

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 January 2007 (25.01.2007)

PCT

(10) International Publication Number
WO 2007/010469 A1

(51) International Patent Classification:

G01N 21/64 (2006.01) G01N 21/21 (2006.01)
G01N 21/55 (2006.01)

(21) International Application Number:

PCT/IB2006/052430

(22) International Filing Date: 17 July 2006 (17.07.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

05106685.0 21 July 2005 (21.07.2005) EP
05111139.1 23 November 2005 (23.11.2005) EP

(71) Applicant (for all designated States except US): **KONINKLIJKE PHILIPS ELECTRONICS N. V.** [NL/NL]; Groenewoudseweg 1, NL-5621 BA Eindhoven (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KLUNDER, Derk Jan Wilfred** [NL/DE]; C/o Philips Intellectual Property &, Standards Gmbh Weisshausstr. 2, 52066 Aachen (DE). **BAKKER, Levinus Pieter** [NL/DE]; C/o Philips Intellectual Property &, Standards Gmbh Weisshausstr. 2, 52066 Aachen (DE). **STALLINGA, Sjoerd** [NL/DE]; C/o Philips Intellectual Property &, Standards Gmbh Weisshausstr. 2, 52066 Aachen (DE).

(74) Agent: **VOLMER, Georg**; Philips Intellectual Property &, Standards Gmbh Weisshausstr. 2, 52066 Aachen (DE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

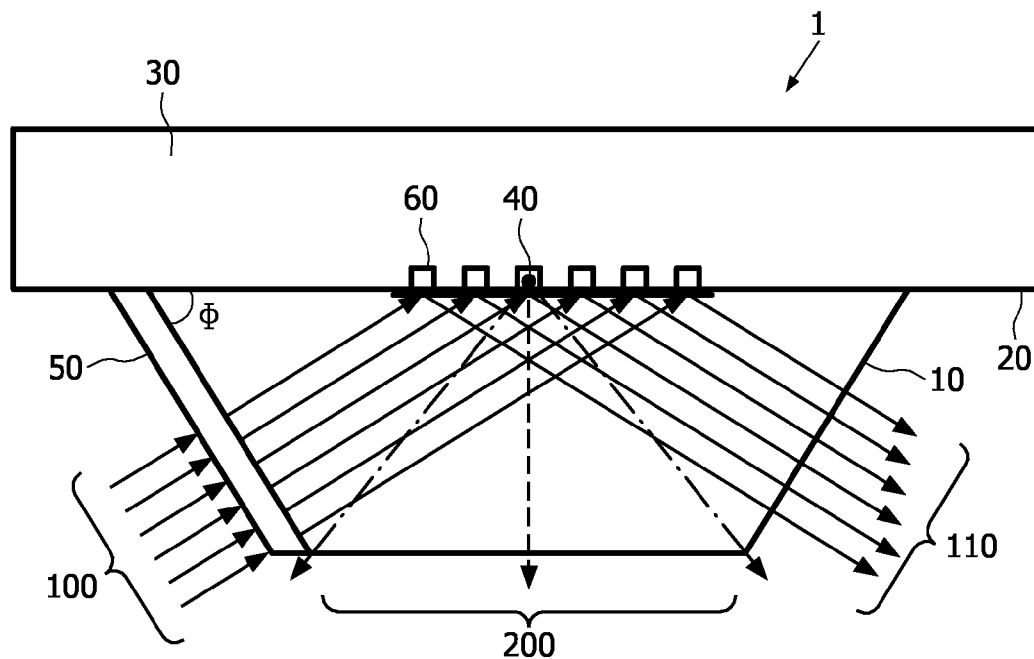
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

[Continued on next page]

(54) Title: DEVICE FOR DETECTION OF EXCITATION USING A MULTIPLE SPOT ARRANGEMENT



(57) Abstract: The invention relates to a device (1) for detection of excitation (110) using a multiple spot arrangement (60), in which a multiple spot generator (50) is matched to the multiple spot arrangement (60) in such a way that light (100) entering the multiple spot generator (50) will be guided to defined areas on the multiple spot arrangement (60).

WO 2007/010469 A1



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Device for detection of excitation using a multiple spot arrangement

5

The present invention is directed to the field of devices for detection of excitation, especially for evanescent field excitation.

In the field of devices for detection of excitation, especially for evanescent field excitation, which are used e.g. as biosensors, it has been common
10 technique to use a prism, which has an interface with the sample (usually an aqueous medium or other fluid). Into the prism, a single spot or collimated beam of light of a certain wavelength is directed under an angle (with respect to the interface between the prism and the sample) larger than the total internal reflection angle. This results in an evanescent field at the interface with the sample. In case of absorption of the evanescent
15 field by species present in the sample, this can result in the excitation of excited states, which can relax to the ground state by e.g., luminescence resulting in the generation of luminescent radiation. Usually the luminescence is caused by a luminescent, mostly fluorescent-labeled biomolecule such as a protein or a DNA or RNA-strand. The luminescence is then measured and the biomolecule identified.

20 However, directing a single spot or collimated beam into the prism bears the following problems:

In case of wide-field illumination; for this case a large area of the interface between the prism and the sample is illuminated by a single (e.g., collimated) beam. When only a few luminophores (this is the general name for a centre which acts
25 as a source of light using the excitation light as input) are present at the interface between the sample and the prism, the volume that can contribute to the background radiation (this volume has order of magnitude of illuminated area times decay length of the evanescent field into the sample) due to e.g., luminophores not bound to the interface or the matrix/carrier (e.g., water) of the sample is significantly larger than the
30 volume that contributes to luminescence of bound luminophores [signal]. As a consequence the signal to background (sometimes called noise) ratio is in most cases poor. An advantage of this arrangement is that a single illumination allows to

measure/probe a large area of the interface between the prism and sample: short measurement times.

In order to increase the signal over background ratio a straightforward and well-known solution is to reduce the illumination area by e.g., using a focused spot.

- 5 Disadvantage of this method is that probing a large area of the interface requires scanning of the single spot over the interface. Moreover, in case of luminescence it is useless to use higher excitation intensity than the saturation intensity, because this will not result in a higher fluorescent signal.

