Device for microorganism analysis

Technical Field

The present invention relates to a device for analyzing a microorganism on a carrier that includes a microorganism to detect a presence of the microorganism.

Background Art

One method for analyzing this kind of carrier is configured to mark the microorganism using a fluorescent or fluorescent material marker and implement an analysis of the fluorescent emitted by the microorganism to detect an existence of the microorganism.

These markers have a characteristic of emitting light with an enzyme in the microorganism only when these are activated in advance.

Generally, these markers include a fluorescent material group and a group that hide or prevent an appearance of fluorescent emission of the fluorescent material group. When the microorganisms exist, the effect of the enzyme therein changes the second group so that the fluorescence of the first group is detected.

Therefore, as illustrated by spectra 53 and 54 in Figure 7, when the microorganisms so marked are subjected to a suitable excitation light, the fluorescent material group is capable of absorbing light energy with an absorption spectrum 53 of which the crest 53' is at a wavelength λ_2 and of discharging that energy in the form of a characteristic fluorescent emission spectrum 54 of which the crest 54' is at a wavelength λ_3, that differs from λ_2.

In order to observe the fluorescence emitted by the microorganisms that exists in such a marker, devices (integrated into a microscope, such as in the United States application US 2007/0153372 and others), already known for microbiological analysis, are equipped with irradiation means adapted to irradiate the carrier of the analysis target in order to detect, by fluorescence, the existence of microorganisms marked by the fluorescent material, and a window

for viewing light coming from the carrier in response to the light emitted by the irradiation means.

Summary of the Invention

Problems to be Resolved by the Invention

The present invention relates to provision of a device for detecting fluorescence, that has better performance and is more practical than a device of the prior art.

Means for Solving the Problem

For that purpose, the present invention provides a device for analyzing a microorganism on a carrier constituted to include a microorganism marked by a fluorescent material, the device characterized in that it is equipped with irradiation means constituted to irradiate the carrier to detect an existence of a microorganism marked using fluorescence, and a window for viewing light emitted by the carrier in response to excitation light generated by the irradiation means. The irradiation means is equipped with two basal portions each mounted with at least one group of the light sources, the light source of that group disposed at a mutually regular distance to form an array for irradiating the carrier. The basal portions are fixed to a frame of the device. One of each side of an opening is formed in the frame to enable light emitted by the carrier to pass through the observation window. The basal portions are mutually inclined to a direction of a predetermined position for receiving the carrier into the device.

The two basal portions (when installed in the device) equipped with light sources pursuant to the device of the present invention attain symmetrical and uniform irradiation of an entire surface of the carrier targeted for irradiation by facing a direction of the carrier targeted for analysis by being mutually inclined, because excitation light generated by the light sources is emitted from both sides of openings formed to enable the observation of the light emitted in response to the excitation of that light.

Disposing the group of light sources in a regular array on each of the two basal portions contributes toward attaining a particularly uniform irradiation of an entire surface of the carrier.

Therefore, such a configuration maintains a uniform irradiation having contrast, and makes it possible to irradiate an even larger sized carrier than the conventional device integrated in a microscope so that it is possible easily to detect an existence of the microorganism with the naked eye or any image sensor.

While making it possible to observe light emitted in response to excitation light generated at a central position, it is also possible particularly to irradiate a microporous membrane, for example.

For reasons of simplicity and convenience of manufacturing and use, preferred characteristics include:

A light source having a same light spectrum as a center of a predetermined wavelength; and/or

The light source is a light-emitting diode.

Pursuant to other preferred characteristics, each basal portion is inclined only an angle of 40° to 50° with regard to a predetermined position for receiving the carrier.

Surprisingly, a range of values [40°-50°] for an incline of the plate with regard to the carrier is demonstrated to give optimum performance for easy reading in order to detect the microorganism.

More specifically, a value in this range appears to minimize the reflection phenomenon (of light reflected on the carrier targeted for analysis) by covering and hiding an effect phenomenon (of light scattered from outside via the observation window).

Said another way, the value in this range attains good image rendering from the points of contrast, uniformity and brightness.

Pursuant to yet another preferred characteristic, a height between a center of each array and a predetermined position to receive the carrier in the device has a correlative relationship with the carrier.

Pursuant to yet another preferred characteristic, a center of each array is positioned at a height of 39.75 mm to 69.75 mm from a predetermined position to receive the carrier.

