

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2013/131928 A1

(43) International Publication Date

12 September 2013 (12.09.2013)

WIPO | PCT

(51) International Patent Classification:

G01N 21/55 (2006.01)

(21) International Application Number:

PCT/EP2013/054446

(22) International Filing Date:

5 March 2013 (05.03.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

106192 5 March 2012 (05.03.2012) PT

(71) Applicant: BIOSURFIT S.A. [PT/PT]; Centro Empresarial de Aveiro, P-3811-501 Aveiro (PT).

(72) Inventor: DE OLIVEIRA GARCIA DA FONSECA, Joao Manuel; Quinta da Nascente, Casais de Baixo, P-2050-360 Azambuja (PT).

(74) Agent: KORENBERG, Alexander, Tal; Kilburn & Strode LLP, 20 Red Lion Street, London, Greater London WC1R 4PJ (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: ENHANCED SURFACE PLASMON RESONANCE METHOD

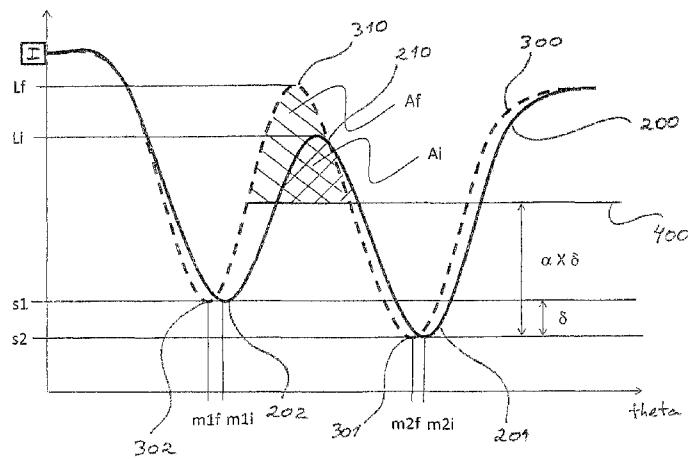


Fig. 2

(57) Abstract: The disclosure relates to processing SPR signals, in particular signals obtained by illuminating a conductive surface with light at two wavelengths. Embodiments involve processing a first and second signal indicative of an intensity of light, received from a conductive layer at which SPR has occurred, as a function of angle of incidence, reflection or diffraction at the layer (depending on whether the incident light beam is received by a detector recording it in reflection or transmission from the conductive layer). The first and second signals each have two dips corresponding to a respective wavelength of the light at a respective angle at which surface plasmon resonance occurs for the respective wavelength and a peak between the two dips. The processing includes deriving a first and second value of a quantity indicative of signal magnitudes in the region of the peak. The method then provides for comparing the first and second values to detect a change in refractive index at the layer after the first signal and before the second signal was captured.

WO 2013/131928 A1

## ENHANCED SURFACE PLASMON RESONANCE METHOD

5 The present invention relates to the detection of Surface Plasmon Resonance effects, in particular to detect changes in refractive index at a conductive surface, for example due to antibody/antigen binding events.

10 The phenomenon of Surface Plasmon Resonance (SPR) can be used to detect minute changes in the refractive index at a surface of a conductive layer as some event occurs near the conductive surface, for example a metal coated surface. In particular it may be used to quantitatively determine a reaction between antigens (targets) and antibodies immobilised on the surface (probes). Surface Plasmon Resonance is due to the oscillation of free electrons which exists at a metal boundary induced by a time varying electric field absorbing photos of an incident light beam. These oscillations are affected by the refractive index of the material adjacent the 15 metal surface and it is this that forms the basis of the sensor mechanism.

20 One of the most common SPR configurations involves the use of a polarized monochromatic light source (e.g. a diode laser) incident on the conductive layer, for example at the metal coated surface with a range of incident angles. In this configuration, one measures the light intensity of the reflected light beam as a function of angle over time while the event to be measured occurs, and detects a change of the angle at which a light intensity minimum occurs as a function of time.

25 For practical applications, SPR detection devices often include a cartridge having a liquid sample to be characterized. In many applications the cartridge is movable, in particular in some applications, the cartridge may rotate while SPR measurements are performed. SPR measurements may detect extremely small changes in a liquid sample (e.g. small concentration of a blood marker bound to corresponding probes), but may also be very sensitive to external effects (e.g. temperature, cartridge tilt and 30 position, bulk effects). The latter can lead to a significant decrease in signal to noise ratios and so limit the application scope of SPR device.

Therefore, it would be desirable to have SPR devices providing better signal to noise ratios when compared to conventional devices. Furthermore, in the case of SPR

devices including movable cartridges, it would be beneficial to have mechanisms for self-referencing and quality controls in order to ensure the quality of output data.

5 The specific description herein relates to a polarized double monochromatic light source incident on a detection zone. The double monochromatic light source has two wavelengths sufficiently similar so to induce the Surface Plasmon resonance effect at two similar incident angles, so that light can be captured for angles at which SPR occurs for both wavelengths, within a practical angular range. Two respective surface plasmons occur simultaneously over a predetermined critical incidence angle range  
10 and it is believed that their effect is combined to result in an increased light adsorption effect. Advantageously, the measurement of light reflection at the critical incidence angle range described herein shows a much greater sensitivity to events occurring in the liquid sample, compared with conventional known Surface Plasmon resonance devices. Furthermore, described herein are self-consistency tests  
15 associated to the double surface plasmon resonance measurements used for quality testing and feedback systems.

In a first aspect, there is provided a method for analysing a Surface Plasmon Resonance Signal as set out in independent claim 1.

20 Some embodiments include processing a first and second signal indicative of an intensity of light, received from a conductive layer at which SPR has occurred, as a function of angle of incidence, reflection or diffraction at the layer (depending on whether the incident light beam is received by a detector recording it in reflection or  
25 transmission from the conductive layer). The first and second signals each have two dips corresponding to a respective wavelength of the light at a respective angle at which surface plasmon resonance occurs for the respective wavelength and a peak between the two dips. The processing includes deriving a first and second value of a quantity indicative of signal magnitudes in the region of the peak. The method then  
30 provides for comparing the first and second values to detect a change in refractive index at the layer after the first signal and before the second signal was captured. Preferably, the step of detecting the change includes deriving a magnitude of the change.