Patent application EP04106477.5 proposes the use of a multiple spot
10 generator that generates an array of spots at the interface with the sample. Each individual spot can be seen as a collection of plane waves propagating towards the sample under different angles. Blocking the angles that are not total internally reflected at the interface with the sample (an would otherwise propagate into the sample, which is an undesired effect) by e.g., a mask with a period array of absorbing material results
15 in an array of spots that are total internally reflected. Disadvantages of this method are:

- The amount of excitation power that can be converted into the evanescent field is limited; portion of the spot propagating under angles not total internally reflected at the interface with the sample is blocked.
- Scanning of the spots requires synchronization of the array of spots and
20 the mask used for blocking.
- Paths of the excitation light and luminescence light propagating in the backward direction overlap which requires a wavelength splitter/filter for separating the luminescence from the excitation light.

Similar arguments like those described above also hold for surface
25 plasmon excitation with the main difference that efficient excitation of the surface plasmon wave requires a large part of the light to be in a small range of angles.

It is therefore an object of the present invention to provide a device,
30 which allows a quicker detection without deterioration in resolution or accuracy and making use of the available excitation power in a more efficient way.

This object is solved by a device according to claim 1 of the present invention. Accordingly, a device for detecting excitation, in particular luminescent excitation in a fluid sample, comprising

- a transparent slab having a slab-sample interface
- 5 - a multiple spot generator provided with the slab, and
- a multiple spot arrangement provided on or in the vicinity of the slab-sample interface

wherein the multiple spot generator is matched to the multiple spot arrangement in such a way that light entering the multiple spot generator will be guided
10 to defined areas on the multiple spot arrangement.

By using such a device, one or more – depending on the actual application - of the following advantages can be reached:

- The conversion of excitation power into evanescent waves is more efficient than in prior art solution; i.e., more power per spot compared with arrangement
15 with mask with blocks and because that maximum useful power per spot is limited by saturation effects dividing the power over multiple spots results in more efficient use of the total power.
- The optical paths of the excitation light (which is the light that enters the slab) and the luminescence (which is the light that is emitted by the sample) are better
20 separated
- The device is usually simpler than those of the prior art
- The device does not require synchronisation of multiple spot arrangement with other optical elements (like a mask used for blocking).

The term “slab” in the sense of the present invention means especially a
25 cuboid or prism-shaped device, which allows a multiple spot arrangement to be formed at an interface between the slab and the sample. According to a preferred embodiment, the slab is a prism; however, for other applications it may be preferred that the slab is cuboid-shaped.

The term “transparent” in the sense of the present invention means
30 especially that the material is essentially transparent (sufficiently low losses) for light having the excitation wavelength and/or the luminescent wavelength.

The term “slab-sample interface” in the sense of the present invention means especially that at least one side of the slab is exposed to the excitation light or is directed towards the sample. This side is then called the interface. In case that surface plasmon resonance is used (as will be described below), there may be a surface plasmon layer provided between the slab and the sample. The “slab sample interface” is then provided with the side of the surface plasmon layer projecting towards the sample.

The term “multiple spot generator ” in the sense of the present invention means especially a device that generates a spot pattern at the slab-sample interface having a well-defined amplitude and phase distribution. Preferably the multiple spot generator determines this way the intensity and angles of incidence with respect to the slab-sample interface.

The term “multiple spot arrangement” in the sense of the present invention means especially a spot pattern at the slab-sample interface having a well-defined amplitude and phase distribution.

The term “defined areas” in the sense of the present invention means especially certain areas on the slab-sample interface, towards which light from the multiple spot generator is directed to form the multiple spot arrangement.

According to a preferred embodiment of the present invention, the multiple spot generator comprises at least one diffractive element and/or a microlens arrangement. These have shown in practice to be suitable implementations for the multiple spot generator.

In case the multiple spot generator comprises a diffractive element it is preferred that the diffractive element employs one or more of the following features:

- The diffractive element is equipped as to generate a multiple spot array at the interface between the slab and the sample.
- The diffractive element should be transparent for excitation light.

In case the multiple spot generator comprises a microlens arrangement it is preferred that the microlens arrangement employs one or more of the following features:

- In case the slab is a prism the microlenses have a varying focal length so that each individual micro-lens generates a spot that is focused on the interface between the slab and the sample.

- In case the slab is cubic shaped the microlenses have a constant focal length and the lens array is illuminated with a tilted (with respect to the plane of the lens array) input beam, such that the spots at the interface have an angle of incidence larger than the total internal reflection angle.

5 According to a preferred embodiment of the present invention, the widths $w_{lens}(q)$ of the lenses in the microlens array are

$$2w_f(q) \leq w_{lens}(q) \leq s$$

whereby the widths $w_{lens}(q)$ of the lenses are selected independently from each other and the $w_f(q)$ is the beam waist of each spot just behind the lens and s is the distance between the spots at the hypotenuse.

10

Preferably, the widths $w_{lens}(q)$ of the lenses in the microlens array are

$$2.5 * w_f(q) \leq w_{lens}(q) \leq s * 0.8$$

whereby the widths $w_{lens}(q)$ of the lenses are selected independently from each other and the $w_f(q)$ is the beam waist of each spot just behind the lens and s is the distance between the spots at the hypotenuse.

15

Preferably $w_f(q)$ and the focal length f_q of each lens (q) in the array are calculated by the following equations:

$$f_q = f_0 + qd \sin(\phi)$$

$$w_f(q) = \sqrt{w_0^2 + \frac{f_q^2 \lambda_p^2}{\pi w_0^2}}$$

with:

20 f_0 being the focal length of the microlens (lens 0) closest to the angle between the interface and the hypotenuse of the prism;

$\pi/2 - \phi$ being the angle between the interface of the slab and the emitted light into the slab (I have taken this from Fig. 2), whereby the angle ϕ preferably is larger than the total internal reflection angle at the interface between slab and sample;

25 and

d being the pitch between the spots at the interface.

s and d are linked by the following equation.

$$s = d \cdot \cos(\phi)$$

According to a preferred embodiment of the present invention, the slab is a prism having a prism-sample interface with angles such that incident excitation light is totally internally reflected on a prism-sample interface. If we assume a plane wave incident normal to the tilted plane of the prism, preferably the angle of the prism ϕ is selected to be as follows:

$$\theta_{TIR} \leq \phi \leq \frac{\pi}{2}$$

with θ_{TIR} being the total internal reflection angle as the minimum angle with respect to the normal of the interface that results in total internal reflection:

$$\theta_{TIR} = \arcsin\left(\frac{n_{sample}}{n_{prism}}\right)$$

with n_{sample} as the refractive index of the sample and n_{prism} the refractive index of the prism.

If the angle is chosen between these margins, the incident light entering the prism will be totally internally reflected. For a(n) (array of) spot(s) the field can be expressed as a sum (integral) of plane waves. Each plane wave has a certain angle with respect to the interface between the slab/prism and the sample. Preferably, the angle of the prism ϕ is chosen somewhat larger than the lower boundary of the abovementioned relation for the prism angle.

