or system, and/or, generally, to a device which is configured for performing calculations or logic operations. The processing unit may be configured for processing basic instructions that drive the computer or system. As an example, the processing unit may comprise at least one arithmetic logic unit (ALU), at least one floating-point unit (FPU), such as a math co-processor or a numeric coprocessor, a plurality of registers, specifically registers configured
- 25 for supplying operands to the ALU and storing results of operations, and a memory, such as an L1 and L2 cache memory. The processing unit may be a multi-core processor. Specifically, the processing unit may be or may comprise a central processing unit (CPU). Additionally or alternatively, the processing unit may be or may comprise a microprocessor, thus specifically the processing unit’s elements may be contained in one single integrated circuitry (IC) chip. Additionally or alternatively, the processing unit may be or may comprise
- 30 one or more application-specific integrated circuits (ASICs) and/or one or more field-programmable gate arrays (FPGAs) or the like. The processing unit specifically may be configured, such as by software programming, for performing one or more evaluation operations. The processing unit may comprise one or more interfaces, such as one or more wireless
- 35 interfaces and/or one or more wire-bound interfaces.

The term “combining the measured at least two at least partially independent sensor signals” as generally used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to generating and/or establishing the multidimensional signal space. Each sensor signal may be embedded into the multidimensional signal space. Each sensor signal may define a single dimension of the multidimensional signal space. For example, in case of two sensor signals, the multidimensional signal space may have two dimensions, one from each sensor signal. The determination of the multidimensional calibration trajectory may comprise combining the full information content of the measured at least two at least partially independent sensor signals. The combination may comprise combining information contents of the separate sensor signals in such a way that the resulting multidimensional calibration trajectory across this multidimensional signal space directly carries the information about the analyte concentrations via the position along the multidimensional calibration trajectory along with a non-vanishing multi-dimensional gradient. Thus, no information contained in the separate multi-line calibration curves is lost or unused and the algorithmic preparation of the final multidimensional calibration trajectory can easily be done in a fully automatic manner. This combination may allow lifting the degeneration between the separate one-dimensional dependencies of signals and concentrations, allowing the direct usage of saturated and/or hooked calibration curves even close to their respective saturated or hook regions.

The multidimensional calibration trajectory may have at least two dimensions. As outlined above, each sensor signal may define a single dimension of the multidimensional signal space. The multidimensional calibration trajectory may be a trajectory through the multidimensional signal space with concentration values varying along the trajectory given by the measured at least two at least partially independent sensor signals. In addition to the concentration varying systematically along the trajectory, the concentration can be represented as an additional dimensional axis. However, such an additional concentration axis is not necessary as it (the additional axis) carries only redundant information about the concentration. For example, for two sensor signals, the additional concentration axis can be used to visualize the concentration. In case of using more sensor signals, a visualization of the concentration may be done by projecting the trajectory to a (possibly several) at most three-dimensional representation(s). For example, in case the analyte sensor comprising a two-line LFIA, the multidimensional calibration trajectory may be a trajectory in a two-dimensional plane. For example, in case the analyte sensor comprising a three-line LFIA, the multidimensional calibration trajectory may be a trajectory in a three-dimensional signal space. Multidimensional calibration trajectories with more than three signals have no immediate visual or

graphical representation, but can mathematically be handled in a way similar to multidimensional calibration trajectories in two- and three-dimensional signal spaces.

5 A multidimensional gradient and/or a total derivative along the multidimensional calibration trajectory may be non-vanishing across a working range. The term “working range” as generally used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a range in which the analyte sensor delivers calibrated measurements of calibration. The term “measurement range”, also
10 denoted as dynamic range, as generally used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a range in which the analyte sensor is able to deliver measurements (values) as a function of varying concentrations. As long as not all sensor signals show constant values at
15 the same time for a given concentration range, the embedding of the individual signals in a higher-dimensional signal space results in a non-vanishing multi-dimensional gradient along the full multidimensional calibration trajectory. However, even in case the prerequisite of a non-vanishing gradient and/or total derivative everywhere along the whole trajectory is not fulfilled, the method may allow a proper calibration over all regions with non-vanishing
20 gradient and may consider the regions of vanishing gradient and/or total derivative as having considerably larger predicted concentration uncertainties.

For known analyte sensors, as described above, a working range and the full measurement range of the analyte sensor may differ due to non-usage of saturated and/or hooked regions.
25 The method according to the present invention may allow for extending the working range of the at least one analyte sensor for detecting the at least one analyte in the sample to the full measurement range. The proposed calibration can rely on hooked (peaked) and/or saturated (asymptotic) individual sensor signal curves, where an invertible calibration trajectory can still be established although some/all sensor signals might show such a behavior as long
30 as the prerequisite that the multidimensional gradient and/or the total derivative along the multidimensional calibration trajectory is non-vanishing is everywhere fulfilled.

The proposed multidimensional combination of signals opens up the route to a hitherto unexplored methodology for the calibration of multi-line assays. It comprises a fundamentally
35 new way of utilizing all signals of multi-line assays, even those showing saturated and/or hooked calibration curves. It does not attempt to construct a univariate calibration curve from the signal values for the derivation of the analyte concentration via inverse prediction.

Step b) may comprise an inverse prediction and/or derivation of at least one analyte concentration from the measured sensor signal-concentration tuples by using at least one mathematical algorithm such as polynomial, spline, or kernel methods. Data used for calibration is usually measured as a limited discrete set of dedicated prepared samples having predefined and known concentrations, i.e. the reference samples. Using only this set would be insufficient for all situations where concentrations have to be derived from measured signals by inverse prediction. To cover the full concentration range in a dense way, the signal-concentration tuples have to be interpolated. This can be done either by individually interpolating the separate signals as a function of concentration and combining the resulting discrete curves in the multi-dimensional signal space, or else the set of measured signal-concentration tuples may directly interpolated in multi-dimensional signal space. In each case the concentration may be parametrically varying along the multi-dimensional trajectory.

The method may comprise storing the multidimensional calibration trajectory in tabulated and/or analytical functional form, e.g. available for subsequent usage in tabulated or analytical functional form depending on the interpolation algorithm used.

The method may comprise considering at least one historic signal. The historic signal may be some signal from the sample which were measured at one time and the result(s) were, e.g. digitally, stored. Then the sample itself is stored and/or frozen for some time and later the sample is one or more of reactivated, thawed, unfrozen. The further additional sensor signals possibly with different methods are obtained. After the final measurement(s) has(ve) been taken, the previously digitally stored historic measurements may be combined with the final set of signals as per the described multi-dimensional calibration method.

