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(54) **Title**: METHOD FOR DETERMINING THE PRESENCE OF A SAMPLE ON A SAMPLE RECEIVING SURFACE

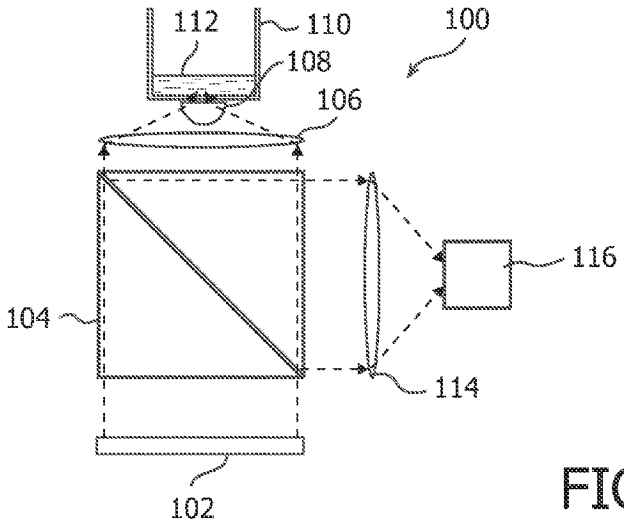

FIG. 1

(57) **Abstract**: A method for determining the presence of a sample on a sample (112) receiving surface (110) is provided. The method comprises the steps of directing an input light beam onto the sample receiving surface using an optical element (108) arranged in near-field contact with the sample receiving surface, determining a reflected light intensity in an output light beam being totally internally reflected inside of the optical element using a light detector (116), and comparing the reflected light intensity with a predetermined light intensity, wherein the result of the comparison is indicative of the presence of the sample on the sample receiving surface.

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Method for determining the presence of a sample on a sample receiving surface

FIELD OF THE INVENTION

The present invention relates to a method for determining the presence of a sample and/or its refractive index, when placed on a sample receiving surface.

BACKGROUND OF THE INVENTION

Micro-fluidic devices are at the heart of most biochip technologies, being used for both the preparation of fluidic, e.g. blood based, samples and their subsequent analysis. Integrated devices comprising biosensors and micro-fluidic devices are known, e.g. under the name DNA/RNA chips, BioChips, GeneChips and Lab-on-a-chip. In particular, high throughput screening on arrays, e.g. micro-arrays, is one of the new tools for chemical or biochemical analysis, for instance employed in diagnostics. These biochip devices comprise small volume wells or reactors, in which chemical or biochemical reactions are examined, and may regulate, transport, mix and store minute quantities of liquids rapidly and reliably to carry out desired physical, chemical, and biochemical reactions and analysis in large numbers. By carrying out assays in small volumes, significant savings can be achieved in time and in costs of targets, compounds and reagents.

Generally, detection of fluorescence signals of a biochip is done using an optical detection system, comprising a light-source, optical components (e.g. optical filters and prism) and detector elements (e.g. a CCD camera), localized in a bench-top/laboratory machine, to quantify the amount of fluorophores present. In a medium with no absorption, i.e. with a purely real refractive index, an electromagnetic wave is evanescent in a particular direction when it maintains a constant phase in that direction but has exponentially decreasing amplitude. In biochips, e.g. use is made of total internal reflection, wherein an excitation beam is totally internally reflected at a surface to which sample particles are attached. At the point of reflection, an evanescent wave is generated with a characteristic decay depth typically of one optical wavelength. Therefore, at the reflecting surface, the light is confined to the surface and it interacts preferentially with sample particles within the decay depth of the surface.

As is disclosed in WO08012703, it is of great value that the optical component are in direct contact with a surface to which the sample to be investigated (i.e. the "bio-interface") are arranged. That is, the direct contact with the optical component may be such that no layer with low index of refraction, e.g. air layer is present between the optical component/element and the detector element. Thereby, the evanescent field may be created by total internal reflection of the excitation radiation in the optical component.

