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(54) Title: FLUORESCENCE MICROSCOPE USING SURFACE PLASMON RESONANCE

(57) Abstract: A fluorescence microscope using a Surface plasmon resonance (SPR) includes a monochromatic light provision unit, an SPR sensor, and a first light detector. The monochromatic light provision unit provides a monochromatic light consistent with an absorption wavelength of a specific fluorophore attached to a sample. The SPR sensor excites surface plasmons to amplify a fluorescence signal of the specific fluorophore. The first light detector detects the fluorescence signal to enable monitoring of a fluorescence intensity of the sample.

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Description

FLUORESCENCE MICROSCOPE USING SURFACE PLASMON RESONANCE

Technical Field

[1] The present invention relates to a fluorescence microscope using Surface plasmon resonance (SPR), for maximizing a fluorescence signal of a bio-sample to which a fluorophore is labeled, using SPR to enable monitoring of the bio-sample.

[2] The invention has been supported by ITR&D Program of MIC/IITA [2006-S-007-02, Ubiquitous Health Monitoring Module and System Development].

Background Art

[3] Fluorescence microscopes make use of the principle that a fluorophore emits fluorescent light when absorbing light of a specific wavelength. The fluorescence microscopes refer to devices for processing a sample with fluorophores (fluorescent dyes) and then irradiating light of an absorption wavelength of the fluorophores into the sample to enable monitoring of the sample by virtue of the fluorophores emitting light. The fluorescence microscopes are mostly used to inspect biological materials.

[4]

[5] FIG. 1 is a schematic diagram illustrating a construction of a conventional fluorescence microscope.

[6] Referring to FIG. 1, the fluorescence microscope includes a first light filter 11, an objective lens 12, a dichroic mirror 13, a second light filter 14, a light receiving unit 15, and a plate 16. The first light filter 11 filters a monochromatic light 10a consistent with an absorption wavelength of a fluorophore that is attached to a sample 17 placed on the plate 16, among a white light 10. The dichroic mirror 13 selects the filtered monochromatic light 10a having the absorption wavelength. The objective lens 12 irradiates the monochromatic light 10a into the sample 17. The second light filter 14 filters light consistent with a emission wavelength of the fluorophore of the sample 17, among light 10b generated from the fluorophore of the sample 17 passing through the objective lens 12 and the dichroic mirror 13. The second light filter 14 provides the filtered light having the emission wavelength to the light receiving unit 15.

[7] The light receiving unit 15 is realized by an ocular lens or a Charge-Coupled Device (CCD). The light receiving unit 15 detects and displays the received light having the emission wavelength of the fluorophore attached to the sample 17 and enables observations of a fluorescence intensity of the sample 17.

[8] SPR sensors are sensors using SPR that occurs when a Transverse Magnetic (TM)-mode polarized light is incident on a thin metal film at an angle satisfying an

SPR condition.

[9] The energy of the incident light is almost absorbed in a surface plasmon mode in an SPR condition that a wave vector of a light source incident on the thin metal film matches with that of the surface plasmons. Therefore, as a result, the intensity of light undergoing the total reflection on a metal surface is minimized. The SPR condition changes due to a minute change of a refractive index of dielectric materials on the metal surface. Biochemical interaction can be quantitatively analyzed by measuring the change of the SPR condition. The SPR sensors have been known as one of the typical non-labeling methods of biosensors that can measure biomolecular interaction without labels such as fluorophores.

[10] Schematic constructions of the SPR sensors are described with reference to FIGS. 2 to 4.

[11] Referring to FIGS. 2 to 4, SPR biosensors each generally include prisms 21a, 21b, and 21c, flat transparent dielectric substrates 22, thin metal films 23, polarizers 25a, 25b, and 25c, and light receiving units 26a, 26b, and 26c. The SPR biosensors are configured to polarize light generated from light sources 24a, 24b, and 24c, in a TM mode by the polarizers 25a, 25b, and 25c, then make the TM-polarized light incident on the thin metal films 23 through the prisms 21a, 21b, and 21c, and then detect light reflected from the thin metal films 23 and emitted to the prisms 21a, 21b, and 21c, by the light receiving units 26a, 26b, and 26c.

[12] Light incident through the prisms 21a, 21b, and 21c induces SPR at the thin metal films 23. Changes of effective refractive indices or effective thickness caused by samples 20 absorbed on the surfaces of the thin metal film 23 are measured by detecting changes of the SPR conditions through the analysis of the reflected light detected in the light receiving units 26a, 26b, and 26c.

[13] In detail, the SPR biosensor of FIG. 2 moves the light source 24a using a motion controller (not shown) and changes an angle of incidence in TM-mode polarizing a monochromatic light generated from the light source 24a by the polarizer 25a and making the TM-mode polarized light incident on the prism 21a. By doing so, the SPR biosensor of FIG. 2 measures a change of the SPR angle caused by a change of an effective refractive index or effective thickness of the sample 20 on the thin metal film 23.

[14] The SPR biosensor of FIG. 3 fixes an angle of incidence of a monochromatic light generated from the light source 24b, while expanding the incident light in a two-dimensional plane form to utilize a two-dimensional light receiving unit such as a CCD. By doing so, the SPR biosensor of FIG. 3 expresses, by a relative difference in grayscale, a change of effective refractive index or effective thickness of the sample 20 appearing at each point (pixel) on the thin metal film 23. In general, the SPR biosensor

is applied in a form of a multi channel sensor system.

[15] The SPR biosensor of FIG. 4 controls a focus of a monochromatic light generated from the light source 24c, using a lens 27 so that the incident light can be normal in all directions to the surface of the prism 21c. Like the SPR biosensor of FIG. 2, the SPR biosensor of FIG. 4 measures, by a change of the SPR angle, a change of an effective refractive index or effective thickness of the sample 20 on the thin metal film 23.