The method may comprise considering at least one additional data source for determining the multidimensional calibration trajectory. For example, a calibration trajectory may be affected by temperature, humidity or other external or environmental interferences. A multitude of calibration trajectories can be established, each of which is representative of a specific external influence. In case of a sufficiently dense continuous coverage of a range of the external interference, the separate trajectory curves can be considered as a trajectory surface.

The method may further comprise determining a concentration corresponding to at least two sensor signals by using the multidimensional calibration trajectory and the measurement unit on at least one measuring sample by identifying a concentration value along the trajectory nearest to a value of the sensor signal using at least one analytical or numerical algorithm.

In a further aspect, a method for detecting at least one analyte in a sample by using at least one analyte sensor is disclosed. The analyte sensor comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals. Each of the independent sensor signals is dependent on a concentration of the analyte.

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The method comprises the following steps that may be performed in the given order. However, a different order may also be possible. In particular, one, more than one or even all of the method steps may be performed once or repeatedly. Further, the method steps may be performed successively or, alternatively, one or more of the method steps may be performed in a timely overlapping fashion or even in a parallel fashion and/or in a combined fashion. The method may further comprise additional method steps that are not listed.

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The method comprises the following steps:

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i. retrieving at least one multidimensional calibration trajectory determined by performing a method for calibrating at least one analyte sensor according to the present invention;

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ii. measuring at least two sensor signals by using the measurement unit on at least one measuring sample, wherein the method comprises determining a concentration corresponding to the respective sensor signals by using the multidimensional calibration trajectory, wherein the determination comprises identifying a concentration value nearest to a value of the sensor signals along the multidimensional calibration trajectory using at least one analytical or numerical algorithm.

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In the method, as outlined above, at least one multidimensional calibration trajectory is determined by performing a method for calibrating at least one analyte sensor according to the present invention, such as according to any one of the embodiments disclosed above and/or according to any one of the embodiments disclosed in further detail below. Thus, for possible embodiments and definitions, reference is made to the description of the method above.

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The method may be computer-implemented.

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The term “retrieving” as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to a process of a system specifically a computer system, of generating data and/or obtaining data from an arbitrary data source, such as from a data storage, from a network or from a further computer

or computer system or cloud, and/or receiving the data, e.g. as user input via a human-machine interface. The retrieving specifically may take place by at least one computer interface, e.g. a communication interface, such as via a port, e.g. a serial or parallel port. The retrieving may comprise several sub-steps, such as the sub-step of obtaining one or more items of primary information and generating secondary information by making use of the primary information, such as by applying one or more algorithms to the primary information, e.g. by using a processor, e.g. of the processing unit. The retrieving may comprise obtaining data from at least one database, e.g. from a cloud, having stored the multidimensional calibration trajectory thereon. The retrieving additionally may comprise obtaining data from one or more of a user input, at least one measurement, at least one calculation, literature, at least one handbook, knowledge, experience and at least one simulation.

As outlined above, the method comprises determining a concentration corresponding to the sensor signals by using the multidimensional calibration trajectory, wherein the determination comprises identifying a concentration value nearest to a value of the sensor signals using at least one analytical or, possibly iterative, numerical algorithm. A concentration measurement with an analyte sensor would deliver multiple, e.g. two, sensor signals, which in turn can be viewed as a single multi-dimensional point in the multidimensional signal space initially generated by the calibration method. Concentrations can be read off from the multidimensional calibration trajectory by finding the concentration value nearest to the new signal point. The term “nearest” as generally used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to as having the smallest orthogonal distance as measured along the straight line perpendicular to the multidimensional calibration trajectory or any other distance metric suitable for the calibration problem at hand. Since it cannot be expected that calibration trajectories in general can be described by closed-form analytical mathematical models, the determination of the associated concentration may use a, possibly iterative, numerical algorithms such as stochastic or gradient-based minimization of the distance to the interpolated calibration trajectory. However, in case of sufficiently simple individual sensor signal curves (e.g. straight lines of constant slope) the multidimensional calibration trajectory will also be simple (a straight line), which means that in this particular case the whole calibration procedure can in principle make use of analytical formulae and algorithms (instead of numerical computations).

The multidimensional signal space and the trajectory therein may be created by combining at least two sensor signals. In order to get a concentration prediction from the trajectory, a

similar number of sensor signals may be used. The individual sensor signal curves may comprise at least one ambiguous region where signal values may occur several times (denoted as “Hook” or „Hook-peak“) at different analyte concentrations or vanishing changes in signal height with concentration indistinguishable within the scope of the measurement accuracy (saturation). Thereby, the proposed method allows using even hooked and/or saturated
5 signal curves for establishing the multidimensional calibration trajectory and, thus, distinguishes from techniques trying to establish one-dimensional univariate uniquely invertible calibration curves from combinations of partial (piecewise) sensor signals.

10 The method may comprise determining a measure of accuracy of the determined corresponding concentration with respect to the multidimensional calibration trajectory by using an orthogonal or any other distance metric between the value of the sensor signals from the multidimensional calibration trajectory. Thus, the distance can be used as a measure of accuracy of the resulting concentration with respect to the pre-established multidimensional
15 calibration curve. A large distance may indicate that the multi-dimensional signal point is far from and not well represented by the multidimensional calibration trajectory, which might indicate an associated large uncertainty of the derived concentration. In reverse, a small or even vanishing distance to the trajectory may indicate that the sensor signals are representative of those already seen during the calibration procedure, indicating an accurate
20 estimation of the concentration with a particularly small or even negligible uncertainty.

The method may comprise at least one failsafe step. The failsafe step may comprise comparing the measure of accuracy with at least one predefined threshold. The determined analyte concentration may be safeguarded in case the predefined threshold is exceeded. For
25 example, the predefined threshold may be given by quality control, regulatory or product marketing requirements. For example, the analyte concentration may be safeguarded in case a measured sensor signal point appears more distant from the pre-established multidimensional calibration trajectory than a predefined secure failsafe-threshold value. For example, the measurement may be flagged as unreliable and/or may be completely discarded. In case
30 of repeatedly determined failures of measurements of the same sample, it can also be used as an indicator that an incorrect sample type has possibly been offered to the analyte sensor, thereby acting as a first-level classifier of allowed or disallowed sample types.