According to a preferred embodiment of the present invention, the multiple spot generator is movable along or with the slab.

By doing so, one or more of the following advantages can be achieved:

- The focus of the multiple spot arrangement can be kept better on the interface between the slab and the sample; in case of a slab that has at least one hypotenuse.
- The alignment of the multiple spot generator with the slab can be improved.
- In case of an interface between the multiple spot generator and the slab (essentially) parallel to the interface between the slab and the sample one can scan (in 2D) the array of focused spots in a plane (essentially) parallel to the interface between the slab and the sample.

According to a preferred embodiment of the present invention, the light that enters the diffractive element and/or the multiple spot generator is bent and/or guided in order to reach the multiple spot arrangement with an angle ϕ (with respect to the normal of the slab-sample interface) towards the slab-sample interface of

$$5 \quad \theta_{TIR} \leq \phi \leq \frac{\pi}{2}$$

with θ_{TIR} being the total internal reflection angle as the minimum angle with respect to the normal of the interface that results in total internal reflection:

$$\theta_{TIR} = \arcsin\left(\frac{n_{sample}}{n_{prism}}\right)$$

with n_{sample} as the refractive index of the sample and n_{prism} the refractive
10 index of the prism.

According to a preferred embodiment of the present invention, the multiple spot generator is moved and/or translated along the hypotenuse of the prism. By doing so, a proper alignment of the spots generated by the multiple spot generator can be achieved in order to properly focus the array of spots on the interface between
15 the prism and the sample.

According to a preferred embodiment of the present invention, the total internal reflection cone of the slab is set to fulfill the equation:

$$\theta_{TIR} \leq \phi \leq \frac{\pi}{2}$$

with θ_{TIR} being the total internal reflection angle as the minimum angle
20 with respect to the normal of the interface that results in total internal reflection:

$$\theta_{TIR} = \arcsin\left(\frac{n_{sample}}{n_{prism}}\right)$$

with n_{sample} as the refractive index of the sample and n_{prism} the refractive
index of the prism.

According to a preferred embodiment, the angle θ_m , which is the
25 maximum angle (relative to the normal of the interface between the slab and the environment) in the slab under which the generated luminescence can still be detected is set to fulfill the equation:

$$\theta_m \leq \arcsin\left(\frac{n_0}{n_p}\right)$$

with n_0 being the refractive index of the environment of the device (usually air) and n_p the refractive index of the prism.

According to a preferred embodiment, with the multiple spot generator
 5 having a width W and the angles ϕ and θ_m being as described above, the thickness of the slab D is set to fulfill the equation:

$$D > D^*$$

$$\text{with } D^* \text{ being } D^* = \frac{W}{\tan(\phi) - \tan(\theta_m)}$$

Preferably, the thickness is set to be:

10 $1.5 * D^* \leq D \leq 100 * D^*$, more preferably $2 * D^* \leq D \leq 10 * D^*$ and most preferred $3 * D^* \leq D \leq 8 * D^*$

According to a preferred embodiment of the present invention, the intensity of the individual spots (generated by the multiple spot generator) at the interface between the slab and the sample is $\geq 1 \mu\text{W}/(\mu\text{m})^2$ and $\leq 1 \text{ mW}/(\mu\text{m})^2$

15 According to a preferred embodiment of the present invention, the intensity of the individual spots (generated by the multiple spot generator) at the interface between the slab and the sample is (slightly) below the saturation intensity of the fluorophore/luminophore.

20 According to a preferred embodiment of the present invention, the multiple spot arrangement is a 1D or 2D arrangement.

According to a preferred embodiment of the present invention, the excitation is done via surface plasmon excitation. Instead of totally internally reflecting the multiple spots at the interface between a prism and a sample (resulting in evanescent fields in the sample), it is also possible to coat the slab interface facing
 25 towards the sample with a surface plasmon layer and excite short-range surface plasmon waves that propagate along the interface of the surface plasmon layer with the sample.

In the sense of the present invention a short range surface plasmon wave is especially a wave which propagates only a few microns along the interface between

the surface plasmon layer and the sample. It is preferred that the propagation length should be at least smaller than the pitch between the spots of the multiple spot arrangement.

According to a preferred embodiment of the present invention, the surface plasmon layer is made out of a material selected out of the group comprising Au, Ag, Cr, Al or mixtures thereof. These materials have shown to be best suitable in practice.

According to a preferred embodiment of the present invention, the real index of the surface plasmon layer (normalized by index of sample) is

$$0 \leq \text{real} \left(\frac{n_{SP}}{n_{sample}} \right) \leq 4$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time;

By doing so, a surface plasmon with practical values for the decay length of the evanescent tail into the sample can be achieved.

According to a preferred embodiment of the present invention, the real index of the surface plasmon layer (normalized by index of sample) is

$$0.01 \leq \text{real} \left(\frac{n_{SP}}{n_{sample}} \right) \leq 1.5$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to a preferred embodiment of the present invention, the imaginary index of the surface plasmon layer (normalized by index of sample) is

$$-7 \leq \text{imaginary} \left(\frac{n_{SP}}{n_{sample}} \right) \leq -0.2$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to a preferred embodiment of the present invention, the imaginary index of the surface plasmon layer (normalized by index of sample) is

$$-3.3 \leq \text{imaginary} \left(\frac{n_{SP}}{n_{sample}} \right) \leq -0.6$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

This has also shown in practice to achieve a surface plasmon with practical values for the decay length of the evanescent tail into the sample.

5 According to a preferred embodiment of the present invention, the thickness of the surface plasmon layer is ≥ 10 nm and ≤ 350 nm, preferably ≥ 70 nm and ≤ 300 nm. These margins have proven themselves to be best suitable in practice.

According to a preferred embodiment of the present invention, the surface plasmon layer is made out of an gold material and the real index of the surface
10 plasmon layer (normalized by index of sample) is

$$0 \leq \text{real} \left(\frac{n_{SP}}{n_{sample}} \right) \leq 3.1$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to a preferred embodiment of the present invention, the
15 surface plasmon layer is made out of an silver material and the real index of the surface plasmon layer (normalized by index of sample) is

$$0 \leq \text{real} \left(\frac{n_{SP}}{n_{sample}} \right) \leq 0.8$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

20 According to a preferred embodiment of the present invention, the surface plasmon layer is made out of an chromium material and the real index of the surface plasmon layer (normalized by index of sample) is

$$2.0 \leq \text{real} \left(\frac{n_{SP}}{n_{sample}} \right) \leq 3.8$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of
25 the light and t the time.

