Device for microbiological analysis

The present invention concerns a device for the microbiological analysis of supports that may contain microorganisms in order to detect the presence or absence of those microorganisms.

One way to analyze such supports consists of detecting the presence of the microorganisms by analysis of the fluorescence emitted by those microorganisms after they have been marked by what are referred to as flurogen or fluorophore markers.

These markers have the particularity of fluorescing only when they have been activated beforehand by an enzyme contained in the microorganisms.

These markers generally comprise a fluorophore group as well as a group capable of concealing or preventing the fluorescence of the fluorophore group from showing. When the microorganisms are present, the effect of the enzyme thereof is to modify that second group in order that the fluorescence of the first group may be detected.

Thus, as illustrated by the spectra 53 and 54 of Figure 7, when the microorganisms so marked are subjected to an appropriate excitation light, the fluorophore 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 releasing that energy in the form of a characteristic fluorescent emission spectrum 54 of which the crest 54' is at a wavelength λ_3, distinct from λ_2.

In order to observe the fluorescence emitted by the microorganisms in presence of such markers, devices are already known for microbiological analysis (integrated into microscopes, such as in the United States application US 2007/0153372), comprising illuminating means adapted to illuminate the support to analyze for detecting, by fluorescence, the presence of microorganisms marked by a fluorophore agent as well as a window for viewing the light coming from the support in response to the light emitted by the illuminating means.

The invention concerns the provision of a device for detecting fluorescence, as for the device of the prior art, while having better performance and being more practical.

To that end, it provides a device for the microbiological analysis of a support adapted to contain microorganisms marked by a fluorophore agent comprising illuminating means adapted to illuminate said support to detect, by fluorescence, the presence of said marked microorganisms as well as a window for viewing the light emitted by said support in response to the excitation light produced by said illuminating means; characterized in that said illuminating means comprise two bases on each of which is mounted at least one group of light sources, with said sources of that group being regularly spaced from each other to form an array for illuminating said support, said bases being fixed to a frame of said device, one on each side of an opening formed in said frame to allow the light emitted by said support to pass through said viewing window, with said bases being inclined towards each other in the direction of a predetermined location for reception of said support in said device.

The two bases provided with light sources of the device according to the invention, by being inclined relative to each other and directed towards the support to analyze (when it is placed in the device), make it possible to obtain symmetrical and homogenous illumination of the whole of the surface of the support to illuminate since the excitation light produced by the light sources is emitted from both sides of the opening which is provided to enable the observation of the light emitted in response to that light excitation.

The fact that those two bases are each provided with a group of light sources arranged in a regular array also contributes to making the illumination of the whole of the surface of that support particularly homogenous.

Such an arrangement thus makes it possible to illuminate supports of larger size than for the devices of the prior art integrated into microscopes while maintaining uniform illumination with a contrast such that it is easily possible to detect the presence of microorganisms with the naked eye or with any other image sensor.

It is in particular made possible to illuminate for example microporous membranes while keeping an optimum and central location of the opening enabling the observation of the light emitted in response to the light excitation produced by the light sources.

According to features that are preferred for reasons of simplicity and convenience for both manufacture and use:

- said light sources each have a same light spectrum centered on a predetermined wavelength; and/or
- said light sources are light emitting diodes.

According to other preferred features, each said base is inclined by an angle between 40° and 50° relative to said predetermined location for reception of said support.

It proves to be the case, surprisingly, that the range of values [40°-50°] for the inclination of the plates relative to the support gave an optimum performance regarding the comfort of reading for the detection of the microorganisms.

More particularly, it appears that within this range of values veil effect phenomena (from diffusion of extraneous light through the viewing window) and reflection phenomena (from the reflection of light on the support to analyze) are significantly minimized.

In other words, it is within this range of values that the best image rendering is obtained, in terms of contrast, homogeneity and brightness.

According to still other preferred features, the height between the center of each array and said predetermined location for reception of said support in said device is a function of said support.

According to still other preferred features, the center of each said array is situated at a height between 39.75 mm and 69.75 mm from said predetermined location for reception of said support.

It has proved to be the case that, given in particular the dimensions that are desirable in practice for the support to analyze, this range of height provided a good compromise enabling at the same time to obtain a compact

device while keeping good performance with regard to contrast and comfort for reading for the easy detection of the microorganisms.