Advantageously, this method is less sensitive to external effects, for example temperature, cartridge tilt and position, and bulk effects and, therefore, can provide improved signal to noise ratios.

5 It will be understood that reference to detecting a change (obtaining a magnitude of the change or merely that a change occurred) in refractive index includes doing so implicitly as the fundamental signal detectable by SPR, even if it is not explicitly calculated or detected. For example, the method may produce a result indicative of e.g. composition or concentration changes in a liquid sample of the conductive layer,  
10 such as targets binding to probes immobilised on the layer, temperature changes or any other changes affecting SPR. Further, the method may be equally applicable whether incident light is transmitted or reflected by the layer to reach a detector and irrespective of whether the beam path passes through a sample in contact with the liquid or not (e.g. by reflection from a surface of the layer not in contact with the  
15 sample). The method is equally applicable as an online method where signals are processed and changes detected as the signals are collected, or offline, where the signals are stored for later processing.

20 In some embodiments, the first signal was recorded before a sample is brought into contact with the layer and the second signal was recorded after the sample is brought into contact with the layer. It will be appreciated that in this way, the method allows a user to detect small changes of refractive index at the conductive layer due to a liquid sample. Preferably, probes are immobilised on the layer and the sample includes targets, such that the change in refractive index is due to targets binding to probes on  
25 the layer (and remaining there after a separate wash). This is particularly advantageous as the method can be used to detect small amounts of target present in a blood sample, blood plasma sample or other liquids.

30 In some embodiments, illumination may be provided at both wavelengths simultaneously and, additionally, the signals are captured at both wavelengths simultaneously. By capturing the signals at both wavelengths simultaneously, the signals required for the above processing can be captured in a simple and efficient way for example using an area detector, CCD array or a suitably placed photo

multiplier tube. Further, due to the simultaneous illumination, it is believed that SPR effects may be enhanced.

5 The quantity indicative of signal magnitudes in the region of the peak may be the height of the peak and/or it may be the area under the signal between the peak and a threshold intensity level. The threshold intensity level may be determined by defining the intensity difference between two SPR dips and a pre-defined multiplicative or additive constant. By providing for a method which considers the change in peak height or area, the method provides for greater sensitivity and increased signal to 10 noise ratios when compared to conventional methods which consider angular shifts in resonance angle or intensity measures locked onto certain angular positions. The measured quantity is directly based on the shape of the curve, rather than being indexed to a pre-defined angular position or relying on angle measurements.

15 Advantageously, the method may additionally provide for checking consistency between the first and second signals. This can be done by comparing at least two of the value of the quantity and the angle of each dip of the first signal to the same at least two of the second signal and checking if the respective changes between the first and the second signals are consistent. Such self-consistency checks may not be 20 available or even possible with conventional Surface Plasmon Resonance techniques. By providing for a self-consistency check, quality assurance can be provided to ensure that the results obtained by the method are reliable. Furthermore, where the method finds that the results are not consistent, feedback control can be used to make adjustments to correct the cause of inconsistency. For example, in some 25 embodiments, the method allows the orientation of the conductive layer to be adjusted to ensure consistency.

30 In some embodiments, the conductive layer is provided on a cartridge. Advantageously this allows the conductive layer to be easily changed; thereby minimizing down time between uses of the Surface Plasmon Resonance device. The cartridge may comprise liquid handling structures to bring a liquid sample into contact with the conductive layer. The cartridge may be rotatable to drive liquid flow. The layer may comprise a grating profile.

The conductive layer may be configured as a suitable diffraction grating to achieve SPR momentum coupling, or a prism configuration may be used to achieve momentum coupling by total internal reflection.

5 In some embodiments, the method includes tuning one or both of the wavelengths (or a difference therebetween) to enhance signal to noise ratios, for example for a given grating configuration or prism coupling arrangement. The tuning may be by means of cooling or heating (or both) element placed in between two laser diodes emitting at respective wavelengths and changing losing behaviour to control  
10 wavelength by temperature control. The diodes may intrinsically emit at the same wavelengths (the difference being established by a temperature difference) or at different wave-lengths (still tuning the difference using temperature).

15 In a second aspect, there is provided a method for detecting a change in refractive index as set out in independent claim 14.

20 Some embodiments comprise simultaneously illuminating a conductive surface with light at two wavelengths and measuring an intensity of light returned from the conductive surface at angles lying between an angle at which a first intensity minimum due to Surface Plasmon Resonance at one of the wavelengths occurs and an angle at which a second intensity minimum due to Surface Plasmon Resonance at the other one of the wavelengths occurs and detecting a change in the refractive index by detecting a change in the measured intensity.

25 In some embodiments, the step of detecting the change includes quantifying a concentration of target molecules in a sample applied to the conductive surface based on change in the measured intensity. The simultaneous illumination may be provided by separate monochromatic sources, such as two lasers, laser diodes or LEDs, combined using a beam splitter (possibly also using suitable filtering). A single  
30 source with suitable spectral lines may be used instead.

In third and fourth aspects there are provided systems configured to implement methods as described above, as set out in independent claim 16 and 29.

Embodiments are now described by way of example only to illustrate aspects and principles of the present disclosure, with reference to the accompanying drawings in which:

5       Figure 1 illustrates a device (1) comprising a rotatable cartridge (10) attached to a motor (50) and an optical module (60); wherein the motor (50) and the optical module (60) are both attached to a base (70). The optical module (60) comprises two laser diodes (62) and (63) aligned perpendicular to each other and a beam splitter (64) which is capable of combining the laser diode beams;

10      Figure 2 illustrates two output light intensity signals detected by the optical detector (80) of device (1) as illustrated in Figure 1, as a function of the incident angle at the optical detector (80); and

          Figure 3 illustrates an operating protocol of a device (1).