The range of this height has been demonstrated when a good compromised scheme was formed to make it possible both to maintain good performance in terms of contrast and ease for reading easily to detect microorganisms, and to attain a compact device, particularly when assuming the actual, preferred dimensions for the carrier targeted for analysis.

Other preferred characteristics include:

At each basal portion, in addition to the group of the light source that is a specified first group, and another group of the light source that is a specified second group, the light source~~s~~ of the second group is installed, arrange at mutually regular distances to form a second array for irradiating the carrier;

Centers of the first and the second arrays match in each basal portion;

The first and the second arrays are combined within a mutual range;

The irradiation means is equipped at the basal portion with a diffuser for light emitted by the light source;

Each diffuser is a glass plate one side thereof having been sandblasted;

The observation window is equipped with a filter composed to enable passage of the longest wavelength;

The observation window is equipped with a plurality of filters;

Each filter is an accessory to a slide slidingly mounted on the frame;

The irradiation means is composed to radiate an entire predetermined position for receiving the carrier; and/or

The carrier targeted for analysis is a microporous membrane.

The characteristics and advantages of the present invention will become clear from the description below given as a preferred, but non-limiting, example, with reference to the accompany drawings.

Brief Description of the Drawings

- Figure 1 is a view of the device pursuant to the present invention;
- Figure 2 is a perspective view of the device showing a filter element of the analysis target beside the device;
- Figure 3 is a perspective view looking from a bottom of the device;
- Figure 4 is a sectional elevation view looking from a central symmetrical plane of the device;
- Figure 5 is a perspective view of one of two printed circuit boards in the device, mounted with two groups of optical diodes each of which lights at a predetermined wavelength;
- Figure 6 is a perspective view of another embodiment of the device pursuant to the present invention; and
- Figure 7 is spectral diagram of different optical members of the device pursuant to the present invention, including a normal gradation of a wavelength along an x-axis, and relative light intensity along a y-axis, showing a spectral diagram of fluorescent material used in the device to mark the microorganism.

Mode for Carrying Out the Invention

A preferred embodiment of the device pursuant to the present invention will now be described using the spectral diagram shown in Figure 7, and using Figures 1 to 5, before describing the operation mode in detail.

The device 1 depicted in Figures 1 to 5 is equipped with a casing 2, irradiation means 3 formed by two irradiation members 4, a sliding drawer 5 and an observation window 7.

The casing has an overall parallelepiped shape, and includes a top wall 10, and four side walls from 11 to 14. A portion of the wall 14 intentionally is not shown to allow the inside of the device to be seen.

Here, the observation window 7 is composed of a low-frequency pass filter 6 (in other words, lowest frequencies, therefore the longest wavelengths can pass therethrough). An opening 9 is formed in the top wall 10 of the casing 2 to receive the filter 6 inside.

As shown below, the inside of this casing 2, its dimensions defined by walls 10 to 14 and 30, forms an analysis that receives the carrier targeted for analysis and shields it from peripheral light.

Here, the filter unit 40 on the carrier targeted for analysis is a microporous membrane 41 that is accessory to a unit commercially sold by the Millipore© company under the trademark Milliflex®. This membrane 41 has a diameter of 55 mm and is circumscribed by the main unit 42 of the filter unit.

The membrane 41 used here is composed of cellulose ester, and has a hole size suited for holding a microorganism whose existence is desired to be detected, of 0.10 microns to 100 microns, in most cases.

The drawer 5 has a main body 30, a collar 31, and a gripping knob 32.

A cylindrically shaped cavity 33 is formed in the main unit 30, disposed to receive the filter unit 40.

Irradiation means 3 will now be described using Figures 3 to 5.

These means 3 are equipped with two individual irradiating members 4.

Each member 4 is equipped with a mounting unit 20 composed of a trapezoidal section, a printed circuit board 21 arranged with two groups 27, and 28 composed of diodes 22 and 23, a diffuser 24 that covers these diodes, and two black partitions 26 disposed between the circuit board 21 edge and the diffuser 24 edge.

The mounting unit 20 is fastened inside the casing 2 on the casing wall 10. At one side, the circuit boards 21 are fastened to the mounting unit 20 at oblique faces thereof, disposed opposite to the wall 10, separated from them, so that the circuit boards are inclined mutually facing a direction of a reception region 33 for the carrier 41.

Therefore, each mounting unit 20 is disposed so each circuit board 21 has a 45° inclination A (Figure 1) with regard the wall 10, and to a predetermined position occupied by the membrane 41 when the unit 40 is inside the device housing 33 (Figure 1).