[16] In the conventional SPR biosensors, the thin metal films 23 are formed not directly on reflection surfaces of the prisms 21a, 21b, and 21c but on the flat type transparent dielectric substrates 22 such as a slide glass or a microscope cover slip having the same refractive indices as the prisms 21a, 21b, and 21c in order to form a Self-Assembled Monolayer (SAM) on a sensor surface or facilitate other biochemical processing and also to use the prisms 21a, 21b, and 21c repeatedly. Then, index-matching oil is introduced between the flat transparent dielectric substrates 22 and the prisms 21a, 21b, and 21c.

[17]

[18] However, the conventional fluorescence microscope may cause an optical background noise because an emission wavelength light and an absorption wavelength light, which are transmitted or reflected by a dichroic mirror, of a fluorophore attached to a sample have the same light path. Thus, there is a disadvantage that removing the optical background noise much depends on performance of the light filter filtering the emission wavelength light or absorption wavelength light.

[19] In order to improve this, the paths of the absorption wavelength light applied to the sample and the emission wavelength light must be isolated completely.

Disclosure of Invention

Technical Problem

[20] The present invention has been made to solve the foregoing problems with the prior art, and therefore the present invention provides a fluorescence microscope using SPR, for improving a Signal to Noise Ratio (SNR) by isolating light paths of an absorption wavelength light of a fluorophore and an emission wavelength light of the fluorophore.

Technical Solution

[21] According to an aspect of the present invention, a fluorescence microscope using a Surface plasmon Resonance (SPR) is provided. The microscope includes a monochromatic light provision unit, an SPR sensor, and a first light detector. The monochromatic light provision unit provides, in a Transverse Magnetic (TM) mode, a monochromatic light of a wavelength consistent with an absorption wavelength of a specific fluorophore that is attached to a sample being in Plasmon evanescent field. The SPR sensor excites surface plasmons by the monochromatic light to amplify a flu-

orescence signal of the specific fluorophore. The first light detector detects the fluorescence signal amplified in the SPR sensor to enable monitoring of a fluorescence intensity of the sample.

[22] The microscope may further include a second light detector for detecting the TM-mode monochromatic light totally reflected from the SPR sensor to enable monitoring of an SPR condition of the SPR sensor.

[23] The monochromatic light provision unit may include a light source for generating light, a first light filter, and a polarizer. The first light filter transmits only the monochromatic light of the wavelength consistent with the absorption wavelength of the specific fluorophore, among the light generated from the light source. The polarizer converts the monochromatic light transmitted by the first light filter into a TM-mode light.

[24] The SPR sensor may include a flat transparent dielectric substrate, a thin metal film, a prism, and a spacer. The thin metal film is formed on top of the flat transparent dielectric substrate and supports surface plasmon. The prism is formed at a bottom of the flat transparent dielectric substrate, makes the transverse magnetic mode monochromatic light, provided from the monochromatic light provision unit to an incidence plane of the prism and being consistent with the absorption wavelength of the fluorophore, incident on the thin metal film to excite the surface plasmon resonance, and emits the monochromatic light reflected from the thin metal film to an emission plane. The spacer is formed on the thin metal film at a predetermined thickness, maintains, by the thickness, a minimal spacing between the fluorophore attached to the sample and the thin metal film, and prevents non-radiative energy transfer from the fluorophore to the thin metal film.

[25] The first light detector may include a second light filter and a light receiving unit. The second light filter is positioned on the SPR sensor and transmits only a monochromatic light of a wavelength consistent with an emission wavelength of the specific fluorophore, among incident light from the SPR sensor. The light receiving unit receives the emission wavelength light transmitting the second light filter and enables monitoring of a fluorescence intensity of the sample.

[26]

Advantageous Effects

[27] As set forth above, a fluorescence microscope according to the present invention has an excellent effect of amplifying a fluorescence signal of a fluorophore using the plasmon evanescent field induced in a SPR sensor and completely isolating an absorption wavelength light of the fluorophore from an observation point using the total internal reflection (TIR), thereby greatly improving SNR.

[28] Also, the fluorescence microscope has an excellent effect of being able to expect a high sensitivity and a small background signal compared to a conventional fluorescence microscope by SNR improvement, and constructs a miniaturized fluorescence microscope adapted to the characteristic of a specific fluorophore.

Brief Description of the Drawings

[29] FIG. 1 is a schematic diagram illustrating a construction of a conventional fluorescence microscope;

[30] FIGS. 2 to 4 are schematic diagram illustrating constructions of conventional SPR sensors;

[31] FIG. 5 is a schematic diagram illustrating a construction of a fluorescence microscope according to an exemplary embodiment of the present invention;

[32] FIG. 6 is a graph showing absorption and emission spectrums of a fluorophore generally used for detection of biomolecules; and

[33] FIGS. 7 and 8 are diagrams illustrating different examples of SPR sensors used for a fluorescence microscope according to an exemplary embodiment of the present invention.

Best Mode for Carrying Out the Invention

[34] Preferred embodiments of the present invention will be described herein below with reference to the accompanying drawings. In the following description, well-known functions or constructions are not described in detail since they would obscure the invention in unnecessary detail.

[35] Throughout the drawings, it should be noted that like reference numbers are used to depict the same or similar elements, features and structures.

[36] Throughout the specification, 'connecting of any part with other part' includes not only 'directly connecting' but also 'indirectly connecting' with another element interposed between them. 'Including any element' signifies not excluding other element but being able to further include other element unless otherwise indicated.

[37]

[38] FIG. 5 is a schematic diagram illustrating a construction of a fluorescence microscope according to an exemplary embodiment of the present invention.