According to a preferred embodiment of the present invention, the surface plasmon layer is made out of an Aluminum material and the real index of the surface plasmon layer (normalized by index of sample) is

$$0.8 \leq \operatorname{real} \left(\frac{n_{SP}}{n_{sample}} \right) \leq 2.1$$

5 with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to a preferred embodiment of the present invention, the surface plasmon layer is made out of an gold material and the imaginary index of the surface plasmon layer (normalized by index of sample) is

10
$$-3.3 \leq \operatorname{imaginary} \left(\frac{n_{SP}}{n_{sample}} \right) \leq -0.6$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to a preferred embodiment of the present invention, the surface plasmon layer is made out of an Silver material and the imaginary index of the surface plasmon layer (normalized by index of sample) is

15

$$-4.5 \leq \operatorname{imaginary} \left(\frac{n_{SP}}{n_{sample}} \right) \leq -2.8$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to a preferred embodiment of the present invention, the surface plasmon layer is made out of an Chromium material and the imaginary index of the surface plasmon layer (normalized by index of sample) is

20

$$-4.5 \leq \operatorname{imaginary} \left(\frac{n_{SP}}{n_{sample}} \right) \leq -1.9$$

with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to a preferred embodiment of the present invention, the surface plasmon layer is made out of an Aluminum material and the imaginary index of the surface plasmon layer (normalized by index of sample) is

$$-7 \leq \text{imaginary} \left(\frac{n_{SP}}{n_{sample}} \right) \leq -5.3$$

5 with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

According to an embodiment of the present invention, the multiple wavelength generator comprises at least one polarization device so that at least one of the spots of the at least one array of spots comprises polarized light. This has for most applications the advantage that reflected light may be blocked by a blocking means as will be described later on.

According to an embodiment of the present invention, the multiple wavelength generator comprises at least one polarization filter that transmits one polarization state (ps1) and does not transmit or reflect another (orthogonal) polarization state (ps2);

According to an embodiment of the present invention, the multiple wavelength generator comprises at least one polarization rotator which rotates the polarization state by a rotation angle of $\delta\phi$ 1 degrees.

According to a preferred embodiment of the present invention, the multiple wavelength generator comprises a blocking means which blocks the light or parts thereof generated by the multiple wavelength generator.

According to a preferred embodiment of the present invention, the multiple wavelength generator comprises a first blocking means which blocks one polarization component (polarization state 2; ps2) of the light generated by the multiple wavelength generator and transmits the other polarization component (polarization state 1; ps1) and a second blocking means which blocks the light reflected by at the slab-sample interface.

According to an embodiment of the present invention, the blocking means comprises a polarization filter and/or a polarization rotator.

According to an embodiment of the present invention, the blocking means comprises at least one polarization rotator that rotates the polarization state by $\delta\phi_2$ degrees such that $|\delta\phi_1 + \delta\phi_2| = 90$ degrees.

According to an embodiment of the present invention, the polarization
5 device polarizes the device to p/s polarized light and the blocking means blocks p/s polarized light and/or the polarization device polarizes the device to s/p polarized light and the blocking means blocks s/p polarized light.

According to an embodiment of the present invention, the device
comprises a first blocking means which comprises a polarization filter that blocks
10 polarization state ps_2 and/or a polarization rotator that rotates the polarization angle by $\delta\phi_1$ degrees and a second blocking means which comprises a polarization filter and/or a polarization rotator that rotates the polarization angle by $\delta\phi_2$ degrees. According to an embodiment of the present invention, the polarization rotators are configured such that $|\delta\phi_1 + \delta\phi_2| = 90$ degrees.

15 A device according to the present invention may be of use in a broad variety of systems and/or applications, amongst them one or more of the following:

- biosensors used for molecular diagnostics
- rapid and sensitive detection of proteins and nucleic acids in complex biological mixtures such as e.g. blood or saliva
- 20 - high throughput screening devices for chemistry, pharmaceuticals or molecular biology
- testing devices e.g. for DNA or proteins e.g. in criminology, for on-site testing (in a hospital), for diagnostics in centralized laboratories or in scientific research
- tools for DNA or protein diagnostics for cardiology, infectious disease
25 and oncology, food, and environmental diagnostics
- tools for combinatorial chemistry
- analysis devices

The aforementioned components, as well as the claimed components and the components to be used in accordance with the invention in the described
30 embodiments, are not subject to any special exceptions with respect to their size, shape, material selection and technical concept such that the selection criteria known in the pertinent field can be applied without limitations.

Additional details, features, characteristics and advantages of the object of the invention are disclosed in the subclaims, the figures and the following description of the respective figures and examples, which --in an exemplary fashion-- show several preferred embodiments of a device according to the invention.

5

Fig. 1 shows a very schematic cross-sectional view of a device according to a first embodiment of the present invention;

Fig. 2 shows a very schematic cross-sectional view of a device
10 according to a second embodiment of the present invention;

Fig. 3 shows a very schematic cross-sectional view of a device according to a third embodiment of the present invention; and

Fig. 4 shows a very schematic cross-sectional view of a device according to a fourth embodiment of the present invention.

Fig.5 is a diagram showing the impact of the thickness of the gold
15 layer on the decay lengths in the direction of propagation.

Fig.6 shows a very schematic cross-sectional view of a device according to a fifth embodiment of the present invention;

20

Fig. 1 shows a very schematic cross-sectional view of a device 1 according to a first embodiment of the present invention. The device comprises a slab 10 in form of a prism, which has an interface 20 with the sample 30. The sample 30 is an aqueous or fluidic medium, in which luminescent, preferably fluorescent-marked
25 biomolecules 40 are present.

The device furthermore comprises a multiple spot generator in form of a diffraction element 50. This diffraction element corresponds with a multiple spot arrangement (as indicated by 60) on the interface 20 of the prism 10. Incident light 100, which enters the prism via the diffraction element is guided and transformed into
30 multiple spots at the interface 20 to the multiple spot arrangement 60; the light total internally reflected so the multiple spot arrangement penetrates into the sample as evanescent light) is then reflected (as indicated by numeral 110). However, in case that

a biomolecule is present in the evanescent part of the multiple spot arrangement, fluorescent light (as indicated by the dotted lines 200) is emitted and may be detected, thus indicating the presence of the biomolecule 40 in the sample 30.

