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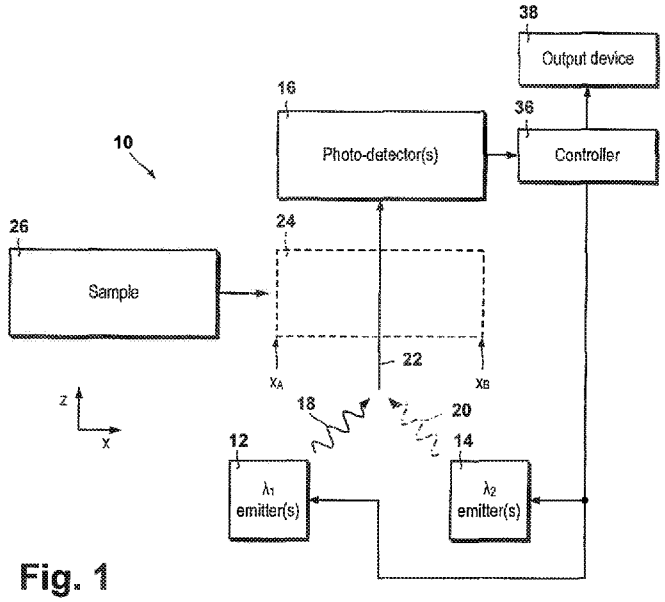

Fig. 1

(57) Abstract: The present invention provides an apparatus comprising: at least one set of emitters, the or each set of emitters comprising a first sub-set of one or more light emitters configured to emit light within a range around a first wavelength and a second sub-set of one or more light emitters configured to emit light within a range around a second wavelength, wherein said first and second sub-sets of one or more light emitters are configured to be simultaneously illuminable, and further wherein: the first sub-set of one or more light emitters is deluminable from an illuminated state over a first period; and the second sub-set of one or more light emitters is deluminable from an illuminated state over a second period. The apparatus further comprises one or more photodetectors arranged such that light from the or each set of emitters reaches the photodetectors via an optical path comprising a sample receiving portion; and a liquid transport path comprising a first end, a second end and a liquid sample receiving region, the liquid transport path configured to transport a liquid sample received in the liquid sample receiving region towards the second end and through the sample receiving portion of the optical path.

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APPARATUS

FIELD

The present invention relates to an apparatus for analytical testing and particularly, but not exclusively, to an optical biosensor.

BACKGROUND

Biological testing for the presence and/or concentration of an analyte may be conducted for a variety of reasons including, amongst other applications, preliminary diagnosis, screening samples for presence of controlled substances and management of long-term health conditions.

Lateral flow devices (also known as "lateral flow immunoassays") are one variety of biological testing. Lateral flow devices may be used to test a liquid sample, such as saliva, blood or urine, for the presence of an analyte. Examples of lateral flow devices include home pregnancy tests, home ovulation tests, tests for other hormones, tests for specific pathogens and tests for specific drugs. For example, EP 0 291194 Al describes a lateral flow device for performing a pregnancy test.

In a typical lateral flow testing strip, a liquid sample is introduced at one end of a porous strip, which is then drawn along the strip by capillary action (or "wicking"). A portion of the lateral flow strip is pre-treated with labelling particles which are activated with a reagent which binds to the analyte to form a complex, if the analyte is present in the sample. The bound complexes and also unreacted labelling particles continue to propagate along the strip before reaching a testing region which is pre-treated with an immobilised binding reagent which binds bound complexes of analyte and labelling particles and does not bind unreacted labelling particles. The labelling particles have a distinctive colour, or other detectable optical or non-optical property, and the development of a concentration of labelling particles in the test regions provides an observable indication that the analyte has been detected. Lateral flow test strips may be based on, for example, colorimetric labelling using gold or latex nanoparticles, fluorescent marker molecules or magnetic labelling particles.

Another variety of biological testing involves assays conducted in liquids held in a container such as a vial, a PCR well/plate, a cuvette or a microfluidic cell. Liquid assays may be measured based on colorimetry or fluorescence. Some liquid-based assays may allow tests to be conducted using very small (e.g. picolitre) volumes. Sometimes, merely determining the

presence or absence of an analyte is desired, i.e. a qualitative test. In other applications, an accurate concentration of the analyte may be desired, i.e. a quantitative test. For example, WO 2008/101732 A1 describes an optical measuring instrument and measuring device. The optical measuring instrument includes at least one source for providing at least one electromagnetic beam to irradiate a sample and to interact with the specimen within the sample, at least one sensor for detecting an output of the interaction between the specimen and the electromagnetic beam, an integrally formed mechanical bench for the optical and electronic components and a sample holder for holding the sample. The at least one source, the at least one sensor, and the mechanical bench are integrated in one monolithic optoelectronic module and the sample holder can be connected to this module.

Another optical measurement system involves irradiating a sample with light at a wavelength around a first wavelength and, at a different time, irradiating the sample with light at a wavelength around a second wavelength. A measured intensity of light received at a photodetector through the sample (i.e. transmission) or reflected from the sample (i.e. reflection) at one of the first or second wavelengths is subtracted from a measured intensity of light received at the photodetector at the other of the first or second wavelengths. This subtraction step allows for removal of background variations in absorbance (due to variations in absorbance of a substrate for holding the sample).

Quantitative detectors for biological testing methods may require optical components such as beamsplitters, lenses, monochromators, filters and so forth. Such components may be complex, expensive and/or bulky, and may have properties which vary considerably with the wavelength of light. Optical components such as beamsplitters, lenses, monochromators, filters and so forth are typically too bulky for integration into a single use, self-contained lateral flow immunoassay test or a self-contained microfluidic assay test.

Biological samples that may contain an analyte of interest may be coloured, for example blood or urine. Conventionally, coloured samples have been treated by filtering out the coloured dye (e.g. filtering full red blood to obtain clear serum) or by introducing a washing/flushing step.

SUMMARY

According to an aspect of the present invention, there is provided an apparatus comprising: at least one set of emitters, the or each set of emitters comprising a first sub-set of one or more light emitters configured to emit light within a range around a first wavelength and a second sub-set of one or more light emitters configured to emit light within a range

around a second wavelength, wherein said first and second sub-sets of one or more light emitters are configured to be simultaneously illuminable, and further wherein:

the first sub-set of one or more light emitters is deluminable from an illuminated state over a first period; and

the second sub-set of one or more light emitters is deluminable from an illuminated state over a second period;

one or more photodetectors arranged such that light from the or each set of emitters reaches the photodetectors via an optical path comprising a sample receiving portion; and

a liquid transport path comprising a first end, a second end and a liquid sample receiving region, the liquid transport path configured to transport a liquid sample received in the liquid sample receiving region towards the second end and through the sample receiving portion of the optical path.

The ability to simultaneously illuminate the emitters and deluminate them at different rates (i.e. independently) means that a time-gated contrast can be achieved between light at the two wavelengths. In other words, the colour of the illuminating light may vary over time following initiation of delumination of the light emitters. This may mean that no filters would be required on either the light emitter side, or the photodetector side, so any impact of misalignment of filters may be avoided, e.g. cross-talk.

The ability to deluminate the first light emitter(s) over a first period and the second light emitter(s) over a second period means that an intensity of light transmitted through the sample, or reflected from the sample may be measured at different times so as to achieve the contrast between light at the two wavelengths. For example, the intensity could be measured when both first and second light emitter(s) are illuminated and measured again when only one of the first and second light emitter(s) is illuminated (i.e. following delumination of the other at the end of the corresponding period).

Thus, the apparatus may include a simplified optical path, which does not require optical components such as filters or monochromators to perform dual wavelength measurements. Thus, the apparatus may be less bulky and simpler to manufacture.

Optionally, first and second sub-sets of one or more light emitters may be deluminable responsive to a change in state of an illumination control signal. Further optionally, the first sub-set of one or more light emitters may deluminate over a period of around 50 nanoseconds. Yet further optionally, the second sub-set of one or more light emitters may deluminate over a period of around 5 microseconds.

Optionally, the illumination control signal oscillates at a frequency:

$$\frac{1}{second\ period} < f \ll \frac{1}{first\ period}.$$

Optionally, the frequency may be between around 100kHz and around 20MHz. Further optionally, the frequency may be between around 150kHz and around 10MHz. Yet further optionally, the frequency may be between around 175kHz and around 5Mhz. Still further optionally, the frequency may be around 200kHz.

Optionally, the second period may differ from the first period by a factor of two or more. Further optionally, the first period may be a function of a first decay rate, which may be a characteristic of a luminescent material of the first sub-set of one or more light emitters. Yet further optionally, the second period may be a function of a second decay rate, which may be a characteristic of a luminescent material of the second sub-set of one or more light emitters. The luminescent material of the first sub-set of one or more light emitters may comprise a fluorescent material. The luminescent material of the second sub-set of one or more light emitters may comprise a thermally activated delayed fluorescence material, or a phosphorescent.

Optionally, the apparatus may further comprise a controller configured to: simultaneously illuminate each sub-set of emitters and to obtain a measured absorbance value using the one or more photodetectors at: a first time prior to the first and second periods commencing; and a second time between expiry of said first period and expiry of the second period; generate an absorbance vector using the measured absorbance values; and determine a concentration vector by multiplying the absorbance vector with a de-convolution matrix.

In other words, the controller may be configured to illuminate the first and second sub-sets of light emitters and obtain a first set of measurements using the photodetectors, initiate delumination of both the first and second sub-sets of light emitters, but obtain a second set of measurements using the photodetectors when one of the sub-sets of light emitters has deluminated but before the other sub-set has deluminated, and to subtract the second set of measurements from the first set of measurements.

Optionally, the controller may be configured to multiply the second set of measurements by a weighting factor before subtracting the second set of measurements from the first set of measurements.

Absorbance measurements obtained using the at least one set of emitters may be deconvoluted (de-mixed) to quantify the concentration of one or more analytes whilst also compensating for optical scattering due to defects or other inhomogeneities of a sample.

The controller may be configured to subtract a signal obtained at a reference wavelength, for example the second wavelength, from a signal obtained when the sample is illuminated with light at both the first and second wavelengths, in order to compensate for optical scattering due to defects or other inhomogeneities in a medium or on a substrate holding the sample.

Thus, using first and second separate, simultaneously illuminable and independent illuminable emitters, absorbance measurements may be corrected using measurements at a reference wavelength. Thus, the apparatus may provide improved signal-to-noise ratio for measurements of one or more analytes.

Optionally, the optical path may be configured such that the one or more photodetectors receive light transmitted through the sample receiving portion of the optical path.

Optionally, the optical path may be configured such that the one or more photodetectors receive light reflected from the sample receiving portion of the optical path.

Optionally, the one or more photodetectors may form an image sensor arranged to image all or a portion of the sample receiving portion of the optical path.

Optionally, the optical path may include a slit arranged on the optical path before the sample receiving portion and the/or each sub-set of emitters may be arranged to illuminate the slit.

Each sub-set of one or more first light emitters and each sub-set of one or more second light emitters may have a cylindrically symmetric angular emission profile, and each pair of first and second sub-sets of light emitters may be arranged such that the slit perpendicularly bisects the pair.

Thus, equal normalised spatial intensity profiles of light at the first and second wavelengths may be provided at the sample receiving portion using a particularly simple and compact arrangement of first and second emitters.

A diffuser may be included between each sub-set of emitters and the slit. The slit may have adjustable width. The slit may have a width between 100 μm and 1 mm inclusive. The slit may have a width between 300 μm and 500 μm inclusive. The light emitters belonging to each sub-set may have Gaussian angular emission profiles.

Optionally, the or each light emitter of the second sub-set may be substantially transparent at the wavelengths emitted by the or each light emitter of the first sub-set and wherein the or each light emitter of the first sub-set may emit light into the optical path through a corresponding light emitter of the second sub-set of one or more light emitters.

Thus, the optical path may be a gap between a second emitter and a photodetector. In this way, optical components such as beamsplitters, lenses, filters, monochromators, diffusers or the like may be omitted.

Thus, the two first and second sub-sets of light emitters may precisely illuminate the same region of the sample (because one overlies the other). This may improve a background subtraction calculation compared with a previous two-wavelength illumination apparatus, because, in the previous apparatus (where light emitters are side-by-side) the light emitters illuminate mostly the same region, but because of their lateral spacing would not necessarily be able to illuminate exactly the same region. Having one light emitter overlying the other may improve the signal-to-noise by providing a more accurate background subtraction (because exactly the same region of the sample may be illuminated in the overlying arrangement).

Optionally, the at least one set of emitters may also include a third sub-set of one or more light emitters configured to emit within a range around a third wavelength. Further optionally, the at least one set of emitters may include a fourth sub-set of one or more light emitters configured to emit within a range around a fourth wavelength.

Optionally, the or each light emitter of the second sub-set may be substantially transparent at the wavelengths emitted by the or each light emitter of the first sub-set and by the or each light emitter of the third sub-set, and wherein the or each light emitter of the first sub-set and the or each light emitter of the third sub-set may emit light into the optical path through a corresponding light emitter of the second sub-set of one or more light emitters.

Transparency at the wavelengths emitted by each other sub-set of emitters may correspond to a transmittance of more than 50%, more than 75%, more than 85%, more than 90% or more than 95%. Transparency at the first wavelength may correspond to a transmittance of more than 50%, more than 75%, more than 85%, more than 90% or more than 95%.

The at least one set of emitters may be arranged into an array including a plurality of pixels. Each pixel may include at least two sub-pixels, with a first set of sub-pixels comprising a light emitter corresponding to the first sub-set and a second set of sub-pixels comprising a light emitter corresponding to the second sub-set.

A plurality of light emitters of the first sub-set and a plurality of light emitters of the second sub-set may be arranged into an array, wherein the light emitters of the first and second sub-sets may alternate in a chessboard configuration.

Thus, the optical path may be a gap between an array of light emitters and a photodetector. In this way, optical components such as beamsplitters, lenses, filters, monochromators or the like may be omitted.

Two, three or four sub-sets of emitters may be interdigitated with one another to form an array.

Optionally, the liquid transport path may take the form of a porous medium. The porous medium may include nitrocellulose or other fibrous materials capable of transporting an aqueous liquid by capillary action, whether inherently or following appropriate surface treatments. The liquid transport path may include at least one microfluidic channel. The microfluidic channel may form a part of a microfluidic device.

Optionally, the liquid transport path may take the form of a lateral flow type strip. Further optionally, the liquid transport path may take the form of the whole, a part, or at least one channel of a microfluidic device.

Optionally, the apparatus may also include at least one output device. The at least one output device may take the form of one or more light emitting diodes, and the controller may be configured to illuminate each light emitting diode in response to a corresponding value of the concentration vector exceeding a predetermined threshold.

Optionally, the at least one output device may take the form of a display element, and the controller may be configured to cause the display element to display one or more outputs in response to determining the concentration vector. Further optionally, the controller may be configured, in response to a value of the concentration vector exceeding a predetermined threshold, to cause the display element to display a corresponding symbol or symbols. The controller may be configured to cause the display element to display one or more values of the concentration vector.

Optionally, the at least one output device may take the form of a wired or wireless communications interface for connection to a data processing apparatus, and the controller may be configured to output the concentration vector to the data processing apparatus via the wired or wireless communications interface.

Optionally, the controller may be configured to normalise absorbance values with respect to a reference calibration absorbance value.

Optionally, light within a range around the first wavelength is strongly absorbed by a tag used in the sample and the light within a range around the second wavelength is not absorbed, or is weakly absorbed, by a tag used in the sample.

Optionally, the wavelength corresponding to each sub-set of emitters may correspond to a peak emission wavelength of the emitters. Each sub-set of emitters may emit light within a range having a full-width at half maximum of no more than 10 nm, no more than 25 nm, no more than 50 nm, no more than 100 nm or no more than 200 nm.

Optionally, the optical path may include no monochromators. The optical path may include no beamsplitters between the sample receiving portion and the photodetector(s). The optical path may include no fibre couplers and/or fibre splitters between the sample receiving portion and the photodetectors.

Optionally, the first and second sub-sets of light emitters and one or more photodetectors may be configured such that, at the sample receiving portion of the optical path, a normalised spatial intensity profile generated by each sub-set of emitters is substantially equal to a normalised spatial intensity profile generated by each other sub-set of emitters. Further optionally, normalised spatial intensity profiles may be substantially equal at an entrance to, an exit from, or on any plane perpendicular to the optical path.

Normalised spatial intensity profiles may be considered to be substantially equal on a plane perpendicular to the path if the normalised intensity values for the first and second wavelengths are within 5%, within 10%, within 15% or within 20% of one other at each point on that plane. Normalised spatial intensity profiles may be considered to be substantially equal on a plane perpendicular to the path if the normalised intensity values for the first and second wavelengths differ, at each point on that plane, by less than two times, less than three times or less than five times the standard error of normalised intensities at the first wavelength or the second wavelength, whichever has the larger standard error.

The wavelengths corresponding to each sub-set of light emitters may be selected in dependence upon the absorbance spectrum of one or more target analytes. The wavelength corresponding to each sub-set of light emitters may be selected such that a target analyte has relatively higher absorbance at said wavelength than at a wavelength corresponding to each other sub-set of light emitters. A target analyte may be any suitable labelling molecule or particles such as, for example, gold nanoparticles.

The first and second wavelengths may be selected in dependence upon the absorbance spectrum of a target analyte. The first and second wavelengths may be selected such that a target analyte has relatively higher absorbance at the first wavelength than at the second wavelength. The ratio of target analyte absorbance at the first and second wavelengths may be at least two, up to and including five, up to and including ten or more than ten. A target

analyte may be any suitable labelling molecule or particles such as, for example, gold nanoparticles.

The wavelengths corresponding to each sub-set of emitters may lie in the range between 300 nm and 1500 nm inclusive. The wavelengths corresponding to each sub-set of emitters may lie in the range between 400 nm and 800 nm inclusive.

Each sub-set of light emitters may include inorganic light emitting diodes. Each sub-set of light emitters may include organic light emitting diodes. Organic light emitting diodes may be solution processed. The apparatus may include a plurality of sub-sets of emitters arranged to form an array. The array may include more emitters in a first direction than in a second, perpendicular direction.

The first sub-set of light emitters may be inorganic light emitting diodes. The first sub-set of light emitters may be organic light emitting diodes. The second sub-set of light emitters may be inorganic light emitting diodes. The second sub-set of light emitters may be organic light emitting diodes. The apparatus may include a plurality of first and second sub-sets of light emitters arranged in an array. The array may include more emitters in a first direction than in a second, perpendicular direction.

The one or more photodetectors may take the form of photodiodes, photo resistors, phototransistors, complementary metal-oxide semiconductor (CMOS) pixels, charge coupled device (CCD) pixels, photomultiplier tubes or any other suitable photodetector. The one or more photodetectors may take the form of organic photodiodes. Organic photodiodes may be solution processed. The apparatus may include a plurality of photodiodes arranged in an array. The array may include more photodiodes in a first direction than in a second, perpendicular direction.

According to another aspect of the present invention, there is provided a method of operating the apparatus. The method includes applying a liquid sample to the liquid sample receiving region of the apparatus.

BRIEF DESCRIPTION OF THE DRAWINGS

One or more specific embodiments in accordance with aspects of the present invention will be described, by way of example only, and with reference to the following drawings in which:

Fig. 1 schematically illustrates an apparatus including first and second light emitters;

Fig. 2 illustrates first and second light emitter illumination and delumination responses to an illumination control signal for controlling illumination and delumination of the first and second light emitters;

Figs. 3a to 3e illustrate waveforms corresponding to the illumination control signal, an inverse of the illumination control signal, an output signal of a photodetector representative of detected intensity of light transmitted through a sample, a signal obtained by multiplying (i.e. convolving) the illumination control signal with the output signal, and a signal obtained by multiplying the inverse of the illumination control signal with the output signal;

Figs. 4 and 5 illustrate determining first and second beam profiles corresponding to first and second emitters;

Fig. 6 illustrates normalised spatial intensity profiles generated by the first and second emitters of the apparatus;

Fig. 7 schematically illustrates a lateral flow test strip;

Fig. 8 illustrates fibres making up a porous strip of a lateral flow test strip;

Fig. 9 illustrates a UV-visible absorbance spectrum of labelling particles used for a lateral flow test strip;

Figs. 10 and 11 illustrate the absorbance of a lateral flow test strip as a function of position, obtained at combined first and second wavelengths, and at a second wavelength alone;

Fig. 12 illustrates a correction performed by subtracting measurements at a second wavelength from measurements made at the combined first and second wavelengths;

Fig. 13 is a process flow diagram for a dual wavelength measurement made using the apparatus;

Fig. 14 illustrates illumination timings for first and second emitters of the apparatus;

Fig. 15 illustrates an apparatus for transmission measurements;

Fig. 16 illustrates an apparatus for reflectance measurements;

Fig. 17 illustrates obtaining image data using an apparatus;

Figs. 18 and 19 illustrate a liquid transport path, which intersects an optical path of an apparatus;

Fig. 20 illustrates a first arrangement for coupling light of first and second wavelengths into an optical path of an apparatus;

Figs. 21 and 22 illustrate normalised spatial intensity profiles generated by the first and second emitters of an apparatus;

Fig. 23 illustrates a second arrangement for coupling light of first and second wavelengths into an optical path of an apparatus;

Fig. 24 illustrates scanning a lateral flow test strip using an elongated light emitting diode array;

Fig. 25 illustrates a third arrangement for coupling light of first and second wavelengths into an optical path of an apparatus;

Fig. 26 illustrates a portion of a first light emitting diode array for an apparatus;

Fig. 27 illustrates a UV-visible absorbance spectrum of a second emitter of an apparatus;

Fig. 28 illustrates a portion of a second light emitting diode array for an apparatus;

Fig. 29 is a schematic cross-section of an apparatus integrated into a lateral flow testing device;

Fig. 30 shows a sample produced using gold nanoparticle inks having different solution optical densities to deposit a number of test lines on a nitrocellulose strip;

Fig. 31 shows variations in the absorbance of a blank nitrocellulose strip measured at combined green and near infrared wavelengths, and near infrared wavelengths;

Fig. 32 illustrates corrected absorbance measurements of a set of test lines deposited on a nitrocellulose strip;

Figs. 33 and 34 compare the apparatus with prior testing devices;

Fig. 35 compares the apparatus with prior testing devices for reading a Troponin lateral flow assay;

Fig. 36 shows experimental and modelling data illustrating the influence of beam profile differences;

Fig. 37A illustrates a portion of a third light emitting diode array for an apparatus;

Fig. 37B illustrates a portion of a fourth light emitting diode array for an apparatus;

Fig. 38 illustrates a typical organic photodetector sensitivity profile and green, red and near infrared light emission profiles typical of organic light emitting diodes;

Fig. 39 illustrates typical absorbance profiles for gold nanoparticles, a blue dye and nitrocellulose fibres;

Fig. 40 illustrate assumed concentration profiles for gold nanoparticles, for a blue dye and for nitrocellulose fibres forming a porous strip;

Fig. 41 illustrates simulated organic photodetector signals obtained based on the data shown in Figures 38 to 40;

Fig. 42 illustrates a simulated organic photodetector signal corresponding to a green organic light emitting diode;

Fig. 43 illustrates a simulated organic photodetector signal corresponding to a near infrared organic light emitting diode;

Figs. 44 and 45 illustrate converting normalised transmission values to absorbance values;

Figs. 46 and 47 illustrate estimating absorbance fingerprint values corresponding to gold nanoparticles and nitrocellulose fibres;

Fig. 48 illustrates analysing a three component simulated system using first and second wavelengths;

Fig. 49 illustrates analysing a three component simulated system using first, second and third wavelengths.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

If the number and complexity of optical components in a quantitative detector could be reduced, then the size and cost of the detector could be reduced. This may be advantageous for handheld or portable testing devices, and for single use home testing kits.

The minimum threshold for detecting an analyte may be improved if the signal-to-noise ratio of the measurement could be improved. Additionally, improvements in the signal-to-noise ratio may also allow for an analyte concentration to be determined with improved resolution.

Referring to Fig. 1, an apparatus 10 includes one or more first light emitters 12, one or more second light emitters 14 and one or more photodetectors 16.

Each first light emitter is configured to emit light 18 within a range around a first wavelength λ_1, and each second light emitter is configured to emit light 20 within a range around a second wavelength λ_2. The first light emitter(s) 12 may take the form of, for example, organic or inorganic light emitting diodes. Similarly, the second light emitter(s) 14 may take the form of, for example, organic or inorganic light emitting diodes. Organic light emitting diodes may be solution processed. If the first light emitter(s) 12 take the form of organic light emitting diodes, the second light emitter(s) 14 need not take the form of organic light emitting diodes and vice versa. The apparatus 10 may include a plurality of first and second light emitters 12, 14 arranged in an array. The array may include more light emitters 12, 14 in a first direction than in a second, perpendicular direction.

The one or more photodetector(s) are sensitive across a broad wavelength range, which includes at least the first and second wavelengths λ_1, λ_2. The photodetector(s) 16 may take the form of, for example, photodiodes, photoresistors, phototransistors, complementary metal-oxide semiconductor (CMOS) pixels, charge coupled device (CCD) pixels, photomultiplier tubes or any other suitable photodetector. Photodiodes may be organic or inorganic. Organic photodiodes may be solution processed. The apparatus 10 may include a plurality of photodetectors 16 arranged in an array. The array may include more photodetectors in a first direction y than in a second, perpendicular direction x.

The first and second light emitters 12, 14 are each coupled to an optical path 22 along which the light 18, 20 travels to reach the photodetector(s) 16. The optical path 22 includes a sample receiving portion 24. The apparatus 10 is arranged to receive a sample 26. When a sample 26 is received into the apparatus 10, the sample, or at least a portion of the sample 26, intersects the sample receiving portion 24 of the optical path 22.

The sample receiving portion 24 of the optical path 22 may be configured to receive a sample 26 in the form of a lateral flow test strip 28 (Fig. 7), or a microfluidic device. When the apparatus 10 is integrated into a lateral flow or microfluidic test, the sample 26 may be already positioned within the sample receiving portion of the optical path 22 before the assay is commenced.

The first light emitter(s) 12 and the second light emitter(s) 14 are simultaneously illuminable, i.e. they are illuminable at the same time. An illumination control signal 30 (see Fig. 2) controls illumination and delumination of the first and second light emitters 12, 14. Illumination of the first and second light emitters 12, 14 may be interspersed with both periods when only one of the first and second light emitters 12, 14 is illuminated and periods when neither of the first and second light emitters 12, 14 is illuminated.

Illumination and delumination of the first and second light emitters 12, 14 will now be described with reference to Fig. 2.

The first light emitter 12 is deluminable from an illuminated state 32 over a first time period τ_1 responsive to a change in state of the illumination control signal 30, e.g. a high-to-low transition of the illumination control signal 30, or a low-to-high transition of the illumination control signal 30.

The second light emitter 14 is deluminable from an illuminated state 34 over a second time period τ_2 responsive to the change in state of the illumination control signal 30, e.g. a high-to-low transition of the illumination control signal 30, or a low-to-high transition of the illumination control signal 30.

In the illustrated example, delumination of the first and second light emitters 12, 14 is initiated when the illumination control signal 30 transitions from a high to low state at time t_1.

The transition of the illumination control signal 30 from a high state to a low state at time t_1 causes the first and second light emitters 12, 14 to be powered-off and the first and second time periods τ_1, τ_2 to begin.

The first and second light emitters 12, 14 comprise different materials that have different lifetimes, or decay rates. In other words, following powering-off, light emitted by the first light emitter 12 will decay at a first decay rate r_1 and the first light emitter 12 will transition from an effective-on state (at time t_1) to an effective-off state (at time t_2) over time period τ_1. Light emitted by the second light emitter 14 will decay at a second decay rate r_2 and the second light emitter 14 will transition from an effective-on state (at time t_1) to an effective-off state (at time t_3) over time period τ_2.

As can be seen, when the illumination control signal 30 transitions from high to low at time t_1, an amount of light emitted by the first light emitter 12 decreases sharply until the first light emitter 12 is deluminated (i.e. effectively-off) at time t_2. Also at time t_1, an amount of light emitted by the second light emitter 14 decreases until the second light emitter 14 is deluminated at time t_3. Due to the difference in decay rates of the materials forming the first and second light emitters 12, 14, the amount of light emitted by the second light emitter 14 following time t_1 decreases more gradually than that of the first light emitter 12. Therefore, the second light emitter 14 emits light at wavelength λ_2 for longer than the first light emitter 12 emits light at wavelength λ_1.