Thus, besides allowing making use of saturated and/or hooked separate calibration curves
35 over the full measurable signal range of an e.g. LFIA, the proposed methods may have other unique advantages currently not available in other standard univariate calibration procedures. These are a built-in way to gain additional information about the accuracy of derived concentrations and, further, a safeguard property, indicating whether a given measurement

likely has signal values incompatible with the previously established multidimensional calibration trajectory and assay performance.

- 5 The method may comprise measuring a plurality of at least partially independent sensor signals by using the measurement unit on the measuring sample. At least two of the measured at least partially independent sensor signals may separately used for determining an analyte concentration. The term “separately used for determining an analyte concentration” as generally used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning.
- 10 ing. The term specifically may refer, without limitation, to determining the analyte concentration from one of the sensor signals (e.g. from a first sensor signal) and determining the analyte concentration from the other one of the sensor signals respectively, e.g. without considering the respective other sensor signal for determining the analyte concentration from the first sensor signal. Each of the sensor signals can be separately used to predict an analyte
- 15 concentration from the crossing of the signals’ hyperplane with the multidimensional calibration trajectory. The distribution of these single-signal-derived concentrations, e.g. in conjunction with the concentration value determined using a distance metric as described above, can be used a measure of the derived concentration accuracy.
- 20 The determination of the analyte concentration may comprise determining a concentration corresponding to the respective sensor signal by using the multidimensional calibration trajectory by identifying a concentration value nearest to a value of the respective sensor signal using the at least one analytical and/or, possibly iterative, numerical algorithm, e.g. as described above.
- 25 The method may comprise determining a combined analyte concentration from several determined analyte concentrations. The determining of the combined analyte concentration may comprise one or more of determining a mean value, a median value and the like.
- 30 The method may comprise outputting the analyte concentration and/or the combined analyte concentration. The term “outputting” as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to the process of making information available to another system, data storage, person
- 35 or entity. As an example, the outputting may take place via one or more interfaces, such as a computer interface, a web interface or a human-machine interface. The outputting, as an example, may take place in one or more of a computer-readable format, a visible format or an audible format.

The methods according to the present invention can make use of multi-line assay signals in a multidimensional signal space. The individual sensor signals may be embedded in a higher multidimensional signal space, where the concentration is systematically and invertibly varying along the multidimensional trajectory. In contrast to methods of prior art in this field the methods not only make use of the full unrestricted information content of all sensor signals in parallel but additionally has significant advantages not found in any other method currently in use to derive analyte concentrations from measured assay line intensities. Although the problems with large working ranges have been encountered decades ago, the current dearth of an applicable approach such as the one described above, which is able to make use of both saturated and/or hooked calibration dependencies, clearly indicates that the described invention is not devisable by simply utilizing ordinary skills and common knowledge in the field.

The application of the described methods is not restricted to signals from LFIA's but can in general be used generally for sensors and signals which might show a saturated and/or hooked behavior in their individual calibration curves. It can even be applied to sensors and signals which do show well-known sigmoidal, non-linear monotonic or even linear calibration curves either as a redundant method in addition to a standard procedure or as the primary method utilizing the additional advantages of accuracy measures and safeguarding.

In a further aspect, a system is disclosed. The system comprises at least one analyte sensor for detecting at least one analyte in a sample. The analyte sensor comprises the measurement unit configured for measuring at least two at least partially independent sensor signals on at least one measuring sample, wherein each of the at least partially independent sensor signals is dependent on a concentration of the analyte. The system is configured for retrieving at least one multidimensional calibration trajectory determined by performing a method for calibrating at least one analyte sensor according to the present invention. The system comprises at least one processing unit configured for determining a concentration corresponding to at least one of the sensor signals by using the multidimensional calibration trajectory. The determining comprises identifying a concentration value nearest to a value of the sensor signal using at least one analytical or numerical algorithm.

For possible embodiments and definitions, reference is made to the description of the methods above.

The term "system" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or

customized meaning. The term specifically may refer, without limitation, to an arbitrary set of interacting or interdependent components parts forming a whole. Specifically, the components may interact with each other in order to fulfill at least one common function. The at least two components may be handled independently or may be coupled or connectable.

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The system may be configured for performing a method for detecting at least one analyte in a sample according to the present invention.

10 The system comprises at least one database configured for storing the multidimensional calibration trajectory in tabulated and/or analytical functional form. The term "database" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term specifically may refer, without limitation, to an organized collection of data, generally stored and accessed electronically from a computer or computer system. The database may
15 comprise or may be comprised by a data storage device. The database may comprise at least one data base management system, comprising a software running on a computer or computer system, the software allowing for interaction with one or more of a user, an application or the database itself, such as in order to capture and analyze the data contained in the data base. The database management system may further encompass facilities to administer the
20 database. The database, containing the data, may, thus, be comprised by a database system which, besides the data, comprises one or more associated applications. The database may be directly physically integrated into a measurement system or at least partially or completely cloud based.

25 The system may comprise at least one communication interface, e.g. at least one user interface. The communication interface may be configured for outputting information, e.g. the analyte concentration as described above. The term "user interface" as used herein is a broad term and is to be given its ordinary and customary meaning to a person of ordinary skill in the art and is not to be limited to a special or customized meaning. The term may refer,
30 without limitation, to a feature of the system which is configured for interacting with its environment, such as for the purpose of unidirectionally or bidirectionally exchanging information, such as for exchange of one or more of data or commands. For example, the user interface may be configured to share information with a user and to receive information by the user. The user interface may be a feature to interact visually with a user, such as a display,
35 or a feature to interact acoustically with the user. The user interface, as an example, may comprise one or more of: a graphical user interface; a data interface, such as a wireless and/or a wire-bound data interface.

In a further aspect a computer program is disclosed comprising instructions which, when the program is executed by the system according to the present invention in one or more of the embodiments enclosed herein, cause the system to perform a method for calibrating at least one analyte sensor according to the present invention in one or more of the embodiments enclosed herein and/or a method for detecting at least one analyte in a sample according to the present invention in one or more of the embodiments enclosed herein. Specifically, the computer program may be stored on a computer-readable data carrier and/or on a computer-readable storage medium.

10 In a further aspect, a computer-readable storage medium is disclosed comprising instructions which, when the instructions are executed by the system according to the present invention, cause the system to perform a method for calibrating at least one analyte sensor according to the present invention and/or a method for detecting at least one analyte in a sample according to the present invention.