[39] Referring to FIG. 5, the fluorescence microscope includes a monochromatic light provision unit 31, an SPR sensor 32, and a first light detector 33. The monochromatic light provision unit 31 provides a monochromatic light (hereinafter, referred to as "absorption wavelength light") of a wavelength that is consistent with an absorption wavelength of a specific fluorophore, in a Transverse Magnetic (TM) mode. The SPR sensor 32 excites surface plasmons by virtue of the absorption wavelength light provided in the TM mode from the monochromatic light provision unit 31 and

amplifies a fluorescence signal of the specific fluorophore in the plasmon evanescent field. The first light detector 33 detects the amplified fluorescence signal and enables observations of a fluorescence intensity of a sample 30. The fluorescence microscope can further include a second light detector 34 for detecting the TM-mode absorption wavelength light undergoing the total reflection in the SPR sensor 32 to enable monitoring of an SPR condition of the SPR sensor 32.

[40] In detail, the monochromatic light provision unit 31 includes a light source 311, a first light filter 312, and a polarizer 313. The first light filter 312 transmits the absorption wavelength light of the specific fluorophore among light generated from the light source 311. The polarizer 313 converts the absorption wavelength light transmitted through the first light filter 312 into a TM-mode light. The light source 311 can be realized by a monochromatic light source or white light source, including a tungsten-halogen lamp (Quartz-Tungsten-Halogen (QTH) lamp), a laser, and a Light Emitting Diode (LED). Also, the light source 311 can employ light sources of several types, such as a point light source, an expanded parallel light source, and a wedge-shaped light source.

[41] The monochromatic light provision unit 31 can further include one or more lenses (a spherical lens or a cylindrical lens) (not shown) located in rear of the polarizer 313 according to need. The one or more lenses convert the TM-mode absorption wavelength light into a two-dimensional expanded parallel light. The absorption wavelength light is incident on the SPR sensor 32 in a form of two-dimensional parallel light.

[42] Here, the absorption wavelength light from the monochromatic light provision unit 31 is incident on the SPR sensor 32 at an SPR angle of the SPR sensor 32.

[43] Thus, the absorption wavelength light incident at the SPR angle from the monochromatic light provision unit 31 induces an excitation of SPR in the SPR sensor 32.

[44] A detailed construction of the SPR sensor 32 is described. The SPR sensor 32 includes a prism 321, a flat transparent dielectric substrate 322, a thin metal film 323, and a spacer 324.

[45] By optical coupling function, the prism 321 makes it go through an absorption wavelength light, which is incident at the SPR angle from the monochromatic light provision unit 31 through an incidence plane, to an interface between the flat transparent dielectric substrate 322 and the thin metal film 323 and emits the absorption wavelength light undergoing the total reflection at the interface to an emission plane. In detail, the prism 321 is desirably formed to have a triangular shape.

[46] The flat transparent dielectric substrate 322 underlies the thin metal film 323 and is in contact at its bottom with one surface of the prism 321 of the triangular shape.

[47] The prism 321 and the flat transparent dielectric substrate 322 can be either coupled through an index matching oil, etc. or can be formed in a same body, e.g. transparent plastic having a high refractive index. The case of integrally forming is described later in detail.

[48] Because the thin metal film 323 is made from metal that supports surface plasmons, SPR occurs by the monochromatic light incident at the SPR angle. In detail, the thin metal film 323 is formed of metal having a negative dielectric constant at a thickness of dozens nanometer (nm) such as aurum (Au), argentums (Ag), copper (Cu), and aluminum (Al). Of the metal, argentums (Ag) which is showing the sharpest SPR peak and aurum (Au) having excellent surface stability are used generally.

[49] The spacer 324 is to prevent non-radiative energy transfer from the fluorophore 303 to the thin metal film 323 that may occur when the fluorophore 303 attached to the sample 30 comes closer to the thin metal film 323 than they are at a specific distance of about 10 nm.

[50]

[51] FIG. 5 shows a sandwich immunoassay method, for example. In FIG. 5, a detection antibody 302 is injected into the sample 30. The detection antibody 302 which is conjugated with a fluorophore 303 can be bound to an antigen 304 that is target molecule, of which the concentration to be determined. A capture antibody 301 is immobilized on top of the spacer 324 of the SPR sensor 32. The capture antibody 301 can be bound to an antigen 304 that is the target molecule, of which the concentration to be determined. Thus, coupling of capture antibody 301 - antigen 304 - detection antibody 302 - fluorophore 303 takes place at a surface of the spacer 324 because of a bio reaction between antibody and antigen.

[52] Regarding the bio reaction, the spacer 324 serves to isolate the fluorophore 303 and the thin metal film 323 such that they do not come closer than a predetermined distance.

[53] Accordingly, the spacer 324 is formed of dielectric medium at a predetermined thickness to provide the minimum spacing distance between the fluorophore 303 and the thin metal film 323. It is desirable that the spacer 324 is set to have a thickness within a range to prevent non-radiative energy transfer of the fluorescence signal while amplifying the fluorescence signal by virtue of the influence of the plasmon evanescent field which decays exponentially in the normal direction to the thin metal film 323.

[54] The spacer 324 must have a scheme facilitating fixing to a surface of the thin metal film 323 and enabling surface orientation, uniform spatial distribution, and easy functionalization of its surface. For example, the spacer 324 can use Self-Assembled Monolayer (SAM), PolyEthylene Glycol (PEG), or dextran.

[55] The first light detector 33 is formed in the opposite direction to the prism 321 on the

basis of the thin metal film 323 of the SPR sensor 32, that is, at a predetermined height from a top of the spacer 324 of the SPR sensor 32 on which the sample 30 is placed. The first light detector 33 detects a fluorescence signal, which is amplified by SPR of the thin metal film 323, of the fluorophore 303 included in the sample 30. For this, the first light detector 33 includes a second light filter 331 and a light receiving unit 332. The second light filter 331 transmits a monochromatic light (hereinafter, referred to as "emission wavelength light") of a wavelength consistent with an emission wavelength of the fluorophore 303, among light generated from the sample 30. The light receiving unit 332 receives the emission wavelength light transmitted through the second light filter 331 and enables observations of a fluorescence intensity of the sample 30. The light receiving unit 332 can be realized by an ocular lens, a Charge-Coupled Device (CCD) that is a two-dimensional light receiving unit, etc. because the fluorescence microscope aims at monitoring of a shape of a target or a bio reaction.