The difference in decay rates of light emitted by the first and second light emitters 12, 14 following powering-off can be used to provide contrasting illuminations of the sample 26. That is, up to time t_2, the sample 26 is illuminated with light emitted by both the first and second emitters 12, 14, and between time t_2 and time t_3, the sample is illuminated with light emitted by the second emitter 14 only. Readings, from the photodetector(s) 16, representative of detected intensities of light transmitted through the sample 26 can be taken at a first time (e.g. time t_A) within the period up to time t_2, and at a second time (e.g. time t_B) within the period between times t_2 and t_3. These readings, at times t_A and t_B, can be used in calculations, discussed later, to determine a concentration of a particle-target complex in a test region of the sample 26.

Optionally, there is a large difference in lifetime/decay rate of the materials forming the first and second light emitters 12, 14 so as to achieve a high contrast between light at

wavelength λ_1 emitted by the first emitter 12 and light at wavelength λ_2 emitted by the second emitter 14.

Optionally the material of the first emitter 12 may be a fluorescent material and/or the material of the second emitter 14 may be a thermally activated delayed fluorescence (TADF) material, or a phosphorescent material.

A period τ_3 (where $\tau_3 = \tau_2 - \tau_1$) between delumination off the first light emitter(s) 12 and delumination of the second light emitter(s) 14 may be used for detecting fluorescence excited by the light 20 from the second light emitter(s) 14.

The apparatus 10 also includes a controller 36. The controller 36 is configured to simultaneously illuminate the first and second light emitters 12, 14 and to initiate delumination of the first and second light emitters 12, 14 (which delumination takes place over time period τ_1 for the first light emitter 12, and over time period τ_2 for the second light emitter 14). This is done by way of the illumination control signal 30 input to the first and second light emitter(s) 12, 14 by the controller 36. The controller 36 is configured to derive absorbance values using transmitted light intensity measurements obtained from the photodetector(s) 16. The controller 36 is also configured to generate an absorbance vector using the derived absorbance values, and to determine a concentration vector by multiplying the absorbance vector with a de-convolution matrix as described hereinafter. The controller may optionally be configured to intersperse illumination of each set of emitters with periods when none of the sets of emitters is illuminated. The controller 36 may be configured to normalise absorbance values with respect to a reference calibration absorbance value.

In the particular case of first and second light emitters 12, 14, the controller 36 is configured to simultaneously illuminate the first and second light emitter(s) 12, 14, and obtain a first set of measurements using the photodetector(s) 16 (e.g. at time t_A, or at any other suitable time during the period up to time t_1). The controller 36 is also configured to initiate simultaneous delumination of the first and second light emitter(s) 12, 14, and obtain a second set of measurements using the photodetector(s) 16 when the second light emitter(s) 14 is/are still illuminated, but the first light emitter(s) 12 is/are deluminated (e.g. at time t_B, or at any other suitable time during the period τ_3 between time t_1 and time t_2). The controller is configured to subtract the second set of measurements from the first set of measurements, as further described hereinafter.

The controller 36 may be configured to multiply the second set of measurements by a weighting factor before subtracting the second set of measurements from the first set of

measurements. Further details of the methods, processes and calculations carried out by the controller 36 are described hereinafter.

The apparatus 10 also includes at least one output device 38. For example, the output device 38 may take the form of one or more light emitting diodes, which are arranged for viewing by a user of the apparatus 10. The controller 36 may be configured to illuminate each light emitting diode in response to a concentration of a specific analyte vector exceeding a predetermined threshold.

In a further example, the output device 38 may take the form of a display element. The controller 36 may be configured to cause the display element to display one or more outputs in response to determining the concentrations of one or more analytes. The controller 36 may be configured, in response to a determined concentration of an analyte exceeding a predetermined threshold, to cause the display element to display a corresponding symbol or symbols. The controller 36 may be configured to cause the display element to display the determined concentrations of one or more analytes.

In another example, the at least one output device 38 may take the form of a wired or wireless communications interface for connection to a data processing apparatus (not shown). The data processing apparatus may take the form of, for example, a mobile telephone, tablet computer, laptop, desktop or a server. The controller 36 may be configured to output the measured concentrations of one or more analytes to the data processing apparatus (not shown) via the wired or wireless communications interface.

Various signals are output by the controller 30, input to the controller 30, and used by the controller 30 to derive further signals. These signals and derived signals are illustrated in Figs. 3a to 3e, in which:

- Fig. 3a illustrates an example waveform representing the illumination control signal 30;
- Fig. 3b illustrates an example waveform 40 representing an inverse of the illumination control signal;
- Fig. 3c illustrates an example waveform 42 representing an output signal of the photodetector(s) 16, which is representative of a detected intensity of light transmitted through a sample;
- Fig. 3d illustrates a waveform 44 representing a signal obtained by multiplying (i.e. convolving) the illumination control signal with the photodetector output signal. That is {output signal*illumination control signal} = $\lambda_1 + \lambda_2$ signal; and

- Fig. 3e illustrates an example waveform 46 representing a signal obtained by multiplying the inverse of the illumination control signal with the output signal. That is {output signal * $\overline{\text{illumination control signal}}$} = "$\lambda_2$ only" signal.

The controller 36 may implement a lock-in amplifier function. With reference to Figs. 3a to 3a, the illumination control signal 30 (first and second light emitters 12, 14 on/off) with 50% duty cycle may be employed with a frequency of:

$$f > 1/(\text{decay time of material of second light emitter(s)14})$$

and

$$f \ll 1/(\text{decay time of material of first light emitter(s)12}).$$

For example, if the lifetime/decay rate of the material of the second light emitter(s) is approximately 5μs, then f > 200kHz.

The λ1 + λ2 signal 44 can be derived by the controller 36 by multiplying the illumination control signal 30 by the output signal 42 of the photodetector(s) 16.

The "λ_2 only" signal 46 (see Fig. 3e), derived by the controller 36 by multiplying the inverse 40 of the illumination control signal by the output signal 42 of the photodetector(s) 16, will be lower than the portion of the $\lambda_1 + \lambda_2$ signal attributable to the transmitted light at λ_2. This factor may need to be taken into account during calculations of absorbance, or concentration, performed by the controller 36.

Since the $\lambda_1 + \lambda_2$ signal 44 and "λ_2 only" signal 46 can be derived by the controller 36:

- a value of the transmitted intensity of light through the sample can be obtained at the time t_A, i.e. during the period when both the first and second light emitter(s) 12, 14 are "ON", which value comprises light intensity of light transmitted through the sample from both the first and second emitter(s) 12, 14; and
- a value of the transmitted intensity of light through the sample can be obtained at the time t_B, i.e. during the period when only the second light emitter(s) 14 is/are emitting light (at wavelength λ_2), which value comprises a light intensity of light transmitted through the sample from the second emitter(s) 14 only.

Thus, a transmitted light intensity value for light containing wavelengths $\lambda_1 + \lambda_2$ is known and a transmitted light intensity value for light at wavelength λ_2 is known. Calculation

of the transmitted light intensity value for light at wavelength λ_1, i.e. a determination of the intensity contribution at time t_a due to light at wavelength λ_1 can be achieved by performing the following calculation:

$$TI\ (\lambda_1\ +\ \lambda_2) - xTI\ (\lambda_2)\ =\ TI(\lambda_1)$$

where $TI(\lambda_1 + \lambda_2)$ is the transmitted intensity of light containing wavelengths $\lambda_1 + \lambda_2$ through the sample 26 (e.g. at time t_A, or at any other suitable time during the period up to time t_1), $TI(\lambda_2)$ is the transmitted intensity of light at wavelength λ_2 through the sample 26 (e.g. at time t_B, or at any other suitable time during the period τ_3 between time t_1 and time t_2), x comprises a correction factor to compensate for a drop in intensity of light at wavelength λ_2 between t_A and t_B, and $TI(\lambda_1)$ is the transmitted intensity of light at wavelength λ_1.

The calculated value for $TI(\lambda_1)$ and the measured value for $TI(\lambda_2)$ can be used by the controller 26 to derive absorbance values, from which a concentration of a particle-target complex in a test region of the sample 26 can be determined (discussed later).

Optionally, a "sample and hold circuit" may be used as an alternative to the above method.

Referring also to Figs. 4 to 6, the first and second light emitters 12, 14 and the optical path 22 are arranged so that a normalised beam profile 48 of light 18 from the first emitter 12 is substantially equal to a normalised beam profile 50 of light 20 from the second emitter 14.

For example, referring in particular to Fig. 4, light 18, 20 introduced into the optical path 22 intersects a sample surface 52 in a first direction x between first and second locations x_A, x_B. Likewise, light 18, 20 introduced into the optical path 22 intersects the sample surface 52 in a second, perpendicular direction y between first and second locations y_A, y_B. For example, the sample surface 52 may be a surface of a lateral flow test strip or a surface of a substrate containing/defining microfluidic channels. The optical path 22 makes an angle θ with the normal 54 to the sample surface 52. The positions x_A, x_B, y_A, y_B bound a notional surface 56 of the sample receiving portion 24, which approximately corresponds to the sample surface 52 in use. When the apparatus 10 is integrated into a lateral flow or microfluidic test, the notional surface 56 may be coincident with a surface of a lateral flow test strip or a surface of a substrate containing defining microfluidic channels. The angle θ is greater than or equal to 0 degrees and less than 90 degrees. The normal 54 is oriented with respect to the sample surface 52 on average, rather than a local normal, which can vary significantly from point-to-point due to surface roughness and/or localised inhomogeneity.

The optical path 22 may be converging or diverging, i.e. the light 18, 20 may form a converging or diverging beam, in which case θ is the angle between a central ray/centre of the optical path 22 and the normal 54.

Referring in particular to Fig. 5, the normalised beam profiles of light 18, 20 from the first and second light emitters 12, 14 may be obtained using a beam profiler 58. The beam profiler 58 is arranged to intersect the optical path 22 in the absence of a sample 26. The beam profiler 58 is arranged at the position where the optical path 22 intersects the notional surface 56 of the sample receiving portion 24 of the optical path 22. The beam profiler 58 is arranged so the centre of the beam profiler 58 corresponds as closely as is practical to the centre of the optical path 22. The beam profiler 58 is arranged with a detection surface 60 oriented perpendicular to the optical path 22, or at least to a centre of the optical path 22. In other words, the beam profiler 58 is rotated by an angle of θ compared to the notional surface 56 of the sample receiving portion 24. In this way, the beam profiler 58 measures the beam profile intensities 48, 50 in a measurement plane 62, which is transverse to the optical path 22 (or the centre thereof). A line of common intersection between the optical path 22, the notional surface 56 of the sample receiving portion 24 and the measurement plane 62 defines the measurement location. When a sample 26 is received into the sample receiving portion 24, the line of common intersection will approximately correspond to the sample surface 52, with deviations depending on the regularity of the sample 26 and the accuracy of placing the sample 26.

The beam profiler 58 measures light 18, 20 intensities in a measurement plane 62, which is rotated by an angle θ about the second direction y with respect to the notional surface 56. Positions on the notional surface 56, for example the bounds x_A, x_B, y_A, y_B of the notional surface 56 of the sample receiving portion 24, are projected onto positions x_A', x_B', y_A', y_B' on the measurement plane 62 according to $x_A' = x_A/\sin\theta$, $x_B' = x_B/\sin\theta$, $y_A' = y_A$ and $y_B' = y_B$. Preferably, a light sensitive area of the beam profiler 58 detection surface 60 is large enough to encompass the projected bounds x_A', x_B', y_A', y_B' of the notional surface 56.

Referring in particular to Fig. 6, the intensity of light from the first light emitter(s) 12 is denoted $I_1(x',y')$ on the x'-y' measurement plane 62. The normalised spatial intensity profile 48 generated by the first light emitter(s) 12 (herein also referred to as the first beam profile 48) may be defined as the ratio of the intensity of light from the first light emitter(s) 12 divided by the summed intensity I_1^{sum} detected by the beam profiler 58, i.e. $I_1(x',y')/ I_1^{sum}$. The normalised spatial intensity profile 50 generated by the second light emitter(s) 14 (herein also referred to as the second beam profile 50) is defined in the same way as $I_2(x',y')/ I_2^{sum}$.

The first and second beam profiles 48, 50 are preferably substantially equal on the measurement plane 62, i.e. on entering the sample receiving portion 24. Preferably, the normalised spatial intensity profiles 48, 50 are substantially equal throughout the sample receiving portion 24 of the optical path 22. However, uniformity throughout the sample receiving portion 24 is not necessary since, in use, the scattering from the sample 26 will be more significant than effects of diverging beam profiles 48, 50.

A number of difference metrics may be used to quantify the extent of differences between the first and second beam profiles 48, 50. For example, a maximum beam profile difference Δ_{max} may be defined according to:

$$\Delta_{max} = \max\left(\left|\frac{I_1(x',y')}{I_1^{sum}} - \frac{I_2(x',y')}{I_2^{sum}}\right|\right) \quad (1).$$

Similarly, an average beam profile difference Δ_{avg} may be defined according to:

$$\Delta_{avg} = \frac{\iint_{y'_A x'_A}^{y'_B x'_B}\left|\frac{I_1(x',y')}{I_1^{sum}} - \frac{I_2(x',y')}{I_2^{sum}}\right| dx'dy'}{(x'_B - x'_A)\times(y'_B - y'_A)} \quad (2)$$

If the output of the beam profiler 58 is an array of intensities corresponding to an array of positions x', y', the integral defined in Equation 2 may be readily converted to a sum in order to determine the average beam profile difference Δ_{avg}.

Alternatively, a root-mean-square (RMS) difference Δ_{RMS} may be defined according to:

$$\Delta_{RMS} = \sqrt{\frac{\iint_{y'_A x'_A}^{y'_B x'_B}\left|\frac{I_1(x',y')}{I_1^{sum}} - \frac{I_2(x',y')}{I_2^{sum}}\right|^2 dx'dy'}{(x'_B - x'_A)\times(y'_B - y'_A)}} \quad (3)$$

If the output of the beam profiler 58 is an array of intensities corresponding to an array of positions x', y', the integral defined in Equation 3 may be converted to a sum to determine the average beam profile difference Δ_{avg}. Difference metrics are not limited to the maximum, average and/or RMS beam profile differences Δ_{max}, Δ_{mean}, Δ_{RMS}, and alternative difference metrics may be defined to quantify the extent of differences between the first and second beam profiles 48, 50.

The first and second light emitters 12, 14 and the optical path 22 are arranged such that the first and second beam profiles 48, 50 are substantially equal on the measurement plane 62. The following description shall refer to an example in which light 18 from the first emitter 12 is used to quantify the sample 26, whilst light 20 from the second light emitter 14 is used as a reference (as explained hereinafter). However, the same principles are applicable if light 20 from the second light emitter 14 is used to quantify the sample 26, whilst light 18 from the first light emitter 12 is used as a reference.

The beam profiles 48, 50 may be considered to be substantially equal when the maximum difference Δ_{max}, average difference Δ_{avg} or RMS difference Δ_{RMS} are less than or equal to an absolute threshold determined by prior experiments. Preferably, whether or not the beam profiles 48, 50 may be considered to be substantially equal can be evaluated by comparing the maximum difference Δ_{max}, average difference Δ_{avg} or RMS difference Δ_{RMS} to a relative threshold determined from the beam profiles 48, 50 themselves.

For example, a first threshold may be based on a fraction of the maximum normalised intensity of light 18 from the first light emitter 12, i.e. $I_1^{max} = \max(I_1(x',y'))$. The first and second beam profiles 48, 50 may be considered to be substantially equal if the maximum difference Δ_{max}, average difference Δ_{avg} or RMS difference Δ_{RMS} is less than or equal to 0.05 x I_1^{max} (≤5%), less than or equal to 0.1 x I_1^{max} (≤10%), less than or equal to 0.2 x I_1^{max} (≤20%) or less than or equal to 0.5 x I_1^{max} (≤50%).

In an ideal case, the first and second beam profiles 48, 50 are equal to each other at each point, i.e. for all x', y' measured by the beam profiler 58. In practice, an alternative determination of whether the first and second beam profiles 48, 50 are sufficiently similar to be regarded as substantially equal may be performed using the inequality:

$$\left|\frac{I_1(x',y')}{I_1^{sum}} - \frac{I_2(x',y')}{I_2^{sum}}\right| \leq f \times \frac{I_1(x',y')}{I_1^{sum}} \quad (4)$$

In which $0 \leq f \leq 0.5$ is a fraction. For example, a value of $f = 0.1$ corresponds to testing whether the difference between first and second beam profiles 48, 50 is less than or equal to 10% of the first beam profile 48. In one example, the first and second beam profiles 48, 50 may be considered to be substantially equal if the inequality of Equation (4) is satisfied for all $x_A' \leq x' \leq x_B'$ and all $y_A' \leq y' \leq y_B'$. Alternatively, the first and second beam profiles 48, 50 may be considered to be substantially equal if the inequality of Equation (4) is satisfied for a threshold percentage of the area measured by the beam profiler 58, for example, if the

inequality of Equation (4) is satisfied for greater than or equal to 90%, greater than or equal to 75% or greater than or equal to 50% of the measured area.

The beam profiler 58 may be any suitable form of beam profiler such as, for example, camera based beam profiler, a translating slit beam profiler, a translating step beam profiler and so forth. The relative sensitivity of the beam profiler 58 to different wavelengths need not be the same at the first and second wavelengths λ_1, λ_2, since any difference should be compensated for through the use of relative spatial intensities.

As described above, a signal obtained using the second light emitter(s) 14 alone ("λ_2 only" signal 46) is subtracted from the calculated signal for the first light emitter(s) 12 alone in order to compensate for optical scattering due to defects or other inhomogeneities in a medium, or substrate which forms part of the sample 26. The subtraction is carried out by the controller 36.

Referring also to Fig. 7, a lateral flow test strip 28 is an example of a sample 26, which may be measured using the apparatus 10.

Lateral flow test strips 28 (also known as "lateral flow immunoassays") are a variety of biological testing kit. Lateral flow test strips 28 may be used to test a liquid sample, such as saliva, blood or urine, for the presence of an analyte. Examples of lateral flow devices include home pregnancy tests, home ovulation tests, tests for other hormones, tests for specific pathogens and tests for specific drugs.

In a typical lateral flow test strip 28, a liquid sample is introduced at one end of a porous strip 64, and the liquid sample is then drawn along the lateral flow test strip 28 by capillary action (or "wicking"). A portion of the lateral flow test strip 28 is pre-treated with labelling particles 68 (Fig. 8), which are activated with a reagent which binds to the analyte to form a complex if the analyte is present in the liquid sample. The bound complexes, and also unreacted labelling particles 68 (Fig. 8) continue to propagate along the lateral flow test strip 28 before reaching a testing region 66, which is pre-treated with an immobilised binding reagent which binds complexes of analyte bound to labelling particles 68 (Fig. 8) and does not bind unreacted labelling particles 68 (Fig. 8). The labelling particles 68 (Fig. 8) have a distinctive colour, or otherwise absorb one or more ranges of ultraviolet or visible light. The development of a concentration of labelling particles 68 (Fig. 8) in the test region 66 may be measured and quantified using the apparatus 10, for example by measuring the optical density of labelling particles 68 (Fig. 8). The apparatus 10 may perform measurements on developed lateral flow test strips 28, i.e. the liquid sample has been left for a pre-set period to be drawn

along the test strip 28. Alternatively, the apparatus 10 may perform kinetic, i.e. dynamic time-resolved measurements of the optical density of labelling particles 68 (Fig. 8).

Referring also to Fig. 8, the porous strip 64 is typically formed from a mat of fibres 70, for example nitrocellulose fibres. Within the test region 66, the immobilised binding reagent binds complexes of analyte and labelling particles 68.

The fibres 70 scatter and/or absorb light across a broad range of wavelengths in an approximately similar way. For example, the proportion of light 18 from the first light emitter(s) 12 that is scattered by fibres 70 is approximately the same as the proportion of light 20 from the second light emitter(s) 14. However, the fibrous porous strip 64 is not uniform, and the density of fibres 70 may vary from point-to-point along the porous strip 64. As explained further hereinafter, such background variations of absorbance, which are due to the inhomogeneity of the porous strip 64, may limit the sensitivity of a measurement, i.e. the minimum detectable concentration of labelling particles 68.

Referring also to Fig. 9, the apparatus 10 may compensate for such background variations of absorbance due to the inhomogeneity of the porous strip 64, provided that the first and second wavelengths λ_1, λ_2 are selected appropriately for the labelling particles 68 used for a lateral flow test strip 28. For example, an ultraviolet-visible spectrum 72 of the labelling particles 68 may be obtained to determine how the absorbance of the labelling particles 68 varies with wavelength/frequency. The first wavelength λ_1, is selected to be a wavelength that is at, or close to, a peak absorbance of the labelling particles 68. The second wavelength λ_2 is selected to be a wavelength that lies substantially away from a peak absorbance of the labelling particles 68. In other words, the first and second wavelengths λ_1, λ_2 are selected such that labelling particles have relatively higher absorbance at the first wavelength λ_1 than at the second wavelength λ_2. The ratio of absorbance between the first and second wavelengths λ_1, λ_2 may be a factor of, for example, at least two, up to and including five, up to and including ten, or more than ten.

The first and second wavelengths λ_1, λ_2 may lie in the range between 300 nm and 1500 nm inclusive. The first and second wavelengths λ_1, λ_2 may lie in the range between 400 nm and 800 nm inclusive.

Referring in particular to Figs. 8 and 9, light 18 from the first light emitter(s) 12, having wavelengths around the first wavelength λ_1, is absorbed by the labelling particles 68, in addition to being scattered and/or absorbed by the fibres 70. By contrast, light 20 from the second light emitter(s) 14, having wavelengths around the second wavelength λ_2, is absorbed by the labelling particles 68 only weakly or not at all.

Referring also to Figs. 10 to 12, a lateral flow test strip 28 may be passed through the sample receiving portion 24 of the optical path 22, and the absorbance values A(x) measured as a function of position x along the porous strip 64 of the lateral flow testing device 28. The absorbance values A(x) are determined based on the difference in transmittance or reflectance when a sample 26 occupies the sample receiving portion 24 and a reference condition, for example, the absence of a sample 26.

The calculated absorbance $A_1(x)$ when the sample 26 is illuminated by light 18 at the first wavelength λ_1 and the calculated absorbance $A_2(x)$ when the sample 26 is illumined by light 20 at the second wavelength λ_2 from the second emitter alone have substantially equal contributions from scattering and/or absorption by the fibres 70 of the porous strip 64. The background level of absorbance varies with position x along the porous strip 64 due to the inhomogeneity of fibre 70 density. Absorbance signals resulting from the labelling particles 68 cannot be reliably detected unless they are at least larger than the background variance that results from inhomogeneity of the porous strip 64. This restricts the lower limit of labelling particle concentration that can be detected using a lateral flow test strip 28. The same background variance also limits the resolution of a quantitative measurement of labelling particle 68 concentration/optical density.

However, since the fibres 70 scatter light at the first and second wavelengths λ_1, λ_2 in approximately the same way, the absorbance values $A_2(x)$ values at the second wavelength λ_2 may be subtracted from the absorbance values $A_1(x)$ from illumination of the sample at the first wavelength λ_1 to reduce or remove the effect of the variations in background absorbance, which result from the inhomogeneous distribution of fibres 70 in the porous strip.

Although in practice some amount of background variance in absorbance will remain when the difference $A_1(x) - A_2(x)$ is obtained, the relative size of the signal that is specific to the labelling particles 68 may be increased, in some cases substantially, with respect to background variations. In this way, the lower limit of labelling particle 68 concentrations/ optical densities that may be detected may be reduced. Similarly, the resolution of a quantitative measurement of labelling particle 68 concentration/optical density may be increased.

Although the normalised spatial intensity profiles, i.e. first and second beam profiles 48, 50 generated by the first and second light emitter(s) are preferably substantially equal for the correction to be effective (as described hereinbefore), the absolute spatial intensity profiles (not shown) need not be equal.

When the absolute intensities of light 18, 20 from the first and second light emitters 12, 14 are not equal, the intensity ratio α of the first and second light emitters 12, 14 may be measured in the absence of a sample 26 and used to perform a weighted correction, i.e. $A_1(x) - \alpha A_2(x)$. Alternatively, the weighting factor α may account for differing sensitivity of the photodetector(s) 16 at the first and second wavelengths λ_1, λ_2.

Through alternately illuminating the sample 26 with light emitted by both the first and second light emitters 12, 14 at the same time, and light emitted by the second light emitter 14 only, the apparatus 10 may include a relatively simple optical path 22 that does not require optical components such as beamsplitters, filters or monochromators to perform dual-wavelength measurements. Thus, the apparatus 10 may be less bulky, simpler and less expensive to manufacture. Additionally, many optical components such as beamsplitters have wavelength dependent properties, which may restrict the choice of wavelengths λ_1, λ_2. By reducing the number of optical components in the optical path 22, or in some examples removing the need for intermediate optical components altogether, the wavelengths λ_1, λ_2 for a dual-wavelength measurement may be less constrained.

Referring also to Figs. 13 and 14, a process of obtaining and correcting absorbance measurements will be described. The process described with reference to Figs. 13 and 14 may be carried out by the controller 36 of the apparatus 10.

A sample 26 is placed so that a region of interest on the sample 26 coincides with the sample receiving portion 24 of the optical path 22 (step S1). When the apparatus 10 is integrated in a self-contained assay including a lateral flow strip or a microfluidic device, this stage may be omitted. The first and second light emitter(s) 12, 14 are turned on for a period of duration δt_1, and the photodetector(s) 16 measure the light 18,20 transmitted through (or reflected from) the sample receiving portion 24 of the path (step S2).

The first light emitter(s) 12 deluminate over a period of duration δt_2 while the second light emitter(s) 14 begin to deluminate over the period δt_2 and continue to deluminate over a further period of duration δt_3. The first light emitter(s) 12 is/are deluminated (i.e. "OFF") at the end of period δt_2. The second light emitter(s) 14 is/are deluminated (i.e. "OFF") at the end of period δt_3. During period δt_3, the photodetector(s) 16 measure the light 20 transmitted through (or reflected from) the sample receiving portion 24 of the path (step S3).

A calculation is performed to determine the transmitted intensity of light at the λ_1 wavelength, i.e. $TI(\lambda_1 + \lambda_2) - xTI(\lambda_2) = TI(\lambda_1)$ (step S4).

Absorbance values for light at wavelength λ_1, i.e. $A_1(x)$, and light at wavelength λ_2, i.e. $A_2(x)$, are calculated (step S5).

The absorbance values $A_2(x)$ determined using the second light emitter(s) 14 are subtracted from the absorbance values $A_1(x)$ determined using the first light emitter 12 according to $A_1(x) - \alpha A_2(x)$, in which α is a weighting factor to account for differences in the absolute intensity of illumination between the first and second wavelengths λ_1, λ_2 and/or differing sensitivity of the photodetector(s) 16 at the first and second wavelengths λ_1, λ_2 (step S6).

If further samples 26 are to be measured, then the next sample 26 may be placed (step S7). Alternatively, if there are additional regions of interest on the same sample 26, for example if the sample 26 is a lateral flow test strip 28 having more than one test region 66, the sample 26 may be repositioned with the next region of interest within the sample receiving portion 28.

Measurement geometries

The apparatus 10 may be configured to use a range of emitter 12, 14 and photodetector 16 geometries.

Referring also to Fig. 15, the optical path 22 may be configured so that the photodetector(s) 16 receive light 18, 20 transmitted through the sample receiving portion 24 of the optical path 22. For measurements in transmission, the light emitter(s) 12, 14 and photodiode(s) 16 may simply be spaced apart by a gap, which corresponds to the optical path 22. The sample receiving portion 24 of the optical path 22 then corresponds to the part of the gap which is occupied by a sample 26 when the sample 26 is received into the apparatus 10.

For example, if a sample 26 in the form of a lateral flow test strip 28 is used, the lateral flow test strip 28 may be arranged with a testing region 66 positioned between the light emitter(s) 12, 14 and photodiode(s) 16. The sample receiving portion 24 of the path 22 corresponds to the thickness of the lateral flow test strip 28, which intersects the optical path 22.

Additional optical components may be included in the optical path 22. For example, the light from the light emitters 12, 14 into the optical path 22 and/or the light from the optical path 22 to the photodiode(s) 16 may be restricted by slits or other apertures. Optionally, a diffuser, one or more lenses and/or other optical components may also be included in the optical path 22.