15 In a further aspect a non-transient computer-readable medium is disclosed including instructions that, when executed by one or more processors, cause the one or more processors to perform a method for calibrating at least one analyte sensor according to the present invention and/or a method for detecting at least one analyte in a sample according to the present invention.

20 As used herein, the terms “computer-readable data carrier” and “computer-readable storage medium” specifically may refer to non-transitory data storage means, such as a hardware storage medium having stored thereon computer-executable instructions. The computer-readable data carrier or storage medium specifically may be or may comprise a storage medium such as a random-access memory (RAM) and/or a read-only memory (ROM).

25 Thus, specifically, one, more than one or even all of method steps as indicated above may be performed by using a computer or a computer network, preferably by using a computer program.

30 Further disclosed and proposed herein is a computer program product having program code means, in order to perform the method according to the present invention in one or more of the embodiments enclosed herein when the program is executed on a computer or computer network. Specifically, the program code means may be stored on a computer-readable data carrier and/or on a computer-readable storage medium.

Further disclosed and proposed herein is a data carrier having a data structure stored thereon, which, after loading into a computer or computer network, such as into a working memory or main memory of the computer or computer network, may execute the method according to one or more of the embodiments disclosed herein.

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Further disclosed and proposed herein is a computer program product with program code means stored on a machine-readable carrier, in order to perform the methods according to one or more of the embodiments disclosed herein, when the program is executed on a computer or computer network. As used herein, a computer program product refers to the program as a tradable product. The product may generally exist in an arbitrary format, such as in a paper format, or on a computer-readable data carrier and/or on a computer-readable storage medium. Specifically, the computer program product may be distributed over a data network.

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Finally, disclosed and proposed herein is a modulated data signal which contains instructions readable by a computer system or computer network, for performing the method according to one or more of the embodiments disclosed herein.

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Referring to the computer-implemented aspects of the invention, one or more of the method steps or even all of the method steps of one or several of the methods according to one or more of the embodiments disclosed herein may be performed by using a computer or computer network. Thus, generally, any of the method steps including provision and/or manipulation of data may be performed by using a computer or computer network. Generally, these method steps may include any of the method steps, typically except for method steps requiring manual work, such as providing the samples and/or certain aspects of performing the actual measurements.

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Specifically, further disclosed herein are:

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- a computer or computer network comprising at least one processor, wherein the processor is adapted to perform one or several of the methods according to one of the embodiments described in this description,
- a computer loadable data structure that is adapted to perform one or several of the methods according to one of the embodiments described in this description while the data structure is being executed on a computer,
- a computer program, wherein the computer program is adapted to perform one or several of the methods according to one of the embodiments described in this description while the program is being executed on a computer,

- a computer program comprising program means for performing one or several of the methods according to one of the embodiments described in this description while the computer program is being executed on a computer or on a computer network,
- 5 - a computer program comprising program means according to the preceding embodiment, wherein the program means are stored on a storage medium readable to a computer,
- a storage medium, wherein a data structure is stored on the storage medium and wherein the data structure is adapted to perform one or several of the methods according to one of the embodiments described in this description after having been
10 loaded into a main and/or working storage of a computer or of a computer network, and
- a computer program product having program code means, wherein the program code means can be stored or are stored on a storage medium, for performing the method according to one of the embodiments described in this description, if the program
15 code means are executed on a computer or on a computer network.

Summarizing and without excluding further possible embodiments, the following embodiments may be envisaged:

- 20 Embodiment 1. A method for calibrating at least one analyte sensor for detecting at least one analyte in a sample, wherein the analyte sensor comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals, wherein each of the independent sensor signals is dependent on a concentration of the analyte, the method comprises the following steps:
- 25 a) measuring at least two at least partially independent sensor signals by using the measurement unit on at least one reference sample having a known analyte concentration;
- b) determining a multidimensional calibration trajectory by combining the measured at least two at least partially independent sensor signals by using
30 at least one processing unit.

- Embodiment 2. The method according to the preceding embodiment, wherein the determination of the multidimensional calibration trajectory comprises combining the full information content of the measured at least two at least partially independent sensor
35 signals.

- Embodiment 3. The method according to any one of the preceding embodiments, wherein the multidimensional calibration trajectory is at least one-dimensional.

- Embodiment 4. The method according to any one of the preceding embodiments, wherein the multidimensional calibration trajectory is a trajectory through a multidimensional signal space with concentration values varying along the trajectory given by the measured at least two at least partially independent sensor signals.
- Embodiment 5. The method according to any one of the preceding embodiments, wherein a multidimensional gradient and/or a total derivative along the multidimensional calibration trajectory is non-vanishing across a working range.
- Embodiment 6. The method according to any one of the preceding embodiments, wherein the measurement unit is configured for generating the at least two at least partially independent sensor signals by using at least a single or a plurality of identical or different diagnostic methods.
- Embodiment 7. The method according to any one of the embodiments 1 to 5, wherein the measurement unit is configured for generating the at least two at least partially independent sensor signals by performing at least two measurements using the same diagnostic methods.
- Embodiment 8. The method according to any one of the preceding embodiments, wherein the measurement unit is configured for generating the at least two at least partially independent sensor signals by using lateral flow immunoassays.
- Embodiment 9. The method according to any one of the preceding embodiments, wherein step a) comprises measuring the at least two at least partially independent sensor signals on a plurality of reference samples.
- Embodiment 10. The method according to any one of the preceding embodiments, wherein step b) comprises an inverse prediction and/or derivation of at least one analyte concentration from the measured sensor signal-concentration tuples by using at least one mathematical algorithm.
- Embodiment 11. The method according to any one of the preceding embodiments, wherein the method comprises storing the multidimensional calibration trajectory in tabulated and/or analytical functional form.

Embodiment 12. The method according to any one of the preceding embodiments, wherein the method comprises considering at least one external data source for determining the multidimensional calibration trajectory.

5 Embodiment 13. The method according to any one of the preceding method embodiments, wherein the sensor signals comprise signal curves having none or at least one ambiguous region.

10 Embodiment 14. The method according to any one of the preceding method embodiments, wherein the method is computer-implemented.

15 Embodiment 15. A method for detecting at least one analyte in a sample by using at least one analyte sensor, wherein the analyte sensor comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals, wherein each of the independent sensor signals is dependent on a concentration of the analyte, the method comprises the following steps:

- i. retrieving at least one multidimensional calibration trajectory determined by performing a method for calibrating at least one analyte sensor according to any one of the preceding embodiments;
- 20 ii. measuring at least two sensor signals by using the measurement unit on at least one measuring sample, wherein the method comprises determining a concentration corresponding to the respective sensor signals by using the multidimensional calibration trajectory, wherein the determination comprises identifying a concentration value nearest to a value of the sensor signals along the multidimensional calibration trajectory using at least one analytical or numerical algorithm.