15      Figure 1 illustrates a device (1) comprising rotatable cartridge (10) which is releasably attachable to a motor (50). The device (1) further comprises an optical module (60). Both the motor (50) and optical module (60) are preferably attached to a base (70). The optical module (60) comprises two laser diodes (62) and (63) which are preferably aligned perpendicular to each other. Preferably, the optical module (60) comprises a beam splitter (64) suitable for combining the laser diode beams.

25      The light beam (100) coming from the beam splitter (64) is focused on a detection zone (30) on the cartridge (10). A grating surface (40) consisting of a grating dielectric with a predetermined grating profile covered by a thin metal layer may be provided on the cartridge (10) to provide a conductive layer acting as a detection surface for generating a Surface Plamon Resonance effect. The light beam (100) is then incident on an optical element (90), for example a lens, and then on an optical detector (80).

30      Preferably, the detection zone (30) may contain a sample liquid with specific biological elements to be quantitatively measured by the device (1). Preferably, the cartridge (10) comprises two plastic parts (11) and (12) which may be bonded together. The cartridge (10) is arranged such that while it is being rotated by action of the motor (50), the light beam (100) can be detected by the optical detector (80).

Figure 2 illustrates two output light intensity signals detected by the optical detector (80) of the device (1) described above in respect of Figure 1, as a function of the incident angle at the optical detector (80). As illustrated in Figure 2, the first light intensity output signal (200) comprises two dips (201) and (202) which correspond to the Surface Plasmon Resonance effect of each light source (62) and (63). Preferably, the device (1) is configurable to allow, a set of parameters to be adjusted; including but not limited to: the grating profile (i.e. grating period, grating height and profile shape), the range of incident angles and the wavelength difference between the two monochromatic sources (62) and (63). As depicted in Figure 2, in between the two Surface Plasmon Resonance dips (201) and (202) there is a light intensity peak (210). Due to the difference of wavelengths of the two monochromatic sources, the two dips (201) and (202) have different light intensity values.

The analytical device (1) may be used to probe or quantify events or elements in a liquid sample placed or flowing into at least one detection zone (30), and measurements maybe performed as a function of time. Preferably the device (1) determines a sensorgram, thereby providing the temporal variation of at least one parameter which affects Surface Plasmon Resonance.

The sensorgram may depict the angle of incidence corresponding to the minimum of the Surface Plasmon Resonance dip (201) of a light intensity output signal as a function of, e.g. incident, angle over time.

Continuing the detailed description of Figure 2, the curve (300) represents the light intensity output signal detected in the optical detector (80) after a period of time when the signals of the curve (200) was detected. A certain event occurring in the liquid sample, for example, the binding of a blood marker with a specific antibody coated on the thin metal layer of the grating surface (40) (and preferably a subsequent wash to remove excess sample),, The change in refractive index of the grating surface (40) associated with the event leads to a change of angular position of the minima of dips (301) and (302) compared to curve (200). Conventional methods relate to quantifying this shift, represented by the angular distance between  $m_{1i}$  and  $m_{1f}$  for one wavelength and the distance between  $m_{2i}$  and  $m_{2f}$  for the other wavelength.

5 The method described herein explores a change of the peak of light intensity of the output light signal (210) to (310), located in between the two Surface Plasmon Resonance dips. This will be noted from Figure 2 which illustrates a much more marked change of the peak (210) to peak (310), when compared to the changes of each Surface Plasmon Resonance dips.

10 The observed fact that both Surface Plasmon resonance dips originated each by one of the monochromatic light sources (62, 63) have significantly different minimum intensity may further be explored in order to enhance the change signal calculated by the device (1). By measuring the difference  $\delta$  in intensity between each dip (201) and (202), defined by s1 and s2 in Figure 2, the device (1) determines a threshold level (400) and calculates the total area of the peak above that threshold. The threshold is in some embodiments calculated as a factor at times the difference  $\delta$ .

15 The area  $A_i$  changes into area  $A_f$  while the event to be detected by the device (1) occurs. Detection of the change in area may be done with a sensitivity increased by many orders of magnitude when compared to the angular shift between  $m_{2i}$  and  $m_{2f}$ , which is the only information used by conventional methods.

20 The analytical method described herein, explores this novel effect, associated with the asymmetric change of each Surface Plasmon Resonance dip for the two-wavelength intensity signal.

25 An additional advantages of the method described herein, is due to the fact that the claimed method measures light which is easier than measuring dark, thereby enabling a further increase in the signal to noise ratio.

30 Further described herein is a method of self-consistency checking provided by the device (1) to overcome quality control issues occurring in conventional Surface Plasmon Resonance devices. One important aspect associated with Surface Plasmon Resonance analytical devices relates to consistency and quality controls.

By exploring known correlations between features detected in the output signal and illustrated in Figure 3, the device (1) may be used with built-in self-consistency checks. These checks may include acceptance and rejection criteria for accepting or rejecting measurements from specific detection zones having non-consistent output signals. In one example of correlations used for self-consistency checks, if the shift or change of the dip m<sub>2</sub> is significantly smaller or larger than the shift or change of m<sub>1</sub> then measurements are not deemed to be self-consistent. In another examples if both shifts of m<sub>1</sub> and m<sub>2</sub> are consistent but the shift of the peak intensity L is significantly smaller or larger when compared to an expected shift obtained from correlation with the shifts of m<sub>1</sub> and m<sub>2</sub>, then measurements are also not deemed to be self-consistent.

Figure 3 illustrates an operating protocol of the device (1).

Non consistency of each output parameter may arise from different effects; for example, variations may occur depending on the orientation or position of the cartridge (10) with respect to the optical module (60), particularly if it is outside an acceptable range. The device (1) described herein may be operated in such a way that measurements of correlated parameters of output data can be used to determine the relative position of the cartridge (10) and/or its orientation with respect to the optical module (60), thereby allowing corrective algorithms for measured shifts to be applied. Furthermore, having determined the position and orientation of the cartridge (10), the device (1) may be arranged in such a manner as to induce feedback correction actions by mechanical actuators.

One specific, embodiment of a device and method is described.