Accordingly, for reliable detection, it is necessary to check if the bio-interface is correctly covered with the sample to be investigated, for example a fluid, and thus if the interface is completely wetted with the sample. This can be checked by external methods, such as using electrode structures to measure the electrical conductivity. A disadvantage is that a separate measurement is needed, increasing cost and complexity of the reader device. Also, the refractive index, n, of the fluid to be investigated provides useful information (in simplest form, e.g. wetting, namely presence of liquid with $n > 1$ instead of air with $n = 1.0$), especially for optical detection methods using evanescent excitation. In the latter case, this refractive index influences the evanescent decay distance, and thus has an effect on the measurement result. For best performance, correction of the measured values for differences in refractive index is necessary. Similar to the wetting detection, the refractive index can be measured using external methods involving additional structures, leading to increased cost and complexity.

There is therefore a need for an simplified and improved method for determining the presence of a sample on a sample and/or its refractive index when placed on a sample receiving surface, e.g. the fluid to be investigated, for providing improved measurement results in relation to for example a biochip.

SUMMARY OF THE INVENTION

According to an aspect of the invention, the above object is met by a method for determining the presence of a sample on a sample receiving surface, the method comprising directing an input light beam onto the sample receiving surface using an optical element arranged in near-field contact with the sample receiving surface, determining a reflected light intensity in an output light beam being totally internally reflected inside of the optical element using a light detector, and comparing the reflected light intensity with a predetermined light intensity, wherein the result of the comparison is indicative of the presence of the sample on the sample receiving surface.

The general concept of the present invention is based on the fact that it is possible to only slightly modify a general set-up for a biochip when e.g. performing evanescent field excitation. That is, by detecting a difference in the amount of light that is reflected back within the optical element between a predetermined case and a present case, it is possible to determine if there is a sample present on the sample receiving surface. The reflected light intensity detected by the light detector will in its most basic case only measure the time-averaged energy flux in a given direction, and accordingly the amount of light that passes back from the optical element (i.e. which is totally reflected) and falls within a given angle for the light detector. It should be noted that the meaning of light in this case covers the wavelength spectrum from electromagnetic spectrum from infrared to ultraviolet.

An advantage with the invention is that it will not be necessary to use external equipment for determining if there is a sample present, thereby allowing for the manufacturing of a measurement device and/or sample receiving surface that is more compact and thus less expensive. The predetermined light intensity may advantageously be determined when no sample is present on the sample receiving surface. Thus, a calibrated light intensity value is provided giving a higher accuracy for the detection.

In a preferred embodiment of the present invention, the step of comparing comprises the step of determining the refractive index of the sample on the sample receiving surface using a ratio comparison between the predetermined light intensity and the reflected light intensity. Accordingly, in this case it may be preferred to have knowledge of also the spatial distribution of the reflected output light beam, and also a predetermined spatial distribution in the case no sample is present on the sample receiving surface. For detecting the spatial distribution of the reflected output light beam, it is possible to use as the light detector at least one of a pixelated detector such as a CCD, linear array and a position sensitive detector. In the case a linear array detector is used, it is possible to detect a one dimensional reflected spatial distribution. However, using a CCD sensor (e.g. a two dimensional simplified camera chip) it would also be possible to determine the two-dimensional spatial distribution of the light reflecting back from the optical element.

It is advantageous to use an objective lens and a solid immersion lens, SIL, as the optical element. Solid immersion lenses are previously known from e.g. optical recording where they are used due to the fact that they can be achieved with numerical apertures greater than one. An SIL is essentially a hemispherical lens that focuses a light beam to a very small spot located just inside the high-refractive index material of the SIL.

The spot size is determined by, among other parameters, the NA (numerical aperture) of the optical element, defined as NA = n * sin(θ), where n is the refractive index of the medium in which the light is focused and θ is the half angle subtended by the focused cone of light in that medium. The maximum NA of an optical element in air or through a plane parallel plate (such as the sample receiving surface) is unity. The NA of a SIL can exceed unity if the light is focused in a material of high refractive index without refraction at the air-medium interface, and accordingly, this is achievable using an SIL. However, it is required that the SIL lens is positioned very close to the sample receiving surface during operation, in the region of 25 – 40 nm, such that the air-gap is made small compared with the wavelength of light to allow photons to "tunnel" across the gap. As a comparison, in relation to optical data storage this associated technique is often referred to as near field recording. Accordingly, the input light beam, e.g. emitted by a diode laser or an LED, may be arranged to have an output wavelength of about 400 to about 1100 nm.