According to still other preferred features:

- on each base there is also mounted, in addition to said group of light sources, designated first group, another group of light sources, designated second group, with said sources of said second group also being regularly spaced from each other to form a second array for illuminating said support.
- for each base, the centers of said first and second arrays coincide;
- said first and second arrays are interlaced within each other;
- said illuminating means comprise, for each base, a diffuser for the light emitted by said sources.
- each diffuser is a plate of glass on which one of the faces is sandblasted;
- said viewing window comprises a filter adapted allow the longest wavelengths to pass;
- said viewing window comprises a plurality of filters;
- said filters belong to a slide slidingly mounted on said frame;
- said illuminating means are adapted to illuminate the whole of said predetermined location for reception of said support; and/or
- said support to analyze is a microporous membrane.

The features and advantages of the invention will appear from the following description, given by way of preferred but non-limiting example, with reference to the accompanying drawings in which:

- Figure 1 is a diagram of a device according to the invention;
- Figure 2 is a perspective view of that device beside which is represented a filter unit to analyze in the device;
- Figures 3 and 4 are respectively a perspective view taken from below and a section view in elevation taken on a median plane of symmetry of that device;
- Figure 5 is a perspective view of one of the two printed circuit boards of that device on which are fixed two groups of light emitting diodes, each emitting light at a predetermined wavelength;

- Figure 6 is a perspective view of another embodiment of the device according to the invention; and

- Figure 7 illustrates the spectral diagrams of different optical members of a device according to the invention as well as the spectral diagram of the fluorophore agent used with that device to mark the microorganisms, with a common scale of wavelengths along the x-axis and a common scale of relative light intensity along the y-axis.

A description will now be given of a preferred embodiment of the device according to the invention with the help of Figures 1 to 5, before detailing its mode of operation with the help of the spectral diagrams illustrated in Figure 7.

The device 1 illustrated in Figures 1 to 5 comprises a casing 2, illuminating means 3 formed from two illuminating members 4, a sliding drawer 5 and a viewing window 7.

The casing is of parallelepiped general shape and has an upper wall 10 and four side walls 11 to 14, a portion of wall 14 being deliberately not shown in order to show the interior of the device.

The viewing window 7 is here constituted by a low-pass filter 6 (that is to say that it allows the lowest frequencies to pass, and thus the longest wavelengths), an opening 9 being formed in the upper wall 10 of the casing 2 to receive that filter 6 therein.

As will be seen below, the interior of this casing 2, delimited by the walls 10 to 14 and 30, forms an analysis chamber isolated from the surrounding light and in which the support to analyze is received.

The support to analyze is here a microporous membrane 41 belonging to a filter unit 40, here a unit commercialized by Millipore© under the trademark Milliflex®. This membrane 41 has a diameter of 55 mm and is surrounded by a body 42 of the filter unit.

The membrane 41 used here is of cellulose ester and has a pore size adapted to retain the microorganisms whose presence it is desired to detect, most often between 0.10 and 100 microns.

The drawer 5 has a body 30, a collar 31 and a grasping lug 32.

In the body 30 there is formed a cylindrical cavity 33 provided to receive a filter unit 40.

The illuminating means 3 will now be described with the help of Figures 3 to 5.

These means 3 comprise two separate illuminating members 4.

Each member 4 comprises a mounting 20 of trapezoidal section, a printed circuit board 21 on which two groups 27 and 28 of diodes 22 and 23 are disposed, a diffuser 24 covering those diodes and two black screens 26 between the edges of the board 21 and those of the diffuser 24.

The mountings 20 are fixed inside the casing 2 on the wall 10 of that casing, while the boards 21 are fixed to the mountings 20 on the inclined faces thereof, that are remote from those disposed against the wall 10 such that those boards are inclined towards each other in the direction of the reception zone 33 for the support 41.

Each mounting 20 is thus provided such that each plate 21 has an inclination A (Figure 1) of 45° relative to the wall 10 and to the predetermined location that the membrane 41 occupies, when the unit 40 is in the housing 33 of the device (Figure 1).

The upper edges 25 of the boards 21 are at a distance from each other equal to 100 mm while those edges 25 are at a distance of 23 mm from the wall 10.

The summits of light emitting diodes 22 and 23 are situated at a distance of 15 mm from the diffusers 24.

These diffusers 24 are here made from a plate of glass on which one of the faces is sandblasted (the face which is turned towards the diodes).

On each printed circuit board, and as illustrated in Figure 5, a first group 27 of diodes is disposed, constituted by sixteen diodes 22 as well as a second group 28 of diodes constituted by four diodes 23.