Upper side edges 25 of the circuit board 21 are mutually placed at a distance equivalent to 100 mm; these edges 25 are separated 23 mm from the wall 10.

Apexes of light emitting diodes 22 and 23 are arranged to be separated 15 mm from the diffusers 24.

These diffusers 24 are made of glass plates one face thereof (a face facing towards the diodes) having been sandblasted.

As shown in Figure 5, a first group 27 of diodes is composed of sixteen diodes 22 disposed on each printed circuit board. Similarly, a second group 28 of diodes 23 is composed of four diodes 23.

The diodes 22 are arranged mutually separated at regular distances. Each diode 22 is arranged in four equidistance rows of four dioxides, separated 16 mm from the nearest diodes 22. Therefore, this group 27 forms a diode array that can uniformly irradiate the membrane 41 when disposed in the casing 2 at the predetermined position.

Diodes 23 are disposed scattered in four corners of a square combined in a center of the diode~~s~~ 22 array. Each diode 23 is disposed in a center of a square disposed with the four diodes 22 separated 32 mm from the adjacent two diodes, in each corner (Figure 5). The center of the array matches an array center 29 composed of diodes 22.

Diodes 22 are manufactured by the LUMILED© company, commercialized under the reference symbol LXHL-PB01, with an emission spectrum 55 shown in Figure 7. The Gaussian distribution format spectrum has a peak 55' at a wavelength λ_1 of 470 nm, and therefore emits a blue light.

Diodes 23 are manufactured by the same company, commercialized under the reference symbol LXHL-PD01, an emission spectrum not shown. The Gaussian distribution format spectrum has a peak at a wavelength of 625 nm, and therefore emits a red light.

These diodes have a solid angle of 90º with a solid radiating angle of 140° and relative strength of 60% for a relative strength value of 25%.

The diodes 23 have substantially four times the emission intensity than that of diodes 22 as there are four times fewer than diodes 22.

The arrays of diodes 22 and 23 are combined to attain the most uniform light that is possible in the filter unit that is irradiated, regardless of whether the light is coming from the diodes 22 or the light is coming from the diodes 23 (in other words, the arrays are nested in each other's ranges).

Each group of diodes forms a light spectrum with a predetermined wavelength as a center, so that it is possible to excite different predetermined types of fluorescent material.

A conductive track on each circuit board 21 is electrically connected (not shown) to an instruction control unit for the device.

Preparing the sample targeted for analysis will now be briefly described.

Before the actual detection step, the worker collects a sample targeted for analysis (that includes a microorganism) by filtering through the membrane 41 in the unit 40.

Any step can be included to touch the microorganism to an appropriate culturing medium once the microorganism is filtered and held in the membrane. This culturing medium is preferably a gel-like medium that can be placed on the membrane after filtration. This step is optional. However, this step makes it possible to obtain a colony of each of the initially filter microorganisms, thereby increasing the number of cells targeted for detection.

Later, the membrane and the microorganism contained therein are placed in contact with a composition to make the microorganism walls permeable. Then, the fluorescent marker is incorporated into the permeable-rendering composition to be able to enter inside the microorganism targeted for detection.

Here, judgment of presence of the microorganism on the sample prepared in this way will now be described based on the device pursuant to the present invention.

At a first step, the drawer 5 is pulled out and the worker places the filter unit 40 into the cavity 33 in the drawer. (In some cases, it is acceptable to cover the membrane with a transparent cover, not shown in the drawing, to protect it from external contamination). Then, in order to place the membrane

41 at a predetermined position in the casing 2 for irradiation, the drawer 5 is pushed until the collar 31 touches the wall 13.

Depending on the fluorescent material used to mark the microorganisms, the worker next selects the corresponding diodes 22 or 23 (the diodes 22 in the example shown in the drawing) to operate (for example using a switch not shown in the drawing) in order uniformly to irradiate an entire surface of the membrane 41 and to excite the fluorescent material supplied for marking, at the correct wavelength.

The diffusers 24 can also impart a particularly uniform irradiation onto the entire surface of the membrane 41, regardless of which diode group is used, in the same way as the space distribution of diodes on the circuit board 21.

In addition to the circuit boards 21 being disposed at each side of the opening 9 to receive the filter 6, light coming from the filter unit 40 lighted in response to the excitation light from the diodes, passes through the filter, as shown in Figure 1, so is possible to observe the light response from the membrane 41 with the naked eye or a camera, through the filter 6.