The cartridge (10) consists of two disk-shape polycarbonate parts of 0.6mm thickness each bonded together, having an outer diameter of 120mm and an inner hole of 15mm diameter centred at the rotational axis of the motor (50). The cartridge contains 30 detection zones (30) each of 0.02mm depth engraved into the upper cartridge part (11) having an average radius of 50mm from the rotational axis and being capable of holding 0.2uL of blood. It will be appreciated that the cartridge need

not be provided in a disk shape and the dimensions and materials of the cartridge described herein are illustrative only.

5 While the cartridge is rotated, the liquid sample, consisting of diluted blood plasma, flows from upstream chambers into downstream chambers passing each detection zone (30). Preferably, the cartridge is rotated at around 25Hz. The lower part (12) of the cartridge (10) may contain a grating at the detection zones (30) which is in some embodiments a sine-trapezoidal shape. More particularly, the grating may have a grating period of 950nm and a grating height of 50nm in some embodiments. The 10 grating is defined in the polycarbonate part (12) and maybe coated by 100nm of gold, wherein antibodies are attached. It will be appreciated that other suitable coating may be provided which allows for antibodies to attach thereto.

15 The optical module (60) contains two diode lasers (62) and (63) in some embodiments. In an illustrative example, the diode lasers emit at 785nm and 808nm, and are aligned perpendicularly. A beam splitter consisting of a glass plate of 0.1mm of thickness and 15nm of reflective metal layer, with the thickness adjusted in order to have ~50% of light transmission at ~800nm of wavelength is provided. An acrylic 20 cylinder (65) preferably focuses the light beam (100) into the grating surface (40) of the detection zone (30). The first order reflective diffraction passes into a polarizer (90) and is incident into the optical detector, preferably a CMOS camera (80). The camera can detect the output light signal over ~3° of angular range, centred at ~55° with respect to a plane of the grating surface (40). It will be appreciated that other 25 suitable specifications and arrangements of diode laser, beam splitter and optical detector may be provided.

30 The motor (50) may be a standard BLDC motor, which can be controlled with rotational speeds between 5Hz and 150Hz. Both the motor (50) and the optical module (60) can be attached to a base (70) and the whole system is preferably temperature controlled by external components.

When the motor is rotated, at 25Hz in one example, a first buffer liquid, which consists of PBS 1X (or any other suitable buffer), in this example, can flow through the detection zones (30) at a roughly constant flow rate. The acquired signal is

represented by the curve (200) in Figure 2. Then the sample diluted blood plasma flows through the detection zone and finally a buffer liquid preferably consisting of PBS 1X flows again through the detection zone (30), washing and making a final baseline signal represented in Figure 2 by the curve (300).

5

The observed change is substantially proportional to the concentration of an analyte having bound to antibodies immobilised on the grating surface of a detection zone (30).

10 The intensities and angular positions of the dips and peaks may be further analysed for self-consistency as described above and the measurement(s) may be accepted or rejected depending on the observed values and criteria.

15 In some embodiments, the two Surface Plasmon Resonance dips are expected to occur centred at respective angles, for example 54.5° and 55.5°. Several experimental effects may lead to different values for the two Surface Plasmon Resonance dips, such as: bending or orientation of the cartridge, temperature of the system, liquid sample concentration, biological coating, etc. In one example, the system is defined with an acceptance criteria as an angular tolerance, e.g. of +/-0.1°, 20 meaning that the measurement is accepted only if the measured dip angles have a difference consistent with the respective angular tolerance, e.g. of 1° +/- 0.1°. In a further example, the acceptance criteria includes also or instead an absolute angle criteria, for example requiring an angular spacing of 1° +/- 0.1° and a centre of each dip at specific angles, e.g. 54.5° and 55.5°, respectively. Measurements would be 25 rejected in this latter case, if the two dips had an angular spacing of 1° +/- 0.1° but were centred around 54.5° and 55.5°, respectively. This could in practice occur if, for example, the cartridge had a tilted orientation with respect to its expected orientation.

30 In particular embodiments, a cartridge orientation tilted at a certain angular value [e.g. 5°] would lead to an equivalent shift of both SPR dips [e.g. the same 5°] due to the corresponding tilt of the conductive layer / detection surface. This tilted orientation could then be easily determined by observing the initial angular positions of the two SPR dips. This tilted orientation may be then considered, in some implementations, irrelevant for the quality of the measurements, provided that there is consistency in

the relative angular position of the dips. In other embodiments, the tilted orientation of the cartridge could be considered important for the acceptance of measurements, even if both SPR dips are observed to be mutually spaced within the acceptable angular range. One could then reject measurements by defining and implementing 5 specific acceptance criteria accordingly.

Furthermore, in specific embodiments, a feedback loop applies commands to action 10 mechanical actuators in order to correct the orientation of the cartridge (and hence the conductive layer / detection surface). For example, a feedback mechanism is in some embodiments implemented as follows: (i) confirm the two SPR dips are spaced within the acceptable range; (ii) find an angular deviation of dip centre position from the expected position, e.g. 2°; (iii) tilt the optical module (60) by the determined deviation to bring the two SPR dips to their expected angular position; (iv) perform further measurements in accordance with the embodiments described above.

15 Several possible correlations for quality control may be derived depending on the application and, each particular implementation of the Surface Plasmon Resonance detection system. Correlations rely on the expected common shapes of optical signals resulting from similar but slightly different two Surface Plasmon Resonance 20 dips. Self-consistency parameters need to be observed in order to accept specific measurements in such embodiments.

In one specific implementation, the SPR dips need to be within a predefined angular 25 position [e.g. 54.5° +/- 0.1° for the first SPR dip and 55.5° +/- 0.1° for the second SPR dip] for the initial SPR measurements prior to passing a sample and the mutual spacing between the two SPR dips need to be within another predefined angular range [e.g. 1.0° +/- 0.1°]. In this case measurements are rejected if they do not comply with the acceptance criteria.

30 In another specific implementation example, additionally or alternatively, the intensity level of the initial peak  $A_i$  between the two SPR deeps need to be a pre-defined fraction of the maximum measured light intensity, for example 70% +/- 1% of the maximum measured light intensity.

Measurements are rejected if they do not comply with all the applicable acceptance criteria, in some embodiments.