In order to distinguish the fluorescent light 200 from the incident light 100 and the reflected light 110, the angle ϕ of the prism 10 and the total cone of reflection are chosen to be as described above

EXAMPLE 1:

The spot arrangement of the first embodiment of the present invention as shown in Fig. 1 and as described above will further be illustrated – in a mere exemplarily way – by the following example 1.

In this example, a 2D array of spots is generated by the multiple spot generator (in this embodiment a diffractive element). The excitation intensity is set to be slightly lower than the saturation intensity.

The 2D array is set to be circular spots with a diameter of 0.5 microns (area per spot of $0.2 \mu\text{m}^2$) for typical saturation intensities of a $1 \mu\text{W}/(\mu\text{m})^2$ up to $1 \text{mW}/(\mu\text{m})^2$, thus resulting in a power per spot of $5 \mu\text{W}$ to 5mW per spot. For a laser/LED/other type of light source with a total power of 10-100 mW this results in a possible number of spots between 2-20000 spots. Assuming an illuminated area of 1mm^2 (at the interface between slab and sample), the area to be scanned by each spot is $50 (\mu\text{m})^2$ up to 0.5mm^2 corresponding with a pitch between the spots of $7 \mu\text{m}$ up to 0.7mm .

It should be noted that it is clear for any person skilled in the art that such a spot arrangement may also be of use for any other embodiment, either those that are described below or that are also apparent to be within the present invention.

Fig. 2 shows a very schematic cross-sectional view of a device 2 according to a second embodiment of the present invention. This device 2 differs from the embodiment as shown in Fig. 1 in that that the slab 10 is rectangular when seen in the cross-sectional view (as shown in Fig. 2) and that a movable diffractive element 50 is used. This diffractive element has a width W . Incident light 100, which enters the prism via the diffraction element 50 is guided to the multiple spot arrangement 60 and is bent by the diffraction element 50 in an angle ϕ which is set to be in the range of:

$$\theta_{TIR} \leq \phi \leq \frac{\pi}{2}$$

The thickness D of the slab 10 is preferably $1.5 * D^* \leq D \leq 10 * D^*$ with D^* being as described above.

In this embodiment, it is preferred that the diffractive element 50 is
 5 designed such that the multiple spot arrangement 60 (and therefore also the structure of the diffractive element 50) is periodic (i.e., each spot has the same distance along the direction of propagation to the interface with the sample). This allows that the scanning of the spots is straightforward and can be performed by translating the diffractive element 50 along the surface of the slab 10.

10 EXAMPLE 2:

The second embodiment of the present invention as shown in Fig. 2 and as described above will further be illustrated – in a mere exemplarily way – by the following example 2.

In this example, the following features were set for the device:

15 Slab: index of refraction $n_p=1.68$
 Sample: measurand (e.g. the molecules to be detected) are in a water environment (i.e., water is the sample) having an index $n_s=1.3$.

Environment: the system is in an air environment having an index of refraction $n_0=1$.

20 Multiple spot generator: Width $W = 1$ mm, angle $\phi=60$ degrees

For the chosen refractive indexes avoiding total internal reflection at the interface between the slab and the environment requires angles $\theta_m < 36.5$ degrees being smaller than the minimum value for ϕ for total internal reflection at the slab-sample interface, which is set to be $\phi > 50.7$ degrees. Taking in account the features of the
 25 multiple spot generator and assuming that θ_m is limited by total internal reflection we find a minimum thickness of the slab $D > 2.08$ mm.

Fig. 3 shows a very schematic cross-sectional view of a device 3 according to a third embodiment of the present invention. In this embodiment 3, surface plasmon resonance is used to cause emittance of the marked biomolecule 40. For these
 30 reasons, the slab 10 is covered with a surface plasmon layer 70. This so called surface

plasmon layer has a refractive index n_{SP} and is preferably made out of a material as described above.

EXAMPLE 3:

The second embodiment of the present invention as shown in Fig. 3 and
5 as described above will further be illustrated – in a mere exemplarily way – by the following example 3.

In this example, the following features were set for the device:

prism: index of refraction $n_p=1.68$
layer: gold layer with a index of refraction: $n_m=0.16-j*3.8$, for a
10 wavelength of 688.8 nm
medium water $n_s=1.3$

In this case, the surface plasmon wave has an effective index $n_{eff}=1.383-j*0.0077$; $(1/e)^2$ decay length of 232 nm in the sample and 27 nm in the gold layer; and a decay length in the direction of propagation of 14 μm .

15 The impact of the thickness of the gold layer on the decay lengths in the direction of propagation is shown in Fig. 5. It can be seen from the picture, that the decay length into the direction of propagation decreases towards the decay length for a semi-infinite gold layer for increasing thickness of the surface plasmon layer.

In the example, a gold layer of 70 nm thickness was chosen, resulting in
20 the following:

- Decay length smaller than 20 microns.
- Reasonably efficient excitation: intensity reflection of 35.6 %, which corresponds with about 50 % of the input power converted into the surface plasmon wave.

25 Fig. 4 shows a very schematic cross-sectional view of a device 4 according to a fourth embodiment of the present invention. This embodiment is similar to that of Fig. 1 except that microlenses 80 were used instead of a diffractive element. For reasons of clarity, only the way of the incident light 100 and the reflected light 110 is shown, although this embodiment works in the same way as that shown in Fig. 1.

30 It should be noted, that microlenses 80 instead of a diffractive element 50 may be used within the embodiments as shown in Figs. 2 and 3, too.

EXAMPLE 4:

The fourth embodiment of the present invention as shown in Fig. 4 and as described above will further be illustrated – in a mere exemplarily way – by the following example 4.

For a prism with index of refraction $n_p=1.68$ and an excitation wavelength of 600 nm we find $\lambda=357.14$ nm, which is the wavelength in the medium of the prism. Taking a prism angle of 60 degrees, a pitch between spots of 100 microns and a focal length of 1 mm, we find:

- a pitch between lenses of lens array of $s=50$ microns.
- a minimum beam waist of 4.5 microns of the Gaussian beam
- 10 a minimum beam waist of 9.0 microns of the projection of the Gaussian beam on the interface between the slab/prism and the sample.

Fig.6 shows a very schematic cross-sectional view of a device according to a fifth embodiment of the present invention. This device furthermore comprises two polarizing means 70a and 70b, of which the polarizing means 70b also serves as
15 blocking means.