In a preferred embodiment of the present invention, the numerical aperture, NA, of the SIL is thus kept high, and preferably above at least 1.35, more preferably above at least 1.60, and most preferably above 1.90. Generally, a NA larger than the refractive index of the sample fluid would be adequate, but a larger NA yields a higher sensitivity. However, presently a NA larger than 1.90 may be complicated to manufacture as it would require special high refractive index plastics and hence becomes rather impractical and expensive. Thus, for determining the refractive index of the sample present on the sample receiving surface, when rays of light with angles corresponding to NA > 1 are emitted towards the sample receiving surface and no sample is present, total internal reflection will occur at the sample receiving surface, resulting in a large amount of reflected light, and thus a high reflected light intensity (i.e. the predetermined light intensity), thus corresponding to NA = 1.0.

However, when a sample is present on the sample receiving surface, all rays up to an NA equal to the refractive index of the sample will be able to propagate into the medium. In other words, the amount of total internally reflected light, reflected at the sample receiving surface, will decrease, and thus a lower reflected light intensity will be monitored. A comparison between these intensities (i.e. the light intensity when no sample present and the light intensity when a sample is present) may be used for determining the refractive index of the sample, and accordingly refractive indices n of samples present on the sample receiving surface can be determined accurately for $n < NA_{SIL}$. A more detailed description will be given below in relation to the attached drawings.

Furthermore, it should be noted that the sample may be a liquid, and the sample receiving surface may be a micro-fluidic device, such as a micro-array comprising a plurality of small volume well or reactors.

The invention further provides an analytical or diagnostic method comprising the method for determining the presence of a sample. By implementing the method in a method for characterization of a sample such as in analytical methods, (immune)assay methods or diagnostic methods, reliability and fault tolerance is increased as it can be verified that the sample is present before actual measurement is done. If sample is not present or only partly present, the method may provide feedback to the user to stop measurement or even top prevent measurement that gives erroneous results and costs time and effort without result.

The invention is embodied by a device for determining the presence of a sample on a sample receiving surface, the method comprising:

- an optical element arranged in near-field contact with the sample receiving surface for directing an input light beam onto the sample receiving surface;

- a light detector for determining a reflected light intensity in an output light beam being totally internally reflected inside of the optical element; and

- means for comparing the reflected light intensity with a predetermined light intensity, wherein the result of the comparison is indicative of the presence of the sample on the sample receiving surface.

Furthermore, the device for determining the presence is preferably part of an analytical or diagnostic apparatus. This latter apparatus benefits in all ways as described here before. An analytical device may be a sensor, detection device, device for performing analysis on e.g chemical, or biological samples of any kind that are compatible with the optical principles of the method.

Means for comparing the reflected light intensity may for example be a computer or IC-chip in any form made using semiconductor methods as long as it is able to handle the required electrical and/or optical signals. The means is preferably permanently programmed or user programmable with a computer program that enables the means to perform the comparison steps of the method of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other aspects of the present invention will now be described in more detail, with reference to the appended drawings showing currently preferred embodiments of the invention, in which:

Fig. 1 is a schematic block diagram illustrating a conceptual set-up of a SIL based detection system using the method according to the present invention,

Fig. 2 is a refractive index measurement system for pupil image measurement using an SIL based lens system,

Figs. 3a – 3d are resulting two-dimensional spatial distributions from the refractive index measurement system of Figure 2, and

Fig. 4 is an alternative single beam, single detector for detecting the presence of a sample on a sample receiving surface according to an alternative embodiment of the present invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention will now be described more fully hereinafter with reference to the accompanying drawings, in which currently preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided for thoroughness and completeness, and fully convey the scope of the invention to the skilled addressee. Like reference characters refer to like elements throughout.

Referring now to the drawings and to figure 1, there is depicted a typical schematic block diagram of a SIL based detection system 100 using a method according to an embodiment of the present invention. The system 100 comprises a light source, such as a laser light source 102 for emitting an input light beam, a beam splitter 104 and a first collimator lens 106 for focusing light emitted by the light source 102 onto a spherically shaped solid immersion lens (SIL) element 108 having a refractive index, n_1, higher than that of air which is arranged in near field contact with a cartridge 110 (having a refractive index n_2) holding a sample material, such as for example a liquid 112 (having a refractive index n_3). The light source 102 is preferably constructed by a small-size light source such as a semiconductor laser. Here, the light source 102 is driven by a driving circuit (not shown) that is installed in a control unit (not shown). It is according to the present invention possible to use a laser light source as well as a non-lasing point source such as a small aperture LED.