In other specific implementation examples, other or additional criteria may be preferable, for example where further additional acceptance criteria are necessary for accepting measurements. Additional relevant acceptance criteria may include, but are not limited to, the relative difference of light intensity of the two SPR dips; the relative angular spacing between the initial peak  $A_i$  and the two SPR dips  $m_{1i}$  and  $m_{2i}$ ; the relative difference of shift of each of the two SPR dips, etc.

10

In some embodiments, the diode lasers (62) and (63) may be identical or emit intrinsically at the same wave length. Further an additional peltier thermoelectrical module is placed in between the two laser diodes in these embodiments, and operated in such a way as to induce a temperature difference between the diodes (62) and (63). This temperature difference leads to a significant difference lasing wavelength, in view of the known dependency of the lasing wavelength with temperature. By varying the temperature differences between the elements (62) and (63), the induced temperature difference may be turned in order to maximize the double surface Plasmon resonance effect described above. The same arrangement may also be used to tune the wavelength difference between two laser diodes intrinsically emitting at the same frequency.

15

In some embodiments, the detection surface has a grating surface of a sinusoidal shape, trapezoidal shape or triangular shape, instead of a sine-trapezoidal shape.

20

Each specific shape of the grating surface will result in a different and characteristic shape of each SPR dip, in particular expected SPR dip width and symmetry. Accordingly, where quality or feedback control as described above is employed, the parameters of acceptance criteria are adjusted for each particular grating implementation.

25

Alternatively, in some embodiments, detection systems have a detection surface with a flat conductive surface and use a prism configuration to achieve the momentum coupling required for SPR to occur. In this later case, the SPR dips have a known

pre-defined shape. Again, if quality or feedback control is used, the acceptance criteria need to be adjusted to this particular implementation.

It will be appreciated that the invention is not limited to any specific type of cartridge 5 dimensions, configurations or materials, nor to a specific number detection zones.

For the avoidance of doubt, the term "microfluidic" is referred to herein to mean devices having a fluidic element such as a reservoir or a channel with at least one dimension below 1 mm.

10

It will also be appreciated that the present invention is not intended to be limited by the particular described embodiments and examples.

Further embodiments are disclosed in the following clauses:

15

1. A Surface Plasmon Resonance (SPR) sensing method comprising the steps of: providing a SPR sensor comprising a SPR supporting sensor surface; contacting a sample to be analysed with said sensor surface and monitoring at least two resonance conditions at said SPR supporting sensor surface by illuminating said sensor surface with two SPR exciting light beam at varying incidence angles and 20 sensing the reflected test light beams;

determining at least one property of said reflected or transmitted test light beam; characterized by

- illuminating said sensor surface with two light beam of similar but not equal wavelengths;

25

- performing the simultaneous measurement of the two SPR dips originated by the two light beams;

- determining the intensity level of each of the two SPR dips;

- determining a threshold level defined by the product of the intensity difference between the two SPR dips and a pre-defined constant;

30

- determining the integrated light intensity existing in between the two SPR dips above the calculated threshold as a function of the incidence angles;

2. A method according to clause 1, wherein the sensor surface includes a grating profile;

3. A method according to clauses 1-2 wherein further determination is performed of the angular positions of each of the two SPR dips, by absolute intensity or centroid method or any other method able to determine light intensity minima as a function of  
5 incidence angle;
4. A method according to clauses 1-3 wherein the sensor surface is contained in a cartridge operated by rotation;
- 10 5. A method according to clauses 1-4 wherein shifts are calculated for some biological event for each of the SPR dips, and additional self-consistency tests are performed with respect to both SPR dips, comparison with pre-defined acceptance/rejection criteria is carried and measurements are then accepted or rejected according to the comparison results;
- 15 6. A method according to clauses 1-4 wherein shifts are calculated for some biological event for each of the SPR dips, and information of position and orientation of the cartridge containing the sensor surface is obtained from the results of both SPR dips, corrective shift functions are applied based on the determined information of position and orientation of the cartridge, and further implementation of feedback on mechanical actuators is carried;

**Claims**

1. A method for analysing a Surface Plasmon Resonance Signal, the method comprising:

5 processing a first signal indicative of an intensity of light, received from a conductive layer at which SPR has occurred, as a function of angle of incidence, reflection or diffraction at the layer, the first signal having two dips corresponding to a respective wavelength of the light at a respective angle at which surface plasmon resonance occurs for the respective wavelength and a peak between the two dips, 10 wherein the processing includes deriving a first value of a quantity indicative of signal magnitude in the region of the peak;

15 processing a second signal indicative of an intensity of light, received from a conductive layer at which SPR has occurred, as a function of angle of incidence, reflection or diffraction at the layer, the second signal having two dips corresponding to a respective wavelength of the light at a respective angle at which surface plasmon resonance occurs for the respective wavelength and a peak between the two dips, wherein the processing includes deriving a second value of a quantity indicative of signal magnitude in the region of the peak; and

20 comparing the first and second values to detect a change in refractive index at the conductive layer after the first signal and before the second signal was captured.

2. The method of claim 1, wherein detecting the change includes deriving a magnitude of the change.

25 3. The method of claim 2, wherein the first signal was recorded before a sample is brought into contact with the layer and the second signal was recorded after the sample is brought into contact with the layer.

30 4. The method of claim 3, wherein probes are immobilised on the layer and wherein the sample comprises targets, such that the change in refractive index is due to targets binding to probes on the layer.

5. The method of any preceding claim, further comprising providing illumination at both wavelengths simultaneously and capturing the signals at both wavelengths simultaneously.

5

6. The method of any preceding claim, wherein the quantity indicative of signal magnitudes in the region of the peak comprises the area under the signal between the peak and a threshold intensity level.

10

7. The method of claim 6, wherein the threshold intensity level is proportioned to a difference between respective signal magnitudes at the dips.

8. The method of any one of claims 1 to 5, wherein the quantity indicative of signal magnitudes in the region of the peak comprises the height of the peak.

15

9. The method of any preceding claim, further comprising checking consistency between the first and second signals by comparing at least two of the value of the quantity and the angle of each dip of the first signal to the same at least two of the second signal.