[56]

[57] A description of operation of the fluorescence microscope is made for the exemplary case where an observer makes observations of the antigen 304 of the sample 30 in a sandwich immunoassay method.

[58] In the example, the detection antibody 302 coupled with the fluorophore 303 and simultaneously coupled with the antigen 304 that is the target molecule is injected into the sample 30. The capture antibody 301 that can be specifically bound to the antigen 304 is fixed to the spacer 324 of the SPR sensor 32. As a result, the bio reaction induces coupling with the surface of the spacer 324 in order of capture antibody 301 - antigen 304 - detection antibody 302 - fluorophore 303.

[59] In this state, an absorption wavelength light of a TM mode having a wavelength consistent with an absorption wavelength of the fluorophore 303 is incident at the SPR angle on the thin metal film 323 through the monochromatic light provision unit 31 to the prism 321 of the SPR sensor 32. If so, SPR appears at a surface of the thin metal film 323. At this time, the plasmon evanescent field induces amplification of a fluorescence signal of the fluorophore 303 positioned at the surface of the spacer 324, that is, an emission wavelength light. The first light detector 33 positioned thereof detects the amplified emission wavelength light.

[60] Based on the thin metal film 323 at which SPR appears, the absorption wavelength light undergoes the total reflection at a bottom of the thin metal film 323 and the emission wavelength light is generated at a top of the thin metal film 323. Thus, paths of the absorption wavelength light and the emission wavelength light are isolated completely. This can cause a reduction of a background signal for a fluorescence signal detected in the first light detector 33, improving a Signal to Noise Ratio (SNR).

[61] The amplification of the fluorescence signal of the fluorophore 303 is achieved by

adjusting the SPR wavelength to the absorption wavelength of the fluorophore 303. The amplification of the fluorescence signal can be also maximized when plasmon resonance is maximized. At this time, a measurement range and sensitivity depend on a measurement target because the SPR angle change is proportional to a refractive index change of dielectric materials on the thin metal film 323 under the same wavelength condition.

[62] The spacer 324 adjusts a distance between the fluorophore 303 and the thin metal film 323 and thus, can prevent following energy transfer to the thin metal film 323. As a result, the amplified fluorescence signal is kept constant.

[63]

[64] The second light detector 34 can receive an absorption wavelength light undergoing the total reflection in the SPR sensor 32, detect a change of the SPR angle in the SPR sensor 32, and can be used to control the fluorescence microscope.

[65] FIG. 6 is a graph showing the spectra of the absorption and emission of a fluorophore generally used for detection of biomolecules. In particular, FIG. 6 shows an absorption spectrum 41 and an emission spectrum 42 for Alexa 647, Invitrogen. The absorption spectrum 41 and the emission spectrum 42 are partially overlapped as shown in FIG. 6. Therefore, an absorption wavelength light can be partially mixed even though a second light filter 14 in FIG. 1 filters out an emission wavelength light. Thus, if paths of the absorption wavelength light and the emission wavelength light are completely isolated according to the present invention, the influence of the absorption wavelength light can be completely removed.

[66] As mentioned above, the fluorescence microscope of the present invention is to amplify a fluorescence signal of a fluorophore using the SPR phenomenon. For this, an SPR sensor 32 can be used as SPR sensors of various types.

[67]

[68] FIGS. 7 and 8 are diagrams illustrating different examples of SPR sensors applicable to a fluorescence microscope according to an exemplary embodiment of the present invention. The SPR sensors used for the fluorescence microscope are described in detail with reference to FIGS. 7 and 8.

[69] Referring first to FIG. 7, the SPR sensor 40 can include a sensor substrate 41, a thin metal film 43, and a spacer 44. A prism 42 is formed in a same body on a bottom surface of the sensor substrate 41. The thin metal film 43 is formed on top of the surface of the sensor substrate 41 positioned in the normal direction to the prism 42 to support surface plasmon. The spacer 44 is fixed onto the thin metal film 43 and adjusts a distance between the thin metal film 43 and a fluorophore so that energy transfer from the fluorophore to the thin metal film 43 does not take place.

[70] Referring to FIG. 8, the SPR sensor 50 includes a flat transparent sensor substrate 51,

a thin metal film 53, a spacer 54, and one or more channels 55. A prism 52 is formed in a same body on a bottom surface of the sensor substrate 51. The thin metal film 53 is formed on top of the surface of the sensor substrate 51 positioned in the normal direction to the prism 52 to support surface plasmon. The spacer 54 is fixed onto the thin metal film 53 and adjusts a distance between the thin metal film 53 and a fluorophore so that energy transfer from the fluorophore to the thin metal film 53 does not take place. The one or more channels 55 are formed on the spacer 54 across the SPR dip band at which a reflected light is minimized by SPR of the thin metal film 53. All or part of the channels 55 is formed of different dielectric materials.

[71] That is, the sensor substrates 41 and 51 are obtained by integrally forming the prism 321 and the flat transparent dielectric substrate 322 of FIG. 5 using the same material. The sensor substrates 41 and 51 can use transparent optical polymer materials of a high refractive index including Polystylene (PS), PolyMethyl MethAcrylate (PMMA), PolyCarbonate (PC), and Cyclic Olefin Copolymer (COC). The sensor substrates 41 and 51 can be formed in a way of such as injection molding. The sensor substrates 41 and 51 make it unnecessary to introduce an index matching oil between the prism 321 and the flat transparent dielectric substrate 322 through a manual work one by one. Thus, a use convenience can be enhanced.