Referring also to Fig. 16, an apparatus 10 may alternatively be configured so that the photodetector(s) 16 receive light reflected from the sample receiving portion 24 of the optical path 22. For example, when the apparatus 10 is arranged to receive samples in the form of

lateral flow test strips 28, the light emitters 12, 14 may be arranged to illuminate a region of interest of a lateral flow test strip 28 received into the apparatus 10 at first angle θ_1, and the photodetector(s) 16 may be arranged to receive light reflected from the lateral flow test strip 28. Light reflected from the porous strip 64 of a lateral test strip 28 will, in general, be scattered into a wide range of different angles due to the largely random orientations of the fibres 70. Consequently, the portion of the optical path 22 between the sample receiving region 24 and the photodetector(s) 16 may be oriented at a second angle θ_2, which does not need to be equal to the first angle θ_1. In some examples, the first and second angles θ_1, θ_2 may be equal. In some examples, the light emitters 12, 14 and photodetector(s) 16 may be arranged in a confocal configuration. Light reflected from the sample 26 may originate from the sample surface 52 or from a depth within the sample 26.

Additional optical components may be included in the optical path 22. For example, the light from the light emitters 12, 14 into the optical path 22 and/or the light from the optical path 22 to the photodetector(s) 16 may be restricted by slits or other apertures. Optionally, a diffuser, one or more lenses and/or other optical components may also be included in the optical path 22.

Referring also to Fig. 17, the apparatus 10 may include a number of photodetectors 16 arranged in an array to form an image sensor 74. For example, the image sensor 74 may form part of a camera. An image sensor 74 may be arranged to image all of, or a portion of, the sample receiving portion 24 of the optical path 22. For example, when a lateral flow test strip 28 is received into an apparatus 10, the image sensor 74 may be arranged to image one or more test regions 20 and the surrounding areas of the porous strip 64. A lateral flow test strip 28 may include one or more pairs 76, each pair 76 including a testing region 66 and a control region 78, and the image sensor 74 may be arranged to image the one or more pairs 76 at the same time. An image captured using the second wavelength λ_2 may be subtracted from an image captured using the first and second wavelengths λ_1, λ_2 in combination in order to compensate for background variance due to inhomogeneity of the fibres 70 making up the porous strip 64. The subtraction may be weighted using a weighting factor α when the absolute intensity of illumination from the first and second light emitters 12, 14 is not substantially equal and/or when the sensitivity of the image sensor 74 differs between the first and second wavelengths λ_1, λ_2.

An image sensor 74 may be used to image transmitted or reflected light. Additional optical components may be included in the optical path 22. For example, the light from the light emitters 12, 14 into the optical path 22 and/or the light from the optical path 22 to the

photodetector(s) 16 may be restricted by slits or other apertures. Optionally, a diffuser, or more lenses and/or other optical components may also be included in the optical path 22.

Referring also to Figs. 18 and 19, the apparatus 10 may also include a liquid transport path 78 for transporting a liquid sample received in a liquid sample receiving region 80 proximate to a first end 82 of the liquid transport path 78 towards a second end 84 of the liquid transport path 78. The liquid transport path 78 intersects the sample receiving portion 24 of the optical path 22.

The liquid transport path 78 may take the form of a porous medium, for example the porous strip 64 of a lateral flow test strip 28. The porous strip 64 may include nitrocellulose or other fibrous materials capable of transporting an aqueous liquid by capillary action. The porous strip 64 may be inherently capable of drawing liquid along the liquid transport path 78 by capillary action. Depending on the fibres used, surface treatments may be performed to permit, or enhance, the transport of liquid along the liquid transport path 78. When the liquid transport path 78 takes the form of a porous strip 64, dry and wet portions of the porous strip are separated by a flow front 86, which propagates along the liquid transport path 78. Even once the flow front 86 has reached the second end 84, liquid may continue to flow along the liquid transport path 78 if the second end 84 is in contact with a reservoir or wicking pad 66 (Fig. 19).

The liquid transport path 78 intersects the sample receiving portion 24 of the optical path 22 and the optical absorbance of the porous strip 64 in the sample receiving portion may be monitored as a function of time. Such measurements may sometimes be referred to as dynamic" or "kinetic" measurements. For example, if a lateral flow test strip 28 is arranged with a test region 66 within the sample receiving portion 24, then the development of the concentration of labelling particles 68 may be tracked as a function of time by measuring the absorbance of the test region 66 at the first and second wavelengths λ_1, λ_2 as a function of time. If a lateral flow test strip 28 includes additional regions of interest, for example control regions 78 or further test regions 66, then the apparatus 10 may be provided with additional pairs of emitters 12, 14 and photodetector(s) 16.

The liquid transport path 78 need not be a porous strip 64 of a lateral flow test strip 28. Alternatively, the liquid transport path 42 may take the form of one or more channels of a microfluidic device.

In this way, dynamic information about the development of an assay may be obtained. Dynamic information may be useful, for example, to check that an assay has behaved as expected or within acceptable bounds for a result to be considered reliable. The intervals δt_1,

δt_2 and δt_3 should be relatively short compared to the timescales on which an assay is developed.

Coupling the first and second emitters to the optical path

There are several different ways to introduce light 18, 20 from the first and second emitters 12, 14 onto the optical path 22 so that the corresponding normalised spatial intensity profiles 48, 50 are substantially equal in the sample receiving portion 24 of the optical path 22.

For example, referring also to Fig. 20, light 18, 20 from the first and second emitters 12, 14 may be introduced onto the optical path 22 through a slit 88 defined by a pair of slit members 90 separated by a gap. The slit members 90 may be, for example, knife edge members. The first and second light emitters 12, 14 are arranged close together with an emission surface thereof at a distance d from the slit 88 entrance. The first and second light emitters 12, 14 may be oriented substantially parallel to one another, for example perpendicular to the slit members 90 defining the slit 88. Alternatively, the first and second light emitters 12, 14 may be oriented to converge on the slit 88.

Each pair of first and second light emitters 12, 14 may be arranged such that the slit 88 perpendicularly bisects the pair of emitters 12, 14, when the arrangement is viewed along a direction perpendicular to the slit members 90 defining the slit 88. For example, if the slit members 90 define the slit in an x-y plane with reference to a set of Cartesian axes, then the slit 88 should perpendicularly bisect each pair of emitters 12, 14 when viewed along the z axis.

Optionally, a diffuser 92 may be arranged at a point between the slit 88 and the emitters 12, 14. One or more lenses (not shown) may also be included to collect and/or focus light 18, 20 from the light emitters 12, 14.

Referring also to Figs. 21 and 22, each of the first and second light emitters 12, 14 may have a substantially similar, cylindrically symmetric angular emission profile. For example, the first and second light emitters 12, 14 may have Gaussian angular emission profiles. Along a line which perpendicularly bisects the central points of the circularly symmetric normalised intensity profiles 48, 50, the values of each normalised intensity profile 48, 50 will be substantially equal, i.e. $I_1(x,y) = I_2(x,y)$ along the perpendicular bisector. In this way, the first and second normalised intensity profiles (beam profiles) 48, 50 may be substantially equal along the length of the slit 88 using a relatively simple and compact optical arrangement.

The slit 88 should be relatively narrow to provide fine spatial resolution and to ensure that the normalised intensity profiles 48, 50 are substantially equal across the width t of the slit 88. The slit may have a width between 100 μm and 1 mm inclusive. Preferably, the slit has a width between 300 μm and 500 μm inclusive.

Coupling light 18, 20 from the first and second light emitters 12, 14 into the optical path 22 through a slit 88 may be used for measurements in transmission or reflection.

Referring also to Fig. 23, in some examples of an apparatus 10, the optical path 22 need not include any conventional optical components. For example, a light emitting diode array 94 may simply be arranged at the other end of a plain optical path 22 to a photodetector 16, i.e. the optical path 22 only includes the sample receiving portion 24. The light emitting diode array 94 includes at least two light emitting diodes, i.e. one first light emitter 12 and one second light emitter 14. The light emitting diode array 94 may be composed of a plurality of light emitting diode pixels of similar dimensions to those found in light emitting diode display devices for computers, televisions and so forth. The light emitting diode array 94 may include a mixture of first and second emitters 12, 14.

Where samples 26 include multiple regions of interest, the sample 26 may be moved in front of the light emitting diode array 94 to scan the sample 26. Alternatively, the light emitting diode array 94 and corresponding photodetector 16 may be moved to scan the sample 26. Alternatively, a light emitting diode array 94 and one or more photodetectors 16 may be arranged corresponding to each region of interest of the sample 26 so that each region may be measured concurrently. That is, the apparatus may comprise at least one set of emitters, with the or each set of emitters comprising a first sub-set of one or more first light emitters 12 and ta second sub-set of one or more second light emitters 14.

A light emitting diode array 94 may be used for measurements in reflection or transmission.

Referring also to Fig. 24, the light emitting diode array 94 may extend in one direction or may be a linear light emitting diode array 94.

For example, when a sample is in the form of a lateral flow test strip 28, which extends longitudinally in a first direction x, transversely in a second direction y and has a thickness in a third direction z, a light emitting diode array 94 may extend for substantially the width of the lateral flow test strip 28 in the transverse y direction and for a relatively short distance in the longitudinal x direction. If the lateral flow test strip 18 is mounted in a sample mounting stage 96 including a window for transmission measurements, then the light emitting diode array 94 may extend for substantially the width of the lateral flow test strip 28.

Alternatively, the lateral flow test strip 28 may be mounted fixedly with respect to the apparatus 10, and a pair of an LED array 94 and a photodetector 16 may be provided corresponding to each test region 66 and/or control region 78.

Referring also to Fig. 25, although additional optical components are not required using a light emitting diode array, it may be advantageous for the light 18, 20 from first and second light emitters 12, 14 forming the light emitting diode array 94 to be passed through a slit 88 defined by slit members 90 before entering the optical path 22. In this way, the spatial resolution of measurements made using a light emitting diode array 94 may be improved.

Optionally, a diffuser 92 may be arranged between the light emitting diode array 94 and the sample receiving portion 24 of the optical path 22. One or more lenses (not shown) may also be included to collect and/or focus light 18, 20 from the light emitting diode array 94.

Referring also to Figs. 26 and 27, one way to implement a single light emitter comprising both the first and second light emitters 12, 14, or a light emitting diode array 94 is to stack the first and second light emitters 12, 14 on top of each other. Each first light emitter 12 takes the form of a light emitting diode with a peak emission at the first wavelength λ_1 and the corresponding second light emitter 14 takes the form of a light emitting diode with a peak emission at the second wavelength λ_2. The first and second light emitters 12, 14 are simultaneously addressable to allow for simultaneous illumination. The first and second light emitters 12, 14 will deluminate at different rates as discussed previously.

The second light emitter 14 may be manufactured using materials that are transparent, or substantially transparent at the first wavelength λ_1. For example, the absorbance 96 of the second light emitter 14 at the first wavelength λ_1 may be relatively low. Absorbance may be considered to be relatively low if it is less than 50%, less than 25%, less than 15%, less than 10% or less than 5% (i.e. transmittance of more than 50%, more than 75%, more than 85%, more than 90% or more than 95%). In this way, the light emitting diode providing the second light emitter 14 may be deposited on top of the light emitting diode providing the first light emitter 12, and the first light emitter 12 may emit light onto the optical path 22 through the second light emitter 14.

This arrangement may be particularly compact for transmission measurements, but may also be used for reflectance measurements. Also, the first and second light emitters may precisely illuminate the same region of the sample (because one overlies the other). This may improve a background subtraction calculation compared with an apparatus where light emitters are side-by-side. In such a “side-by-side” arrangement, the light emitters illuminate

mostly the same region, but because of their lateral spacing would not necessarily be able to illuminate exactly the same region. Having one light emitter overlying the other may improve the signal-to-noise by providing a more accurate background subtraction (because exactly the same region of the sample may be illuminated in the overlying arrangement).

Referring also to Fig. 28, another option for a light emitting diode array 94 is to arrange a plurality of first and second light emitters 12, 14 into an array in which the first and second light emitters 12, 14 alternate in a "chess-board" pattern. When the individual light emitters 12, 14, or pixels, of the light emitting diode array 94 are made small, for example comparable with pixels of a light emitting diode display or television, the normalised spatial intensity profiles 48, 50 generated by the first and second light emitters 12, 14 may be substantially uniform and equal to one another at distances more than a few times the typical pixel dimensions. For example, the pixel pitch of the light emitting diode array 94 may be within the range from 5 μm to 300 μm inclusive. The differences between the normalised spatial intensity profiles 48, 50 may be further reduced by arranging a diffuser 92 between the "chess-board" light emitting diode array 94 and the sample receiving portion 24 of the optical path 22. The first and second light emitters 12, 14 are simultaneously addressable to allow for simultaneous illumination. The first and second light emitters 12, 14 will deluminate at different rates as discussed previously.

This arrangement may be particularly compact for transmission measurements, but may also be used for reflectance measurements.

Referring also to Fig. 29, the apparatus 10 may be integrated into a self-contained, single use lateral flow testing device 98.

The lateral flow testing device 98 includes a porous strip 64 divided into a sample receiving portion 100, a conjugate portion 102, a test portion 104 and a wick portion 106. The porous strip 64 is received into a base 108. A lid 110 is attached to the base 108 to secure the porous strip 64 and cover parts of the porous strip 64 that do not require exposure. The lid 110 includes a sample receiving window 112, which exposes part of the sample receiving portion 100 to define the liquid sample receiving region 80. The lid and base 108, 110 are made from a polymer such as, for example, polycarbonate, polystyrene, polypropylene or similar materials.

The base 108 includes a recess 114 into which a pair of light emitting diode arrays 94 are received. Each light emitting diode array 94 may be configured as described hereinbefore. The lid 110 includes a recess 116 into which a pair of photodetectors 16 are received. The photodetectors 16 may take the form of photodiodes. One pair of a light emitting diode array

94 and a photodiode 16 are arranged on opposite sides of a testing region 66 of the porous strip 64. The second pair of a light emitting diode array 94 and a photodiode are arranged on opposite sides of a control region 78 of the porous strip 64. Slit members 90 separate the light emitting diode arrays 94 from the porous strip 64 to define narrow slits 88 with widths in the range between 300 μm to 500 μm inclusive. The slit members 90 define slits 88, which extend transversely across the width of the porous strip 64. For example, if the porous strip 64 extends in a first direction x and has a thickness in a third direction z, then the slits 88 extend in a second direction y. Further slit members 90 define slits 88 which separate the photodiodes 16 from the porous strip 64. The slits 88 may be covered by a thin layer of transparent material to prevent moisture entering into the recesses 114, 116. Material may be considered to be transparent to a particular wavelength λ if it transmits more than 75%, more than 85%, more than 90% or more than 95% of the light at that wavelength λ. A diffuser 92 may optionally be included between each light emitting diode array 94 and the corresponding slit 88.

A liquid sample 118 is introduced to the sample receiving portion 100 through the sample receiving window 112 using, for example, a dropper 120 or similar implement. The liquid sample 118 is transported along the liquid transport path 78 towards the second end 84 by a capillary, or wicking, action of the porosity of the porous strip 100, 102, 104, 106. The sample receiving portion 100 of the porous strip 28 is typically made from fibrous cellulose filter material.

The conjugate portion 102 has been pre-treated with at least one particulate labelled binding reagent for binding an analyte that is being tested for, to form a labelled particle-analyte complex (not shown). A particulate labelled binding reagent is typically, for example, a nanometre- or micrometre-sized label particle 68 that has been sensitised to specifically bind to the analyte. The particles provide a detectable response, which is usually a visible optical response such as a particular colour, but may take other forms. For example, particles may be used that are visible in infrared, which fluoresce under ultraviolet light, or which are magnetic. Typically, the conjugate portion 102 will be treated with one type of particulate labelled binding reagent to test for the presence of one type of analyte in the liquid sample 118. However, lateral flow devices 98 may be produced that test for two or more analytes using two or more particulate labelled binding reagents concurrently. The conjugate portion 102 is typically made from fibrous glass, cellulose or surface modified polyester materials.

As the flow front 86 moves into the test portion 104, labelled-particle-analyte complexes and unbound label particles are carried along towards the second end 84. The test

portion 104 includes one or more testing regions 66 and control regions 78, which are monitored by a corresponding light emitting diode array 94 and photodiode 16 pair. A testing region 66 is pre-treated with an immobilised binding reagent, which specifically binds the label particle-target complex and which does not bind the unreacted label particles. As the labelled-particle-analyte complexes are bound in the testing region 66, the concentration of the label particles 68 in the testing region 66 increases. The concentration increase may be monitored by measuring the absorbance of the testing region 66 using the corresponding light emitting diode array 94 and photodiode 16. The absorbance of the testing region 66 may be measured once a set duration has expired since the liquid sample 118 was added. Alternatively, the absorbance of the testing region 66 may be measured continuously or at regular intervals as the lateral flow strip is developed.

To provide distinction between a negative test and a test that has simply not functioned correctly, a control region 78 is often provided between the testing region 66 and the second end 84. The control region 78 is pre-treated with a second immobilised binding reagent which specifically binds unbound label particles and which does not bind the labelled-particle-analyte complexes. In this way, if the lateral flow testing device 98 has functioned correctly and the liquid sample 118 has passed through the conjugate portion 102 and test portion 104, the control region 78 will exhibit an increase in absorbance. The absorbance of the control region 78 may be measured by the second pair of a light emitting diode array 94 and a photodiode 16 in the same way as for the testing region 66. The test portion 104 is typically made from fibrous nitrocellulose, polyvinylidene fluoride, polyethersulfone (PES) or charge modified nylon materials. All of these materials are fibrous, and as such the sensitivity of the absorbance measurements may be improved by subtracting the measurements obtained using the second wavelength λ_2 to correct for inhomogeneity of the porous strip 64 material.

The wick portion 106 provided proximate to the second end 84 soaks up liquid sample 118, which has passed through the test portion 104 and helps to maintain through-flow of the liquid sample 118. The wick portion 106 is typically made from fibrous cellulose filter material.

Although not shown in Fig. 29, the self-contained lateral flow testing device 98 also includes the controller 36, which is mounted in the base 108 or the lid 110. The lateral flow testing device 98 may also include one or more output devices 38 integrated into the base 108 or lid 110 such that a user may see the output device(s) 38 in use.

Illustrative experimental data

The preceding discussion may be better understood with reference to illustrative experimental data. The apparatus 10 described herein is not limited to the specific conditions and samples used to obtain illustrative experimental data.

Referring to Figures 1, 7 and 30, test samples were prepared by depositing test lines 122 of gold nanoparticle ink onto blank porous strips 64 made from nitrocellulose. Gold nanoparticles are one type of labelling particle 68 used in lateral flow test strips 28. Each test line 122 was deposited using gold nanoparticle ink of a different solution optical density. The solution optical density of the gold nanoparticle ink, OD, may be considered to be a measure of the density of gold nanoparticles in the corresponding test line 122. For example, the test sample shown in Fig. 30 included eight test lines 122a, ... , 122h deposited using gold nanoparticle inks having solution ODs of 15, 100, 25, 7, 5, 2, 0.8 and 0.1 respectively. Each test line 122a, ... , 122h is 1.0±0.5 mm wide and the centre-to-centre spacing of test lines 122a, ... , 122h is 2.0±0.5 mm.

Referring also to Fig. 31, absorbance measurements were conducted for a blank nitrocellulose porous strip 64 and the variations of optical density ΔOD are shown as a function of position x along the blank porous strip 64. In this example, substantially equal beam profiles 48, 50 were provided using an integrating sphere (not shown) and first and second light emitters 12, 14 in the form of light emitting diodes were coupled to a first port of the integrating sphere, and the light from a second port of the integrating sphere illuminated the blank strip. The photodetector 16 was disposed on the other side of the blank porous strip 64 and optical densities (absorbance) were measured in transmission. The first light emitting diode 12 emitted green light 18 (dashed line) and the second light emitting diode 14 emitted light 20 at near infra-red (NIR) wavelengths (dotted line). The beam profiles 48, 50 were substantially uniform and substantially equal due to multiple reflections inside the integrating sphere (not shown).

The measurements were obtained by moving the blank nitrocellulose porous strip 64 through a gap between the photodiode 16 and the light emitting diodes 12, 14 and recording the output signal of the photodiode 16 as a function of the distance. The blank nitrocellulose porous strip 64 was moved using a stepper motor.

The measurements were obtained by illuminating the green light emitting diode 12 and the NIR light emitting diode 14 at different times.

It may be observed that the inhomogeneities in the transmittance of the blank nitrocellulose strip 64 are reproducible over a wide wavelength range, since the

measurements at the green and near-infrared wavelengths are substantially similar. Subtracting measurements made at a second wavelength may substantially correct for the background inhomogeneity of the porous strip 64. For example, for absorbance measurements $A_1(x)$ obtained with a green light emitting diode, the range of ΔOD was more than 0.008, whereas the difference $A_1(x) - A_2(x)$ (solid line) has a range of ΔOD of ≈ 0.001. This represents a substantial decrease in the background signal, and consequently lower optical densities of labelling particles 68 may be resolvable.

The gold nanoparticles used for the test lines 122, which are commonly used as labelling particles 68 in lateral flow test strips 28, are known to absorb strongly in the green, but only relatively weakly in the infrared. Therefore, one example of an apparatus as described herein may compare the difference in signals obtained using green and near-infrared organic light emitting diodes. The same approach may also be used with an imaging camera approach.

Referring also to Fig. 32, a test sample including test lines 122 (containing, for example, gold nanoparticles) was measured using green light (dashed line) and NIR light (dotted line). The test sample used included test lines 122 deposited using inks having solution optical densities of 0.006, 0.01, 0.03, 0.06 and 0.1. The corrected signal (solid line) obtained by subtracting the NIR signal from the green and NIR combined signal displays reduced background variability, which allows the signals resulting from the test lines 122 to be resolved. It is observed that the test lines 122 deposited using inks having solution optical densities of 0.006,0.01, 0.03, 0.06 and 0.1 would be effectively unresolvable using green light alone, yet can be readily distinguished using the corrected signal.

Referring also to Fig. 33 a comparison is shown between measurements using the difference between absorbance ΔOD at combined green and NIR wavelengths (solid line), absorbance ΔOD measured using only the green light (dashed line) and absorbance ΔOD measured using a commercially available handheld lateral flow device reader (chained line). The commercial handheld reader was an Optricon (RTM) Cube-Reader (RTM). The different measurement series have been shifted in the y-axis direction to improve readability of the figure. It may be observed that the corrected, dual wavelength measurement allows resolution of the fainter lines corresponding to inks having solution optical densities of OD = 0.1 and lower.

Referring also to Fig. 34, the limiting optical density (LOD), i.e. the smallest resolvable change in absorbance as a function of gold nanoparticle density was determined using test line 122 for the difference between absorbance ΔOD at combined green and NIR

wavelengths and at NIR wavelengths alone (solid line), absorbance ΔOD measured using only the green light (dashed line), absorbance ΔOD measured using a commercially available benchtop lateral flow device reader (chained line) and the absorbance ΔOD measured using the handheld lateral flow device reader (chained line). The commercial benchtop reader was a Qiagen (RTM) ESEQuant (RTM) lateral flow reader. The LOD of ~0.01 to 0.02 (DOD) observed with commercial readers or single wavelength absorbance measurements is limited by inhomogeneity of the nitrocellulose porous strip 64, which masks test lines 122 printed on the porous strip 64. For the dual wavelength (solid line) measurements, the effect of nitrocellulose thickness variation can be reduced down to LOD ~1.4×10^{-3} with the use of two LEDs, or to a LOD of ~5×10^{-4} using an integrating sphere to illuminate the test lines 122.

Referring also to Fig. 35, experimental data obtained by scanning a lateral flow test strip 28 for performing a Troponin assay are shown for the commercially available handheld reader (chained line), the commercially available benchtop reader (dotted line), a simple transmission reader (dashed line) using a green light emitting diode arranged opposite to an photodiode, and an example of the apparatus 10 (solid line). The apparatus 10 used in this case operated in transmission mode, the first light emitter 12 was a green light emitting diode and the second light emitter 14 was a near-infrared light emitting diode. The different measurement series have been shifted in the y-axis direction to improve readability of the figure.

It may be observed that measurements obtained using the example apparatus 10 have substantially reduced background noise compared to a single wavelength organic light emitting diode/organic photodiode pair. Although the test region 66 and control region 78 are well resolved in this illustrative data, the reduced background noise may allow the apparatus 10 to detect lower concentrations than the single wavelength (green only) device.

Referring also to Fig. 36, measurements and modelling results on the absorbance variation ΔOD of a blank nitrocellulose porous strip 64 are shown. The y-axis (ΔOD) is optical density variation along the porous strip 64, i.e. the maximum - minimum of ΔOD for the porous strip 64. The increasing x-axis direction corresponds to increasing similarity of the first and second beam profiles 48, 50.

Data corresponding to three experimental measurements are shown (triangles, solid line is a fitting line). The left-most, or least equal point corresponds to ΔOD measured with no correction using the second emitter, i.e. the NIR wavelengths. The rightmost, or most equal point corresponds to ΔOD measured using an integrating sphere (not shown). The third

(middle) experimental point corresponds to ΔOD measured using a simple (side-by-side) pair of inorganic LEDs emitting green and NIR light respectively. The values of ΔOD measured using a pair of light emitting diodes is three times higher than ΔOD measured using the integrating sphere (not shown), which may be attributable to a degree of difference between the first and second beam profiles 48, 50. However, the measurements using the pair of light emitting diodes are also ~4.5 times lower than ΔOD measured with only the green wavelengths.

Data corresponding to the results of modelling of the ΔOD achievable for different beam profiles of first (green) and second (NIR) emitters 12, 14 are also shown (open circles, dashed line is a fitting line). Modelling was performed by convolving experimentally measured ΔOD data corresponding to a blank porous strip 64 with different beam profiles A, B, C and D shown schematically in Fig. 36. A first set of beam profiles A corresponds to single wavelength measurement (i.e. NIR illumination profile is absent), and represents a minimum uniformity (or maximum difference). A set of beam profiles D corresponds to identical first and second beam profiles 48, 50, and represents maximum uniformity. The beam profiles Band C represent intermediate situations in which the first and second beam profiles 48, 50 exhibit differences.

The measured data corresponding to the integrating sphere (not shown) is larger than the modelled value of zero. This may be attributable to the beam profiles not being perfectly identical, or may possibly be attributable to deviations from the simple nitrocellulose thickness variation model, which is employed for correction by subtracting the absorbance values measured using the second colour from the absorbance values measured using the combination of the first and second colours. The value of ΔOD ~5e-4 for the integrating sphere (not shown) measurements is nonetheless substantially reduced in comparison to the single wavelength value $\Delta OD > 0.06$.

Modifications

It will be appreciated that many modifications may be made to the embodiments hereinbefore described. Such modifications may involve equivalent and other features which are already known in the design, manufacture and use of analytical test devices and which may be used instead of or in addition to features already described herein. Features of one embodiment may be replaced or supplemented by features of another embodiment.

Although examples have been described in relation to lateral flow test strips 28, the present methods and apparatus can also be used with other types of sample 26 with minimal modifications.

For example, an apparatus 10 may include an optical path 22, which has a sample receiving portion 24 adapted to receive a microfluidic channel or channels (not shown) perpendicularly to the optical path 22. The microfluidic channel(s) (not shown) can be in the form of one or more lengths of tubing or one or more channels machined into polymeric material. The microfluidic channel(s) (not shown) may be dimensioned to enable capillary transport of a liquid sample. Measurements at the second wavelength λ_2 can be used to compensate for scattering or absorption from defects of or contamination on the walls of the microfluidic channel(s) (not shown).

Expansion to more than one analyte

For some tests, it may be desirable to detect and quantify the concentrations of two or more analytes in the same sample concurrently. Additionally or alternatively, many samples which may contain one or more analytes of interest may be coloured, for example blood. Other samples may display a range of colours depending on a concentration of, for example, urine or other biologically derived substances or by-products.

The methods and apparatus described hereinbefore can be adapted to detect two or more analytes in a single sample, whether the sample is coloured or substantially clear.

In general, concentrations of N-1 different analytes may be determined whilst correcting for inhomogeneity of a porous strip 64, or other such source of background scattering, by sequentially illuminating the sample receiving portion 24 using N different wavelengths. Each of the N wavelengths may be provided by a corresponding set of one or more light emitters. The controller 36 may illuminate each of the N sets of one or more light emitters according to a sequence, such that only one set of emitters is emitting light at any given time. Some of the N-1 analytes may not be of direct interest, for example, some of the N-1 analytes may be substances or compositions which provide the colouration of a sample. However, accounting for analytes providing coloration of a sample can allow more accurate detection and quantification of analytes of interest contained in the sample.

Referring also to Figs. 37A and 37B, the second or third arrangements (Figs. 26, 28) for coupling light into an optical path 22, using an LED array 94, may be readily adapted for more than two sets of light emitters.

Referring in particular to Fig. 37A, an LED array 94 may include a number of pixels 124, each of which includes a first light emitter 12, a second light emitter 14 and a third emitter 126 in the form of LED sub-pixels. This arrangement may be combined with the arrangement illustrated in Fig. 26 such that the second light emitter 14 overlies the first light emitter 12 and the third light emitter 126 overlies the second light emitter 14.