25 Embodiment 16. The method according to the preceding embodiment, wherein the method comprises determining a measure of accuracy of the determined corresponding concentration with respect to the multidimensional calibration trajectory by using a distance metric between the value of the sensor signal from the multidimensional calibration trajectory.

30 Embodiment 17. The method according to the preceding embodiment, wherein the method comprises at least one failsafe step, wherein the failsafe step comprises comparing the measure of accuracy with at least one predefined threshold, wherein the determined analyte concentration is safeguarded in case the predefined threshold is exceeded.

Embodiment 18. The method according to any one of the two preceding embodiments, wherein the method comprises measuring a plurality of at least partially independent sensor signals by using the measurement unit on the measuring sample, wherein at least two of the measured at least partially independent sensor signals are separately used for determining an analyte concentration.

Embodiment 19. The method according to the preceding embodiment, wherein the determining of the analyte concentration comprises determining a concentration corresponding to the respective sensor signal by using the multidimensional calibration trajectory by identifying a concentration value nearest to a value of the respective sensor signal using at least one analytical or numerical algorithm.

Embodiment 20. The method according to any one of the two preceding embodiments, wherein the method comprises determining a combined analyte concentration from the determined analyte concentrations.

Embodiment 21. The method according to any one of the preceding method embodiments, wherein the method is computer-implemented.

Embodiment 22. A system comprising at least one analyte sensor for detecting at least one analyte in a sample, wherein the analyte sensor comprises the measurement unit configured for measuring at least two at least partially independent sensor signals on at least one measuring sample, wherein each of the at least partially independent sensor signals is dependent on a concentration of the analyte, wherein the system is configured for retrieving at least one multidimensional calibration trajectory determined by performing a method for calibrating at least one analyte sensor according to any one of the preceding embodiments, wherein the system comprises at least one processing unit configured for determining a concentration corresponding to at least one of the sensor signals by using the multidimensional calibration trajectory, wherein the determining comprises identifying a concentration value nearest to a value of the sensor signal using at least one analytical or numerical algorithm.

Embodiment 23. The system according to the preceding embodiment, wherein the system is configured for performing a method for detecting at least one analyte in a sample according to any one of embodiments 15 to 21.

Embodiment 24. The system according to any one of the preceding embodiments referring to a system, wherein the analyte sensor comprises lateral flow immunoassays.

Embodiment 25. The system according to any one of the preceding embodiments referring to a system, wherein the system comprises at least one database configured for storing the multidimensional calibration trajectory in tabulated and/or analytical functional form.

Embodiment 26. The system according to any one of the preceding embodiments referring to a system, wherein the system comprise at least one user interface.

Embodiment 27. A computer program comprising instructions which, when the program is executed by the system according to any one of the preceding embodiments referring to a system, cause the system to perform a method for calibrating at least one analyte sensor according to any one of embodiments 1 to 14 and/or a method for detecting at least one analyte in a sample according to any one of embodiments 15 to 21.

Embodiment 28. A computer-readable storage medium comprising instructions which, when the instructions are executed by the system according to any one of the preceding embodiments referring to a system, cause the system to perform a method for calibrating at least one analyte sensor according to any one of embodiments 1 to 14 and/or a method for detecting at least one analyte in a sample according to any one of embodiments 15 to 21.

Embodiment 29. A non-transient computer-readable medium including instructions that, when executed by one or more processors, cause the one or more processors to perform a method for calibrating at least one analyte sensor according to any one of embodiments 1 to 14 and/or a method for detecting at least one analyte in a sample according to any one of embodiments 15 to 21.

Short description of the Figures

Further optional features and embodiments will be disclosed in more detail in the subsequent description of embodiments, preferably in conjunction with the dependent claims. Therein, the respective optional features may be realized in an isolated fashion as well as in any arbitrary feasible combination, as the skilled person will realize. The scope of the invention is not restricted by the preferred embodiments. The embodiments are schematically depicted in the Figures. Therein, identical reference numbers in these Figures refer to identical or functionally comparable elements.

In the Figures:

- Figure 1 shows an embodiment of a method for calibrating at least one analyte sensor for detecting at least one analyte in a sample;
- Figure 2 shows an embodiment of a method for detecting at least one analyte in a sample by using at least one analyte sensor; and
- Figures 3A to 3D shows a schematic representation of determining a multidimensional calibration trajectory and 3D representation of the multidimensional calibration trajectory.
- Figure 4 shows a schematic representation of multidimensional calibration trajectory consisting of three signals, where none of the axes is representing the concentration.

Detailed description of the embodiments

Figure 1 shows an exemplary embodiment of a method for calibrating at least one analyte sensor for detecting at least one analyte in a sample. The analyte sensor, not depicted here, comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals. Each of the independent sensor signals is dependent on a concentration of the analyte.

The method comprises the following steps that may be performed in the given order. However, a different order may also be possible. For example, one, more than one or even all of the method steps may be performed once or repeatedly. Further, the method steps may be performed successively or, alternatively, one or more of the method steps may be performed in a timely over-lapping fashion or even in a parallel fashion and/or in a combined fashion. The method may further comprise additional method steps that are not listed.

The method comprises the following steps:

- a) (110) measuring at least two at least partially independent sensor signals (112, 114) by using the measurement unit on at least one reference sample having a known analyte concentration;

- b) (116) determining a multidimensional calibration trajectory (118) by combining the measured at least two at least partially independent sensor signals (112, 114) by using at least one processing unit.