[72] The thin metal films 43 and 53 are formed of metal easily emitting electrons by an external stimulus and having a negative dielectric constant at a thickness of dozens nanometer (nm) such as aurum (Au), argentums (Ag), copper (Cu), and aluminum (Al). Of the metal, argentums (Ag) which is showing the sharpest SPR peak and aurum (Au) having excellent surface stability are used generally.

[73] The prisms 42 and 52 irradiate an incident light into the thin metal films 43 and 53 for excitation of SPR and emit light reflected from the thin metal films 43 and 53. The prisms 42 and 52 are formed to have triangular shapes.

[74] In detail, the sensor substrates 41 and 51 can be formed in the same shape and size as those of a standard glass slide format for convenient use. For example, the sensor substrates 41 and 51 are realized in a rectangular shape. That is, the prisms 42 and 52 are integrally formed at one-side bottom surfaces of the sensor substrates 41 and 51 each having a rectangular shape and a predetermined thickness. The thin metal films 43 and 53 are formed on the top surfaces of the sensor substrates 41 and 51 positioned thereon in the normal directions to the prisms 42 and 52.

[75] The spacers 44 and 54 provide the minimum spacing distance between the fluorophore and the thin metal films 43 and 53 in order to prevent non-radiative energy transfer of a fluorescence signal to the thin metal films 43 and 53. The non-radiative energy transfer may occur when the fluorophore that is an indication material for a target material intended for observation comes closer to the thin metal films 43 and 53

than they are at a specific distance of about 10 nm. It is desirable that the spacers 44 and 54 are set to have a thickness within a range to prevent non-radiative energy transfer of the fluorescence signal of the fluorophore while amplifying the fluorescence signal by virtue of the influence of the plasmon evanescent field which decays exponentially in the normal direction to the thin metal films 43 and 53. The spacers 44 and 54 are realized by Self-Assembled Monolayer (SAM), PolyEthylene Glycol (PEG), or dextran to facilitate fixing to a surface of the thin metal film 323 and enable surface orientation, uniform spatial distribution, and easy functionalization of its surface.

[76] In the SPR sensor 40 of FIG. 7, biomolecules specifically bound to target materials are fixed to a surface of the spacer 44. Then, a sample with a fluorophore coupling with target materials is inputted onto the fixed biomolecules. By doing so, the biomolecules fixed to the spacer 44 are coupled with the target materials of the sample.

[77] In this state, an absorption wavelength light of the fluorophore is applied in a TM mode to the SPR sensor 40 which is placed at an inspection position in the fluorescence microscope of FIG. 5. Then, the SPR sensor 40 generates SPR so that a fluorescence signal of the fluorophore positioned in the plasmon evanescent field is amplified. This enables monitoring of the target materials having the attached fluorophore and coupled to the surface of the spacer 44 of the SPR sensor 40, through the first light detector 33.

[78] The SPR sensor 50 of FIG. 8 further includes one or more channels 55 formed on the spacer 54. All or part of the channels 55 is formed of dielectric materials having a different refractive index, that is, formed of biomolecules specifically bound to different target molecules. The one or more channels 55 each can be coupled with the different target materials. Thus, the SPR sensor 50 of FIG. 8 enables making concurrent observations of the different target materials.

[79]

[80] While the present invention has been shown and described in connection with the exemplary embodiments, it will be apparent to those skilled in the art that modifications and variations can be made without departing from the spirit and scope of the invention as defined by the appended claims.

Claims

[1] A fluorescence microscope using Surface plasmon Resonance comprising:
a monochromatic light provision unit for providing, in a transverse magnetic mode, a monochromatic light of a wavelength consistent with an absorption wavelength of a specificfluorophore;
a surface plasmon resonance sensor for exciting surface plasmon resonance by the monochromatic light to amplify a fluorescence signal of the specific fluorophore that is attached to a sample being in Plasmon evanescent field; and
a first light detector for detecting the fluorescence signal amplified in the surface plasmon resonance sensor to enable monitoring of a fluorescence intensity of the sample.

[2] The microscope of claim 1, wherein the monochromatic light provision unit provides the transverse magnetic mode monochromatic light adjusted to the surface plasmon resonance angle of the surface plasmon resonance sensor.

[3] The microscope of claim 1, further comprising:
a second light detector for detecting the transverse magnetic mode monochromatic light totally reflected from the surface plasmon resonance sensor to enable monitoring of a surface plasmon resonance condition of the surface plasmon resonance sensor.

[4] The microscope of claim 1, wherein the monochromatic light provision unit comprises:
a light source for generating light;
a first light filter for transmitting only the monochromatic light of the wavelength consistent with the absorption wavelength of the specific fluorophore, among the light generated from the light source; and
a polarizer for converting the monochromatic light transmitted through the first light filter into a transverse magnetic mode light.

[5] The microscope of claim 1, wherein the surface plasmon resonance sensor comprises:
a flat transparent dielectric substrate;
a thin metal film formed on top of the flat transparent dielectric substrate and supporting surface plasmon;
a prism formed at a bottom of the flat transparent dielectric substrate, making the transverse magnetic mode monochromatic light, provided from the monochromatic light provision unit to an incidence plane of the prism and being consistent with the absorption wavelength of the fluorophore, incident on the thin metal film to excite the surface plasmon resonance, and emitting the

monochromatic light reflected from the thin metal film to an emission plane; and a spacer formed on the thin metal film at a predetermined thickness, maintaining, by the thickness, a minimal spacing between the fluorophore attached to the sample and the thin metal film, and preventing non-radiative energy transfer from the fluorophore to the thin metal film.

[6] The microscope of claim 1, wherein the first light detector comprises:
a second light filter positioned on the surface plasmon resonance sensor and transmitting only a monochromatic light of a wavelength consistent with a emission wavelength of the specific fluorophore, among incident light from the surface plasmon resonance sensor; and
a light receiving unit for receiving the emission wavelength light transmitting the second light filter and enabling monitoring of a fluorescence intensity of the sample.