The control unit may also be used for performing the determination method according to the present invention. Thus, the input light beam, emitted from the light source 102, is directed to the cartridge 110 through the beam splitter 104 and the first collimator lens 106. The first collimator lens 106 has a function for shaping light emitted from the light source 102 into parallel light rays. The light rays that have passed through the beam splitter 104 are directed onto the inner surface of the cartridge 110 by the SIL element 108 so as to form a fine spot thereon. The spot diameter is determined by, among other parameters, the NA (numerical aperture) of the SIL element 108, defined as NA = n * sin(θ), where n is the refractive index of the medium in which the light is focused and θ is the half angle subtended by the focused cone of light in that medium. The NA of the SIL element 108 can exceed unity if the light is focused in a material of high refractive index without refraction at the air-medium interface, and accordingly, this is achievable using the SIL element 108. However, it is required that the SIL lens is positioned very close to the bottom surface of the cartridge 110 during operation, in the region of a distance, d, around approximately 25 – 50 nm, such that the air-gap is made small compared with the wavelength of light to allow photons to "tunnel" across the gap, i.e. near field arrangement of the SIL element 108 in relation to the cartridge 110. Additionally, the SIL element 108 may be made of a glass material such as SF6, and the application of the SIL element 108 having a high refractive index makes it possible to miniaturize the spot diameter converged on the surface of the cartridge 110. Thus, according to the invention, the numerical aperture, NA, of the SIL element 108 may thus be kept high, and preferably above at least 1.60, and more preferably above 1.90.

The system 100 further comprises a second collimator lens 114 for focusing an output light beam being totally internally reflected at the sample receiving surface 110 to a detector, such as a photo detector 116, for detecting a light intensity in the output light beam. Here, light reflected at the sample receiving surface 110 proceeds in a direction reversed to the above-mentioned direction, and is returned to the beam splitter 104 through the first collimator 106. Then, it is reflected by the beam splitter 104, and made incident on the photo detector 116. In other words, light that is totally internally reflected at the sample receiving surface 110is read by the photo detector 116. Also, it should be noted that the SIL element 108 may be integrated into the cartridge 110, or can be a part of a lens arrangement formed by the SIL element 108 and the first collimator lens 106, preferably having a numerical aperture, NA, above 1.90.

Next, a detailed explanation will be given of the total internal reflection inside of the SIL element 108, and the principle of near field arrangement of the SIL element 108 in

relation to the cartridge 110. That is, figure 2 is essentially a detailed cross-sectional view that schematically shows the functionality of the SIL element 108 in relation to the cartridge 110 and the sample material, e.g. the liquid 112. Additionally, besides determining the presence of the liquid 112 on the cartridge 110, it is also possible to determine a refractive index for the liquid 112 based on pupil image measurement using the system 100.

Accordingly, by upgrading the detector to a detector suitable for measuring also spatial distribution (one or two dimensional), such as a linear array or a CCD sensor, it is also possible to detect the spatial distribution of the light that is totally internally reflected at the sample receiving surface 110. The reflected spatial light intensity (for example when using a two dimensional detector) will be illustrated as a pupil image which will be possible to use for determining the presence of a liquid on the bottom surface of the cartridge 110, and/or for determining the refractive index of the liquid 112 (i.e. if a liquid 112 is present). Figures 3a to 3d illustrate different resulting two-dimensional spatial distributions (i.e. pupil images) with liquid 112 not present (fig. 3a) and with liquid 112 present (fig. 3b to 3d), respectively, and will be referred to in relation to figure 2. It should be noted that the linear array or a CCD sensor may be the same detector as used for the biological measurement. Similarly, the light source used may be the same as used for bio-detection.

As can be seen in figure 2, light emitted by the light source 102 in the direction of the SIL element 108 will leave the SIL element 108, as discussed above, as long as the distance, d, (around approximately 25 – 50 nm) of the air gap between the SIL element 108 and the cartridge 110 is well below the wavelength of the light emitted by the light source 102 and the refractive index, n_2, of the cartridge 110 is high enough. This regardless of the presence of the liquid 112. Accordingly, total internal reflection takes place at the interface of the cartridge 110 and the liquid 112, and here the angle, θ, at which total internal reflection occurs, depends on the refractive index, n_3, of the liquid 112 (air versus sample fluid).