5 The analyte, as an example, may be selected from the group consisting of glucose, cholesterol, triglycerides, lactate, vitamin D, drugs of abuse, therapeutic drugs, hormones, cardiac markers and metabolites in general. Additionally or alternatively, however, other types of analytes may be determined and/or any combination of analytes may be determined. The sample may be an arbitrary sample such as a biological sample. The sample may be a liquid
10 sample. The sample may be an aqueous sample. The sample may comprise or may be suspected to comprise at least one analyte. The sample may be a sample of bodily fluid. The sample may be selected from the group consisting of: a physiological fluid, including whole blood, serum, plasma, saliva, ocular lens fluid, lacrimal fluid, cerebrospinal fluid, sweat, interstitial fluid, urine, saliva, milk, ascites, mucus, synovial fluid, peritoneal fluid, and amniotic fluid; lavage fluid; tissue, cells, or the like.
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The analyte sensor may be configured for detecting quantitatively or qualitative at least one analyte. In an embodiment, determining is qualitative, semiquantitative, or quantitative determination. Qualitative determination may relate to determining whether the value, e.g. of
20 a parameter, is above a predetermined threshold value, e.g. a detection limit or a physiologically relevant threshold value. Semiquantitative determination may be assigning a measured value to a pre-established category, e.g. "low", "medium", or "high" concentration. In an embodiment, determining is quantitative, i.e. is determining a value of a quantitative measure of a parameter. The measurement unit may be configured for generating at least two at
25 least partially independent sensor signals 112, 114. For example, the analyte sensor may comprise two or more lateral flow immunoassays. The analyte sensor may comprise multi-line assays having at least two test lines. The test line may comprise at least one test chemical for detecting the analyte. The test lines may have identical or different test chemicals comprised therein.

30 For example, the analyte sensor may comprise at least one test strip. The test strip may comprise at a plurality of test lines. Each of the test lines comprise at least one component, which changes at least one detectable property when the analyte is present in the sample, e.g. an optical detectable property such as a color change. The test strip may be suited for an ex-vivo measurement, specifically an in-vitro measurement.
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For example, the measurement unit may be configured for an optical detection. The optical detection may comprise a detection of a reaction using an optical test chemical, such as a

color change test chemical which changes in color in the presence of the analyte. The color change specifically may depend on the amount of analyte present in the sample. Techniques for determining the analyte by optical detection and e.g. analyzing color of the spot on the test filed are generally known to the skilled person.

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For example, the measurement unit may be configured for an electrochemical detection. The analyte sensor may be or may comprise at least one electrochemical sensor. The electrochemical sensor may comprise at least one enzyme configured for performing at least one redox reaction in the presence of the analyte to be detected, wherein the redox reaction may be detected by electrical means. For example, the electrochemical detection reaction may be detected by comparing one or more electrode potentials, such as a potential of a working electrode with the potential of one or more further electrodes such as a counter electrode or a reference electrode.

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The sensor signal 112, 114, e.g. as shown in Figures 3A and 3B, may comprise a signal curve. The signal curve may refer to a dependence of a detector response changing with a varying stimulus, e.g. under control of the experimenter. The signal curve may be one or more of concentration vs signal amplitude or phase, angle, time lag, or otherwise appropriately derived or transformed values. The signal curve may comprise a hooked (peaked) and/or saturated (asymptotic) curve. For example, the signal curve may comprise at least one ambiguous region, e.g. depicted with circles in Figures 3A and 3B. The ambiguous region may comprise signal values occurring several times („Hook-peak“) at different analyte concentrations. The ambiguous region may exhibit ambiguities due to changes in signal height which are that low such that these cannot be distinguished within the scope of the measurement accuracy. The proposed method may allow for using even hooked and/or saturated signal curves and, thus, distinguishes from techniques trying to establish one-dimensional univariate calibration curves from combinations of partial (piecewise) sensor signals.

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The at least partially independent sensor signals 112, 114 may be independent in at least one dimension of a multidimensional signal space, wherein each of the sensor signals 112, 114 represents an axis of the multidimensional signal space 120, see Figure 3C. None of the axis of the multidimensional signal space 120 may represent the values of the analyte concentrations directly.

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Step a) 110 may comprise measuring the at least two at least partially independent sensor signals 112, 114 on a plurality of reference samples. The plurality of reference samples may be a limited discrete set of dedicated prepared samples having predefined and known analyte concentrations.

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The at least two at least partially independent sensor signals may be generated by using at least a single or a plurality of identical or different diagnostic methods. For example, the measurement unit may be configured for generating the at least two at least partially independent sensor signals 223, 224 by using at least two different diagnostic methods, e.g. by using at least one optical detection and at least one electrochemical detection.

For example, the measurement unit may be configured for generating the at least two at least partially independent sensor signals 112, 114 by performing at least two measurements using the same diagnostic methods.

For example, the measurement unit may be configured for generating the at least two at least partially independent sensor signals 112, 114 by using lateral flow immunoassays, e.g. at least two successively arranged lateral flow immunoassays or an assay with at least two test lines.

The multidimensional calibration trajectory 118 may be a trajectory within the multidimensional signal space 120 which can be used for transforming one or more measured sensor signals 112, 114 into one or more concentration values.

The combination of the measured at least two at least partially independent sensor signals 112, 114 is shown in Figures 3A to 3C. In Figure 3D, a 3D representation of the multidimensional calibration trajectory 118 is depicted. Each sensor signal 112, 114 may define a single dimension of the multidimensional signal space 120. For example, as shown in Figures 3, in case of two sensor signals 112, 114, the multidimensional signal space 120 may have two dimensions, one from each sensor signal. The determination of the multidimensional calibration trajectory 118 may comprise combining the full information content of the measured at least two at least partially independent sensor signals 112, 114. The combining may comprise combining information contents of the separate sensor signals 112, 114 in such a way that the resulting multidimensional calibration trajectory 118 across this multidimensional signal space 120 directly carries the information about the analyte concentrations via the position along the multidimensional calibration trajectory 118 along with a non-vanishing multidimensional gradient. Thus, no information contained in the separate multi-line calibration curves is lost or unused and the algorithmic preparation of the final multidimensional calibration trajectory can easily be done in a fully automatic manner. This com-

bination may allow lifting the degeneration between the separate one-dimensional dependencies of signals and concentrations, allowing the direct usage of saturated and/or hooked calibration curves even close to their respective saturated or hook regions.

5 The multidimensional calibration trajectory 118 may have at least two dimensions. The multidimensional calibration trajectory 118 may be a trajectory through the multidimensional signal space 120 with concentration values varying along the trajectory given by the measured at least two at least partially independent sensor signals 112, 114. In addition to the concentration varying systematically along the trajectory, the
10 concentration “conc.” can be represented as an additional dimensional axis, e.g. as shown in Figure 3D. However, such an additional concentration axis is not necessary as it (the additional axis) carries only redundant information about the concentration. For example, for two sensor signals, the additional concentration axis can be used to visualize the concentration. In case of using more sensor signals, a visualization of the
15 concentration may be done by projecting the trajectory to a (possibly several) at most three-dimensional representation(s). For example, in case the analyte sensor comprising a two-line LFIA, the multidimensional calibration trajectory may be a trajectory in a two-dimensional plane. For example, in case the analyte sensor comprising a three-line LFIA, the multidimensional calibration trajectory may be a trajectory in a three-
20 dimensional signal space, as shown in Figure 4, with independent sensor signals 112, 113, 114. Multidimensional calibration trajectories with more than three signals have no immediate visual or graphical representation, but can mathematically be handled in a way similar to multidimensional calibration trajectories in two- and three-dimensional signal spaces.