[7] The microscope of claim 4, wherein the light source is realized by a monochromatic light source or white light source comprising a quartz-tungsten-halogen lamp, a laser, and a light emitting diode.

[8] The microscope of claim 4, wherein the monochromatic light provision unit further comprises:
one or more lens located in rear of the polarizer and converting the transverse magnetic mode monochromatic light into a two-dimensional expanded parallel light.

[9] The microscope of claim 5, wherein the surface plasmon resonance sensor further comprises:
one or more channels formed at a top of the spacer and whose part or all is formed of different dielectric materials each coupled with different target materials.

[10] The microscope of claim 5, wherein the prism is formed to have a triangular shape.

[11] The microscope of claim 5, wherein the prism and the flat transparent dielectric substrate are formed of the same material in a body.

[12] The microscope of claim 5, wherein the thin metal film is formed of one of the metal group consisting of aurum (Au), argentums (Ag), copper (Cu), and aluminum (Al) emitting electrons by an external stimulus and having a negative dielectric constant.

[13] The microscope of claim 5, wherein the spacer is formed of materials facilitating fixing to a surface of the thin metal film and enabling surface orientation, uniform spatial distribution, and easy functionalization of its surface.

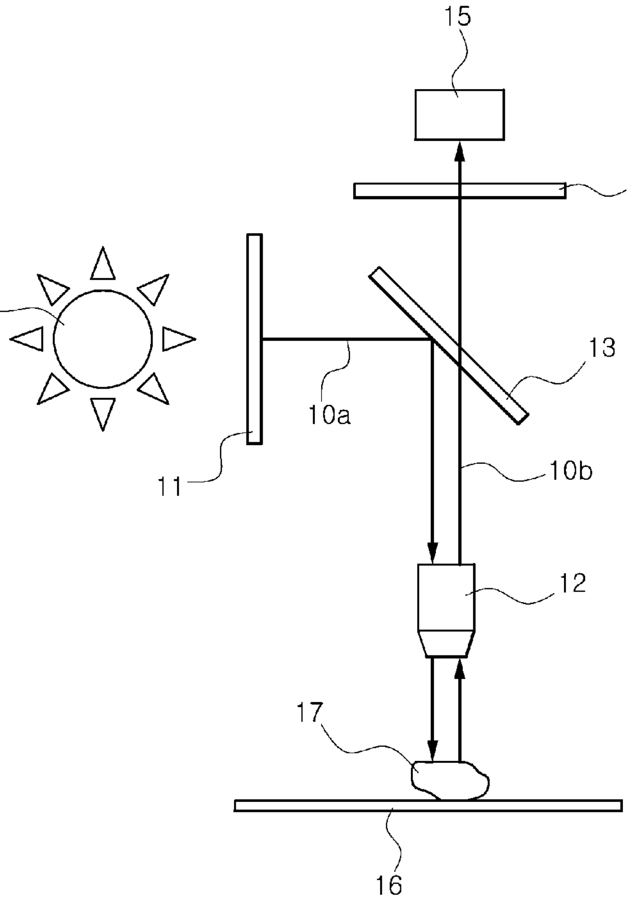
[14] The microscope of claim 6, wherein the light receiving unit is realized by an

ocular lens or a charge-coupled device that is a two-dimensional light receiving unit.

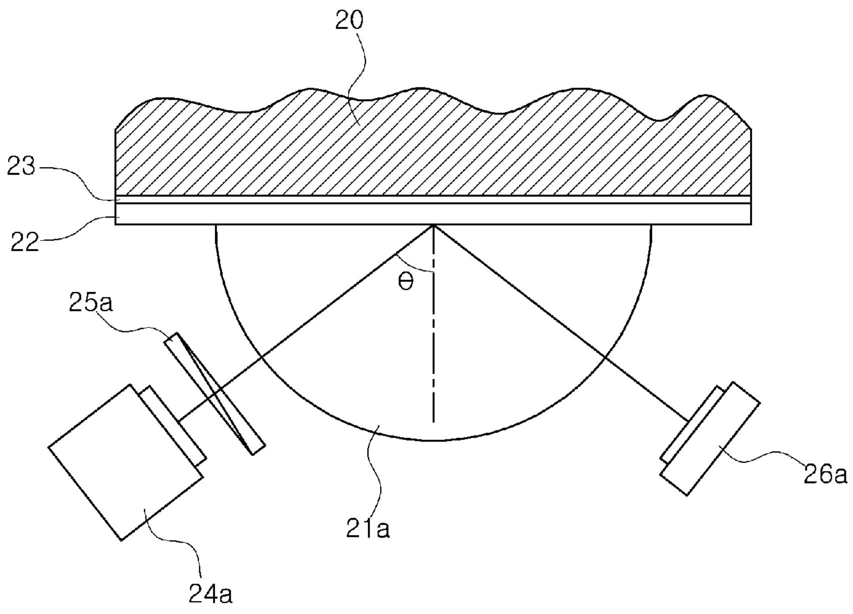
[15] The microscope of claim 11, wherein the prism and the flat transparent dielectric substrate are realized by transparent optical polymer of a high refractive index comprising polystylene, polymethyl methacrylate, polycarbonate, and cyclic olefin copolymer.

[16] The microscope of claim 13, wherein the spacer is realized by one of self-assembled monolayer, polyethylene glycol, and dextran.