Referring in particular to Fig. 37B, an LED array 94 may include a number of pixels 128, each of which includes a first light emitter 12, a second light emitter 14, a third emitter 126 and a fourth emitter 130 in the form of LED sub-pixels. This arrangement may be combined with the arrangement illustrated in Fig. 26 such that the second light emitter 14 overlies the first light emitter 12, the third light emitter 126 overlies the second light emitter 14, and the fourth light emitter 130 overlies the third light emitter 126.

Method of extracting analyte concentrations

A sample may in general include N-1 analytes. The method of extracting concentrations for the N-1 analytes includes sequential illumination using light emitted from N sets of one or more light emitters. Each set of light emitters emits light centred around a different wavelength. The number N-1 of analytes is one less than the number N of sets of emitters to allow correction for scattering from the background inhomogeneities of a porous strip 64, microfluidic channel(s), or any similar source of background scattering. Some of the analytes may be substances or compositions which give rise to the coloration of a sample. Quantifying substances or compositions which give rise to sample colouration may not be of direct interest, however, it can allow more sensitive detection and/or more accurate quantification of one or more analytes of interest contained within a coloured sample such as urine, blood, wine, cooking oils and so forth.

For light of the n^{th} wavelength λ_n out of N-1 wavelengths, the absorbance through the sample receiving portion 24 is denoted $A(\lambda_n)$. In general, the absorbance $A(\lambda_n)$ corresponds to a range of wavelengths which spans the n^{th} wavelength λ_n. For example, $A(\lambda_n)$ may be calculated based on the integral of intensity across a wavelength range.

The total absorbance A(An) may be viewed as the sum:

$$A(\lambda_n) = s(\lambda_n) + \sum_{i=1}^{N-1} \varepsilon_i(\lambda_n) c_i \quad (5)$$

in which $s(\lambda_n)$ is the absorbance at the n^{th} wavelength λ_n due to scattering from background inhomogeneity of the porous strip 64 or other source of background scattering, c_i is the concentration of the i^{th} analyte out of N-1 analytes and $\varepsilon_i(\lambda_n)$ is a coefficient relating the concentration c_i to the absorbance of the i^{th} analyte out of N-1 analytes at the n^{th} wavelength λ_n. The concentrations c_i are expressed in units of absorbance (optical density) corresponding to a reference wavelength, for example, the 1st wavelength λ_1. Thus, the coefficients $\varepsilon_i(\lambda_n)$ are each a ratio of the absorbance of the i^{th} analyte between the 1^{st} and n^{th} wavelengths λ_1, λ_2.

Measurement of absorbance may be direct, for example, in a transmission geometry by obtaining measurements with and without a sample 26 present within the sample receiving portion 24.

Alternatively, when the sample 26 is a lateral flow test strip 28, the absorbance values $A(\lambda_n)$ may be obtained from an image or scan covering a testing region 66 and surrounding regions of untreated porous strip 64. Alternatively, the absorbance values $A(\lambda_n)$ may be obtained by reference to a measurement of transmission/reflection obtained before a liquid sample is introduced to the lateral flow test strip.

Referring also to Figs. 38 to 49 a method of obtaining absorbance values $A(\lambda_n)$, also referred to as absorbance "fingerprints" from a lateral flow strip 66 is explained with reference to theoretically modelled organic photodetector (OPD) signals.

Referring in particular to Fig. 38, the model for generating theoretical OPD signals is based on a representative OPD absorption profile 130, which is a function of wavelength λ, in combination with representative LED emission profiles 132, 134, 136, which are each functions of wavelength λ. The first LED emission profile 132 corresponds to a typical green OLED, the second LED emission profile 134 corresponds to a typical red OLED, and the third LED emission profile 136 corresponds to a typical near infrared (NIR) OLED.

Referring in particular to Fig. 39, further inputs to the model for generating theoretical OPD signals include representative absorption profiles 138, 140, 142 for gold nanoparticles, a blue dye and nitrocellulose fibres 70 respectively. The first absorption profile 138 is a wavelength λ dependent function corresponding to the absorbance of gold nanoparticles. The second absorption profile 140 is a wavelength λ dependent function corresponding to the absorbance of the blue dye. The third absorption profile 142 is a wavelength λ dependent function corresponding to the absorbance of nitrocellulose fibres 70 forming a porous strip 64.

Referring in particular to Fig. 40, further inputs to the model for generating theoretical OPD signals include assumed concentration profiles 144, 146, 148 of gold nanoparticles, blue

dye and nitrocellulose fibres respectively. In the model, it is assumed that the lateral flow test strip 28 is back-illuminated and that the light 10 transmitted through the lateral flow test strip 28 is imaged using a number of OPDs forming an image sensor 74. The x-axis of Fig. 40 is distance in units of pixels of the image sensor 74. Equivalent information could be modelled or measured by scanning a single OPD along the length of the lateral flow test strip 28 (in which case the distance units would be, for example, mm rather than pixels). The first assumed concentration profile 144, plotted against the primary Y-axis (range 0 to 1.2), corresponds to a position dependent concentration of gold nanoparticles. The second assumed concentration profile 146, plotted against the primary Y-axis (range 0 to 1.2), corresponds to a position dependent concentration of blue dye. The third assumed concentration profile 148, plotted against the secondary Y-axis (range 0.9 to 1.02), corresponds to a position dependent concentration of nitrocellulose fibres 70. The third assumed concentration profile 148 includes fluctuations of the nitrocellulose fibre 70 concentration (meaning the density such, for example, fibre volume fraction) with position along the porous strip 64. Also indicated in Fig. 40 is an illumination profile 150 representing a varying illumination intensity at different positions along the length of a lateral flow test strip 28. The illumination profile 150 is assumed to be the same for modelled green, red and NIR OLEDS.

Referring in particular to Fig. 41, simulated OPD signals 152, 154, 156 corresponding to green, red and NIR OLEDs respectively may be estimated based on the emission profiles 132,134,136, illumination profile 150, concentration profiles 144, 146, 148 and absorbance profiles 138, 140, 142. Noise generated based on pseudo-random numbers was added to simulated OPD signals 152, 154, 156 to simulate OPD noise.

Referring in particular to Fig. 42, a simulated green OPD signal 152b is shown, which is calculated for a case in which the blue dye concentration profile 146 is zero everywhere.

As a first step in extracting green absorbance values, a slowly varying background profile 158, plotted against the primary Y-axis (range 0 to 4500), is fitted to the simulated green OPD signal 152b, plotted against the primary Y-axis (range 0 to 4500). The background profile 158 represents an approximation to the average intensity, T_o, transmitted by the nitrocellulose fibres 70 of the porous strip 64. The simulated green OPD signal 152b represents the transmitted intensity, T, through the porous strip 64 and the gold nanoparticles. A normalised green transmission profile 160 is calculated as T/T_o, plotted against the secondary Y-axis (range 0 to 1.2). It may be observed that the normalised green transmission profile 160 retains fluctuations resulting from the point-to-point fluctuations in the nitrocellulose fibre 70 concentration profile 148.

Referring in particular to Fig. 43, a simulated NIR OPD signal156b is shown which is calculated for a case in which the blue dye concentration profile 146 is zero everywhere. As a first step in extracting IR absorbance values, a slowly varying background profile 158, plotted against the primary Y-axis (range 0 to 4500) is fitted to the simulated NIR OPD signal 156b, plotted against the primary Y-axis (range 0 to 4500). Given the present modelling assumptions, the background profile 158 is the same for green and NIR data, however, in practice the background profile 158 may vary for different light emitters 12, 14, 126. A normalised NIR transmission profile 162 is calculated as T/T_o, plotted against the secondary Y-axis (range 0 to 1.2).

Referring in particular to Figs. 44 and 45, the normalised transmission profiles 160, 162 are converted to absorbance values according to the formula $A = -\log_{10}(T/T_o)$. A first simulated absorbance profile 164 is obtained corresponding to the green OLED and comprising green absorbance values $A_G(x)$ at pixel position x. A second simulated absorbance profile 166 comprising NIR absorbance values $A_{NIR}(x)$ is obtained corresponding to the NIR OLED. The absorbance values calculated in this fashion are more strictly viewed as changes in absorbance relative to a perfectly uniform nitrocellulose strip having the same concentration (density/fibre volume fraction) as the average concentration (density/fibre volume fraction) of the porous strip 64. Such values may also be referred to as delta-optical density or ΔOD values. Although the calculation has been outlined with reference to a transmission geometry, analogous calculations may be performed for a reflection geometry.

Referring in particular to Figs. 46 and 47, the estimate of absorbance fingerprint values is illustrated. Both of Figs. 46 and 47 are scatter plots of the green simulated absorbance profile 164 plotted against the X-axis and the NIR simulated absorbance profile 166 against the Y-axis. Each data point 168 represents a pair of a green absorbance value $A_G(x)$ and a NIR absorbance value $A_{NIR}(x)$ at a particular position x of a simulated lateral flow device 28.

Two distinct correlations may be observed in Figs. 46 and 47 having different slopes. A first correlation is most easily seen in Fig. 47 and has approximately unitary slope. This corresponds to the nitrocellulose fibres, the interaction of which with green and NIR wavelengths is essentially the same in the model, and also in practice. By examining the extremal data points 170 of the first correlation, a pair of absorbance values attributable to the fluctuations in the nitrocellulose fibre 70 concentration profile 148, also referred to as the absorbance "fingerprint" of the nitrocellulose fibres 70, may be estimated as $A_{NC}(\lambda_G) \approx 0.01$, $A_{NC}(\lambda_{NIR}) \approx 0.01$, or alternately $A_{NC} \approx (0.01, 0.01)$ using a vector notation.

A second correlation is most easily seen in Fig. 46 and has a much shallower slope representing the relatively strong response of the green light to the gold nanoparticles in comparison to the relatively weak response of the NIR light to the gold nanoparticles. In a similar fashion to the first correlation, for the second correlation an absorbance "fingerprint" corresponding to the gold nanoparticles may be estimated as $A_{NC}(\lambda_G) \approx 1$, $A_{NC}(\lambda_{NIR}) \approx 0.02$, or alternately $A_{AU} \approx (1, 0.02)$ using a vector notation, based on the extremal points 170 and subtracting the signal due to variations in the nitrocellulose fibre 70 concentration profile 148. This method of estimating absorbance fingerprints may be extended to three or more wavelength bands of light, for example, by using 3D plots or N-dimensional analysis methods.

The method of obtaining absorbance values described with reference to the simulated OPD signals 152, 154, 156 is expected to be equally applicable to measured data, whether obtained in transmission or reflection geometries.

Other methods of obtaining absorbance values may be used. Absorbance values measured according to any suitable method may be analysed in accordance with equations (6) to (13), (10b) to (13b) and/or equation (10c) as set out hereinafter.

In the general case, for light of the n^{th} wavelength λ_n out of N-1 wavelengths, the absorbance (however measured) through the sample receiving portion 24 is denoted $A(\lambda_n)$. If the absorbance $A(\lambda_n)$ is measured at each wavelength λ_n, then an absorbance column vector may be defined as:

$$\mathbf{A} = \begin{pmatrix} A(\lambda_1) \\ A(\lambda_2) \\ \vdots \\ A(\lambda_N) \end{pmatrix} \tag{6}$$

For example, the absorbance values $A(\lambda_n)$ may be absorbance fingerprint values obtained as described hereinbefore with reference to Figs. 46 and 47.

Similarly, a concentration column vector may be defined as:

$$\mathbf{c} = \begin{pmatrix} c_1 \\ c_2 \\ \vdots \\ c_{N-1} \\ c_S \end{pmatrix} \tag{7}$$

in which the concentration c_s corresponding to the background absorbance $s(\lambda_n)$ is a dummy concentration which is set to the background absorbance at the reference wavelength, for example $s(\lambda_1)$ at the 1st wavelength A_1. The use of the dummy concentration in equivalent units to the analyte concentrations c_i maintains appropriate scaling of measured absorbance values throughout the calculations described hereinafter. In practice, as explained hereinafter, calibration of the method typically includes obtaining measurements of the background scattering without any analytes, so obtaining a suitable value for the dummy concentration c_s is not problematic. The absorbance vector **A** may be expressed in terms of the coefficients $\varepsilon_i(\lambda_n)$, background absorbance $s(\lambda_n)$ and concentration vector **c** using a matrix equation:

$$\begin{pmatrix} A(\lambda_1) \\ A(\lambda_2) \\ \vdots \\ A(\lambda_{N-1}) \\ A(\lambda_N) \end{pmatrix} = \begin{pmatrix} \varepsilon_1(\lambda_1) & \varepsilon_2(\lambda_1) & \cdots & \varepsilon_{N-1}(\lambda_1) & s(\lambda_1) \\ \varepsilon_1(\lambda_2) & \varepsilon_2(\lambda_2) & \cdots & \varepsilon_{N-1}(\lambda_2) & s(\lambda_2) \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ \varepsilon_1(\lambda_{N-1}) & \varepsilon_2(\lambda_{N-1}) & \cdots & \varepsilon_{N-1}(\lambda_{N-1}) & s(\lambda_{N-1}) \\ \varepsilon_1(\lambda_N) & \varepsilon_2(\lambda_N) & \cdots & \varepsilon_{N-1}(\lambda_N) & s(\lambda_N) \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \\ \vdots \\ c_{N-1} \\ c_s \end{pmatrix} \quad (8)$$

A=Mc

in which **M** is a square matrix having coefficients $M_{ij} = \varepsilon_j(\lambda_i)$ for $1 \leq j \leq N\text{-}1$ and $M_{iN} = s(\lambda_i)$. By inverting the matrix **M**, unknown concentrations c_i of analytes may be determined from the measured absorbance values $A(\lambda_n)$ at each wavelength λ_n:

$$\mathbf{c} = \mathbf{M}^{-1}\mathbf{A} \quad (9)$$

In order to apply Equation (9), it is necessary to know the coefficients M_{ij} of the matrix **M**, so that the inverse $\mathbf{M}^{-1}$ may be calculated. When evaluating Equation (9), a value calculated corresponding to the background scattering "concentration" would ideally be equal to the dummy concentration c_s. In practical circumstances, the value calculated corresponding to the background scattering "concentration" may deviate from the dummy concentration c_s. The size of the deviation may provide an indication of variations between different porous strips 64, microfluidic channels, and so forth. A large deviation may provide an indication of possible problems with a particular sample or with the calibration of the matrix **M** coefficients M_{ij}.

The coefficients M_{ij} of the matrix **M** may be determined in advance from experimental measurements using samples with known concentrations c_i of each analyte. A measured set of absorbance values $A_1(\lambda_n)$ with a first calibration sample represented by the

reference absorbance vector $\mathbf{A}_1$ and the corresponding concentrations c_i^1 by the calibration concentration vector $\mathbf{c}_1$. In general, for N wavelengths λ_1, ..., λ_N, a number N of calibration samples and measurements are required. A fingerprint matrix F is defined using the set of reference absorbance vectors $\mathbf{A}_1$, ..., $\mathbf{A}_N$ by setting the coefficients of each reference absorbance vector $\mathbf{A}_1$, ..., $\mathbf{A}_N$ as the coefficients for a corresponding column of the fingerprint matrix F:

$$F = \begin{pmatrix} \uparrow & \uparrow & & \uparrow \\ \mathbf{A}_1 & \mathbf{A}_2 & \cdots & \mathbf{A}_N \\ \downarrow & \downarrow & & \downarrow \end{pmatrix}$$

$$F = \begin{pmatrix} A_1(\lambda_1) & A_2(\lambda_1) & \cdots & A_N(\lambda_1) \\ A_1(\lambda_2) & A_2(\lambda_2) & \cdots & A_N(\lambda_2) \\ \vdots & \vdots & \ddots & \vdots \\ A_1(\lambda_N) & A_2(\lambda_N) & \cdots & A_N(\lambda_N) \end{pmatrix} \quad (10)$$

The entries of the fingerprint matrix F may constitute absorbance fingerprint values estimated as described in relation to Figs. 46 and 47. However, the entries need not be absorbance fingerprint values, and in general the entries of the fingerprint matrix F may be absorbance values measured or obtained according to any suitable method. The corresponding calibration concentration vectors $\mathbf{c}_1$, …, $\mathbf{c}_N$ may be set as the columns of a calibration matrix C:

$$C = \begin{pmatrix} \uparrow & \uparrow & & \uparrow \\ \mathbf{c}_1 & \mathbf{c}_2 & \cdots & \mathbf{c}_N \\ \downarrow & \downarrow & & \downarrow \end{pmatrix} \quad (11)$$

and the fingerprint matrix F and calibration matrix C are related according to:

$$F=MC \quad (12)$$

The coefficients M_{ij} of the matrix M can then be calculated as $M = FC^{-1}$, and the coefficients of the inverse matrix M^{-1} can be calculated as $M^{-1} = C\ F^{-1}$. Thus, a set of unknown concentrations c_i represented by a concentration vector $\mathbf{c}$ may be recovered using CF^{-1} as a deconvolution matrix (also referred to as a de-mixing matrix) for the measured absorbance values $A(\lambda_n)$ represented by an absorbance vector $\mathbf{A}$ according to:

$$\mathbf{c} = CF^{-1}\mathbf{A} \quad (13)$$

In this way, a set of unknown concentrations $\mathbf{c}_1$, …, $\mathbf{c}_{N-1}$ of N-1 analytes may be reconstructed from measurements of the absorbance values $A(\lambda_1)$, ... , $A(\lambda_N)$ at N wavelengths, λ_1, ..., λ_N emitted from the corresponding N sets of light emitters. The absorbance values $A(\lambda_1)$, ... , $A(\lambda_N)$ may be in the form of absorbance fingerprint values obtained as described in relation to Figs. 46 and 47, or may be absorbance values obtained or estimated using any other suitable method.

The actual physical concentration or number density of each analyte, for example in units of number.cm^{-3}, can be estimated from the reconstructed concentrations c_1, …, c_{N-1} (i.e. absorbance values at the reference wavelength) using the Beer-Lambert law with the path length through the sample receiving portion 24 and an attenuation coefficient for the i^{th} analyte at the reference wavelength (for example the 1st wavelength λ_1). If the attenuation coefficient for the i^{th} analyte is not known at the reference wavelength, then the coefficients $M_{ij} = \varepsilon_j(\lambda_i)$ (calculated by inverting the deconvolution (de-mixing) matrix to obtain $M = FC^{-1}$) may be used to convert the concentration (absorbance) c_i at the reference wavelength to an absorbance at a wavelength for which the attenuation coefficient is known.

Equivalently, since $\mathbf{A}^T = \mathbf{c}^T M^T$, an alternative fingerprint matrix G may be defined by setting the coefficients of each reference absorbance vector $\mathbf{A}_1$, ..., $\mathbf{A}_N$ as the coefficients for a corresponding row of the alternative fingerprint matrix G:

$$G = \begin{pmatrix} \leftarrow & \mathbf{A}_1 & \rightarrow \\ \leftarrow & \mathbf{A}_2 & \rightarrow \\ \leftarrow & \vdots & \rightarrow \\ \leftarrow & \mathbf{A}_N & \rightarrow \end{pmatrix} \qquad (10b)$$

and the corresponding calibration concentration vectors $\mathbf{c}_1$, …, $\mathbf{c}_N$ may be set as the rows of an alternative calibration matrix D:

$$D = \begin{pmatrix} \leftarrow & \mathbf{c}_1 & \rightarrow \\ \leftarrow & \mathbf{c}_2 & \rightarrow \\ \leftarrow & \vdots & \rightarrow \\ \leftarrow & \mathbf{c}_N & \rightarrow \end{pmatrix} \qquad (11b)$$

and the alternative fingerprint matrix G and alternative calibration matrix D are related according to:

$$G=DM^T \quad (12b)$$

Thus, a set of unknown concentrations c_i represented by a concentration vector **c** may equivalently be recovered using $G^{-1}D$ as a deconvolution matrix for the measured absorbances $A(\lambda_n)$ represented by an absorbance vector $\mathbf{A}^T$ according to:

$$\mathbf{c} = \mathbf{A}^T G^{-1} D \quad (13b)$$

It is preferable for each analyte to have an absorbance peak which corresponds to one of the illumination wavelengths $\lambda_1, ..., \lambda_N$. It is preferred for the absorbance peaks corresponding to the N -1 types of analyte to avoid substantially overlapping. If the absorbance spectra of analytes are too similar, this may lead to errors in determining the analyte concentrations c_j. In practice, the number of analytes may be limited by the distinguishability of spectra.

In some examples, it may be convenient to normalise absorbance values with respect to a single reference calibration value, for example, $A_1(\lambda_1)$. For example, with normalisation relative to $A_1(\lambda_1)$, a normalised fingerprint matrix F_n may be expressed as:

$$F = \begin{pmatrix} 1 & \frac{A_2(\lambda_1)}{A_1(\lambda_1)} & \cdots & \frac{A_N(\lambda_1)}{A_1(\lambda_1)} \\ \frac{A_1(\lambda_2)}{A_1(\lambda_1)} & \frac{A_2(\lambda_2)}{A_1(\lambda_1)} & \cdots & \frac{A_N(\lambda_2)}{A_1(\lambda_1)} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{A_1(\lambda_N)}{A_1(\lambda_1)} & \frac{A_2(\lambda_N)}{A_1(\lambda_1)} & \cdots & \frac{A_N(\lambda_N)}{A_1(\lambda_1)} \end{pmatrix} \quad (10c)$$

Each of Equations 6 to 13 and Equations 10b to 13b may be normalised in this manner, to allow absorbance and concentration values to be expressed as fractions with respect to a reference calibration value, for example $A_1(\lambda_1)$.

Determination of concentration and calibration matrix values

The calibration is simplified in the case that pure (or substantially pure) samples of the N-1 different analytes having known concentration c_i are available for testing in reference conditions, for example, supported on a porous strip 64. One of the calibration samples should correspond to only the background scattering $s(\lambda_n)$, e.g. the porous strip 64. In this case, determining the calibration matrix is simplified, since the determination of the concentration c_i for each analyte at the reference wavelength can be simplified. For example,

if the N^{th} calibration sample includes only the background scattering, then a calibration concentration c_i^o of the i^{th} calibration sample ($1 \leq i \leq N-1$), which includes the pure (or substantially pure) i^{th} analyte, using the 1^{st} wavelength λ_1, as the reference wavelength, may be approximated as:

$$c_i^o = A_i(\lambda_1) - A_N(\lambda_1) \quad (14)$$

In which $A_i(\lambda_1)$ is the measured absorbance of the pure or substantially pure sample of the i^{th} analyte at the 1st wavelength. The calibration matrix C may be written as:

$$C = \begin{pmatrix} c_1^o & 0 & \cdots & 0 & 0 \\ 0 & c_2^o & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & c_{N-1}^o & 0 \\ c_s & c_s & c_s & c_s & c_s \end{pmatrix} \quad (15)$$

In which the dummy concentration $c_s = A_N(\lambda_1)$. In this special case, the calculation of the deconvolution matrix CF^{-1} may be simplified.

The calibration matrix C and the calculation of the deconvolution matrix CF^{-1} may be simplified further if the absorbance of pure (or substantially pure) samples of the different analytes may be tested under conditions in which the background scattering is very low or negligible. Under these optimum conditions, the calibration matrix is diagonal, and reference concentration values may be directly set to measured absorbance values at the reference wavelength:

$$C = \begin{pmatrix} A_1(\lambda_1) & 0 & \cdots & 0 & 0 \\ 0 & A_2(\lambda_1) & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & A_{N-1}(\lambda_1) & 0 \\ 0 & 0 & 0 & 0 & c_s \end{pmatrix} \quad (16)$$

In which the dummy concentration $c_s = A_N(\lambda_1)$. Each of Equations 14 to 16 may be normalised to a reference calibration absorbance value, for example $A_1(\lambda_1)$, as explained hereinbefore.

Application to one analyte and background scattering

The method of extracting optical densities for N-1 analytes using N illumination wavelengths may be applied to verify the previously applied result for a single analyte with simultaneous illumination at first and second wavelengths λ_1, λ_2 and subsequent illumination at the second wavelength λ_2 i.e. $A_{1+2}(x)$-$A_2(x)$.

Simulations were conducted using the model described hereinbefore with reference to Figs. 38 to 41 in a case where the blue dye concentration profile 146 was equal to zero at every position. The resulting simulated OPD signals 152b, 156b and simulated absorbance profiles are as shown in Figs. 42, 43 and 45. The concentration values were chosen corresponding to absorbance fingerprint values, and taking the values corresponding to the green OLED as reference values. A first simulated calibration sample corresponding to gold nanoparticles having an optical density of OD = 1 may be represented in the method by the concentration vector $\mathbf{c}_{Au}{}^{T} = (1, 0)$ and the corresponding absorbance vector is $\mathbf{A}_{Au}{}^{T} = (1, 0.02)$. The relevant absorbance values were obtained as absorbance fingerprint values as described hereinbefore with reference to Figs. 46 and 47. A second simulated calibration sample, corresponding to a blank porous strip 64 in the form of a nitrocellulose strip, may be represented in the method by the absorbance vector $\mathbf{A}_{NC}{}^{T} = (0.01, 0.01)$, so that the dummy concentration $c_s = 0.01$ and the corresponding concentration vector is $\mathbf{c}_{NC}{}^{T} = (0, 0.01)$. The relevant absorbance values were obtained as absorbance fingerprint values as described hereinbefore with reference to Figs. 46 and 47. Thus, taking the green OLED wavelength range (see Fig. 38) as the reference, the calibration matrix C and fingerprint matrix F according to Equations 11, 12 and 16 may be written as:

$$C = \begin{pmatrix} 1 & 0 \\ 0 & 0.01 \end{pmatrix} \quad F = \begin{pmatrix} 1 & 0.01 \\ 0.02 & 0.01 \end{pmatrix} \tag{17}$$

The deconvolution (de-mixing) matrix CF^{-1} of Equation 14 may be calculated by inverting the fingerprint matrix F:

$$CF^{-1} = \begin{pmatrix} 1 & 0 \\ 0 & 0.01 \end{pmatrix} \begin{pmatrix} 1.020 & -1.020 \\ -2.041 & 102.041 \end{pmatrix} \tag{18}$$

$$CF^{-1} = \begin{pmatrix} 1.020 & -1.020 \\ -0.020 & 1.020 \end{pmatrix}$$

and substituting the deconvolution (de-mixing) matrix CF^{-1} into Equation 14 yields:

$$\begin{pmatrix} c_{Au} \\ c_{NC} \end{pmatrix} = \begin{pmatrix} 1.020 & -1.020 \\ -0.020 & 1.020 \end{pmatrix} \begin{pmatrix} A_{green} \\ A_{NIR} \end{pmatrix} \quad (19)$$

Thus, the concentration c_{Au} of gold nanoparticles, in this example expressed in terms of absorbance in OD, is given as $c_{Au} = 1.02(A_{green} - A_{NIR})$, which is essentially the same result applied hereinbefore.

Application to one analyte and background scattering with a coloured dye

Simulations were also conducted using the model described hereinbefore with reference to Figs. 38 to 41 in a case where the blue dye concentration profile 146 was as shown in Fig. 40. The resulting simulated OPD signals 152, 154, 156 are shown in Fig. 41. The concentration values were chosen as absorbance values using the green LED emission wavelengths as reference.

Referring also to Fig. 48, application of the simple two-colour method to absorbance values obtained based on the simulated OPD signals 152, 154, 156 leads to inaccuracy in determining the absorbance due to the gold nanoparticles when only the green and NIR simulated OPD signals 152, 156 are considered.

The total, summed absorbance 174 is represented by a solid line. The estimated gold nanoparticle concentration 176 is represented by a dotted line. The estimated background scattering from the nitrocellulose strip 178 is represented by the dashed line.

In particular, the presence of the blue dye leads to errors in the estimated gold nanoparticle concentration 176. In particular, the baseline absorbance around the location of the gold nanoparticles is distorted by absorbance of the blue dye. The problem is that there are three unknowns in the concentration values, namely, the gold nanoparticle concentration c_{Au}, the blue dye concentration c_{dye}, and the background scattering c_{NC} from the nitrocellulose strip. Using green and NIR OLEDs, there are only two measurements. The solution is to increase the number of wavelength ranges to three.