In the present embodiment, the light 100, after passing through the diffraction element 50a, passes a polarizing means 70a in form of a polarization filter that transmits one polarization state (ps_1) and does not transmit or reflect the other (orthogonal) polarization state (ps_2); light having polarization state ps_2 is absorbed by
20 the polarization filter. Subsequently, the light enters a polarization rotator which rotates the polarization state by a rotation angle of $\delta\phi_1$ degrees.

In case that a biomolecule 40 is present in the evanescent part of the multiple spot arrangement, fluorescent light (as indicated by the dotted lines 200) is emitted and may be detected, thus indicating the presence of the biomolecule 40 in the
25 sample 30.

However, most of the light 100 will be reflected (numeral 110) towards the second blocking means 70b. This second blocking means 70b also comprises a polarization rotator that rotates the polarization state by $\delta\phi_2$ degrees such that
 $|\delta\phi_1+\delta\phi_2|=90$ degrees. However, due to the presence of a polarization filter in the
30 blocking means 70b, too, this light will then be blocked and not leave the blocking means (as indicated by the dotted lines 110). As a result, the light that is reflected within the prism 10 is blocked from leaving the device.

The particular combinations of elements and features in the above detailed embodiments are exemplary only; the interchanging and substitution of these teachings with other teachings in this and the patents/applications incorporated by reference are also expressly contemplated. As those skilled in the art will recognize, variations, modifications, and other implementations of what is described herein can occur to those of ordinary skill in the art without departing from the spirit and the scope of the invention as claimed. Accordingly, the foregoing description is by way of example only and is not intended as limiting. The invention's scope is defined in the following claims and the equivalents thereto. Furthermore, reference signs used in the description and claims do not limit the scope of the invention as claimed.

CLAIMS:

5

1. A device for detecting excitation, in particular luminescent excitation in a fluid sample, comprising

- a transparent slab having a slab-sample interface
- 10 - a multiple spot generator provided with the slab, and
- a multiple spot arrangement provided on or in the vicinity of the slab-sample interface

wherein the multiple spot generator is matched to the multiple spot arrangement in such a way that light entering the multiple spot generator will be guided
15 to defined areas on the multiple spot arrangement.

20

2. The device according to claim 1, whereby the multiple spot generator is comprises at least one diffractive element and/or a microlens arrangement and/or is movable along or with the slab.

25

3. The device according to claim 1 or 2, whereby the slab is a prism having a prism-sample interface with angles such that incident excitation light is totally internally reflected on a prism-sample interface and whereby preferably the angle of the prism ϕ is selected to be as follows:

$$\theta_{TIR} \leq \phi \leq \frac{\pi}{2}$$

with θ_{TIR} being the total internal reflection angle as the minimum angle with respect to the normal of the interface that results in total internal reflection:

$$\theta_{TIR} = \arcsin\left(\frac{n_{sample}}{n_{prism}}\right)$$

4. A device according to any of the claims 1 to 3, whereby the total internal reflection cone ϕ of the slab is

$$\theta_{TIR} \leq \phi \leq \frac{\pi}{2}$$

5. A device according to any of the claims 1 to 4, whereby the intensity of the individual spots (generated by the multiple spot generator) at the interface between the slab and the sample is $\geq 1 \mu W/(\mu m)^2$ and $\leq 1 mW/(\mu m)^2$.

6. A device according to any of the claims 1 to 5, whereby the slab has a thickness D of

$$D > D^*$$

$$\text{with } D^* \text{ being } D^* = \frac{W}{\tan(\phi) - \tan(\theta_m)}$$

whereby W is the width of the multiple spot generator and θ_m is the maximum angle (relative to the normal of the interface between the slab and the sample) in the slab under which the generated luminescence can still be detected.

7. A device according to any of the claims 1 to 7, whereby the widths $w_{lens}(q)$ of the lenses in the microlens array are

$$2w_f(q) \leq w_{lens}(q) \leq s$$

whereby the widths $w_{lens}(q)$ of the lenses are selected independently from each other and the $w_f(q)$ is the beam waist of each spot just behind the lens and s is the distance between the spots at the hypotenuse.

8. A device according to any of the claims 1 to 8, whereby the detection is done via surface plasmon excitation.

9. A device according to any of the claims 1 to 8, whereby the real index of the surface plasmon layer (normalized by index of sample) is

$$0 \leq \text{real} \left(\frac{n_{SP}}{n_{sample}} \right) \leq 4$$

and/or the imaginary index of the surface plasmon layer (normalized by index of sample) is

$$-7 \leq \text{imaginary} \left(\frac{n_{SP}}{n_{sample}} \right) \leq -0.2$$

5 with a time dependence of $\exp(j*\omega*t)$ with ω the angular frequency of the light and t the time.

10. A system incorporating a device according to any of the Claims 1 to 9 and being used in one or more of the following applications:

- 10 - biosensors used for molecular diagnostics
- rapid and sensitive detection of proteins and nucleic acids in complex biological mixtures such as e.g. blood or saliva
- high throughput screening devices for chemistry, pharmaceuticals or molecular biology
- 15 - testing devices e.g. for DNA or proteins e.g. in criminology, for on-site testing (in a hospital), for diagnostics in centralized laboratories or in scientific research
- tools for DNA or protein diagnostics for cardiology, infectious disease and oncology, food, and environmental diagnostics
- tools for combinatorial chemistry
- 20 - analysis devices