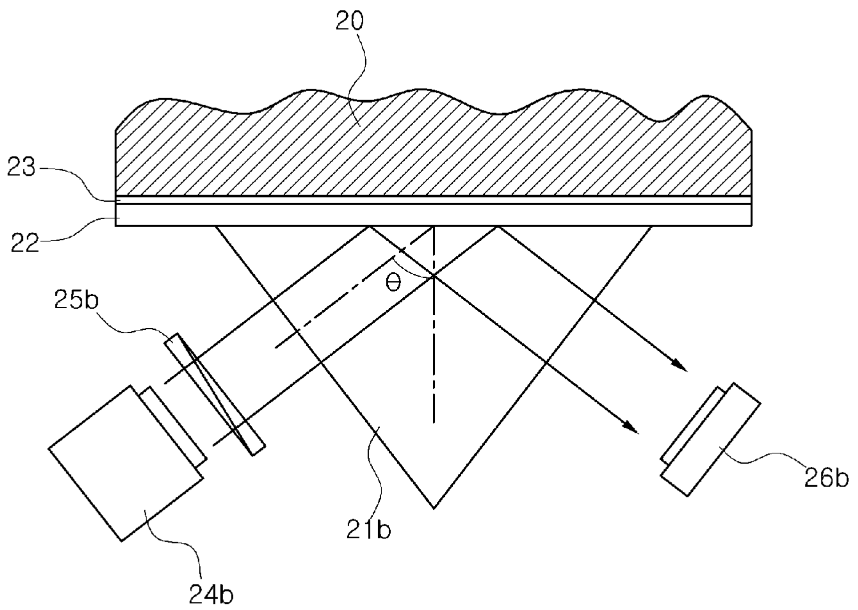
[Fig. 1]



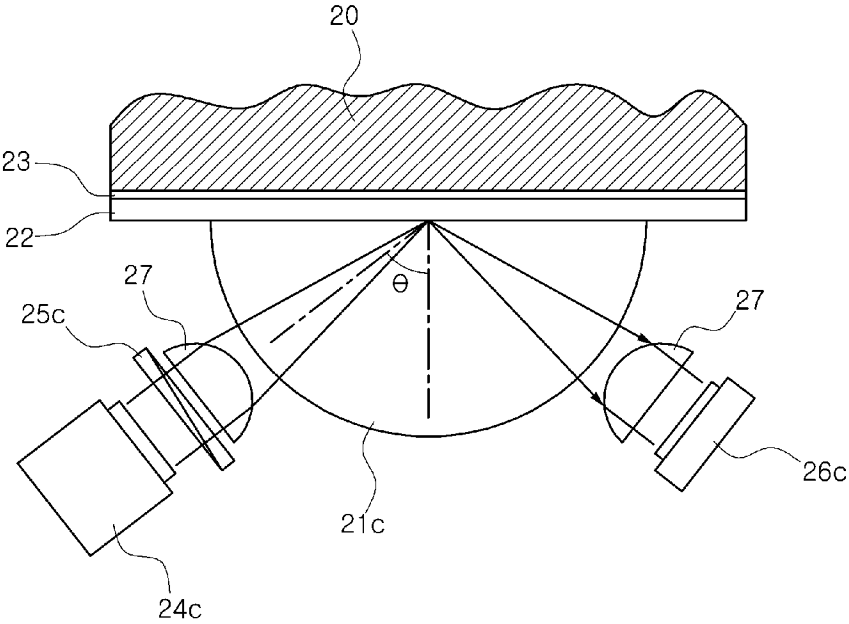
[Fig. 2]



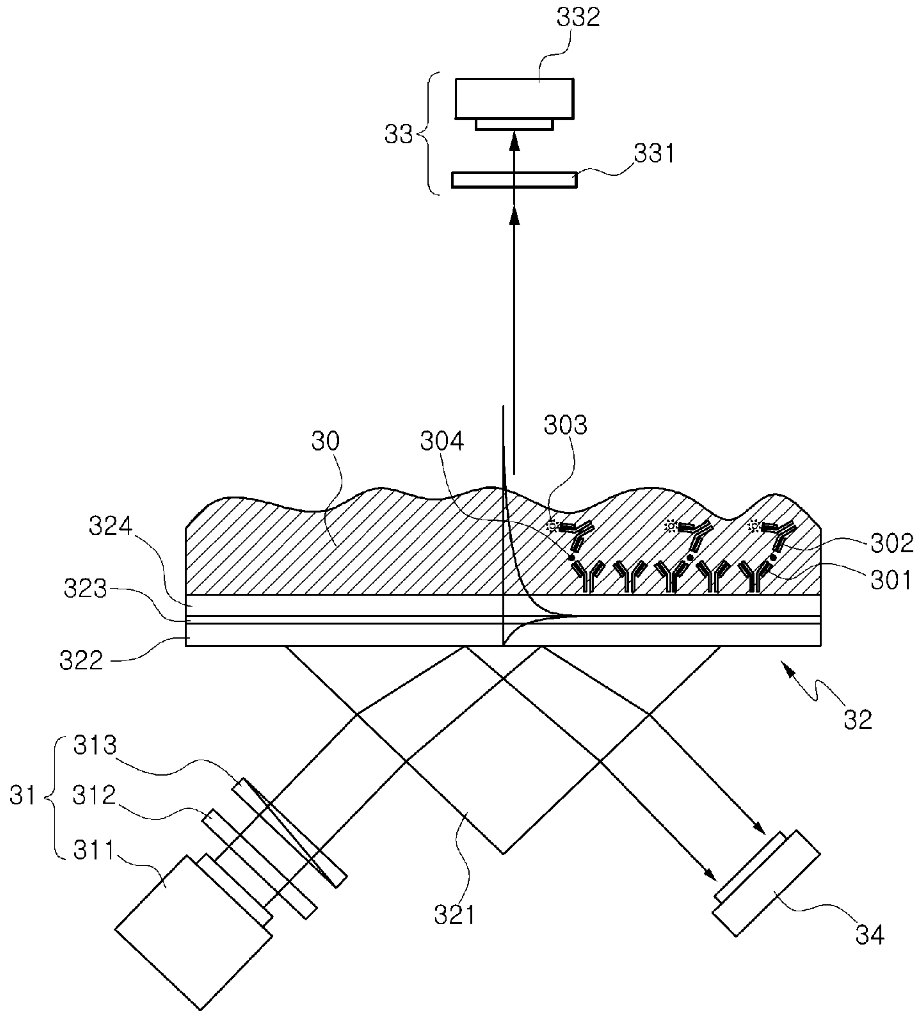
[Fig. 3]