The light response obtained by the existence of the microorganism will now be described in detail using the different spectral diagrams shown in Figure 7.

In Figure 7, spectrum 55 uses the Gaussian format. The peak 55' that corresponds to the maximum light strength value, that corresponds to the spectrum of excitation light (here, the spectrum is formed by diode 22) is a peak at a wavelength λ_1 which is equivalent to 470 nm.

Spectra 53 and 54 respectively correspond to the absorption spectrum of the fluorescent material and optical spectrum selected here in order to mark microorganisms that exist on the membrane.

The fluorescent material shown here is 5-6 CFDA (Carboxy-Fluorescein-Di-Acetate).

The absorption spectrum 53 has a peak 53' at a wavelength λ_2 that is larger than λ_1 and the emission spectrum 54 has a peak 54' at a wavelength λ_3 that is larger than λ_2.

In the example depicted in the drawing, λ_2 is equivalent to 492 nm; and λ_3 is equivalent to 517 nm, the difference between λ_1 and λ_2 is smaller than the difference between λ_2 and λ_3.

Excitation wavelength λ_1 was intentionally selected to be smaller than λ_2 so that the peak 55' of the spectrum 55 is offset to an opposite side of the spectrum 54 for the peak 53' of the spectrum 53. This offset was selected so that the light coming from the irradiation means has sufficient energy to excite the fluorescent material without causing significant parasitic interference with the light emitted by the fluorescent material in response to that excitation light.

The spectrum 56 is that of the filter 6 of the observation window. Basically, the cut-off frequency was selected to allow light emitted by the fluorescent material to pass through (spectrum 54), and to stop light of a shorter wavelength, particularly light coming from the diodes 22 after being reflected in the unit 40, (here, that is approximately 55 nm).

The output filter (a colored filter) is slightly selected to enable an adequate quantity of light to return to the user's eyes giving a generally bright scene, and therefore it ensures a level with a contrast that is suitable for observation using the naked eye, and is easy to observe.

When the excitation light coming from the irradiation means 3 irradiates the membrane 41 on the filter unit 40, each position on the membrane where a microorganisms was marked by the fluorescent material can be seen in the form of a bright spot that is smaller (a few hundreds of microns) than is directly observable with the naked eye from the filter 6.

The values of the angle A and height H provide an optimum state for reading the bright spot on the membrane 41 regardless of whether it is by the naked eye or a camera.

These values have been determined by minimizing parasitic brightness on the black mark and finding positions A and H that have the brightest fluorescent band, for the different values of angle A and height H, in addition to the black mark and fluorescent yellow mark for the control film.

In this way, the range [40°-50°] for the angle A gives maximum light intensity for the fluorescent marks, without parasitic light actually on the black

mark, and therefore was surprisingly verified to correspond to optimum conditions from the viewpoint of ease-of-reading.

Therefore, to have a predetermined safety margin for movement when actually mounting the device, the average value of 45° was selected here.

With regard to the value H for height, it has a correlative relationship with a size of the object to be irradiated. Different tested configurations in a range of values for a membrane having a diameter of 55 mm and a value range for the angle A between 40° and 50° are shown, the best results were obtained for a height range between 39.75 and 69.75 mm. Similarly here, in the example, an average value of 54.75 mm was selected.

The device is also configured to radiate other membrane sample types by adjusting the height H. General, optical carriers (on a top surface or in a volume) are configured to include a microorganism and it is preferred to detect existence using fluorescent light.

Another embodiment of the device is shown in Figure 6.

Generally speaking, the same reference numbers, increased by 100, are used for the same components.

The device 101 has the same characteristics as device 1, except that it does not have the drawer 5 (the filter unit 40 is placed directly on the table, for example), and the observation window 107 is formed from a rectangular slide 108 disposed opposing the wall 110 of the casing 102, and is formed with four filters from 106 to 106''' contained in openings that correspond to each slide 108.

Each optical filter is a low-frequency filter. Therefore, low frequencies (long wavelengths) are able to pass therethrough. The cut-off frequency is different from other cut-off frequencies.

The slide 108 is engaged to a guide rail (not shown in the drawing) on the device so that one randomly selected filter of the four filters 106 to 106''' matches the opening 109 in the wall 110.

Therefore, it is possible to select one that has the most appropriate cut-off frequencies from the four usable filters (for example to attain an optimum

contrast) that corresponds to the selected fluorescent material and operating diodes.