20

10. The method of any preceding claim, further comprising comparing respective values of one or more parameters measured from the first signal, the second signal, or both to expected respective values of the one or more parameters and, preferably, rejecting the signals if the respective values do not match, and, additionally or alternatively, adjusting an orientation of the conductive layer to make the respective values match.

25

11. The method of any preceding claim, wherein the conductive layer is provided on a cartridge comprising liquid handling structures for bringing a sample into contact with the conductive layer.

30

12. The method of claim 11, wherein the cartridge is rotatable to drive liquid flow in the cartridge.

13. The method of any preceding claim, wherein the conductive layer comprises a 5 grating profile.

14. A method of detecting a change in refractive index, the method comprising:

simultaneously illuminating a conductive layer with light at two wavelengths;

measuring an intensity of light returned from the conductive surface at angles

10 lying between an angle at which a first intensity minimum due to Surface Plasmon Resonance at one of the wavelengths occurs and an angle at which a second intensity minimum due to Surface Plasmon Resonance at the other one of the wavelengths occurs; and

detecting a change in the refractive index at the conductive layer by detecting

15 a change in the measured intensity.

16. The method of any preceding claim, wherein the detecting the change includes quantifying a concentration of target molecules in a sample applied to the conductive surface based on change in the measured intensity.

20

17. A system for analysing a Surface Plasmon Resonance Signal, the system comprising:

means for processing a first signal indicative of an intensity of light, received from a conductive layer at which SPR has occurred, as a function of angle of 25 incidence, reflection or diffraction at the layer, the first signal having two dips corresponding to a respective wavelength of the light at a respective angle at which surface plasmon resonance occurs for the respective wavelength and a peak between the two dips, wherein the means for processing a first signal are arranged to derive a first value of a quantity indicative of signal magnitudes in the region of the peak;

means for processing a second signal indicative of an intensity of light, received from a conductive layer at which SPR has occurred, as a function of angle 30

of incidence, reflection or diffraction at the layer, the second signal having two dips corresponding to a respective wavelength of the light at a respective angle at which surface plasmon resonance occurs for the respective wavelength and a peak between the two dips, wherein the means for processing a second signal are 5 arranged to derive a second value of a quantity indicative of signal magnitude in the region of the peak; and

means for comparing the first and second values to detect a change in refractive index at the conductive layer after the first signal and before the second signal was captured.

10

17. The system of claim 16, wherein the means for comparing the first and second values to detect a change is arranged to derive a magnitude of the change.

15

18. The system of claim 17, wherein means for processing a first signal is arranged to record the first signal before the sample is brought into contact with the layer and wherein the means for processing the second signal is arranged to record the second signal after the sample is brought into contact with the layer.

20

19. The system of claim 18, wherein probes are immobilised on the layer, and wherein the sample comprises targets, such that the change in refractive index is due to targets binding to probes on the layer.

25

20. The system of any of claims 16 to 19, further comprising means for simultaneously providing illumination at both wavelengths and, preferably, means for simultaneously capturing the signals at both wavelengths.

30

21. The system of any of claims 16 to 20, wherein the means for processing the first and second signals are arranged to derive the quantity indicative of signal magnitudes in the region of the peak based on the area under the signal between the peak and a threshold intensity level.

22. The system of claim 21, wherein the threshold intensity level is proportional to a difference between respective signal magnitudes at the dips.

23. The system of any of claims 16 to 20, wherein the means for processing the first and second signals derive the quantity indicative of signal magnitudes in the region of the peak based on the height of the peak.

5

24. The system of any of claims 16 to 23, further comprising means for checking consistency between the first and second signals by comparing at least two of the value of the quantity and the angle of incidence of each dip of the first signal to the same at least two of the second signal.

10

25. The system of any of claims 16 to 24, further comprising means for comparing respective values of one or more parameters measured from the first signal, the second signal, or both to expected respective values of the one or more parameters and, preferably one or both of means for rejecting the signals if the respective values do not match and means for adjusting an orientation of the conductive layer to make the respective values match.

15

26. The system of any of claims 16 to 25, wherein the conductive layer is provided on a cartridge comprising liquid handling structures for bringing a sample into contact with the conductive layer.

20

27. The system of claim 26, comprising means for rotating the cartridge to drive liquid flow in the cartridge.

25

28. The system of any of claims 16 to 27, wherein the conductive layer comprises a grating profile.

29. A system for detecting a change in refractive index, the system comprising:

30

a light source arrangement for simultaneously illuminating a conductive layer with light at two wavelengths;

a detector arrangement for measuring an intensity of light returned from the conductive surface at angles lying between an angle at which a first intensity minimum due to Surface Plasmon Resonance at one of the wavelengths occurs and

an angle at which a second intensity minimum due to Surface Plasmon Resonance at the other one of the wavelengths occurs; and

a processor for detecting a change in the refractive index at the conductive layer by detecting a change in the measured intensity.

5

30. A system as claimed in claim 29, wherein the processor is arranged to implement a method as claimed in any one of claims 1 to 13.

10 31. The system of any of claims 16 to 29, the means for detecting or the processor being arranged to quantify a concentration of target molecules in a sample applied to the conductive surface based on change in the measured intensity.

15 32. A system as claimed in any one of claims 16 to 30, wherein the light source arrangement comprises two laser diodes, means for combining light emitted from the two laser diodes and means for tuning the wavelength emitted by each laser diode.

33. A system as claimed in claim 31, wherein the means for tuning comprise a cooling or heating element disposed between the laser diodes or next to one of the laser diodes.