25 A multidimensional gradient and/or a total derivative along the multidimensional calibration trajectory 118 may be non-vanishing across a working range. As long as not all sensor signals show constant values at the same time for a given concentration range, the embedding of the individual signals in a higher-dimensional signal space may result in a non-vanishing
30 multi-dimensional gradient along the full multidimensional calibration trajectory 118. However, even in case the prerequisite of a non-vanishing gradient and/or total derivative everywhere along the whole trajectory is not fulfilled, the method may allow a proper calibration over all regions with non-vanishing gradient and may consider the regions of vanishing gradient and/or total derivative as having considerably larger predicted concentration uncertainties.
35 ties.

For known analyte sensors, as described above, a working range and the full measurement range of the analyte sensor may differ due to non-usage of saturated and/or hooked regions.

The method according to the present invention may allow for extending the working range of the at least one analyte sensor for detecting the at least one analyte in the sample to the full measurement range. The proposed calibration can rely on hooked (peaked) and/or saturated (asymptotic) individual sensor signal curves, where an invertible calibration trajectory
5 can still be established although some/all sensor signals might show such a behavior as long as the prerequisite that the multidimensional gradient and/or the total derivative along the multidimensional calibration trajectory 118 is non-vanishing is fulfilled.

Step b) 116 may comprise an inverse prediction and/or derivation of at least one analyte
10 concentration from the measured sensor signal-concentration tuples by using at least one mathematical algorithm such as polynomial, spline, or kernel methods. Data used for calibration is usually measured as a limited discrete set of dedicated prepared samples having predefined and known concentrations, i.e. the reference samples. Using only this set would be insufficient for all situations where concentrations have to be derived from measured
15 signals by inverse prediction. To cover the full concentration range in a dense way, the signal-concentration tuples have to be interpolated. This can be done either by individually interpolating the separate signals as a function of concentration and combining the resulting discrete curves in the multi-dimensional signal space 120, or else the set of measured signal-concentration tuples may directly interpolated in multi-dimensional signal space. In each
20 case the concentration may be parametrically varying along the multi-dimensional trajectory 118.

The method, e.g. in step b) 116, may comprise storing the multidimensional calibration trajectory 118 in tabulated and/or analytical functional form, e.g. available for subsequent use
25 in tabulated or analytical functional form depending on the interpolation algorithm used.

Figure 2 shows an embodiment of a method for detecting at least one analyte in a sample by using at least one analyte sensor.

30 The analyte sensor comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals. Each of the independent sensor signals is dependent on a concentration of the analyte. The method comprises the following steps that may be performed in the given order. However, a different order may also be possible. In particular, one, more than one or even all of the method steps may be performed once or
35 repeatedly. Further, the method steps may be performed successively or, alternatively, one or more of the method steps may be performed in a timely overlapping fashion or even in a parallel fashion and/or in a combined fashion. The method may further comprise additional method steps that are not listed.

The method comprises the following steps:

- i. (122) retrieving at least one multidimensional calibration trajectory 118 determined by performing a method for calibrating at least one analyte sensor according to the present invention;
- ii. (124) measuring at least two sensor signals by using the measurement unit on at least one measuring sample, wherein the method comprises determining a concentration corresponding to the respective sensor signals by using the multidimensional calibration trajectory 118, wherein the determination comprises identifying a concentration value nearest to a value of the sensor signals along the multidimensional calibration trajectory using at least one analytical or numerical algorithm.

A concentration measurement with an analyte sensor would deliver multiple, e.g. two, sensor signals, which in turn can be viewed as a single multi-dimensional point in the multidimensional signal space 120 initially generated by the calibration method. Concentrations can be read off from the multidimensional calibration trajectory 118 by finding the concentration value nearest to the new signal point. The concentration value nearest to the new signal point may be the concentration value having the smallest orthogonal distance as measured along the straight line perpendicular to the multidimensional calibration trajectory 118 or any other suitable distance metric. Since it cannot be expected that calibration trajectories in general can be described by closed-form analytical mathematical models, the determination of the associated concentration may use possibly iterative numerical algorithms. However, in case of sufficiently simple individual sensor signal curves (e.g. straight lines of constant slope) the multidimensional calibration trajectory will also be simple (a straight line), which means that in this particular case the whole calibration procedure can in principle make use of analytical formulae and algorithms (instead of numerical computations).

The method may comprise determining a measure of accuracy of the determined corresponding concentration with respect to the multidimensional calibration trajectory 118 by using an orthogonal or other distance metric between the value of the sensor signal from the multidimensional calibration trajectory 118. Thus, the distance can be used as a measure of accuracy of the resulting concentration with respect to the pre-established multidimensional calibration curve. A large distance may indicate that the multi-dimensional signal point is far from and not well represented by the multidimensional calibration trajectory 118, which might indicate an associated large uncertainty of the derived concentration. The method may comprise at least one failsafe step. The failsafe step may comprise comparing the measure of accuracy with at least one predefined threshold. The determined analyte concentration

may be safeguarded in case the predefined threshold is exceeded. For example, the predefined threshold may be a threshold range of e.g. $\pm 10\%$, or any other value suitable for the calibration problem at hand. Threshold values may come, among others, from regulatory requirements or limits of empirically determined distributions suitably characterizing the calibration task. For example, the analyte concentration may be safeguarded in case a measured sensor signal appears more distant from the pre-established multidimensional calibration trajectory than a predefined secure failsafe-threshold value. For example, the measurement may be flagged as unreliable and/or may be discarded. In case of repeated determined failures of measurements of the same sample, it can also be used as an indicator that an incorrect sample type has possibly been offered to the analyte sensor, thereby acting as a first-level classifier of allowed or disallowed sample types.

Thus, besides allowing making use of saturated and/or hooked separate calibration curves over the full measurable signal range of an LFIA, the proposed methods may have other unique advantages currently not available in other standard univariate calibration procedure. These are a built-in way to gain additional information about the accuracy of derived concentrations and, further, a safeguard property, indicating whether a given measurement likely has signal values incompatible with the previously established multidimensional calibration trajectory and assay performance.