In the one embodiment not shown, the filter 6 is a bandpass filter, not a low-frequency filter, with the passband centering on the wavelength λ_2.

In another embodiment, not shown, the printed circuit board 21 includes either a single group of diodes arranged at regular distances, or a group of three or more diodes. Each group of diodes is composed to irradiate the carrier 41 at different wavelengths. The array of each diode group has a regular shape, like a square, diamond, a triangle or the like.

In still another embodiment, not shown, the diode is replaced by another type of local and single light source, such as a chemiluminescent light spot, and/or the printed circuit board is replaced by another type of basal portion that can support a light source, such as glass or ceramic plate and the like.

Lastly, it should be noted that such an irradiating system can also be obtained with different versions for many other applications such as analysis by microscope, scanning of a biochip, reading of a plate by fluorescence, cytometry, transilluminators, or real-time reading of PCR. Geometrical configurations of devices for each of these applications would be a version specific to that particular application. The frame of the device to which the basal portion 21 may be a hoop that encloses an optical system of a microscope, for example, and does not necessary need to be a casing like the casing shown in the drawing.

The present invention is not limited to the embodiments herein described and can include any variant form thereof.

CLAIMS

1. A device for microorganism analysis on a carrier (41) composed to include a microorganisms marked by a fluorescent material, the device equipped with irradiation means (3) composed to irradiate the carrier (41) for detection of an existence of the marked microorganism; and an observation window (7 ; 107) for viewing a light emitted by the carrier (41) in response to excitation light generated by the irradiation means (3); characterized in that

the irradiation means (3) is equipped with two basal portions (21), at least one light-source (22) group (27) is mounted to each basal portion (21); the light- source (22) group (27) is disposed at mutually regular distances to form an array for irradiating the carrier (41), the basal portions (21) are fastened to frames (2 ; 102) on the device, the basal portions (21) are arranged at both sides of holes (9 ; 109) formed in the frames (2 ; 102) to enable passage through the observation windows (7 ; 107), light emitted by the carrier (41); the basal portions (21) mutually inclined toward a direction of a determined position to receive into the device the carrier (41); and

the irradiation means (3) is equipped at each basal portion (21) with a diffuser (24) for light emitted by the light source (22), wherein only the diffuser (24) exists between the light source (22) and the basal portion (21) being irradiated.

2. The device according to claim 1, further characterized in that the light sources (22) have a same light spectrum (55) centering on a predetermined wavelength (λ_1).

3. The device according to claim 2, characterized in that the light source is a light-emitting diode (22).

4. The device according to any one of claims 1 to 3, characterized in that each basal portion (21) is inclined only an angle (A) of 40° to 50° with regard to a predetermined position for receiving the carrier (41).

5. The device according to any one of claims 1 to 4, characterized in that a height (H) between a center (29) of the array (27) of each basal portion

and the predetermined position for receiving into the device the carrier (41) corresponds to a size of the carrier (41).

6. The device according to claim 5, characterized in that the center (29) of the array (27) is positioned at a height (H) of 39.75 mm and 69.75 mm from the predetermined position for receiving the carrier (41).

7. The device according to any one of claims 1 to 6, characterized in that in addition to the group (27) of the light source (22), specified as a first group (27), another group (28) of a light source (23), specified as the second group is also mounted, the light source (23) of the second group is disposed at each basal portion (21) at mutually regular distances to form a second array for irradiating the carrier (41).

8. The device according to claim 7, characterized in that centers (29) of the first and the second arrays (27, 28) match, for each basal portion (21).

9. The device according to claim 8, characterized in that the first and the second arrays (27, 28) are mutually combined.

10. The device according to claim 1, characterized in that each diffuser (24) is a glass plate one side thereof being sandblasted.

11. The device according to any one of claims 1 to 10, characterized in that the observation windows (7 ; 107) are equipped with filters (6 ; 106) composed to enable a longest wavelength to pass therethrough.

12. The device according to claim 11, characterized in that the observation window (107) is equipped with a plurality of filters (106, 106', 106'', 106''').

13. The device according to claim 12, characterized in that the filters (106, 106', 106'', 106''') are accessories to a slide (108) slidably mounted on the frame (102).

14. The device according to any one of claims 1 to 13, characterized in that the irradiation means (3) is configured to irradiate an entire predetermined position that receives the carrier (41).

15. The device according to claim 14, characterized in that the carrier targeted for analysis is a microporous membrane (41).