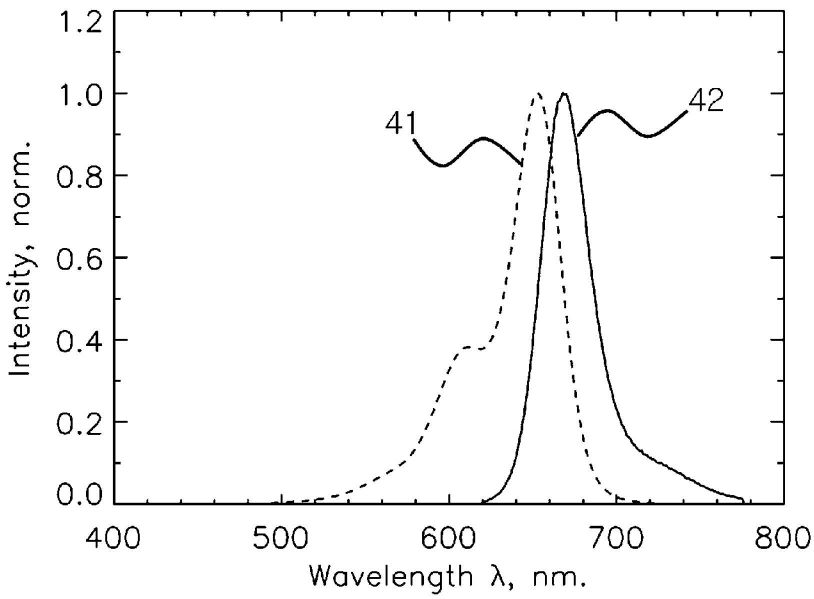
[Fig. 4]



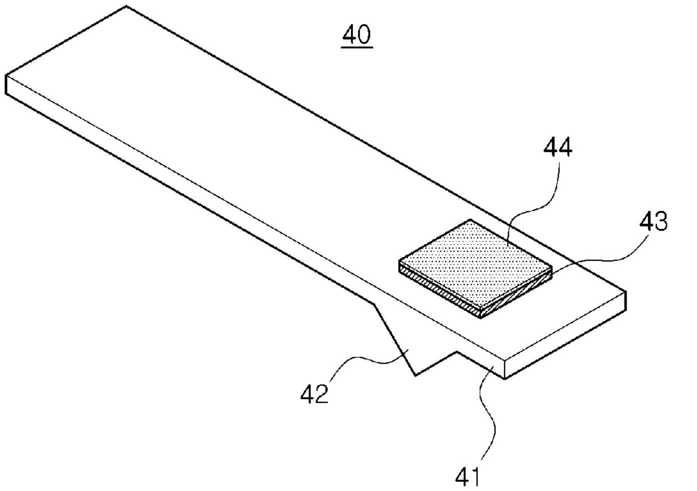
[Fig. 5]



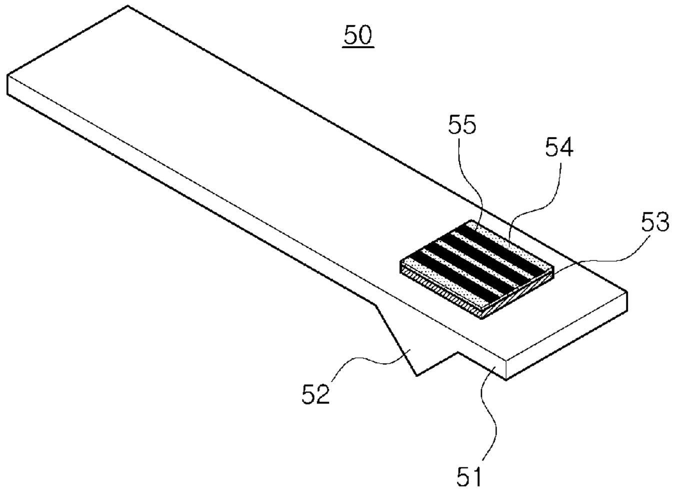
[Fig. 6]


1.2
1.0
0.8
0.6
0.4
0.2
0.0
Intensity, norm.
41
42
400
500
600
700
800
Wavelength λ, nm.

[Fig. 7]



[Fig. 8]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/003114

A. CLASSIFICATION OF SUBJECT MATTER

G02B 21/06(2006.01)i

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)
IPC 8 G01B 7/30

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models since 1975
Japanese Utility models and applications for Utility models since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKIPASS (KIPO internal) & keywords: "SPR", "sensor", and "fluorescence"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 6194223 B1 (RUPERT HERRMANN et al.) 27 February 2001 see claims 1-8, figure 1	1-4,6-8,14 5,9-13,15,16
A	US 6268125 B1 (ELAINE A PERKINS) 31 July 2001 see claims 1-6, figures 1-4	1-16
A	MOTOHIRO FURUKI et al., Surface plasmon resonance sensors utilizing microfabricated channels, Sensors and Actuators B, September 2001, Vol. 79, pages 63-69. See Abstract, pages 63-66	1-16

☐ Further documents are listed in the continuation of Box C.　　☒ See patent family annex.

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"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
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"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
21 NOVEMBER 2008 (21.11.2008)	**21 NOVEMBER 2008 (21.11.2008)**

Name and mailing address of the ISA/KR	Authorized officer
Korean Intellectual Property Office Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	KYONG Chon Su Telephone No. 82-42-481-8434

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2008/003114

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