The deconvolution (de-mixing) matrix method may be applied if all three of the simulated OPD signals 152, 154, 156 are utilised. A first simulated calibration sample, corresponding to gold nanoparticles having an optical density of OD = 1, may be represented in the method by the concentration vector $\mathbf{c}_{Au}^{T}$ = (1, 0, 0) (c_{Au}, c_{dye}, c_{NC}) and the corresponding absorbance vector is $\mathbf{A}_{Au}^{T}$ = (1, 0.17, 0.02) (green, red, NIR). The relevant absorbance values were obtained as absorbance fingerprint values according to a method analogous to that described hereinbefore with reference to Figs. 46 and 47. A second

simulated calibration sample, corresponding to the blue dye, may be represented in the method by the concentration vector $\mathbf{c}_{dye}^{T}$ = (0, 0.024, 0) and the corresponding absorbance vector is $\mathbf{A}_{Au}^{T}$ = (0.024, 0.89, 0). The relevant absorbance values were obtained as absorbance fingerprint values according to a method analogous to that described hereinbefore with reference to Figs. 46 and 47. A third simulated calibration sample, corresponding to a blank porous strip, has an absorbance vector of $\mathbf{A}_{NC}^{T}$ = (0.01, 0.01, 0.01), so that the dummy concentration c_s = 0.01 and the corresponding concentration vector is $\mathbf{c}_{NC}^{T}$ = (0, 0, 0.01). The relevant absorbance values were obtained as absorbance fingerprint values according to a method analogous to that described hereinbefore with reference to Figs. 46 and 47. Thus, taking the green wavelength as reference wavelength, the calibration matrix C and fingerprint matrix F according to Equations 11, 12 and 16 may be written as:

$$C = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0.024 & 0 \\ 0 & 0 & 0.01 \end{pmatrix} \quad F = \begin{pmatrix} 1 & 0.024 & 0.01 \\ 0.17 & 0.89 & 0.01 \\ 0.02 & 0 & 0.01 \end{pmatrix} \qquad (20)$$

The deconvolution (de-mixing) matrix CF^{-1} of Equation 14 may be calculated by inverting the fingerprint matrix F:

$$CF^{-1} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 0.024 & 0 \\ 0 & 0 & 0.01 \end{pmatrix} \begin{pmatrix} 1.025 & -0.028 & -0.997 \\ -0.173 & 1.128 & -0.956 \\ -2.049 & 0.055 & 101.994 \end{pmatrix} \qquad (21)$$

$$CF^{-1} = \begin{pmatrix} 1.025 & -0.028 & -0.997 - 1.020 \\ -0.004 & 0.027 & -0.023 \\ -0.02 & 0.001 & 1.020 \end{pmatrix}$$

and substituting the deconvolution (de-mixing) matrix CF^{-1} into Equation 14 yields:

$$\begin{pmatrix} c_{Au} \\ c_{dye} \\ c_{NC} \end{pmatrix} = \begin{pmatrix} 1.025 & -0.028 & -0.997 \\ -0.004 & 0.027 & -0.023 \\ -0.02 & 0.001 & 1.020 \end{pmatrix} \begin{pmatrix} A_{green} \\ A_{red} \\ A_{NIR} \end{pmatrix} \qquad (22)$$

Thus, the concentration c_{Au} of gold nanoparticles, in this example expressed in terms of absorbance in OD, is given as $c_{Au} = (1.025A_{green} - 0.028A_{red} - 0.997A_{NIR})$.

Referring also to Fig. 49, the total, summed absorbance 174 is represented by a solid line. The estimated gold nanoparticle concentration 176 is represented by a dotted line. The

estimated background scattering from the nitrocellulose strip 178 is represented by the dashed line. The estimated concentration of the blue dye 180 is represented by the chained line.

It can be seen that applying the method of sequential measurements at three different wavelengths (green, red and NIR) is expected to allow for clear separation of the absorbance due to the gold nanoparticles, blue dye and the nitrocellulose strip. In particular, the estimated gold nanoparticle concentration 176 and the estimated concentration of the blue dye 180 are expected to be separable.

The hereinbefore described deconvolution (de-mixing) method may be carried out by the controller 36 of the apparatus 10.

Although claims have been formulated in this application to particular combinations of features, it should be understood that the scope of the disclosure of the present invention also includes any novel features or any novel combination of features disclosed herein either explicitly or implicitly or any generalization thereof, whether or not it relates to the same invention as presently claimed in any claim and whether or not it mitigates any or all of the same technical problems as does the present invention.

The applicant hereby gives notice that new claims may be formulated to such features and/or combinations of such features during the prosecution of the present application or of any further application derived therefrom.

CLAIMS

1. An apparatus comprising:

at least one set of emitters, the or each set of emitters comprising a first sub-set of one or more light emitters configured to emit light within a range around a first wavelength and a second sub-set of one or more light emitters configured to emit light within a range around a second wavelength, wherein said first and second sub-sets of one or more light emitters are configured to be simultaneously illuminable, and further wherein:

the first sub-set of one or more light emitters is deluminable from an illuminated state over a first period; and

the second sub-set of one or more light emitters is deluminable from an illuminated state over a second period;

one or more photodetectors arranged such that light from the or each set of emitters reaches the photodetectors via an optical path comprising a sample receiving portion; and

a liquid transport path comprising a first end, a second end and a liquid sample receiving region, the liquid transport path configured to transport a liquid sample received in the liquid sample receiving region towards the second end and through the sample receiving portion of the optical path.

2. An apparatus according to claim 1, wherein the first and second sub-sets of one or more light emitters are deluminable responsive to a change in state of an illumination control signal.

3. An apparatus according to claim 2, wherein said illumination control signal oscillates at a frequency:

$$\frac{1}{second\ period} < f \ll \frac{1}{first\ period}$$

4. An apparatus according to any one of the preceding claims, wherein said second period differs from said first period by a factor of two or more.

5. An apparatus according to any one of the preceding claims, wherein the first period is a function of a first decay rate and the second period is a function of a second decay rate.

6. An apparatus according to claim 5, wherein the first decay rate is a characteristic of a luminescent material of the first sub-set of one or more light emitters and the second decay rate is a characteristic of a luminescent material of the second sub-set of one or more light emitters.

7. An apparatus according to claim 6, wherein the luminescent material of the first sub-set of one or more light emitters comprises a fluorescent material.

8. An apparatus according to claim 6 or 7, wherein the luminescent material of the second sub-set of one or more light emitters comprises a phosphorescent material.

9. An apparatus according to any one of the preceding claims, further comprising a controller configured to:

simultaneously illuminate each sub-set of emitters and to obtain a measured absorbance value using the one or more photodetectors at:

a first time prior to said first and second periods commencing; and

a second time between expiry of said first period and expiry of said second period;

generate an absorbance vector using the measured absorbance values; and

determine a concentration vector by multiplying the absorbance vector with a de-convolution matrix.

10. An apparatus according to any one of the preceding claims, wherein the optical path is configured such that the one or more photodetectors receive light transmitted through the sample receiving portion of the optical path.

11. An apparatus according to any one of claims 1 to 9, wherein the optical path is configured such that the one or more photodetectors receive light reflected from the sample receiving portion of the optical path.

12. An apparatus according to any one of the preceding claims, wherein the one or more photodetectors form an image sensor arranged to image all or a portion of the sample receiving portion of the optical path.

13. An apparatus according to any one of the preceding claims, wherein the optical path further comprises a slit arranged before the sample receiving portion, and further wherein the or each set of emitters is arranged to illuminate the slit.

14. An apparatus according to any one of the preceding claims, wherein the or each light emitter of the second sub-set of one or more light emitters is substantially transparent at the wavelengths emitted by the or each light emitter of the first sub-set of one or more light emitters and wherein the or each light emitter of the first sub-set of one or more light emitters emits light into the optical path through a corresponding light emitter of the second sub-set of one or more light emitters.

15. An apparatus according to any one of the preceding claims, wherein the at least one set of emitters further comprises a third sub-set of one or more light emitters configured to emit light within a range around a third wavelength.

16. An apparatus according to claim 15, wherein the or each light emitter of the second sub-set of one or more light emitters is substantially transparent at the wavelengths emitted by the or each light emitter of the first sub-set of one or more light emitters and by the or each light emitter of the third sub-set of one or more light emitters, and wherein the or each light emitter of the first sub-set of one or more light emitters and the or each light emitter of the third sub-set of one or more light emitters emits light into the optical path through a corresponding light emitter of the second sub-set of one or more light emitters.

17. An apparatus according to any one of the preceding claims, wherein the liquid transport path comprises a lateral flow type strip.

18. An apparatus according to any one of the preceding claims, wherein the liquid transport path comprises the whole, a part, or at least one channel of a microfluidic device.

19. An apparatus according to claim 9, and any one of claims 10 to 18 when dependent on claim 9, further comprising at least one output device.

20. An apparatus according to claim 19, wherein the at least one output device comprises one or more light emitting diodes, and wherein the controller is configured to illuminate the

or each light emitting diode in response to a corresponding value of the concentration vector exceeding a predetermined threshold.

21. An apparatus according to claim 20, wherein the at least one output device comprises a display element, and wherein the controller is configured to cause the display element to display one or more outputs in response to determining the concentration vector.

22. An apparatus according to claim 21, wherein the controller is configured, in response to a value of the concentration vector exceeding a predetermined threshold, to cause the display element to display a corresponding symbol or symbols.

23. An apparatus according to claim 21 or 22, wherein the controller is configured to cause the display element to display one or more values of the concentration vector.

24. An apparatus according to any one of claims 19 to 23, wherein the at least one output device comprises a wired or wireless communications interface for connection to a data processing apparatus, and wherein the controller is configured to output the concentration vector to the data processing apparatus via the wired or wireless communications interface.

25. An apparatus according to any one of the preceding claims, wherein the light within a range around a first wavelength is strongly absorbed by a tag used in a sample and the light within a range around a second wavelength is not absorbed, or is weakly absorbed, by a tag used in the sample.

26. An apparatus according to any one of the preceding claims, wherein the at least one set of emitters and one or more photodetectors are configured such that, at the sample receiving portion of the optical path, a normalised spatial intensity profile generated by each sub-set of emitters is substantially equal to a normalised spatial intensity profile generated by each other sub-set of emitters.

27. A method of operating an apparatus according to any preceding claim, the method comprising applying a liquid sample to the liquid sample receiving region.

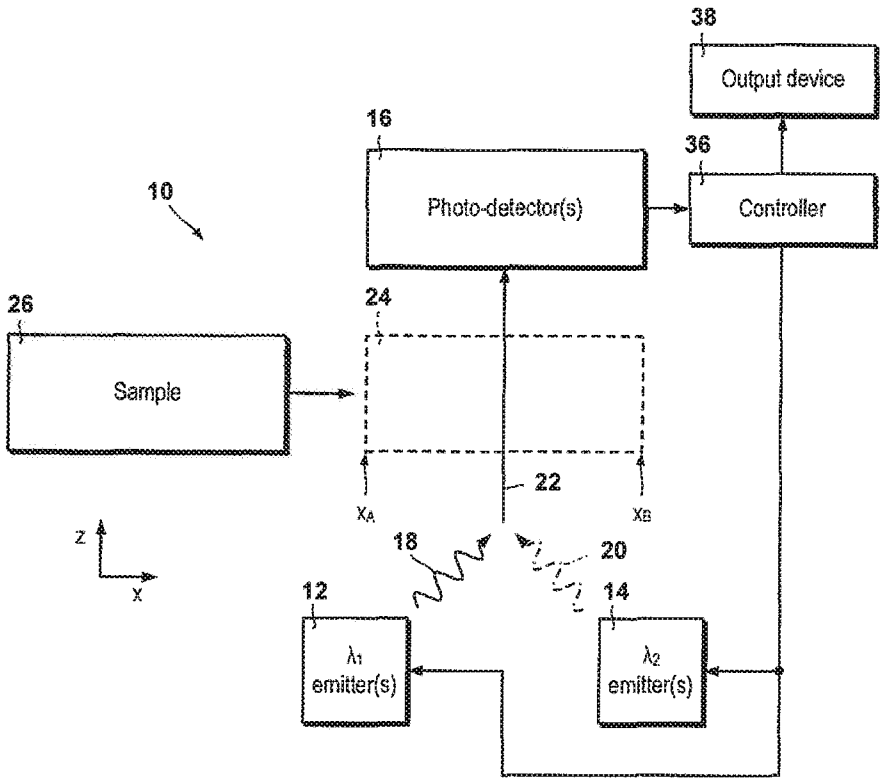
38
Output device
16
Photo-detector(s)
36
Controller
10
26
Sample
24
22
x_A
x_B
z
x
18
20
12
λ_1 emitter(s)
14
λ_2 emitter(s)

Fig. 1

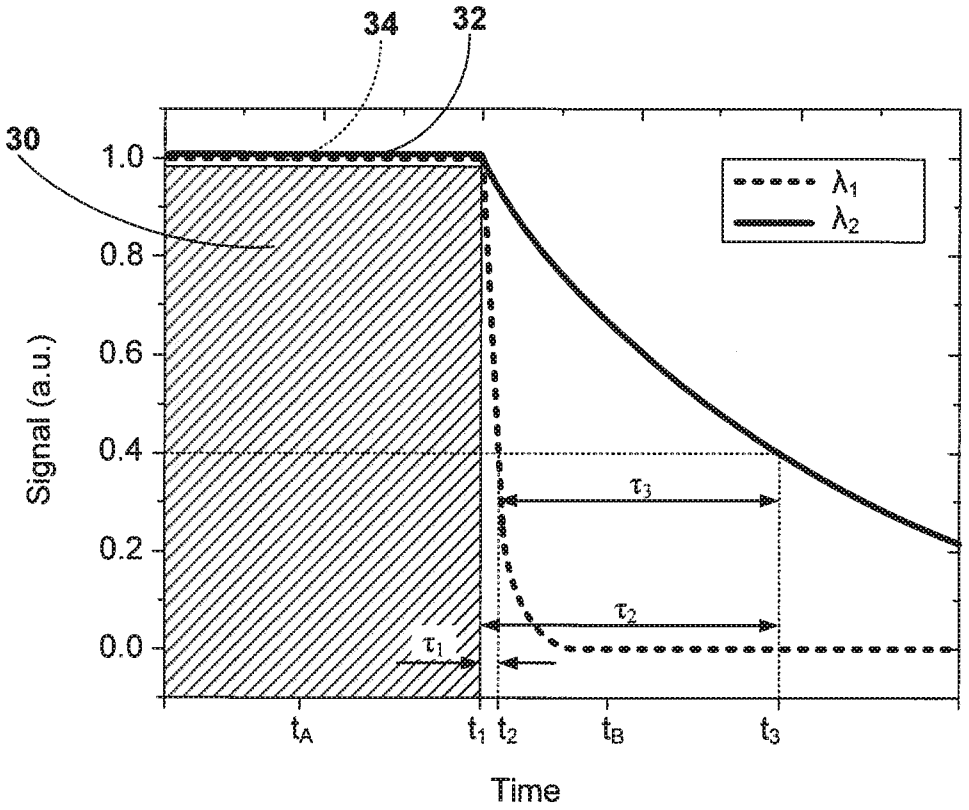
34
32
30
1.0
0.8
0.6
0.4
0.2
0.0
Signal (a.u.)
λ_1
λ_2
τ_3
τ_2
τ_1
t_A
t_1
t_2
t_B
t_3
Time

Fig. 2

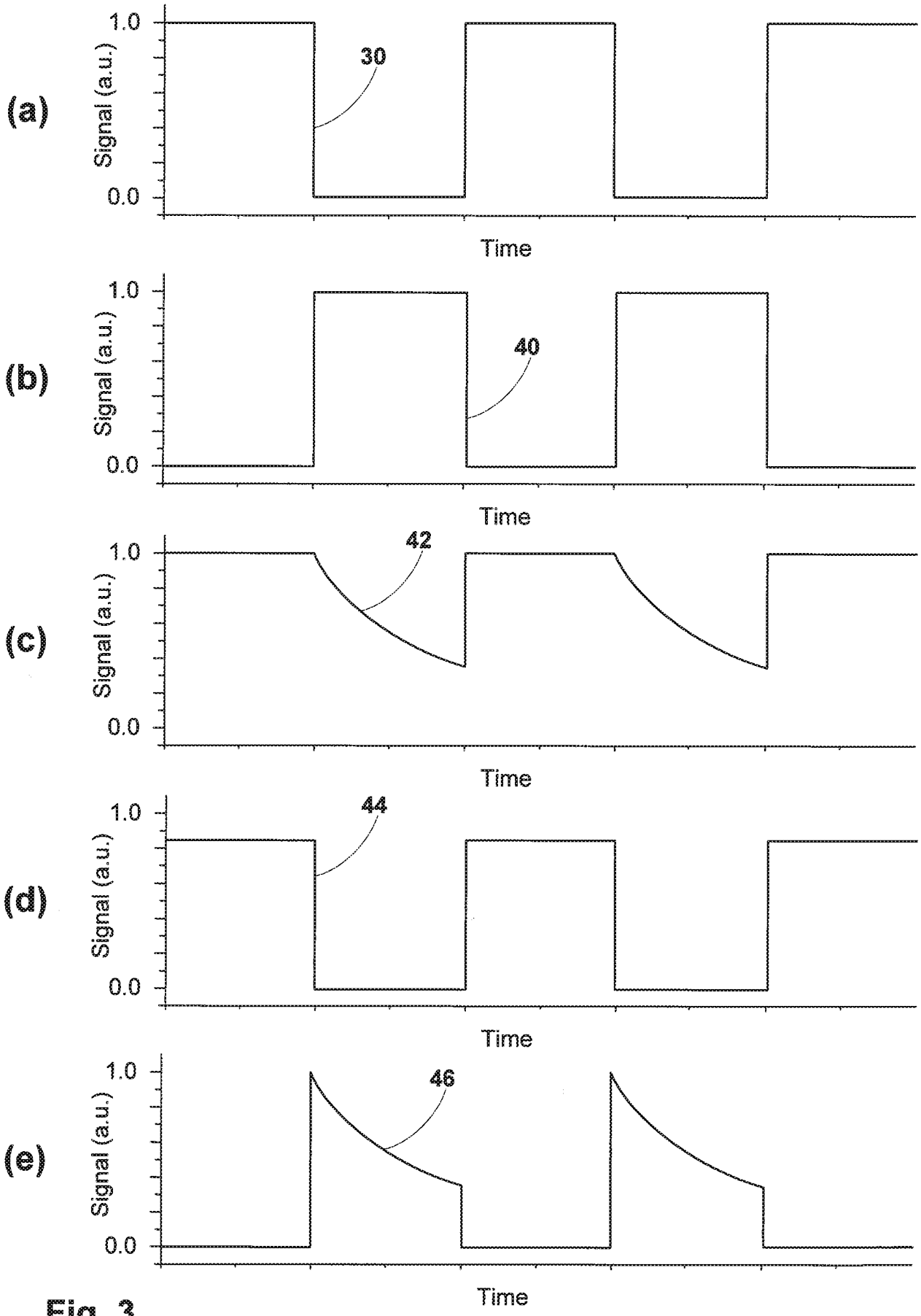
(a)
1.0
0.0
Signal (a.u.)
30
Time
(b)
1.0
0.0
Signal (a.u.)
40
Time
(c)
1.0
0.0
Signal (a.u.)
42
Time
(d)
1.0
0.0
Signal (a.u.)
44
Time
(e)
1.0
0.0
Signal (a.u.)
46
Time

Fig. 3

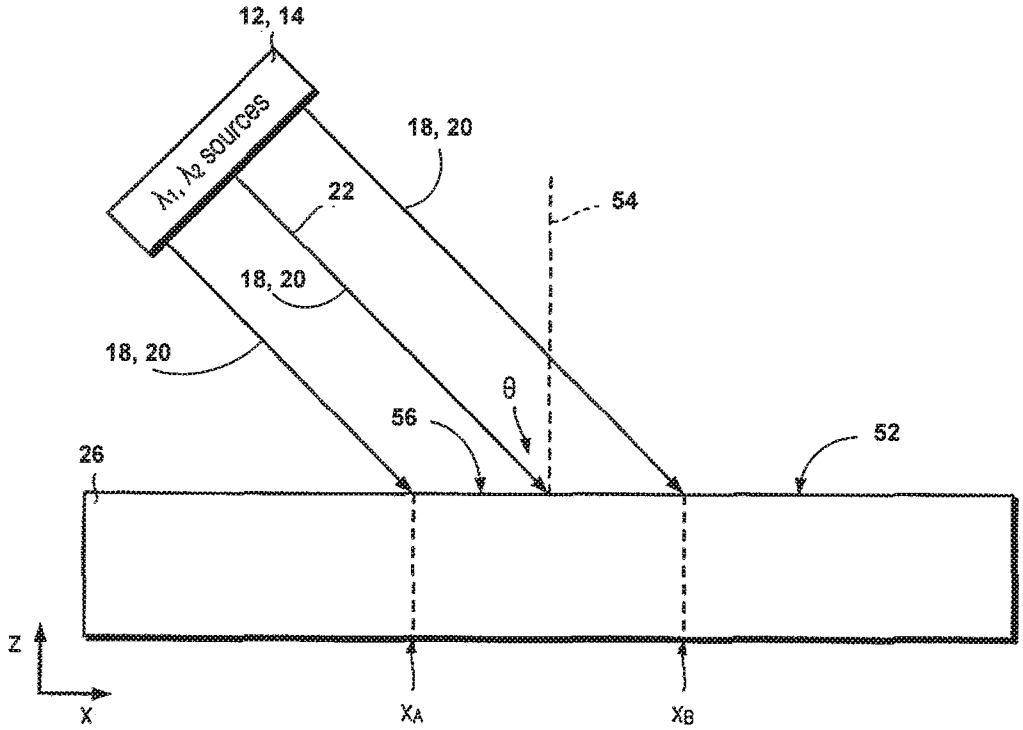
12, 14
λ1, λ2 sources
18, 20
22
54
18, 20
18, 20
θ
56
52
26
z
x
XA
XB

Fig. 4

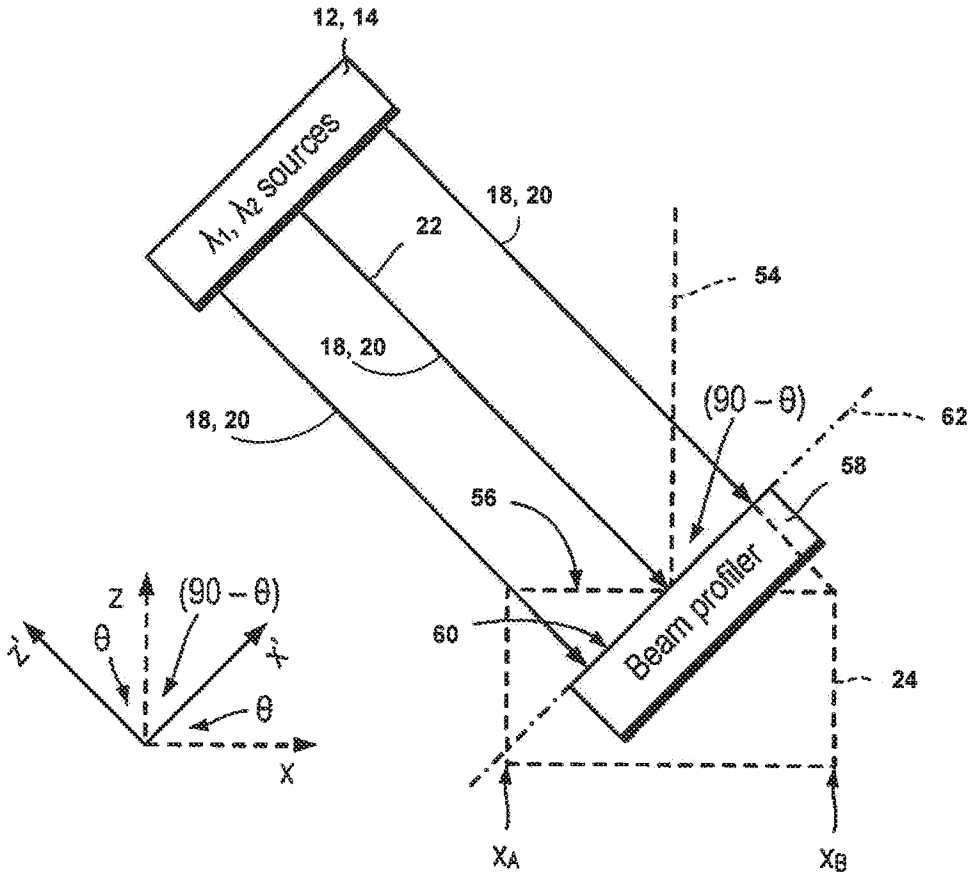
12, 14
λ1, λ2 sources
18, 20
22
54
18, 20
18, 20
(90 – θ)
62
58
56
Beam profiler
60
24
z
(90 – θ)
z'
θ
x'
θ
x
XA
XB

Fig. 5

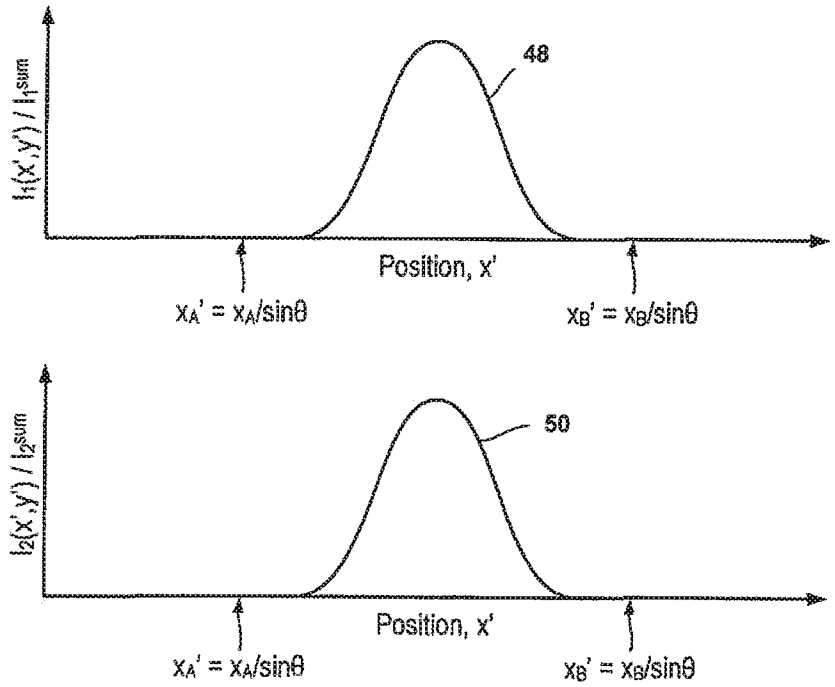
$I_1(x',y') / I_1^{sum}$
48
Position, x'
$x_A' = x_A/\sin\theta$
$x_B' = x_B/\sin\theta$
$I_2(x',y') / I_2^{sum}$
50
Position, x'
$x_A' = x_A/\sin\theta$
$x_B' = x_B/\sin\theta$

Fig. 6

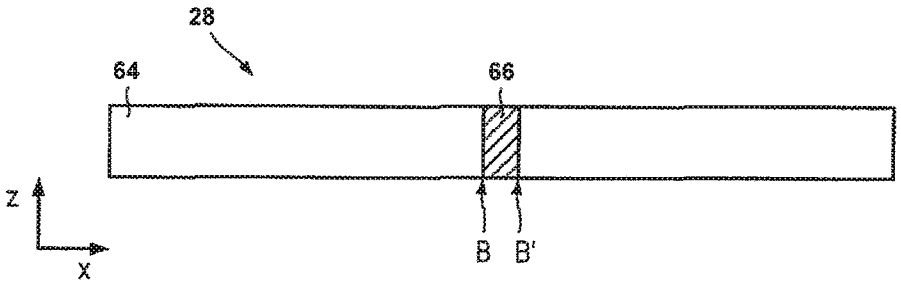
28
64
66
Z
X
B
B'