1/3

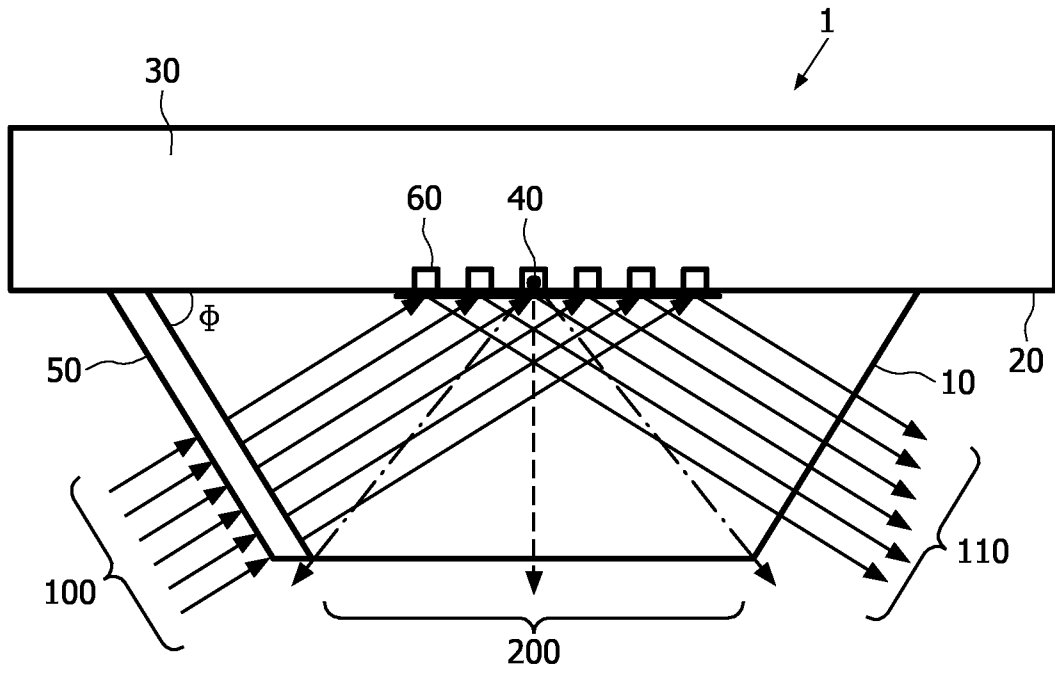


FIG. 1

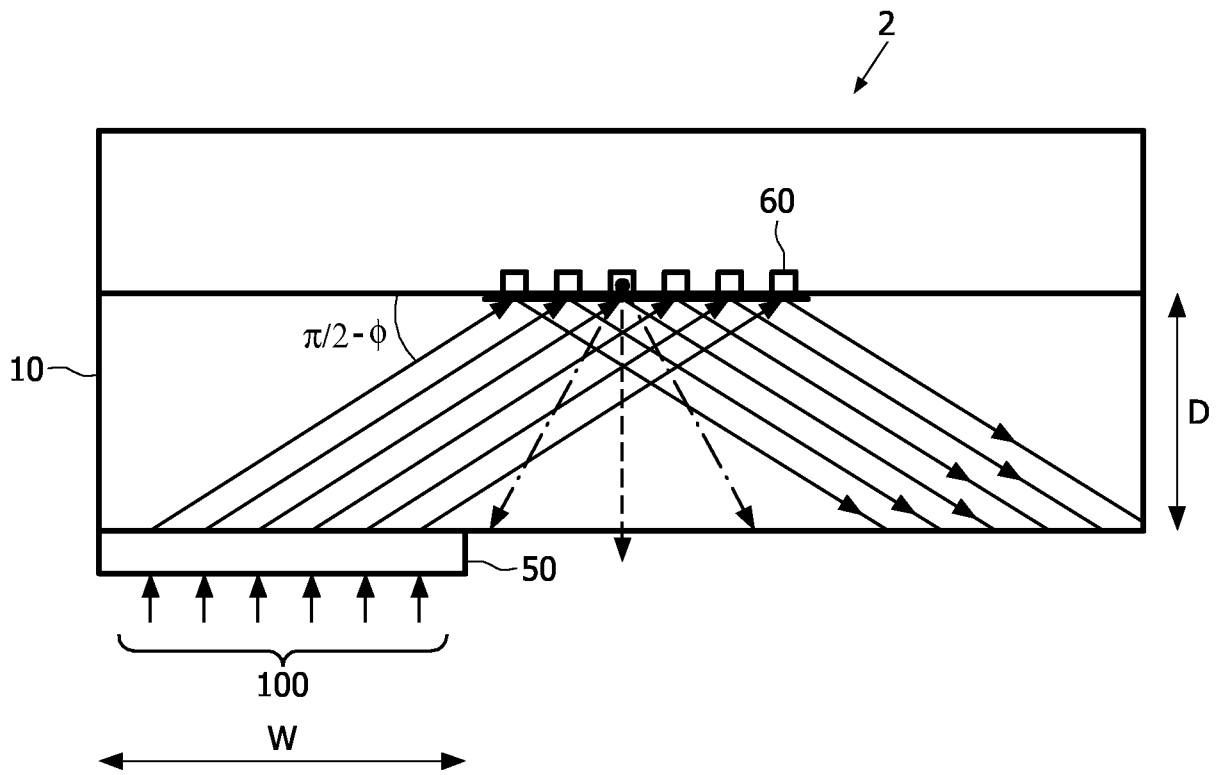


FIG. 2

2/3

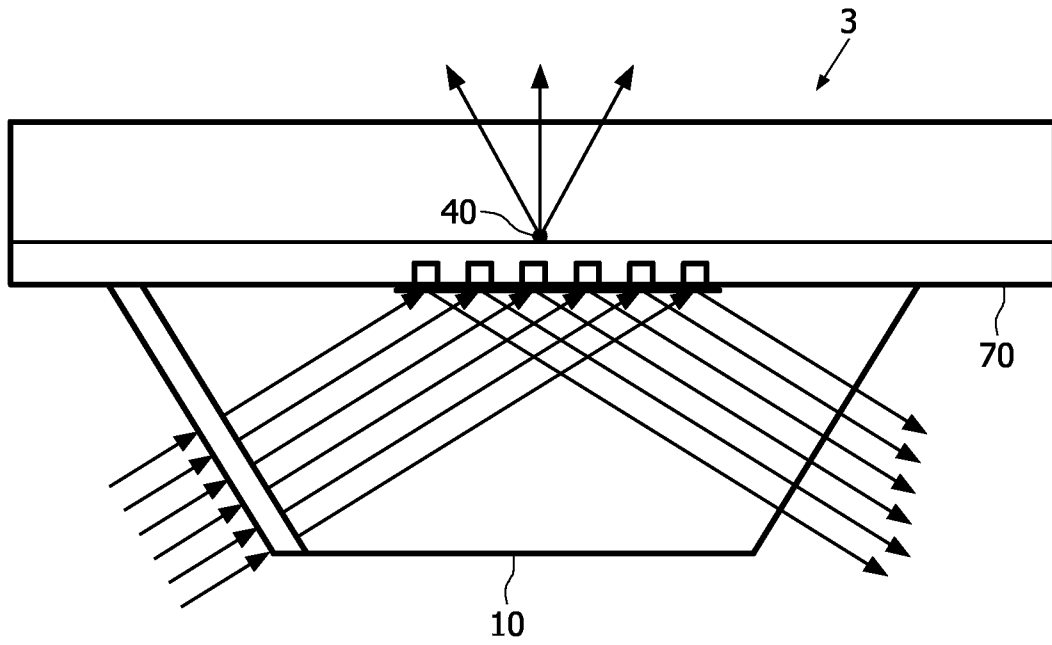


FIG. 3

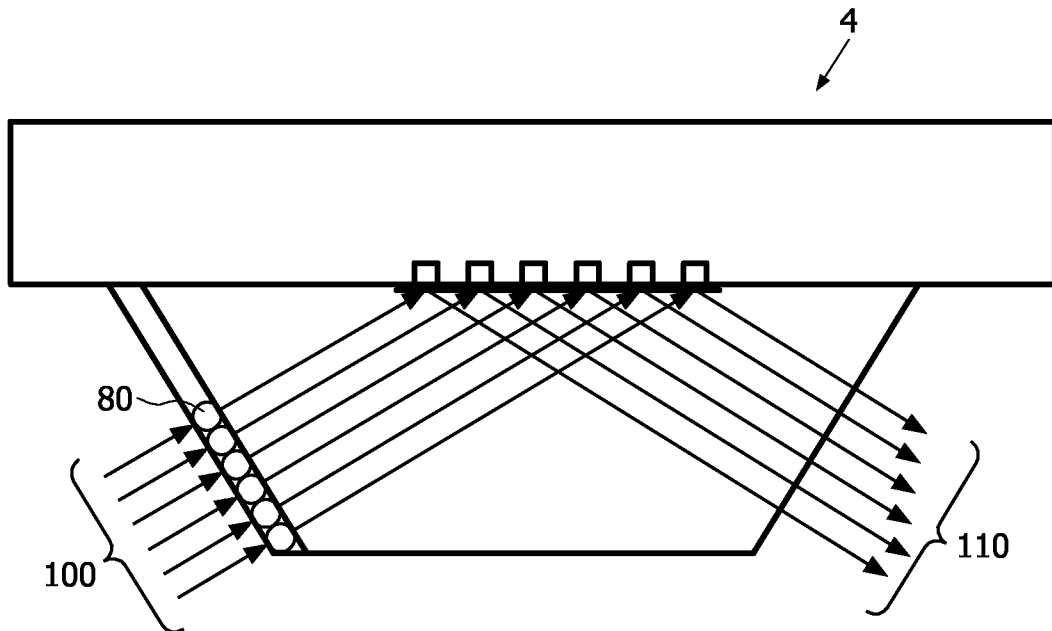


FIG. 4

3/3

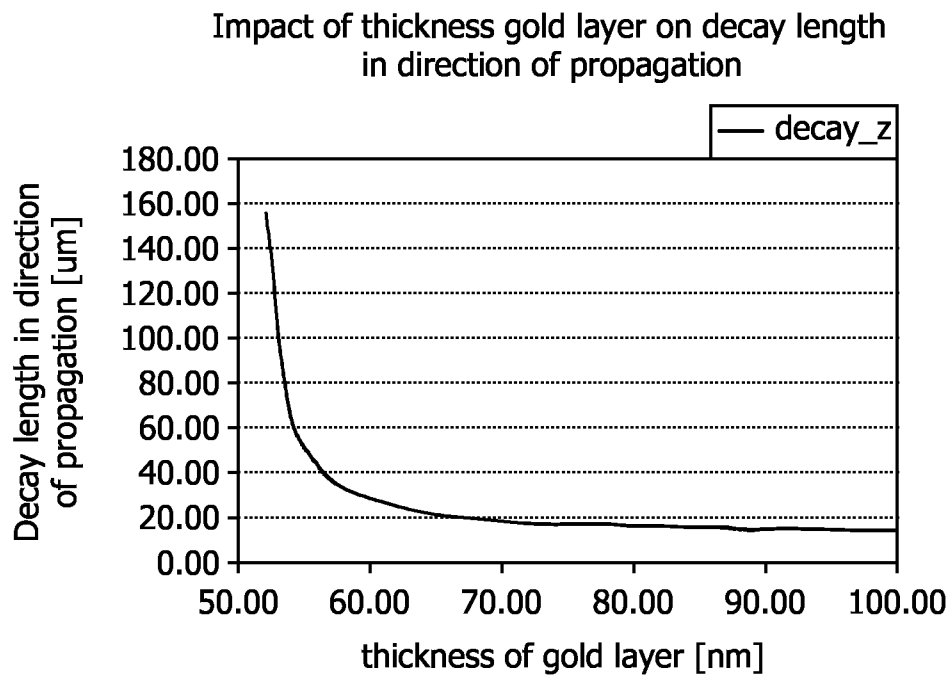


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2006/052430

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/64 G01N21/55 G01N21/21

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, 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	DE 100 17 824 A1 (TILL I D GMBH [DE]) 18 October 2001 (2001-10-18) paragraphs [0001], [0002], [0018], [0025]; figures 1-3	1-7, 10
X	WO 00/58715 A2 (UNIV GLASGOW [GB]; COOPER JONATHAN MARK [GB]; BENOIT VINCENT [GB]; AIT) 5 October 2000 (2000-10-05) page 1 - page 4 page 9 - page 13; figures 1,2,9	1,2,8-10
X	US 6 300 638 B1 (GROGER HOWARD P [US] ET AL) 9 October 2001 (2001-10-09) column 9, line 46 - column 10, line 38; figures 1,28	1,3,4,10
	----- -/--	

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 document 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

11 December 2006