20

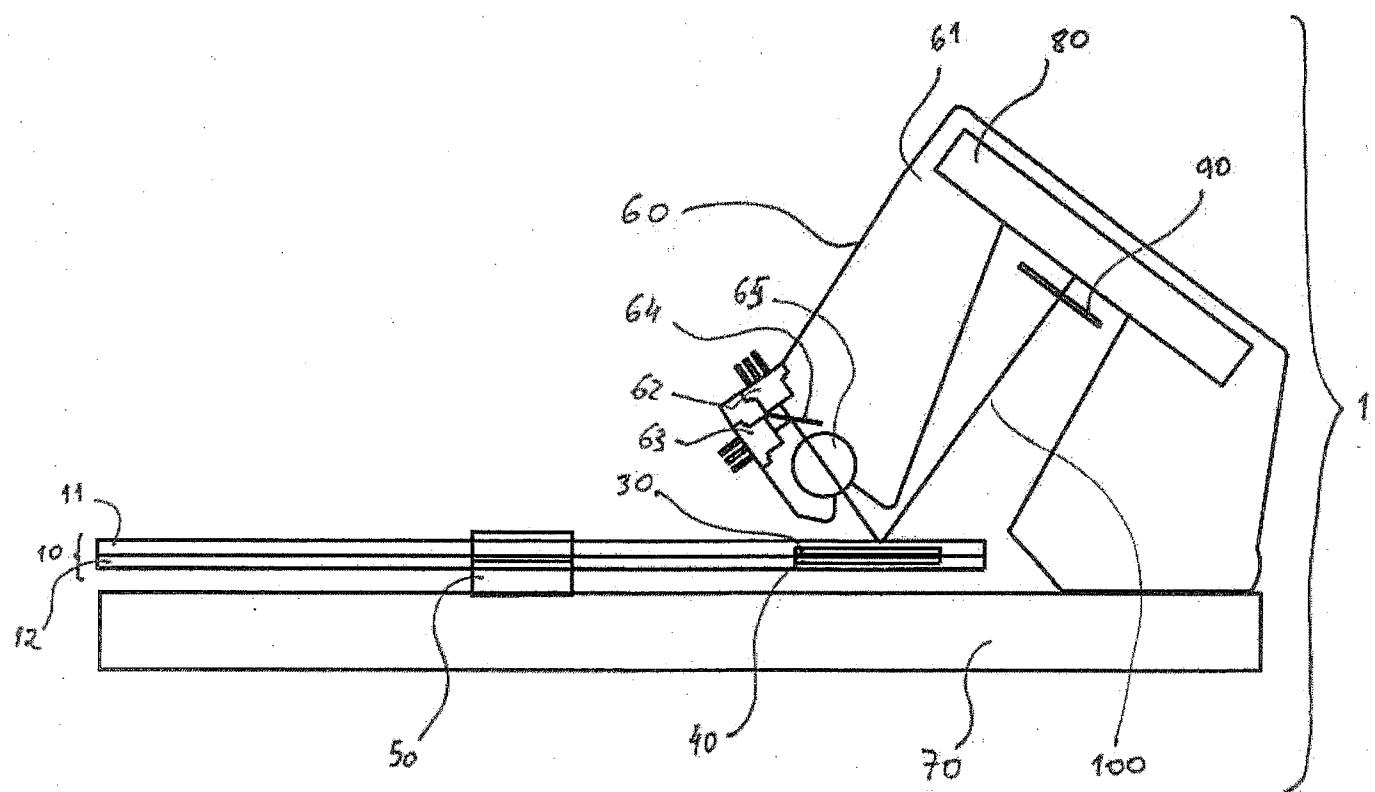


FIG. 1

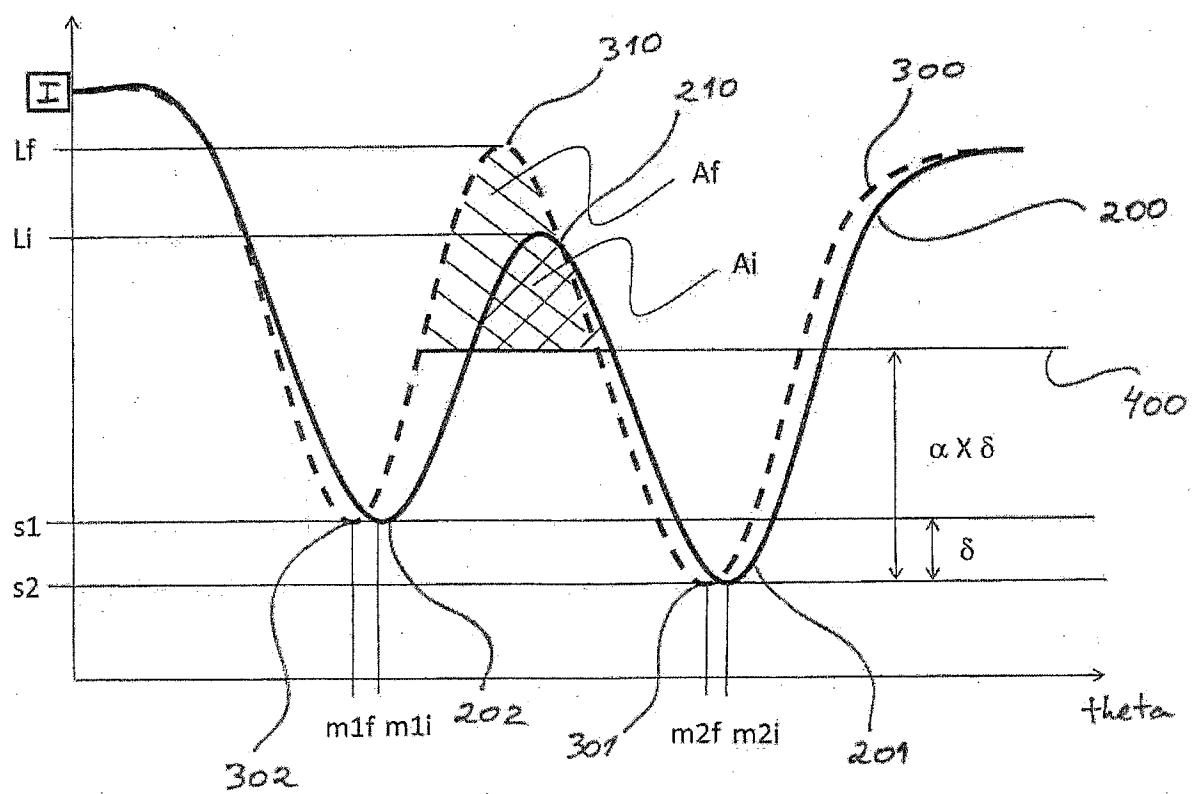


Fig. 2

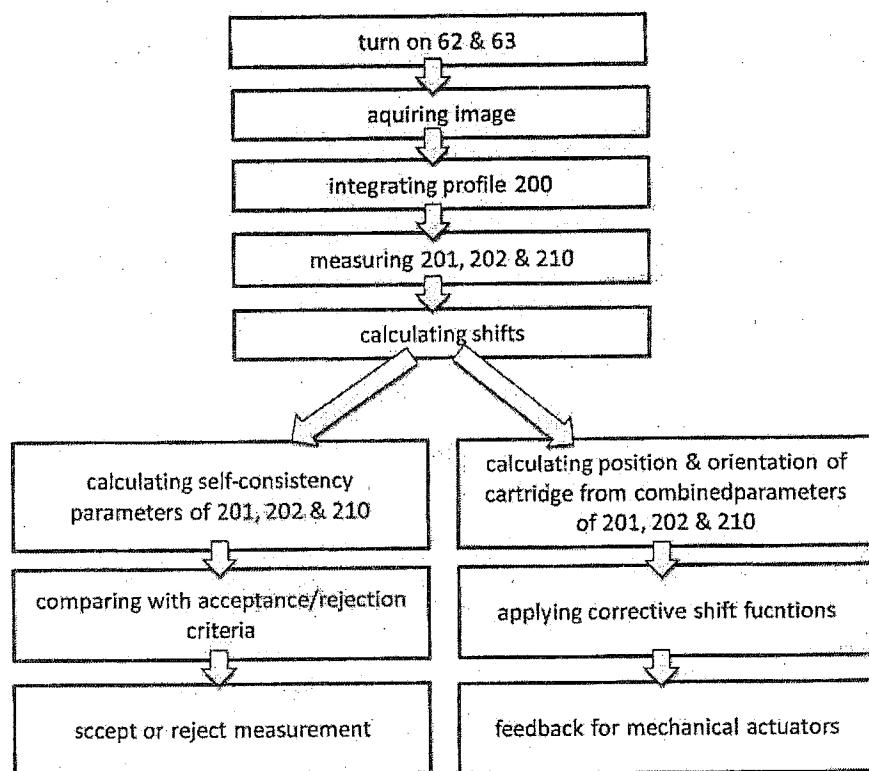


Fig. 3

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/EP2013/054446

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. G01N21/55  
ADD.

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, WPI Data, INSPEC

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/212102 A1 (NUZZO RALPH G [US] ET AL) 4 September 2008 (2008-09-04)	1-5, 13-20, 28-33
Y	paragraph [0003] paragraph [0012] - paragraph [0013] paragraph [0033] paragraph [0034] paragraph [0036] - paragraph [0037] paragraph [0040] paragraph [0042] paragraph [0118] paragraph [0039] ----- EP 1 684 063 A1 (SAMSUNG ELECTRONICS CO LTD [KR]) 26 July 2006 (2006-07-26) the whole document paragraph [0001] paragraph [0014] - paragraph [0015] ----- -/-	11,12, 26,27 11,12, 26,27
Y		-/-

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance  
"E" earlier application or patent but published on or after the international filing date  
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
"O" document referring to an oral disclosure, use, exhibition or other means  
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 2013	18/06/2013

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

Authorized officer

Stadlmeyer, R

## INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/054446

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 198 17 472 A1 (BIOTUL BIO INSTR GMBH [DE]) 28 October 1999 (1999-10-28) column 1, line 1 - line 7 column 7, line 37 claim 21 claim 29 ----- US 2010/271632 A1 (JOHANSEN KNUT [SE]) 28 October 2010 (2010-10-28) paragraph [0004] paragraph [0009] paragraph [0011] paragraph [0030] paragraph [0031] paragraph [0033] - paragraph [0034] ----- US 2009/231590 A1 (NAYA MASAYUKI [JP] ET AL) 17 September 2009 (2009-09-17) paragraph [0007] paragraph [0032] paragraph [0039] paragraph [0040] paragraph [0083] paragraph [0084] paragraph [0087] - paragraph [0088] paragraph [0117] paragraph [0133] ----- EP 1 967 844 A1 (OMRON TATEISI ELECTRONICS CO [JP]) 10 September 2008 (2008-09-10) paragraph [0009] - paragraph [0010] paragraph [0146] - paragraph [0147] paragraph [0155] ----- US 2008/037022 A1 (NISHIKAWA TAKEO [JP] ET AL) 14 February 2008 (2008-02-14) paragraph [0001] paragraph [0044] - paragraph [0045] paragraph [0049] paragraph [0050]; figure 8 -----	29,31 29,31 29,31 29,31 29,31 1,14,16, 29

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/EP2013/054446
---

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
US 2008212102	A1 04-09-2008	US 2008212102 A1 WO 2008030666 A2			04-09-2008 13-03-2008
EP 1684063	A1 26-07-2006	EP 1684063 A1 JP 2006201163 A KR 20060084499 A US 2006187459 A1			26-07-2006 03-08-2006 24-07-2006 24-08-2006
DE 19817472	A1 28-10-1999	AU 3927999 A DE 19817472 A1 WO 9954713 A2			08-11-1999 28-10-1999 28-10-1999
US 2010271632	A1 28-10-2010	EP 2232241 A1 US 2010271632 A1 WO 2009082353 A1			29-09-2010 28-10-2010 02-07-2009
US 2009231590	A1 17-09-2009	EP 1943500 A1 US 2009231590 A1 WO 2007037520 A1			16-07-2008 17-09-2009 05-04-2007
EP 1967844	A1 10-09-2008	CN 101261227 A EP 1967844 A1 JP 2008216055 A US 2008218761 A1			10-09-2008 10-09-2008 18-09-2008 11-09-2008
US 2008037022	A1 14-02-2008	CN 1918467 A US 2008037022 A1 WO 2005078415 A1			21-02-2007 14-02-2008 25-08-2005