The diodes 22 are regularly spaced from each other and disposed in four equidistant rows of four diodes, such that each diode 22 is situated at a distance of 16 mm from the diodes 22 directly neighboring thereto. This group 27 thus forms an array of diodes making it possible to uniformly illuminate the

membrane 41 when it is disposed in the casing 2 at its predetermined location, the center 29 of that array being situated at a height H of 54.75 mm from that predetermined location (Figure 1).

The diodes 23 are distributed in the four corners of a square interlaced in the center of the array of diodes 22, each diode 23 being situated at a distance of 32 mm from the two diodes 23 neighboring thereto and at the center of a square at the corners of which are situated four diodes 22 (Figure 5). The center of this array coincides with the center 29 of the array constituted by the diodes 22.

The diodes 22 are diodes from the company LUMILED© commercialized under the reference LXHL-PB01 and of which the emission spectrum 55 is illustrated in Figure 7, this spectrum of Gaussian distribution form having a crest 55' at the wavelength λ_1 of 470 nm, thus emitting in blue.

The diodes 23 are diodes from the same company commercialized under the reference LXHL-PD01, of which the emission spectrum, not illustrated in the drawings, is also of Gaussian distribution form and has a crest at the wavelength of 625 nm thus emitting in red.

These diodes have a solid angle of emission of 140° for a relative intensity value of 25% and a solid angle of emission of 90° for a relative intensity value of 60%.

The diodes 23 have an emission power substantially four times greater than that of the diodes 22 such that they are four times less numerous than the diodes 22.

The arrays of diodes 22 and 23 are interlaced (that is to say nested within each other) so as to obtain the most homogenous light possible at the filter unit to be illuminated, whether the light comes from the diodes 22 or from the diodes 23.

Each group of diodes produces a light spectrum centered on a predetermined wavelength so as to be able to excite different predetermined types of fluorophore.

The conductive tracks on each board 21 are electrically linked to a command and control unit for the device (not shown).

The preparation of a sample to analyze will now briefly be described.

Prior to the actual detection step, the operator collects a sample to analyze (which may contain microorganisms) by filtration through a membrane 41 of a unit 40.

Once the microorganisms have been filtered and retained on the membrane, an optional step of growing the microorganisms in contact with an appropriate growth medium may be included. This growth medium is preferably a gel medium on which the membrane is deposited after filtration. This step, which is optional, enables colonies of each of the microorganisms initially filtered to be obtained, which increases the number of cells to detect.

The membrane and the microorganisms that it contains are then placed in contact with a composition for rendering the walls of the microorganisms permeable and the fluorophore markers are then incorporated into the permeable-rendering composition in order to enter the inside of the microorganisms to detect.

A description will now be given of the implementation of the determination of the presence or absence of microorganisms on a sample so prepared on the basis of the device according to the invention.

In a first phase, the drawer 5 is pulled and the operator places a filter unit 40 (which may possibly be covered by a transparent cover not illustrated to protect the membrane from exterior contamination) in the cavity 33 of that drawer. The drawer 5 is then pushed until the collar 31 abuts the wall 13 such that the membrane 41 is disposed in its predetermined location within the casing 2 in order to be illuminated.

Depending on the fluorophore which was used to mark the microorganisms, the operator next selects (for example via a switch not illustrated in the drawings) the corresponding diodes 22 or 23 to turn on (the diodes 22 in the example illustrated) so as to uniformly illuminate the whole of the surface of the membrane 41 and to excite, at the right wavelength, the fluorophore that served for the marking.

The diffusers 24 as well as the spatial distribution of the diodes on the boards 21 make it possible to have a particularly homogenous illumination of the whole surface of the membrane 41, whatever the group of diodes used.

As the boards 21 are disposed one on each side of the opening 9 receiving the filter 6, the light coming from the filter unit 40 which is emitted in response to the excitation light from the diodes passes through that filter as illustrated in Figure 1 such that it is possible to observe the light response of the membrane 41 through the filter 6, with the naked eye or else via a camera.

The light response obtained in the presence of microorganisms using the different spectral diagrams illustrated in Figure 7 will now be detailed.

In Figure 7, the spectrum 55 is of Gaussian form and corresponds to the spectrum of the excitation light (here the spectrum produced by the diodes 22) and which has a peak of which the crest 55' corresponding to the maximum light intensity value is at the wavelength λ_1 equal to 470 nm.

The spectra 53 and 54 respectively correspond to the absorption spectrum and to the emission spectrum of the fluorophore chosen here to mark the microorganisms present on the membrane.