Fig. 7

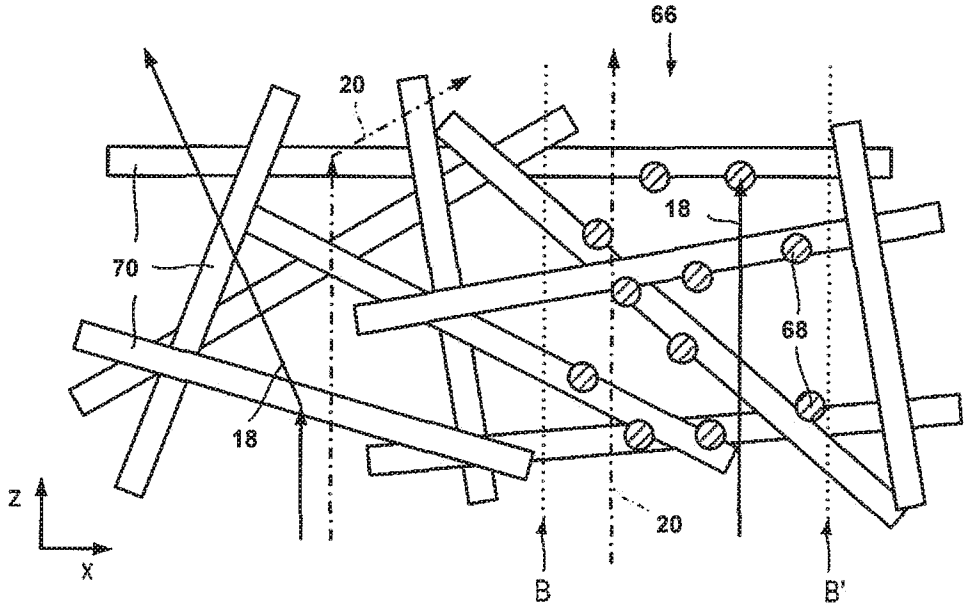

Fig. 8

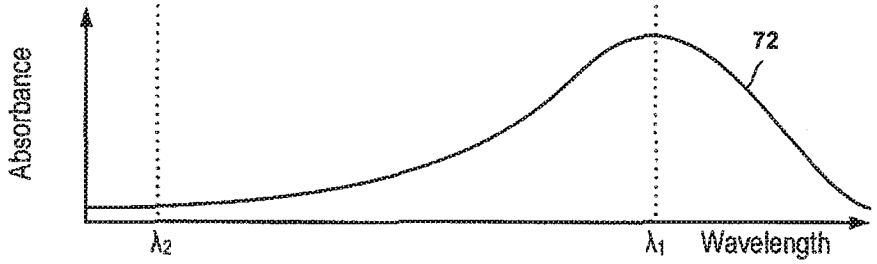

Fig. 9

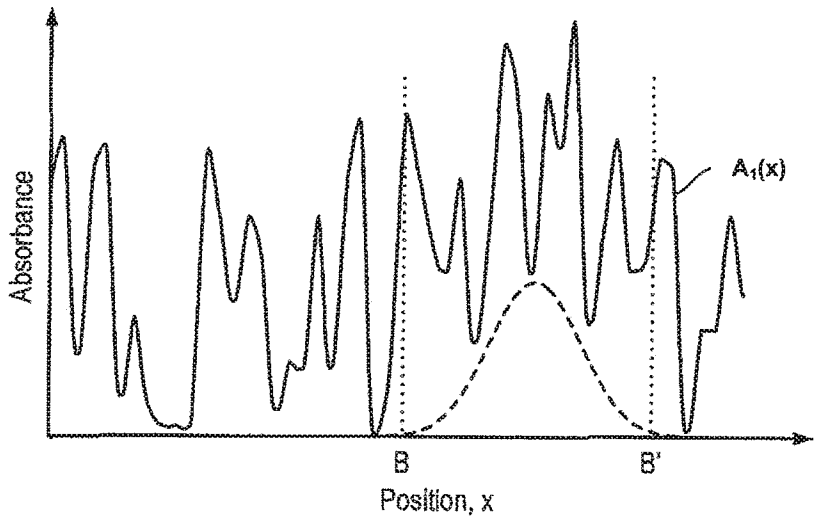

Fig. 10

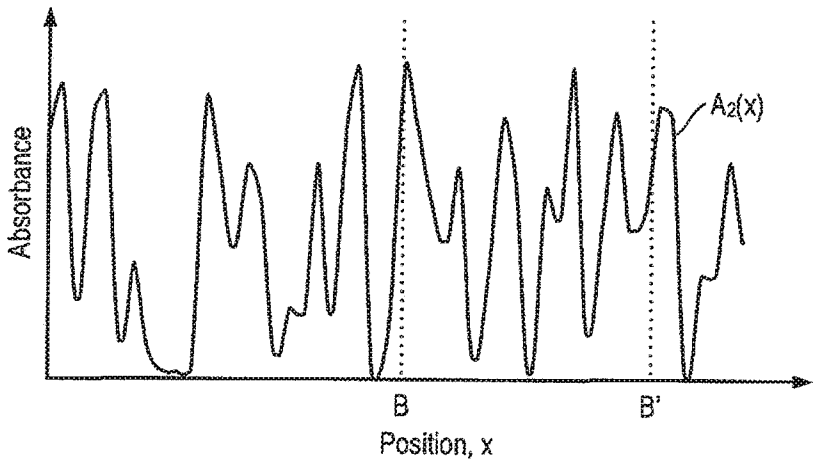

Fig. 11

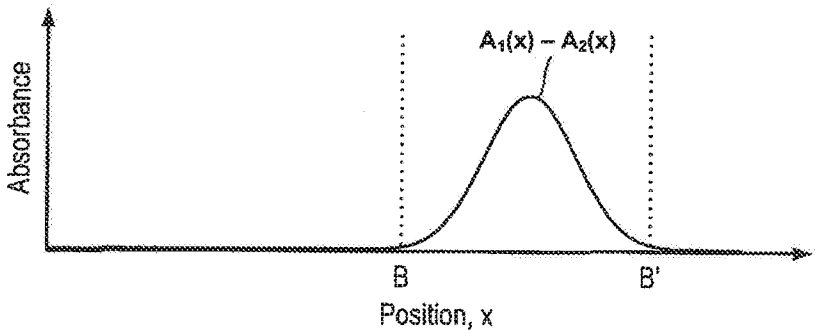

Fig. 12

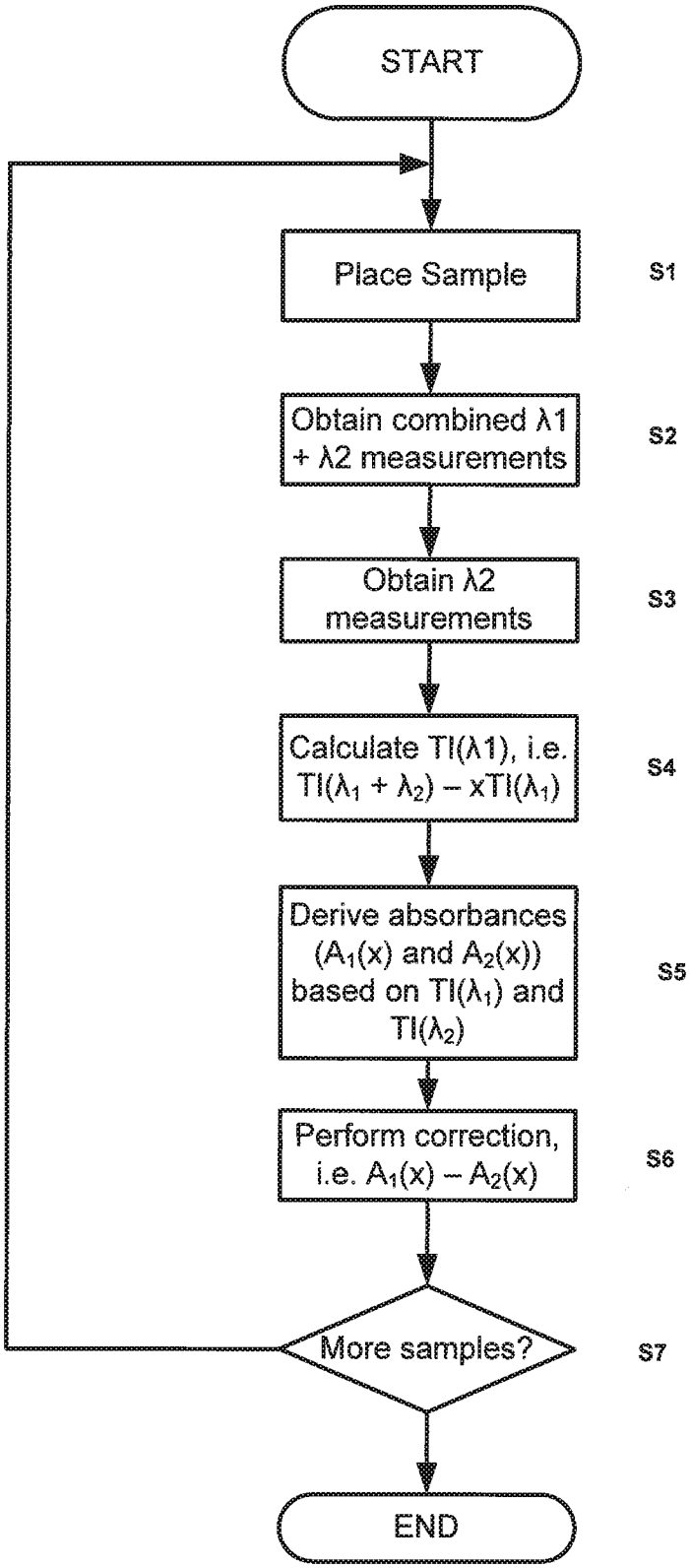
START
Place Sample
S1
Obtain combined λ1 + λ2 measurements
S2
Obtain λ2 measurements
S3
Calculate TI(λ1), i.e. TI(λ1 + λ2) – xTI(λ1)
S4
Derive absorbances (A1(x) and A2(x)) based on TI(λ1) and TI(λ2)
S5
Perform correction, i.e. A1(x) – A2(x)
S6
More samples?
S7
END

Fig. 13

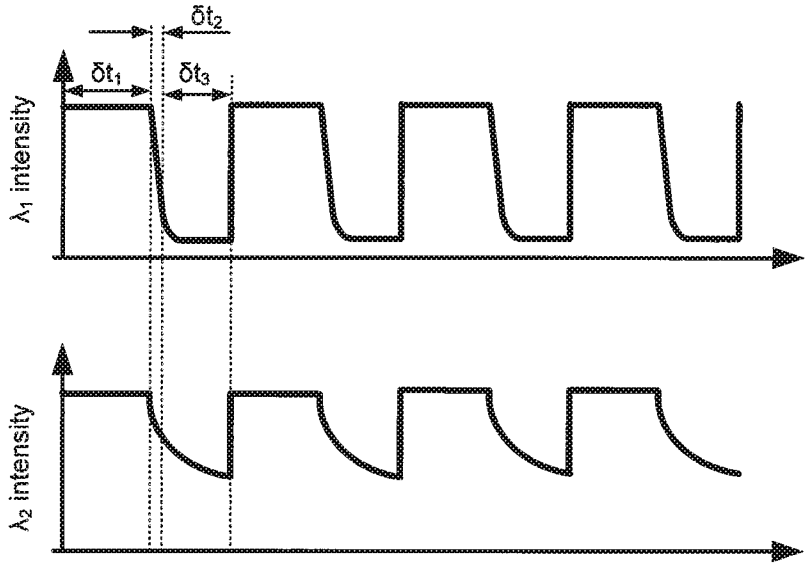
δt2
δt1
δt3
λ1 intensity
λ2 intensity

Fig. 14

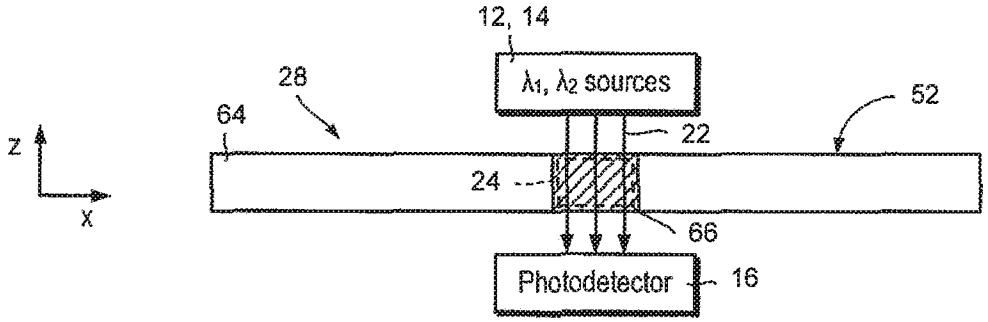
12, 14
λ1, λ2 sources
28
64
52
22
24
66
Photodetector
16
z
x

Fig. 15

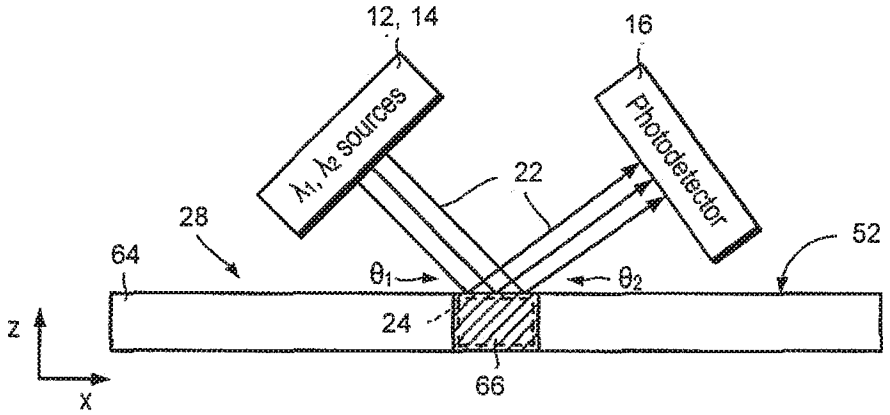
12, 14
16
λ1, λ2 sources
Photodetector
22
28
52
64
θ1
θ2
24
66
z
x

Fig. 16

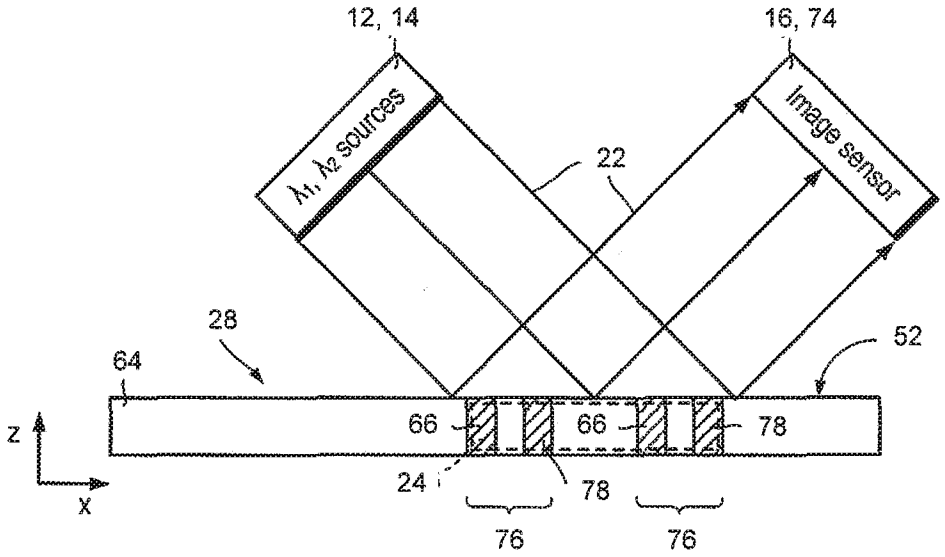
12, 14
16, 74
λ1, λ2 sources
Image sensor
22
28
52
64
66
66
78
24
78
76
76
z
x

Fig. 17

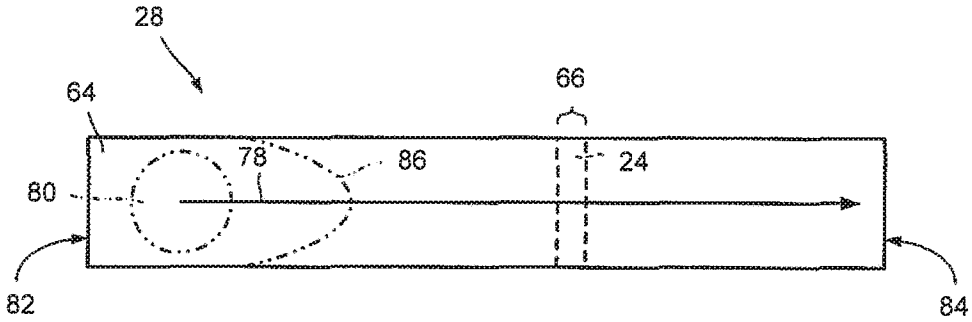

Fig. 18

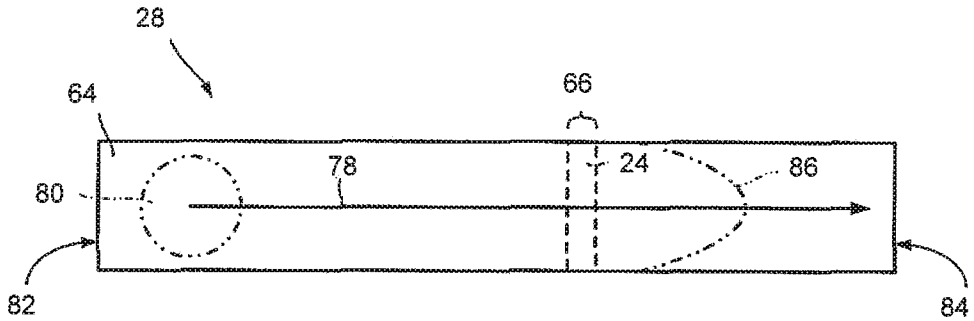

Fig. 19

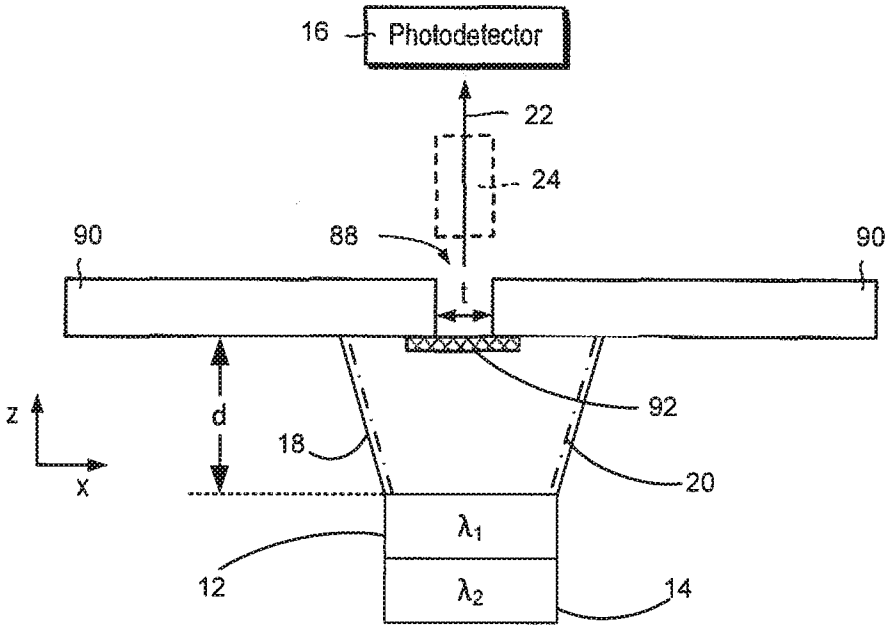

Fig. 20

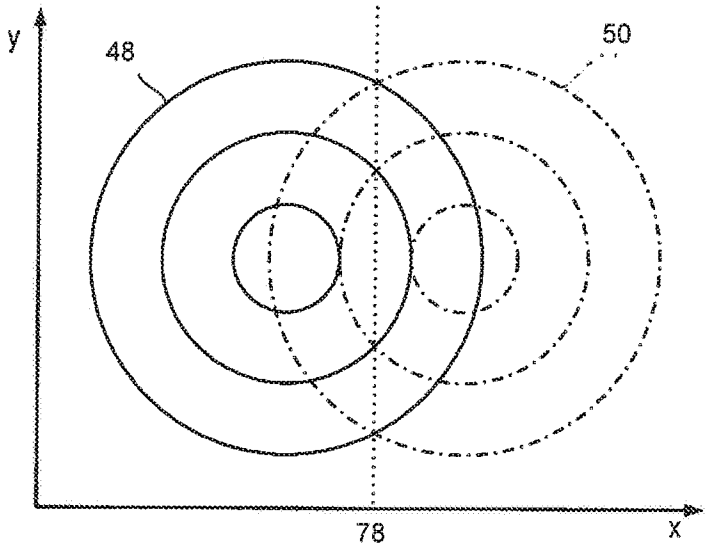

Fig. 21

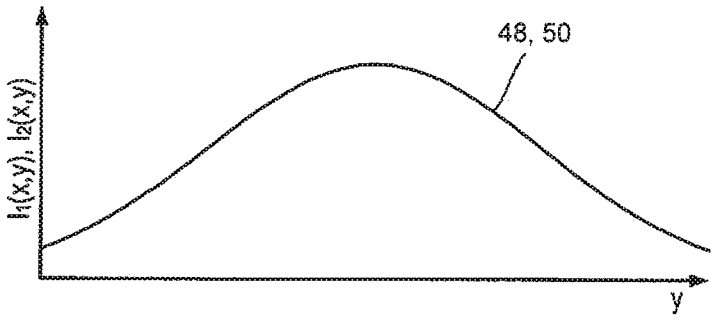

Fig. 22

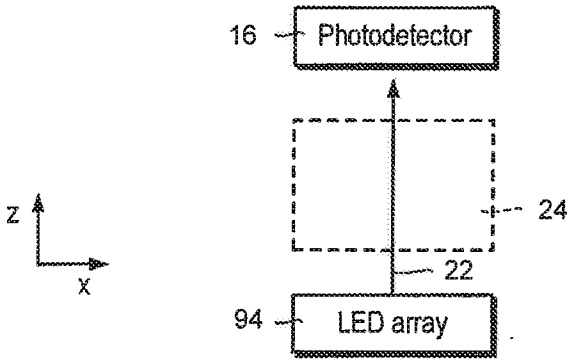

Fig. 23

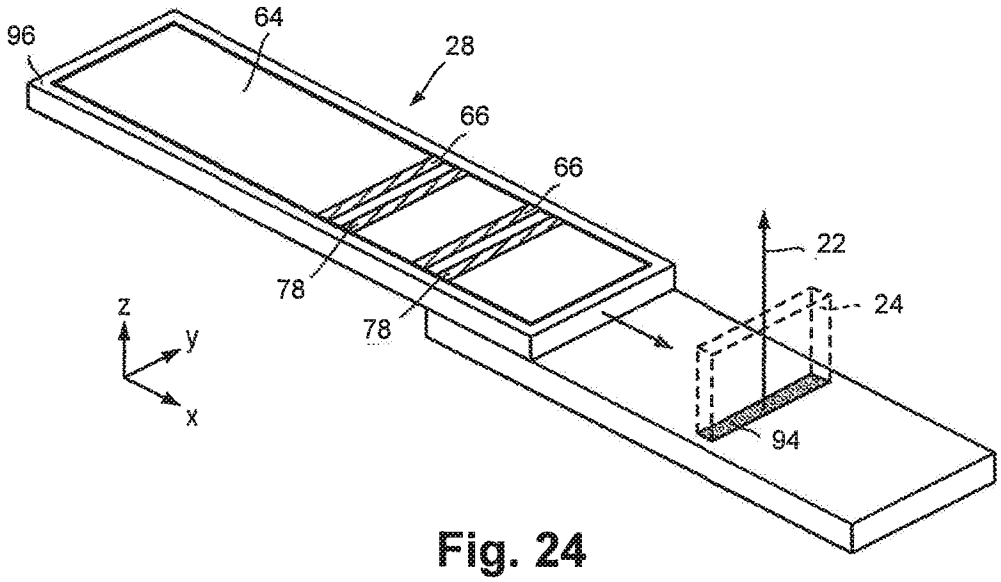

Fig. 24

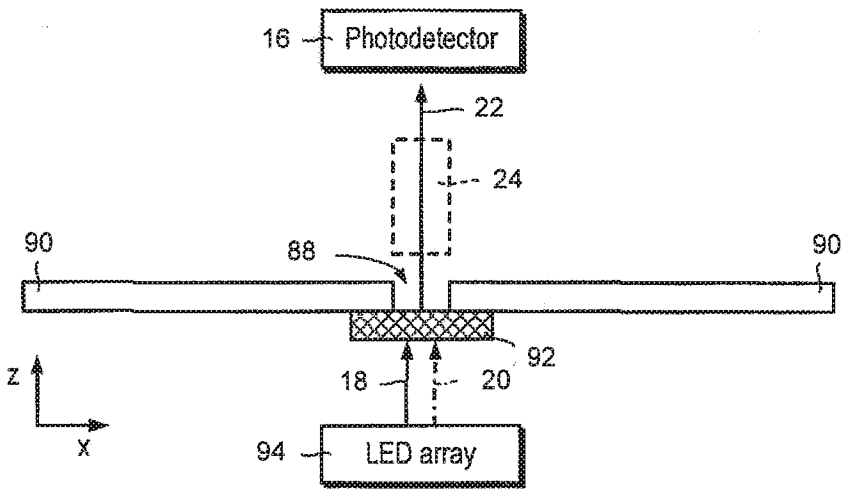

Fig. 25

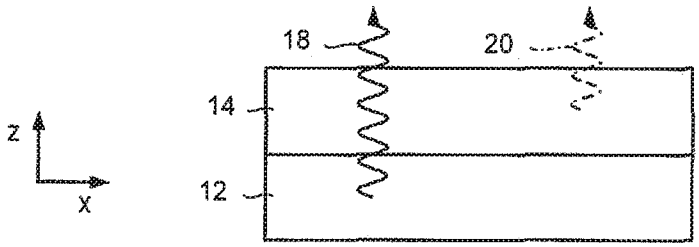

Fig. 26

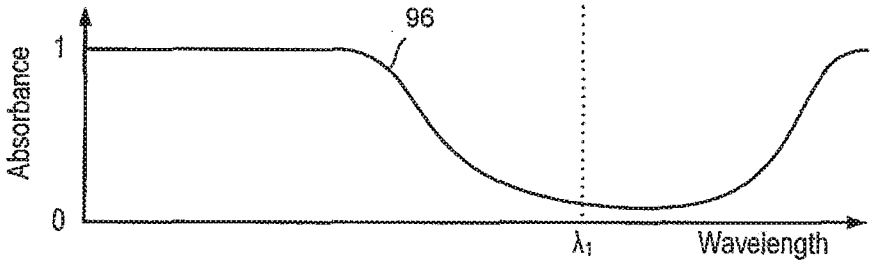

Fig. 27

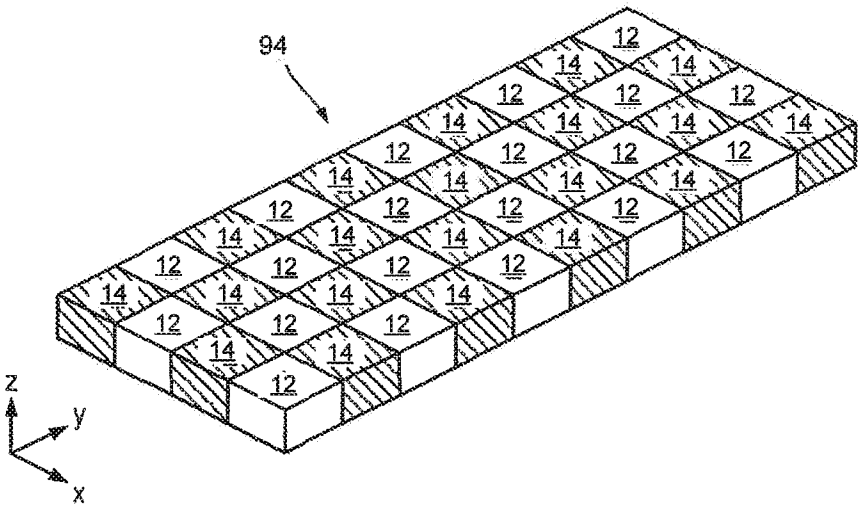

Fig. 28

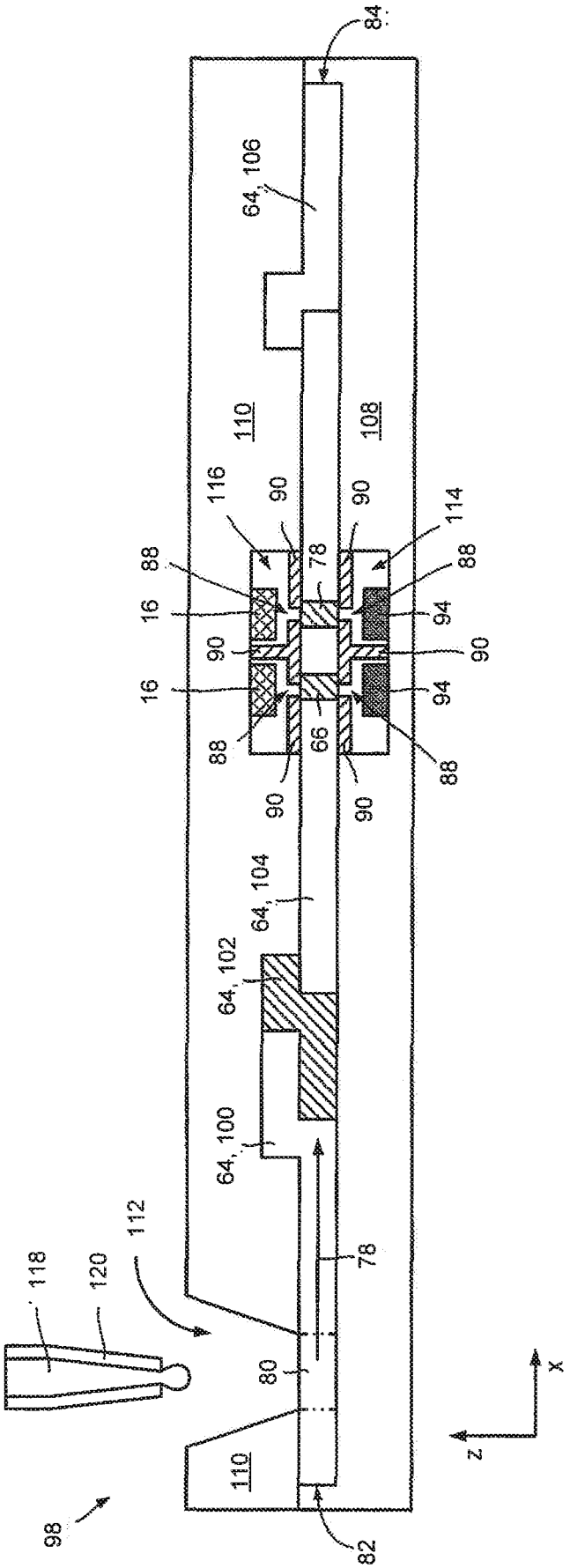
84
64, 106
110
108
116
90
78
114
88
16
94
66
64, 104
64, 102
64, 100
112
118
120
80
82
98
z
x