In figure 3a, no liquid 112 is present, and the inner disk radius 308 corresponds to NA=1.0. That is, light with a numerical aperture, NA, smaller than 1 will be able to leave the interface of the cartridge 110 and the liquid 112 and propagate into the liquid 112. Accordingly, this light will not be totally reflected back and thus resulting in a small dark disk in the center of the reflected pupil image.

However, when the liquid 112 is present on the surface of the cartridge 110, all rays up to an NA equal to the refractive index of the liquid 112 will be able to propagate into the liquid 112. In other words, the central dark disk will increase, as is illustrated in

figure 3b. The larger disk radius 306 (normalized to the radius at NA=1) thereby directly gives the refractive index, n_3, of the liquid 112. Accordingly, since these radii can be determined very precisely, fast and accurate measurements of refractive index are possible. In this way, the presence of materials with a refractive index larger than one can be easily detected, i.e. the presence of a liquid 112 inside of the cartridge 110. Moreover, the refractive indexes of different liquids present in for example a plurality of different cartridges can be determined fast and accurately as long as the refractive index of the liquid is smaller than the numerical aperture of the SIL element 108.

Figure 3c illustrates the presence of another type of liquid 112 present on the surface of the cartridge 110. This different liquid 112 has a higher refractive index, n_3, than the liquid 112 in figure 3b, and accordingly, the central dark disk will increase even further, resulting in a radius 306'.

Turning to figure 3d which illustrates the presence of still another liquid 112 on the surface of the cartridge 110. This still different liquid 112 has an even higher refractive index, n_3, than the liquid 112 in figure 3c, and accordingly, the central dark disk will increase even further, resulting in a radius 308. In this case, the refractive index, n_3, of the liquid 112 equals the refractive index, n_2, of the cartridge, and accordingly, this is the maximum possible refractive index that can be measured.

However, it should be noted that the outer bright ring of the aperture pupil image is effectively due to total internal reflection of light rays at the exit surface of the SIL element 108. These light rays being subject to total internal reflection propagate at an angle exceeding the critical angle, θ_c^1, for total internal reflection at the interface 108 – 110. Due to this large propagation angle, this totally reflected light appears as the outer bright ring of the aperture pupil image. The inner, rather dark, circular shaped region of the central aperture pupil image is due to transmission of input light beam through the SIL element 108 towards the cartridge 110. These transmitted light beams propagate at an angle below the critical angle, θ_c, of total internal reflection. Due to the optical arrangement of the system 100, the boundary between the inner and outer circular shaped regions in the aperture pupil image is governed by the angle of total internal reflection of the SIL element 108, i.e. the numerical aperture, NA, equals one. The outer boundary of the bright, outer ring shaped region is governed by the angle of the marginal rays, i.e. the numerical aperture of the objective system, e.g. NA =1.9.

For simple wetting detection, for illumination with a numerical aperture, NA, smaller than the refractive index of the liquid 112, a bright ring of light will be reflected for

an empty cartridge, but hardly any light will be reflected when it is filled with e.g. water (light will be transmitted). This difference can also be easily detected using an integrating detector, which will be further disclosed below.

Figure 4 illustrates an alternative single beam, single detector for detecting the presence of a sample on a sample receiving surface, e.g. liquid 112 present on the bottom of the cartridge 110, according to an alternative embodiment of the present invention. In this embodiment, wetting detection is added to a standard configuration by adding a separate, simple, photodiode 402, positioned such that it monitors the intensity at a specific angle between the critical angles for glass or plastic to air ($\theta_{c\ air} = 40.2°$) and to fluid ($n = 1.33$ gives $\theta_{c\ fluid} = 59.1°$) at this position, light will totally internally reflect if the cartridge is empty.

When there is a liquid 112 in the cartridge 110 (below a reflection spot) the reflected intensity will drop. Placing a pinhole 404 (or similar structure to shield light from other directions) at the location of the reflected beam 406 with photodiode 402 behind it, results in a high signal when the light source is on and there is no liquid in the region below the reflection area. As soon as liquid 112 is present, the beam will partly transmit into the liquid 112 as indicated by the dotted arrow 408, resulting in a lower detector signal which indicates good wetting. Even when the injected liquid is highly dispersive and/or absorbing, the intensity at the detector will still drop due to the presence of the liquid. In this way, wetting is still correctly detected.