Date of mailing of the international search report

18/12/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Duijs, Eric

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2006/061783 A (KONINKL PHILIPS ELECTRONICS NV [NL]; KLUNDER DERK JAN WILFRED [DE]; VA) 15 June 2006 (2006-06-15) the whole document	1,2,10
P,X	WO 2006/064465 A2 (KONINKL PHILIPS ELECTRONICS NV [NL]; KLUNDER DERK JAN WILFRED [DE]; VA) 22 June 2006 (2006-06-22) the whole document	1,2,10
A	LOHMANN A W ET AL: "Making an array illuminator based on the Talbot effect" APPLIED OPTICS, OSA, OPTICAL SOCIETY OF AMERICA, WASHINGTON, DC, US, vol. 29, no. 29, 10 October 1990 (1990-10-10), pages 4337-4340, XP002314360 ISSN: 0003-6935 the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2006/052430

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 10017824	A1	18-10-2001	NONE
WO 0058715	A2	05-10-2000	AU 3440900 A 16-10-2000 EP 1166092 A2 02-01-2002 JP 2002540423 T 26-11-2002
US 6300638	B1	09-10-2001	AU 2344600 A 05-06-2000 WO 0029820 A2 25-05-2000
WO 2006061783	A	15-06-2006	NONE
WO 2006064465	A2	22-06-2006	NONE