Fig. 29

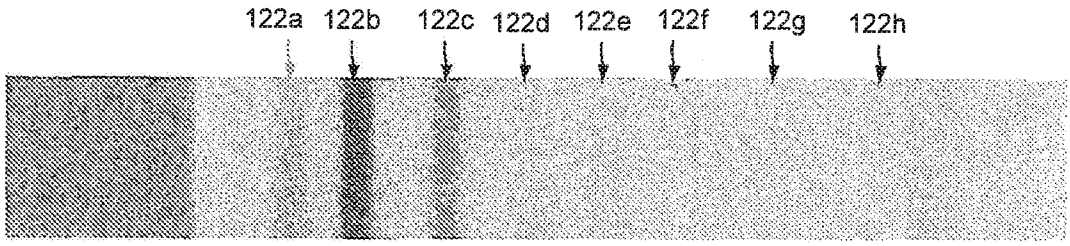
122a
122b
122c
122d
122e
122f
122g
122h

Fig. 30

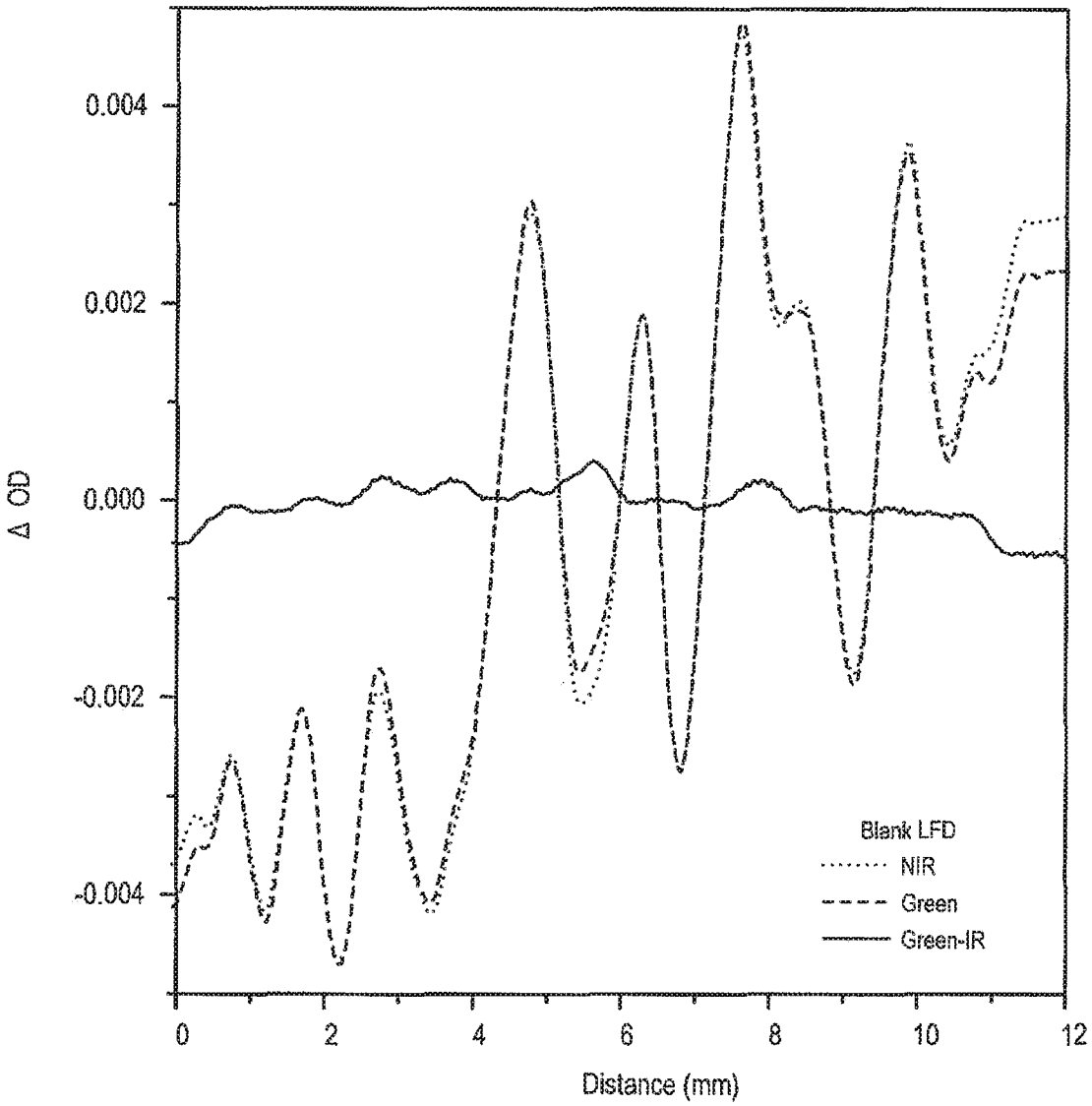
0.004
0.002
0.000
-0.002
-0.004
Δ OD
0
2
4
6
8
10
12
Distance (mm)
Blank LFD
NIR
Green
Green-IR

Fig. 31

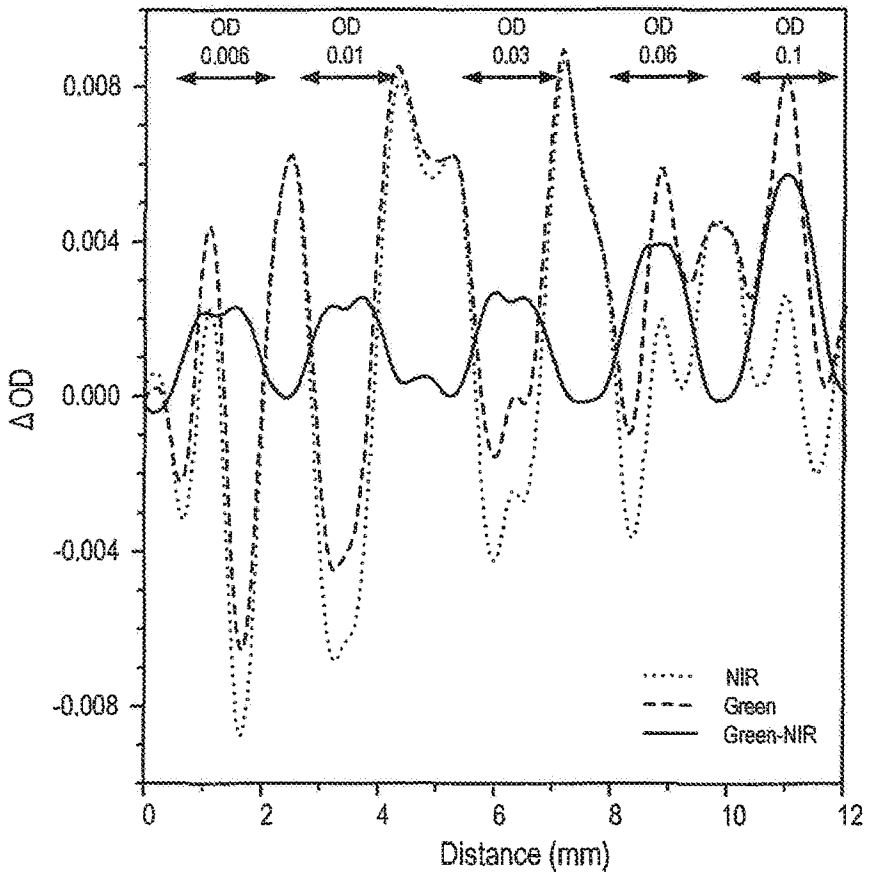
OD 0.006
OD 0.01
OD 0.03
OD 0.06
OD 0.1
0.008
0.004
0.000
-0.004
-0.008
ΔOD
NIR
Green
Green-NIR
0
2
4
6
8
10
12
Distance (mm)

Fig. 32

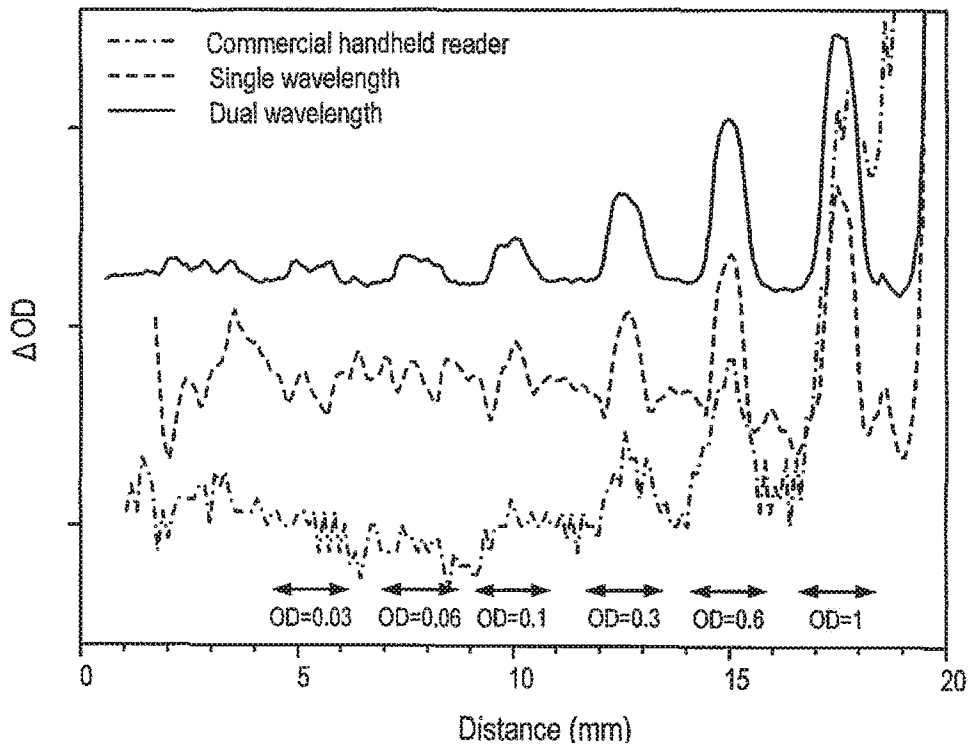
Commercial handheld reader
Single wavelength
Dual wavelength
ΔOD
OD=0.03
OD=0.06
OD=0.1
OD=0.3
OD=0.6
OD=1
0
5
10
15
20
Distance (mm)

Fig. 33

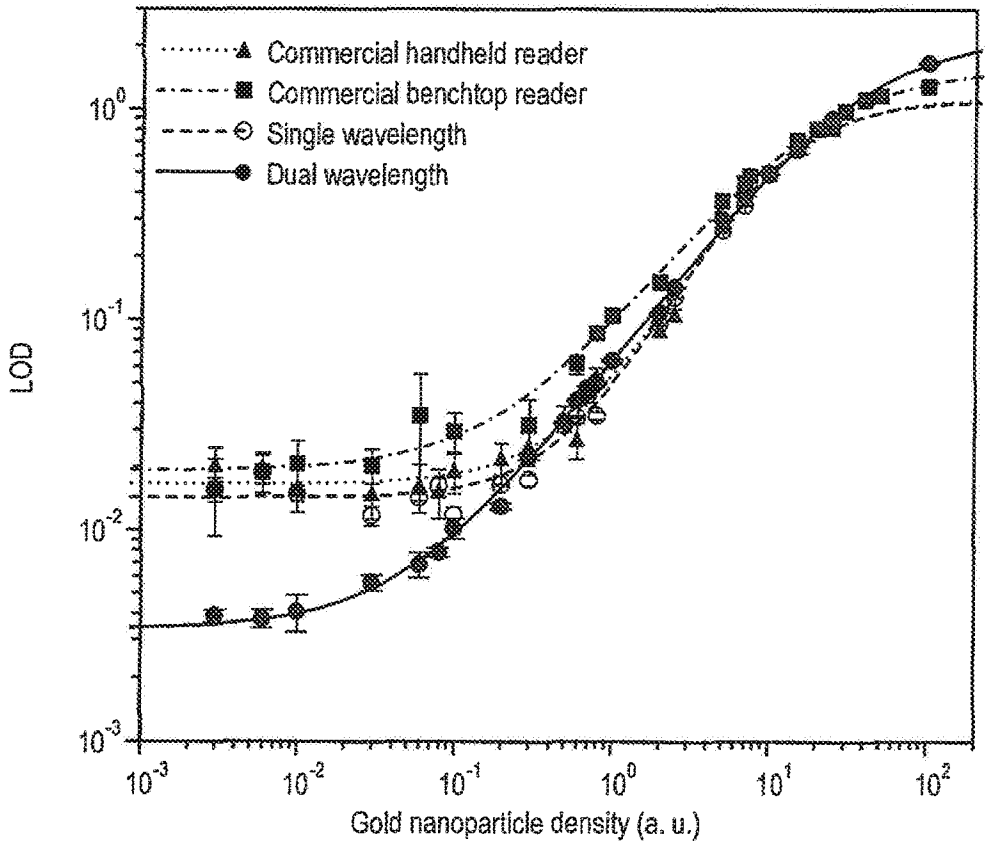
Commercial handheld reader
Commercial benchtop reader
Single wavelength
Dual wavelength
LOD
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
Gold nanoparticle density (a. u.)

Fig. 34

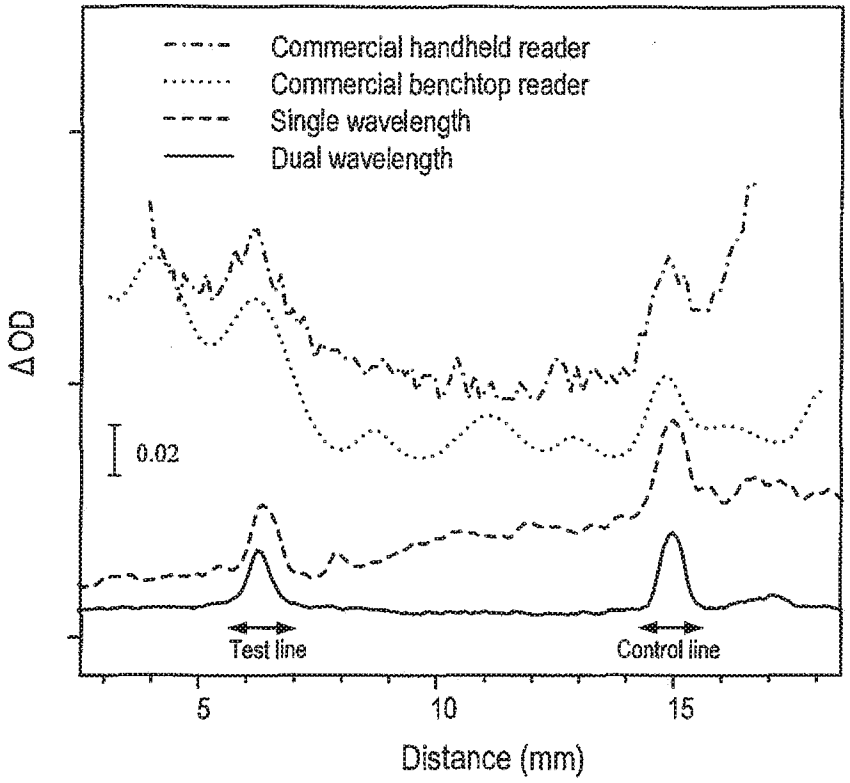
Commercial handheld reader
Commercial benchtop reader
Single wavelength
Dual wavelength
ΔOD
0.02
Test line
Control line
5
10
15
Distance (mm)

Fig. 35

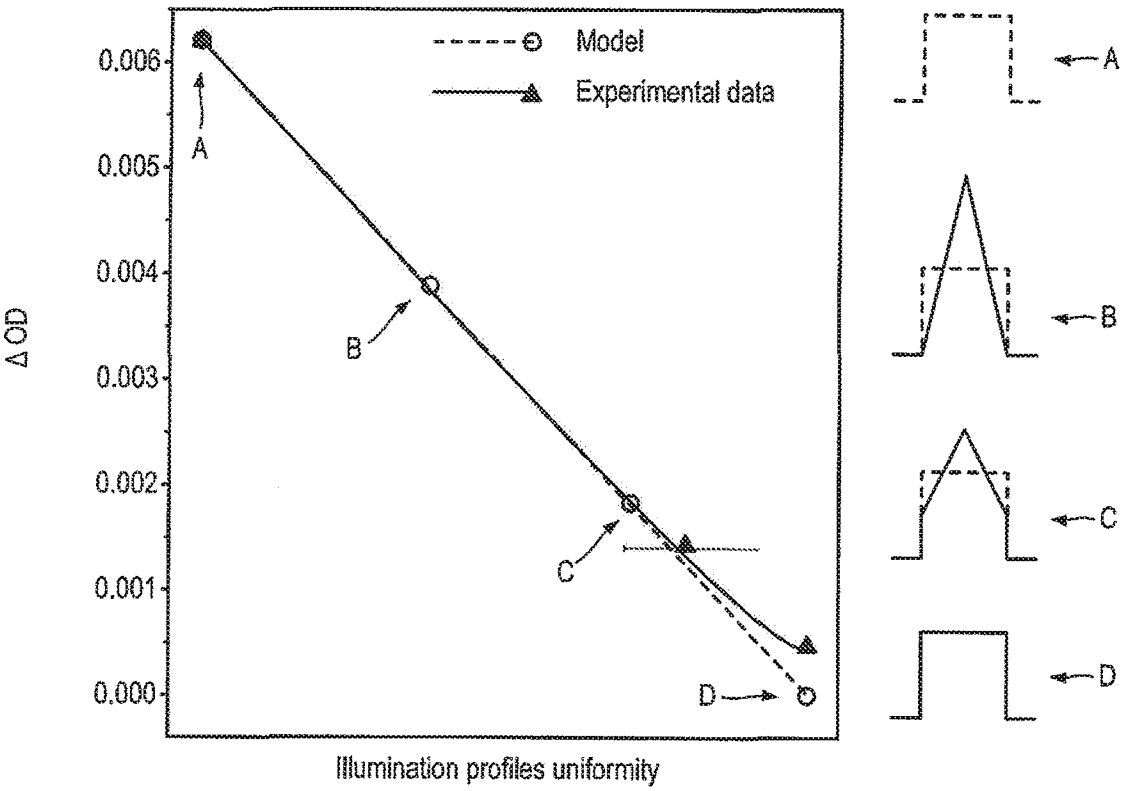
Model
Experimental data
0.006
0.005
0.004
0.003
0.002
0.001
0.000
Δ OD
A
B
C
D
Illumination profiles uniformity

Fig. 36

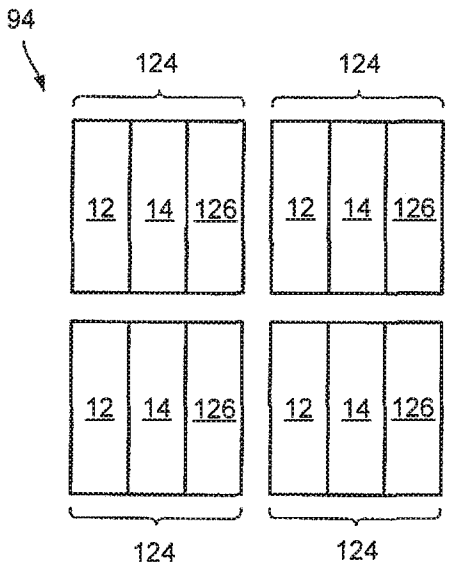

Fig. 37A

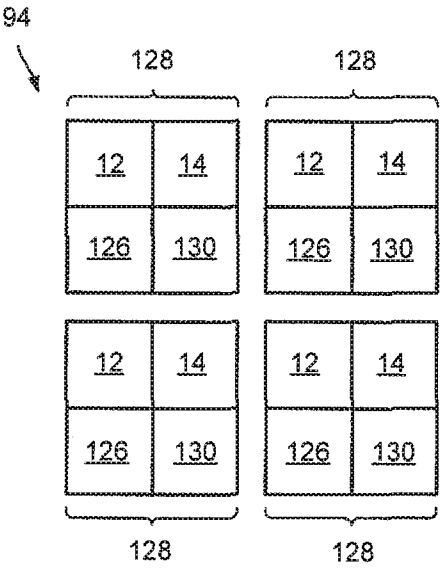

Fig. 37B

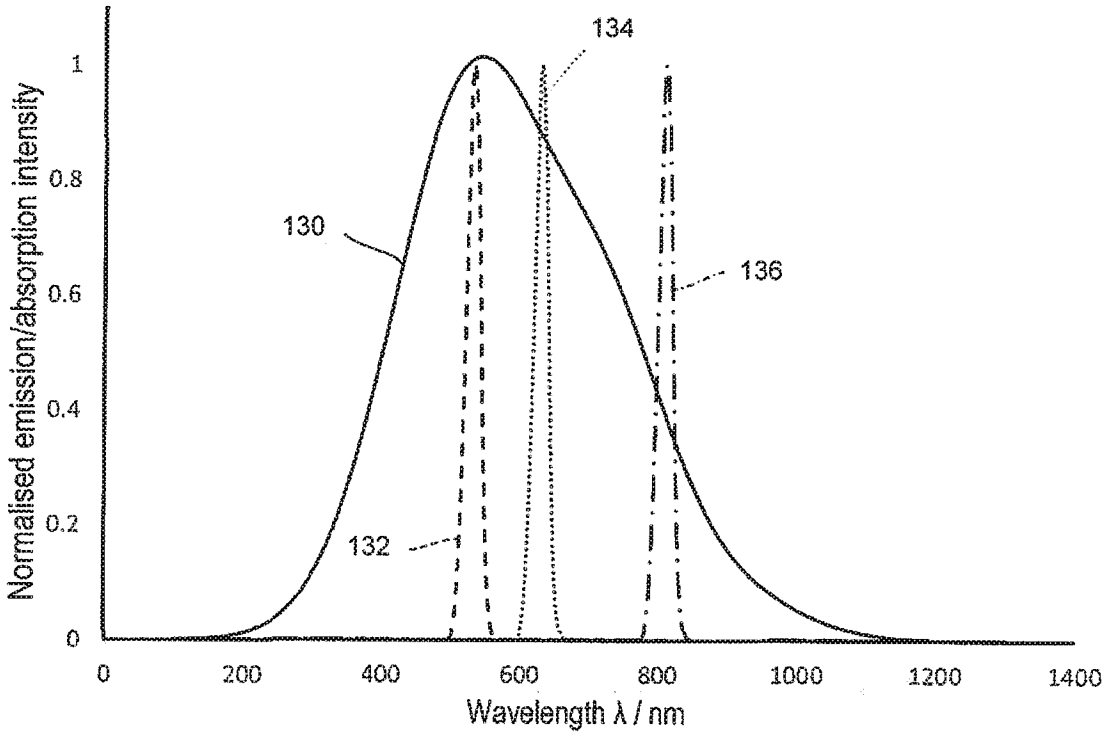
Normalised emission/absorption intensity
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400
Wavelength λ / nm
130
132
134
136

Fig. 38

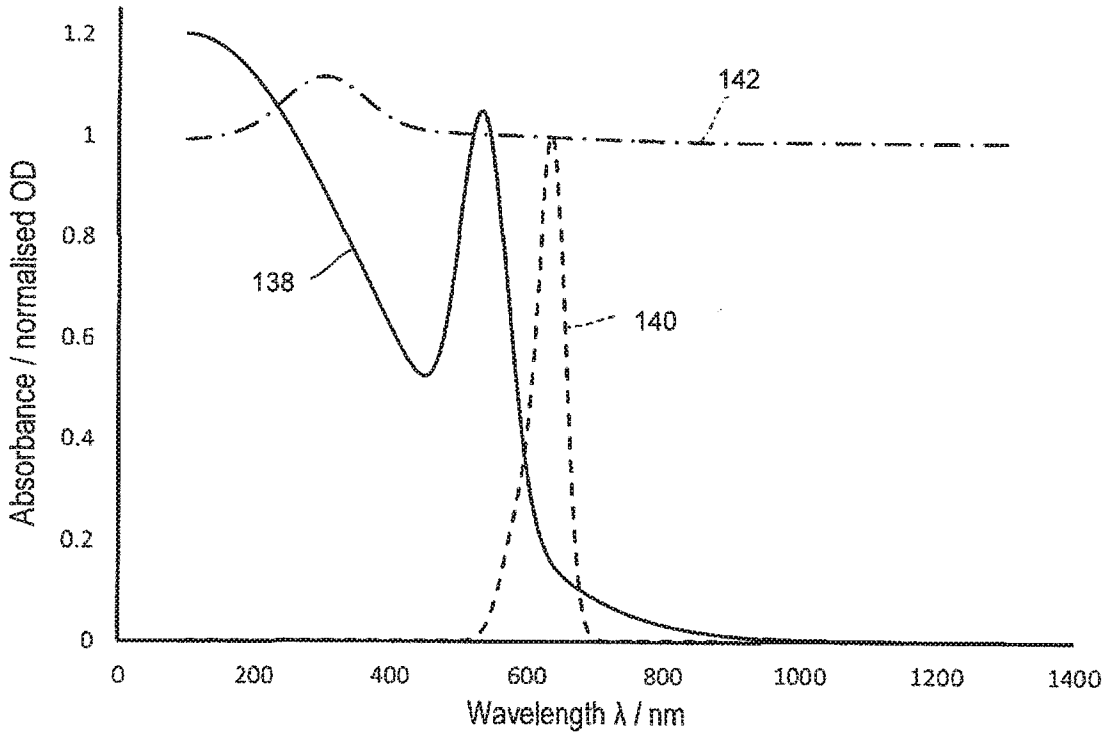
Absorbance / normalised OD
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400
Wavelength λ / nm
138
140
142