The skilled addressee realizes that the present invention by no means is limited to the preferred embodiments described above. On the contrary, many modifications and variations are possible within the scope of the appended claims. For example, even though the SIL element has been shown as to have a spherical shape, it is possible to allow the outer shape of the SIL element to have an outer curved surface having any one of various shapes, such as a parabolic surface, an elliptical shape and an aspherical shape.

Additionally, in many cases, but certainly for evanescent excitation, rays with $\theta > \theta_{c\ fluid}$ ($NA > n_{fluid}$) should be used (or at least included) for bio-detection. That is, to combine these two different ranges, two main options are possible including a first case of reducing NA temporarily for wetting measurement, and a second case wherein different light source with other wavelength and beam diameter (corresponding to reduced NA) are used. In case of fluorescence detection it is advantageous to choose the wavelength for the light source close to or larger than the fluorescence wavelength, so that this beam can also pass through the dichroic filter. However, it may also be advantageous to employ a separate light

source with a different wavelength when using the dichroic filter (such as used for fluorescent evanescent excitation).

CLAIMS:

1. A method for determining the presence of a sample on a sample receiving surface, the method comprising:

- directing an input light beam onto the sample receiving surface using an optical element arranged in near-field contact with the sample receiving surface;

- determining a reflected light intensity in an output light beam being totally internally reflected inside of the optical element using a light detector; and

- comparing the reflected light intensity with a predetermined light intensity, wherein the result of the comparison is indicative of the presence of the sample on the sample receiving surface.

2. Method according to claim 1, further comprising the step of determining the predetermined light intensity when no sample is present on the sample receiving surface.

3. Method according to any one of claims 1 – 2, wherein the step of comparing comprises the step of determining the refractive index of the sample on the sample receiving surface using a ratio comparison between the predetermined light intensity and the reflected light intensity.

4. Method according to any one of the preceding claims, wherein the optical element comprises an objective lens and a solid immersion lens, SIL.

5. Method according to claim 4, wherein the SIL has a numerical aperture, NA, of about at least 1.35, more preferably above at least 1.60, and most preferably above 1.90.

6. Method according to any one of the preceding claims, wherein the sample is a liquid, and the sample receiving surface is a micro-fluidic device.

7. Method according to any one of claims 4 – 6, wherein the light detector is further adapted to detect a reflected spatial distribution of the output light beam, and the

method comprises the step of comparing the reflected spatial distribution and a predetermined spatial distribution.

8. Method according to claim 7, wherein the reflected and the predetermined spatial distributions are two-dimensional spatial distributions.

9. Method according to any one of the preceding claims, wherein the light detector is at least one of a CCD, a linear array and a position sensitive detector.

10. Method according to any one of the preceding claims, wherein the input light beam is emitted by at least one of a diode laser or a or an LED having an output wavelength of about 400 to about 1100 nm.

11. An analytical or diagnostic method comprising the method according to any one of the preceding claims.

12. A device for determining the presence of a sample on a sample receiving surface, the method comprising:

- an optical element arranged in near-field contact with the sample receiving surface for directing an input light beam onto the sample receiving surface;
- a light detector for determining a reflected light intensity in an output light beam being totally internally reflected inside of the optical element; and
- means for comparing the reflected light intensity with a predetermined light intensity, wherein the result of the comparison is indicative of the presence of the sample on the sample receiving surface.