The method may comprise measuring a plurality of at least partially independent sensor signals by using the measurement unit on the measuring sample. At least two of the measured at least partially independent sensor signals may separately used for determining an analyte concentration. Each of the sensor signals can be separately used to predict an analyte concentration from the crossing of the signals' hyperplane with the multidimensional calibration trajectory 118. The distribution of these single-signal-derived concentrations, e.g. in conjunction with the concentration value determined using the distance as described above, can be used a measure of concentration accuracy.

The determining of the analyte concentration may comprise determining a concentration corresponding to the respective sensor signal by using the multidimensional calibration trajectory 118 by identifying a concentration value nearest to a value of the respective sensor signal using the at least one analytical and/or numerical algorithm, e.g. as described above.

The method may comprise determining a combined analyte concentration from the determined analyte concentrations. The determining of the combined analyte concentration may comprise one or more of determining a mean value, a median value and the like.

The method may comprise outputting the analyte concentration and/or the combined analyte concentration. As an example, the outputting may take place via one or more interfaces, such as a computer interface, a web interface or a human-machine interface. The outputting, as an example, may take place in one or more of a computer-readable format, a visible format
5 or an audible format.

List of reference numbers

110	step a)
112	at least partially independent sensor signal
113	at least partially independent sensor signal
114	at least partially independent sensor signal
116	step b)
118	multidimensional calibration trajectory
120	multidimensional signal space
122	step i.
124	step ii.

Claims

1. A method for calibrating at least one analyte sensor for detecting at least one analyte
5 in a sample, wherein the analyte sensor comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals, wherein each of the independent sensor signals is dependent on a concentration of the analyte, the method comprises the following steps:
 - a) (110) measuring at least two at least partially independent sensor signals
10 (112, 114) by using the measurement unit on a plurality of reference sample having a known analyte concentration;
 - b) (116) determining a multidimensional calibration trajectory (118) by combining the measured at least two at least partially independent sensor signals (112, 114) by using at least one processing unit.
- 15 2. The method according to the preceding claim, wherein the determination of the multidimensional calibration trajectory (118) comprises combining the full information content of the measured at least two at least partially independent sensor signals (112, 114).
- 20 3. The method according to any one of the preceding claims, wherein the multidimensional calibration trajectory (118) is at least one dimensional.
4. The method according to any one of the preceding claims, wherein the multidimensional calibration trajectory (118) is a trajectory through a multidimensional signal
25 space with concentration values varying along the trajectory (118) given by the measured at least two at least partially independent sensor signals.
5. The method according to any one of the preceding claims, wherein a multidimensional
30 gradient and/or a total derivative along the multidimensional calibration trajectory (118) is non-vanishing across a working range.

6. The method according to any one of the preceding claims, wherein the measurement unit is configured for generating the at least two at least partially independent sensor signals (112, 114) by using at least a single or a plurality of identical or different diagnostic methods.
7. The method according to any one of the preceding claims, wherein the measurement unit is configured for generating the at least two at least partially independent sensor signals (112, 114) by using lateral flow immunoassays.
8. The method according to any one of the preceding claims, wherein step b) comprises an inverse prediction and/or derivation of at least one analyte concentration from the measured sensor signal-concentration tuples by using at least one mathematical algorithm.
9. A method for detecting at least one analyte in a sample by using at least one analyte sensor, wherein the analyte sensor comprises at least one measurement unit configured for generating at least two at least partially independent sensor signals, wherein each of the independent sensor signals is dependent on a concentration of the analyte, the method comprises the following steps:
 - i. (122) retrieving at least one multidimensional calibration trajectory (118) determined by performing a method for calibrating at least one analyte sensor according to any one of the preceding claims;
 - ii. (124) measuring at least two sensor signals by using the measurement unit on at least one measuring sample, wherein the method comprises determining a concentration corresponding to the respective sensor signals by using the multidimensional calibration trajectory (118), wherein the determination comprises identifying a concentration value nearest to a value of the sensor signals along the multidimensional calibration trajectory (118) using at least one analytical or numerical algorithm.
10. The method according to the preceding claim, wherein the method comprises determining a measure of accuracy of the determined corresponding concentration with respect to the multidimensional calibration trajectory (118) by using a distance metric between the value of the sensor signal from the multidimensional calibration trajectory (118), wherein the method comprises at least one failsafe step, wherein the failsafe step comprises comparing the measure of accuracy with at least one predefined threshold, wherein the determined analyte concentration is safeguarded in case the predefined threshold is exceeded.

11. The method according to the preceding claim, wherein the method comprises measuring a plurality of at least partially independent sensor signals by using the measurement unit on the measuring sample, wherein at least two of the measured at least partially independent sensor signals are separately used for determining an analyte concentration, wherein the determining of the analyte concentration comprises determining a concentration corresponding to the respective sensor signal by using the multidimensional calibration trajectory (118) by identifying a concentration value nearest to a value of the respective sensor signal using at least one analytical or numerical algorithm.
12. The method according to any one of the two preceding claims, wherein the method comprises determining a combined analyte concentration from the determined analyte concentrations.
13. A system comprising at least one analyte sensor for detecting at least one analyte in a sample, wherein the analyte sensor comprises the measurement unit configured for measuring at least two at least partially independent sensor signals (112, 114) on at least one measuring sample, wherein each of the at least partially independent sensor signals (112, 114) is dependent on a concentration of the analyte, wherein the system is configured for retrieving at least one multidimensional calibration trajectory (118) determined by performing a method for calibrating at least one analyte sensor according to any one of the preceding claims, wherein the system comprises at least one processing unit configured for determining a concentration corresponding to at least one of the sensor signals by using the multidimensional calibration trajectory (118), wherein the determining comprises identifying a concentration value nearest to a value of the sensor signal using at least one analytical or numerical algorithm.
14. A computer program comprising instructions which, when the program is executed by the system according to any one of the preceding claims referring to a system, cause the system to perform a method for calibrating at least one analyte sensor according to any one of claims 1 to 8 and/or a method for detecting at least one analyte in a sample according to any one of claims 9 to 12.
15. A non-transient computer-readable medium including instructions that, when executed by one or more processors, cause the one or more processors to perform a method for calibrating at least one analyte sensor according to any one of claims 1 to 8 and/or a

method for detecting at least one analyte in a sample according to any one of claims 9 to 12.