Fig. 39

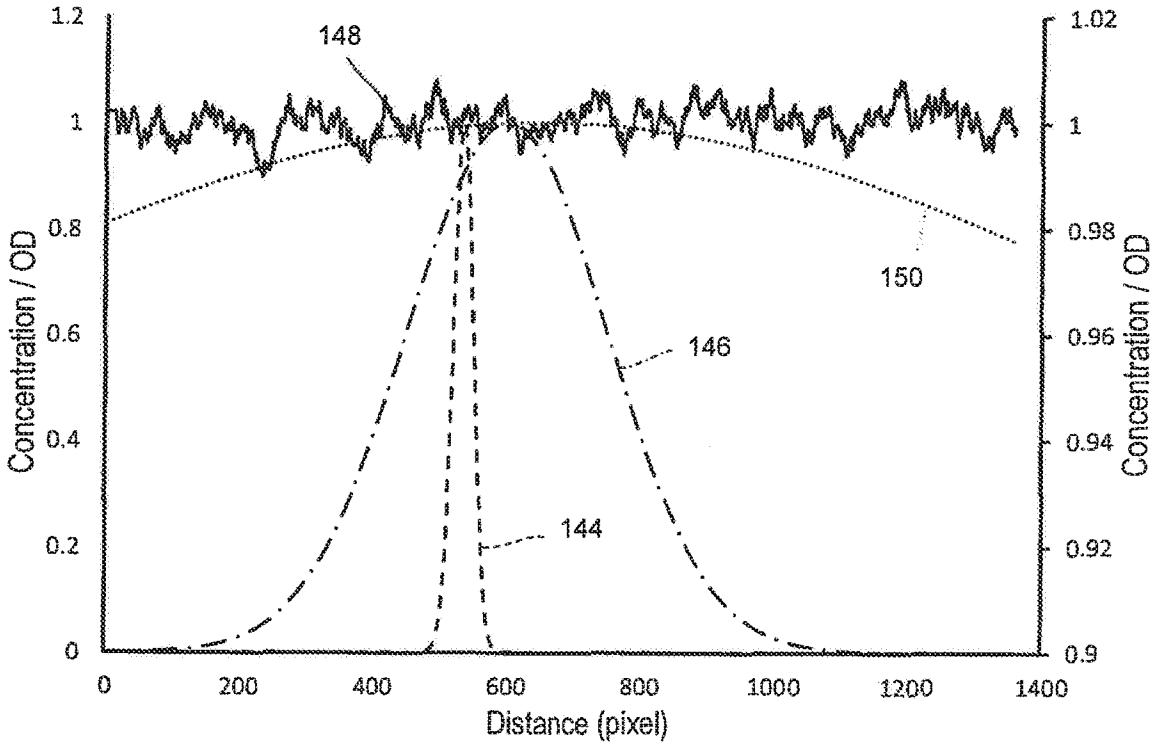
148
150
146
144
Concentration / OD
Concentration / OD
Distance (pixel)
1.2
1
0.8
0.6
0.4
0.2
0
1.02
1
0.98
0.96
0.94
0.92
0.9
0
200
400
600
800
1000
1200
1400

Fig. 40

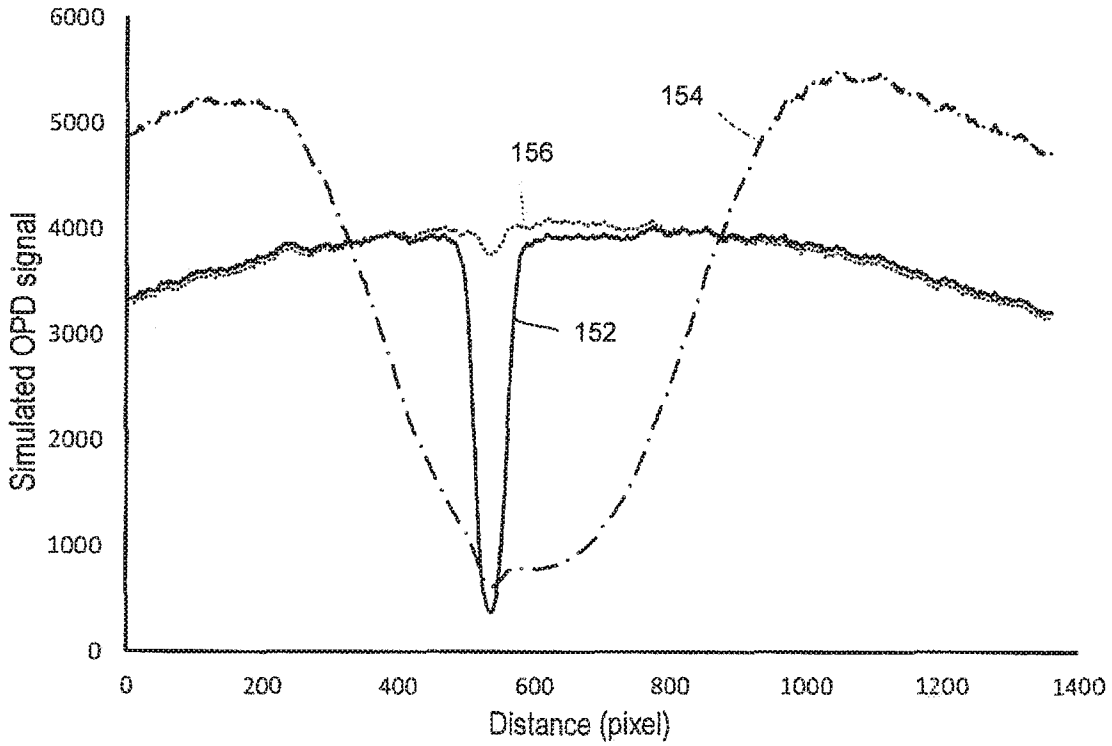
154
156
152
Simulated OPD signal
Distance (pixel)
6000
5000
4000
3000
2000
1000
0
0
200
400
600
800
1000
1200
1400

Fig. 41

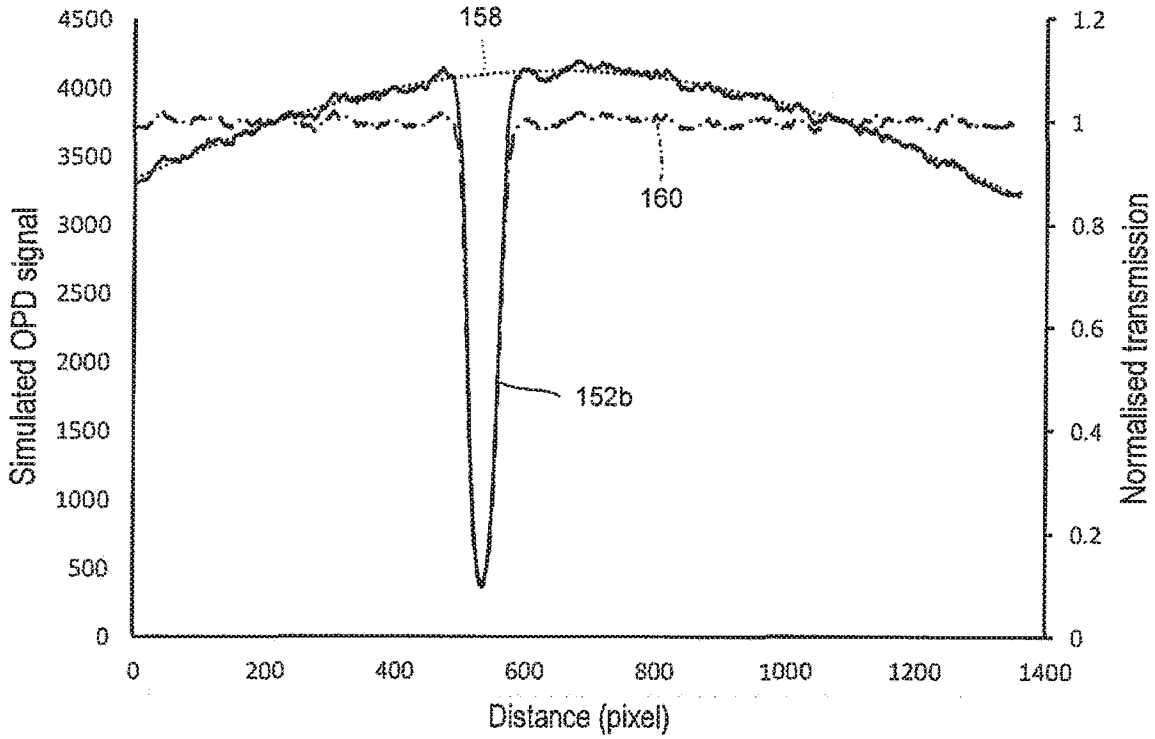
158
160
152b
Simulated OPD signal
Normalised transmission
Distance (pixel)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400

Fig. 42

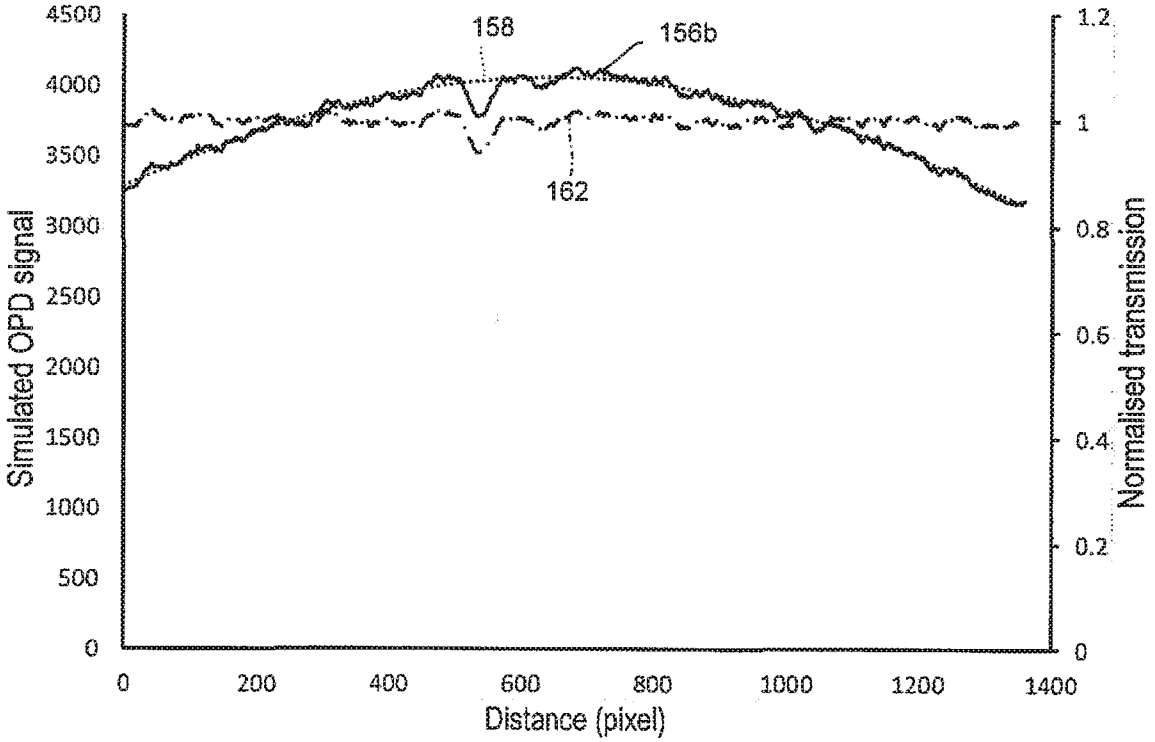
158
156b
162
Simulated OPD signal
Normalised transmission
Distance (pixel)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400

Fig. 43

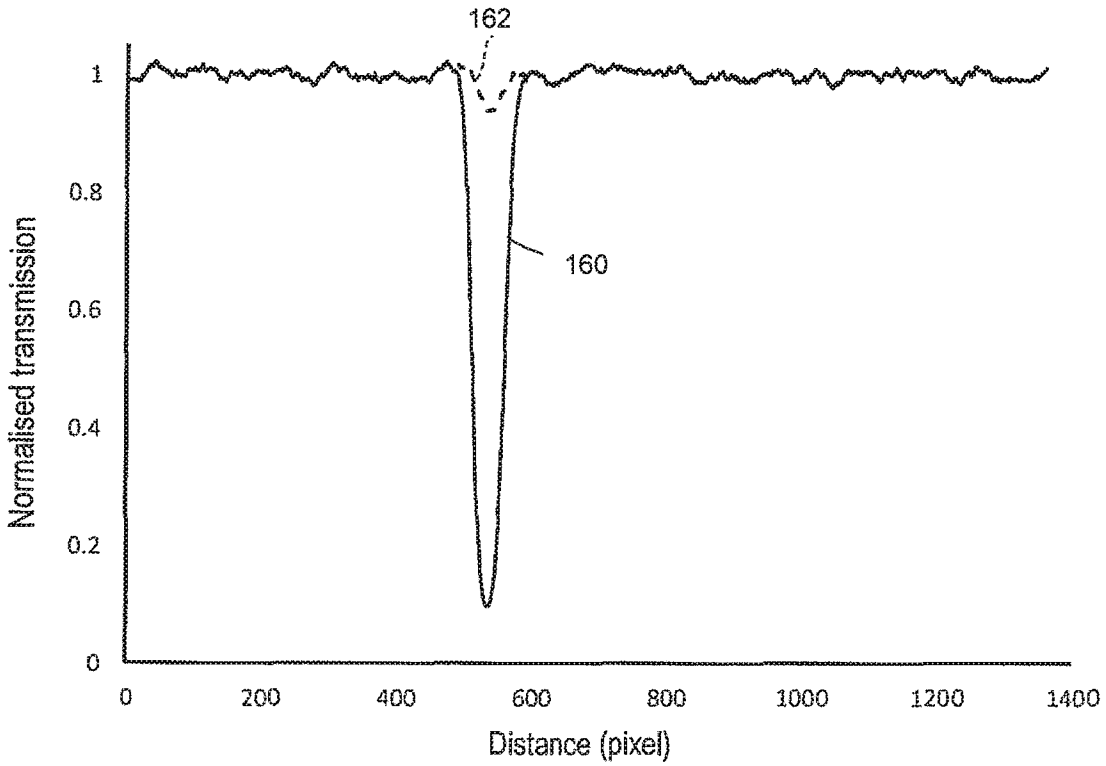
162
160
Normalised transmission
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400
Distance (pixel)

Fig. 44

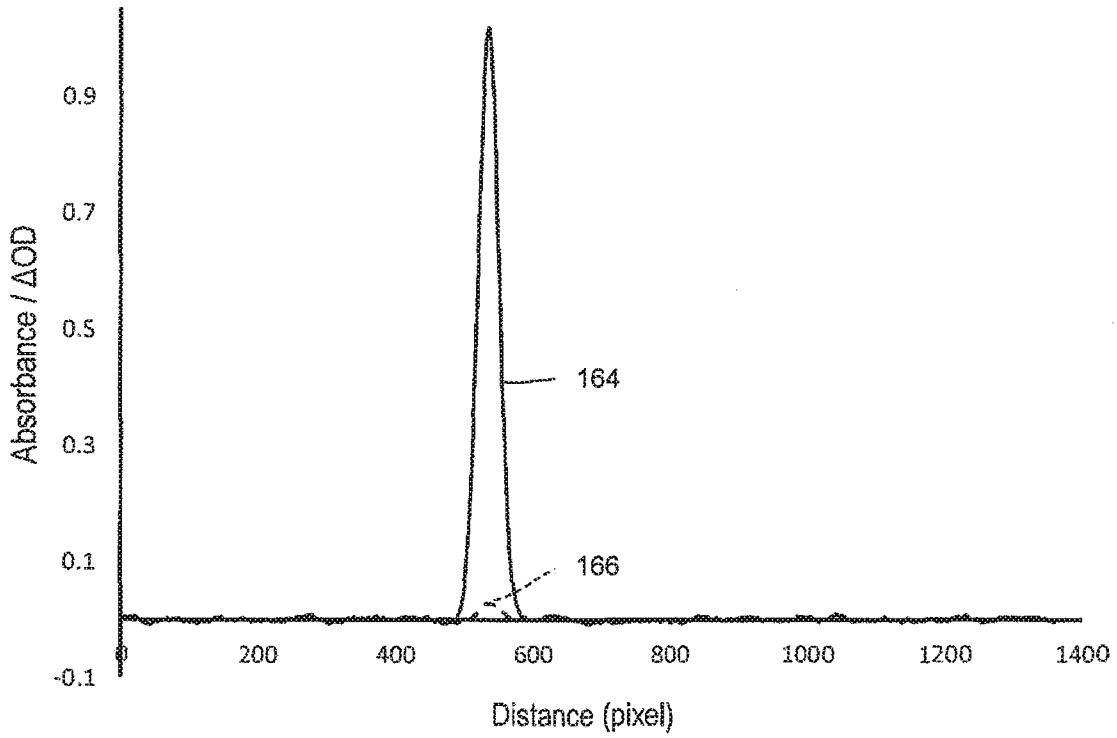
164
166
Absorbance / ΔOD
0.9
0.7
0.5
0.3
0.1
-0.1
200
400
600
800
1000
1200
1400
Distance (pixel)

Fig. 45

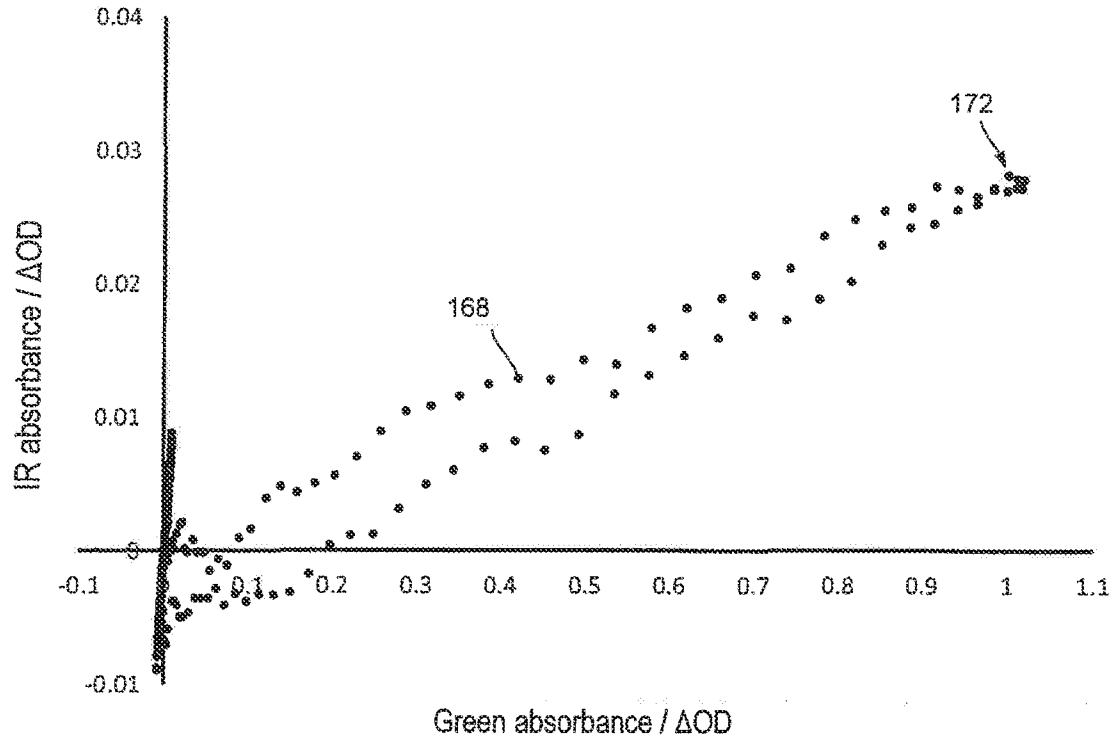
0.04
0.03
0.02
0.01
0
-0.01
IR absorbance / ΔOD
-0.1
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
Green absorbance / ΔOD
168
172

Fig. 46

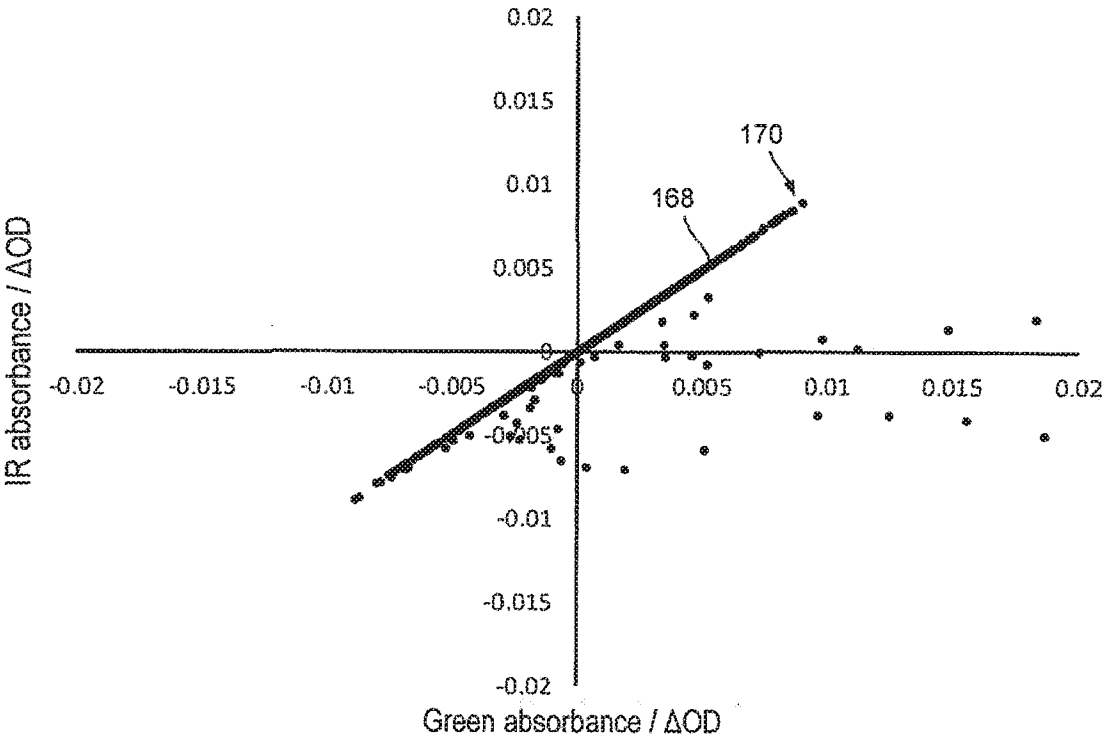
0.02
0.015
0.01
0.005
0
-0.005
-0.01
-0.015
-0.02
IR absorbance / ΔOD
-0.02
-0.015
-0.01
-0.005
0.005
0.01
0.015
0.02
Green absorbance / ΔOD
168
170

Fig. 47

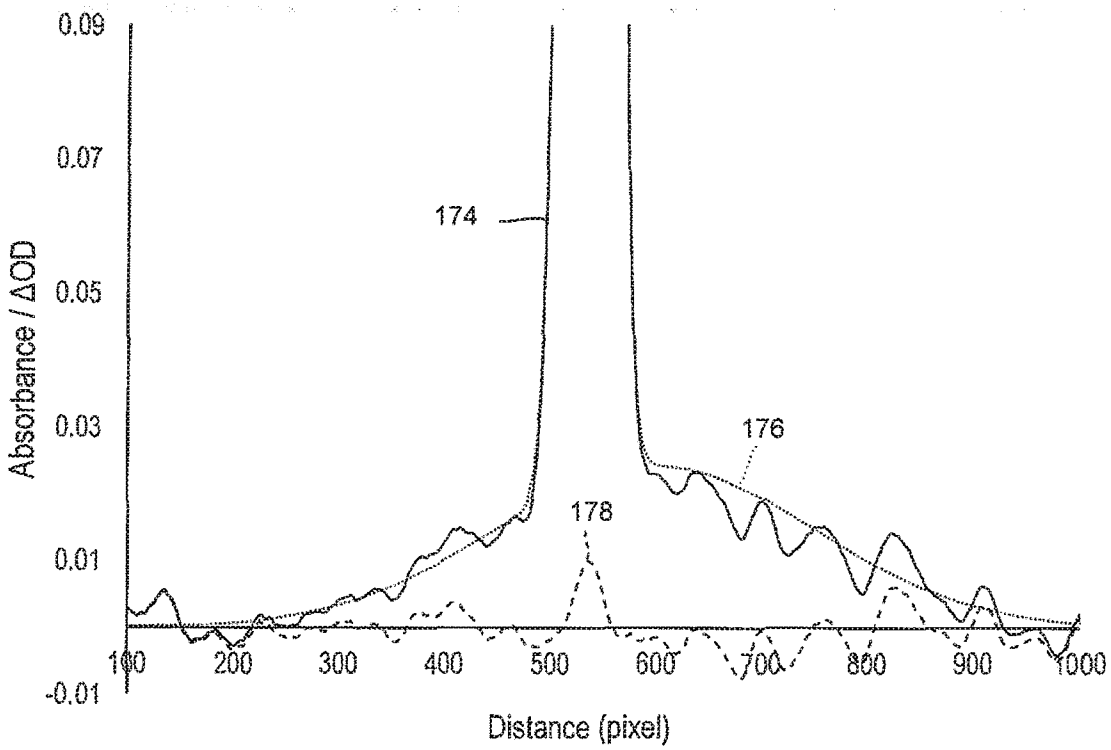
0.09
0.07
0.05
0.03
0.01
-0.01
Absorbance / ΔOD
174
176
178
100
200
300
400
500
600
700
800
900
1000
Distance (pixel)

Fig. 48

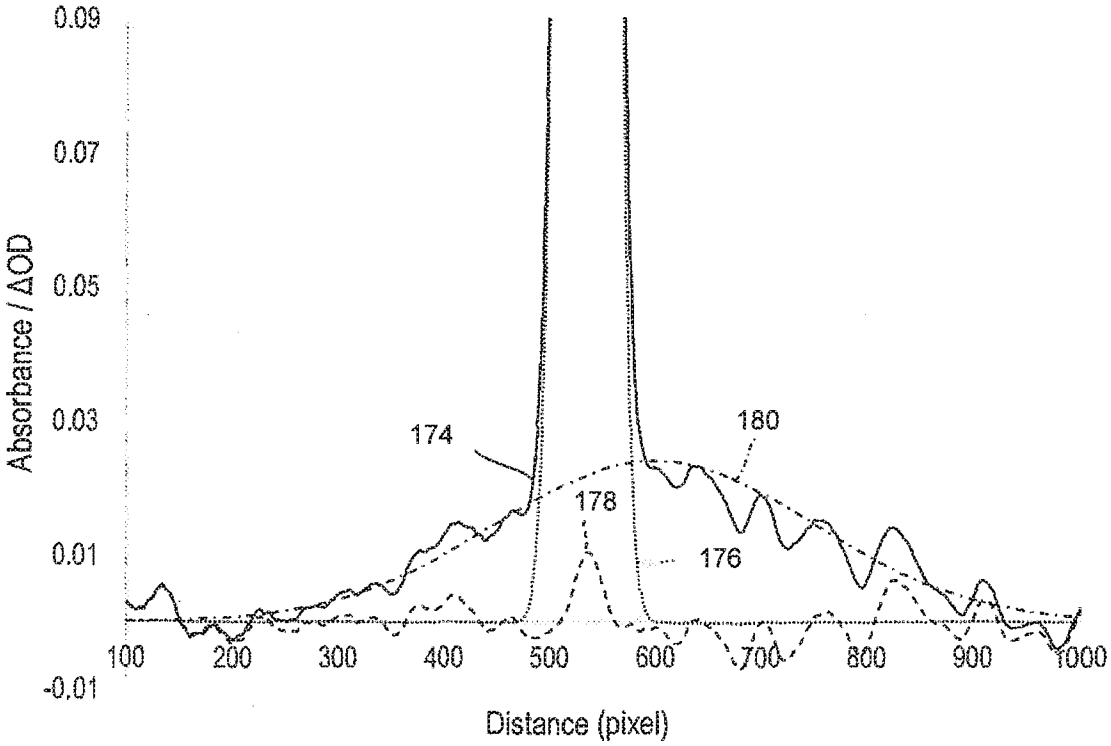
0.09
0.07
0.05
0.03
0.01
-0.01
Absorbance / ΔOD
174
180
178
176
100
200
300
400
500
600
700
800
900
1000
Distance (pixel)

Fig. 49

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/053688

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/31
ADD. G01N21/77 G01N21/84

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2013/003043 A1 (HOENES JOACHIM [DE] ET AL) 3 January 2013 (2013-01-03)	1-8,10, 11, 13-16, 25-27
Y	paragraphs [0002], [0003], [0021] - [0024], [0026], [0030] - [0032] figures 1-3 table 1	9,12, 17-24
Y	US 5 014 216 A (STAFFORD ROGER A [US] ET AL) 7 May 1991 (1991-05-07) the whole document	9,19-24
Y	WO 2017/203239 A2 (MOLECULAR VISION LTD [GB]) 30 November 2017 (2017-11-30) paragraph [0005]; claim 6	12,17

-/--

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

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 February 2019	01/03/2019

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

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/141527 A1 (DING ZHONG [US] ET AL) 22 May 2014 (2014-05-22) paragraphs [0015], [0016]	18
X	US 2015/153272 A1 (EHRING HANNO [SE] ET AL) 4 June 2015 (2015-06-04) paragraphs [0041] - [0043] figure 3	1,27
X	US 2015/177141 A1 (YEO YEONG-BAE [KR] ET AL) 25 June 2015 (2015-06-25) paragraphs [0005], [0057], [0061], [0066] figures 1-3	1,27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2018/053688

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