13. An analytical or diagnostic apparatus comprising the device of claim 12.

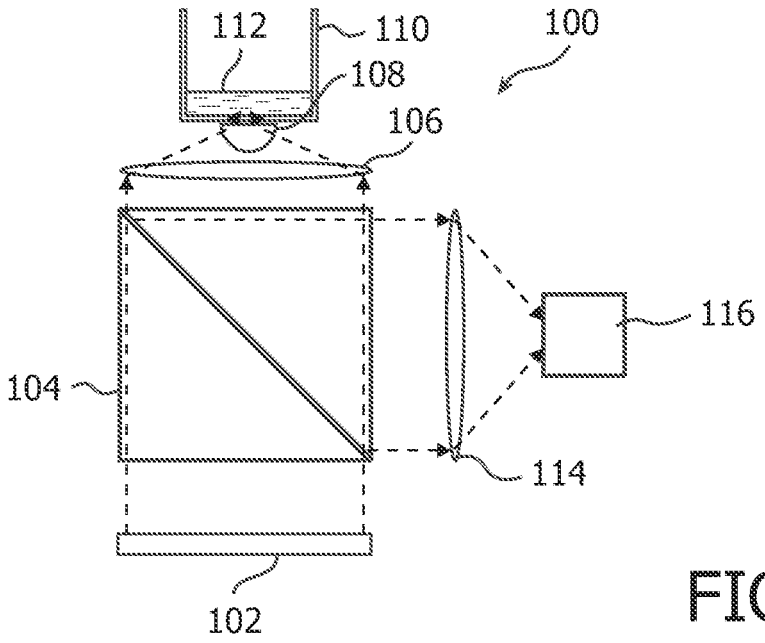

FIG. 1

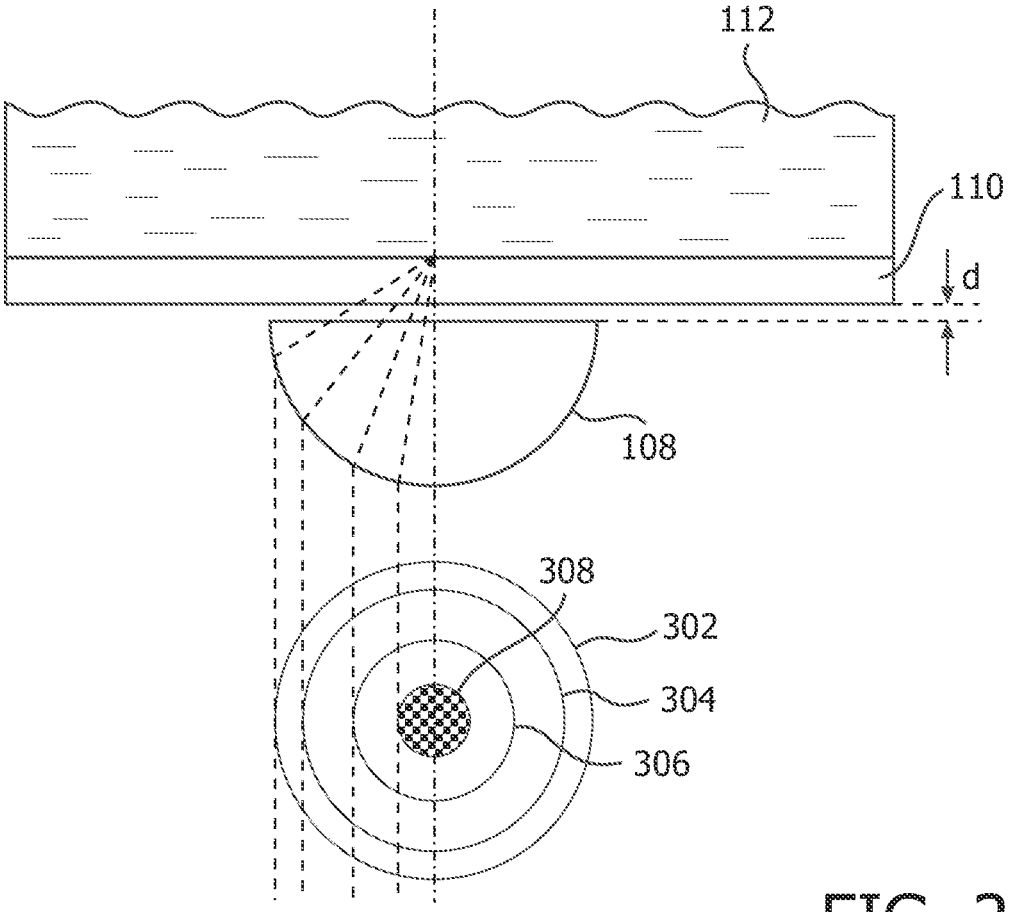

FIG. 2

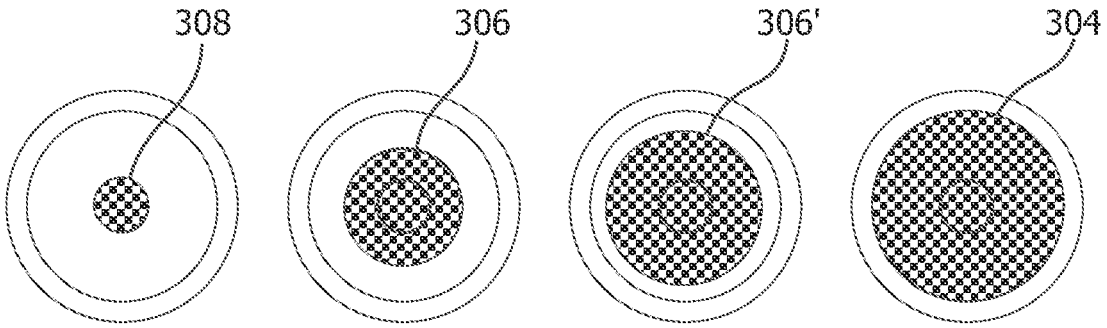

FIG. 3a FIG. 3b FIG. 3c FIG. 3d

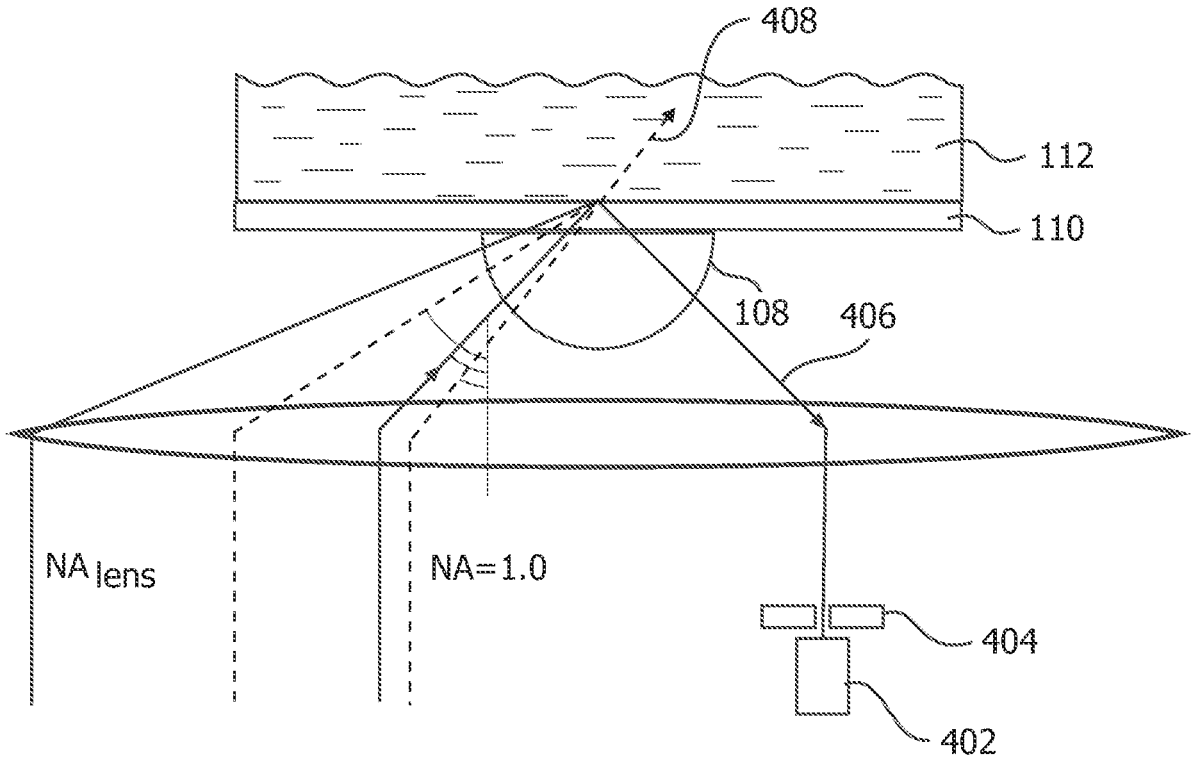

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/053842

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

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

B. FIELDS SEARCHED

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G01N B60F

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)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 180 415 B1 (SCHULTZ SHELDON [US] ET AL) 30 January 2001 (2001-01-30) * col. 1, lines 17-26; col. 5, lines 46-59; col. 8, lines 19-27; col. 15, line 22 to col. 18, line 43; col. 20, line 13 to col. 22, line 31; figures 3,5 *	1-5,7-13
X	US 2002/021443 A1 (VENKATASUBBARAO SRIVATSA [US] ET AL) 21 February 2002 (2002-02-21) paragraphs [0026], [0027]; figures 2,4	1-3,9, 10,12
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2009	08/01/2010

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2009/053842

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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