The fluorophore represented here is 5-6 CFDA (Carboxy-Fluorescein-Di-Acetate).

The absorption spectrum 53 has a crest 53' at the wavelength λ_2 greater than λ_1 and the emission spectrum 54 has a crest 54' at the wavelength λ_3 greater than λ_2.

In the example illustrated λ_2 is equal to 492 nm and λ_3 to 517 nm, the difference between λ_1 and λ_2 here being less than the difference between λ_2 and λ_3.

The excitation wavelength λ_1 is deliberately chosen to be less than λ_2 such that the crest 55' of the spectrum 55 is offset relative to the crest 53' of the spectrum 53, on the opposite side to the spectrum 54. This offset is chosen in order for the light coming from the illuminating means to have sufficient energy to excite the fluorophore without that light causing significant parasitic interference with that emitted by the fluorophore in response to that excitation light.

The spectrum 56 is that of the filter 6 of the viewing window, it cut-off frequency being chosen (here of the order of 550 nm) to let through essentially the light emitted by the fluorophore (spectrum 54) and to stop the light at shorter wavelengths, in particular those coming from the diodes 22 after reflection on the unit 40.

This output filter (a colored filter) is weakly selective to allow a sufficient quantity of light to return to the eyes of the user giving a generally bright scene and which is thus comfortable to observe while ensuring a level of contrast which is adapted for observation with the naked eye.

When the excitation light coming from the illuminating means 3 illuminates the membrane 41 of the filter unit 40, each location of that membrane having microorganisms marked by the fluorophore is rendered visible in the form of a bright spot of small size (a few hundreds of microns) directly observable with the naked eye coming out of the filter 6.

The values of the angle A and of the height H provide an optimum rendering for the reading of the bright spots on the membrane 41, whether with the naked eye or via a camera.

These values have been determined by applying a black mark and a fluorescent yellow mark to a control membrane and by seeking, for different values of that angle A and of that height H, the configurations A, H for which the brightness of the fluorescent band is maximum while minimizing the parasitic brightness on the black mark.

It has thus surprisingly proved that the range [40°-50°] for the angle A gave both a maximum light intensity for the fluorescent mark and practically an absence of parasite light for the black mark, which thus corresponds to an optimum in terms of reading comfort.

So as to have a certain safety margin relative to the play in mounting the device it is thus the average value of 45° which has been chosen here.

As regards the height value H, this is a function of the size of the object to illuminate, the different configurations tested have shown that for a membrane of a diameter of 55 mm and for a value range for the angle A between 40° and 50°, the best results were obtained for a height range between

39.75 and 69.75 mm. Similarly here, it is the average value of 54.75 mm which was chosen in this example.

The device is also adapted, by adjusting the height H, to illuminate types of sample other that membranes, and generally any support adapted to contain microorganisms (whether on a surface or in a volume) and whose presence it is desired to detect by fluorescence.

Another embodiment of this device is represented in Figure 6.

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

The device 101 has the same features as the device 1 apart from it lacking the drawer 5 (the filter unit 40 here being directly placed on a table for example) and for the fact that the viewing window 107 is here formed from a slide 108 of rectangular form disposed against the wall 110 of the casing 102 and from four filters 106 to 106‴, each of the filters being housed in a corresponding opening of the slide 108.

Each optical filter is a low-pass filter, thus allowing the low frequencies to pass (the longest wavelengths) and of which the cut-off frequency is distinct from the other cut-off frequencies.

The slide 108 is engaged in a guide rail (not illustrated) of that device so as to be able to place any chosen one of the four filters 106 to 106‴ in register with the opening 109 of the wall 110.

It is thus possible, depending on the fluorophore chosen and on the diodes which are turned on, to select from the four available filters the one which has the most adapted cut-off frequency (to obtain the best contrast for example).

In one embodiment not illustrated, the filter 6 is not a low-pass filter but a band-pass filter of which the passband is centered on the wavelength λ_2.

In still another embodiment not illustrated, the printed circuit boards 21 have either a single group of regularly spaced diodes, or more than two groups of diodes, each group of diodes being adapted to illuminate the support 41 with different wavelengths, the arrays of each group of diodes having regular shapes, such as a square, diamond, triangle, etc.

In still another embodiment not illustrated, the diodes are replaced by any other type of localized and unitary light source such as chemiluminescent light spots and/or the printed circuit boards are replaced by any other type of base able to support light sources such as glass or ceramic plates.

Lastly, it will be noted that such an illuminating system may also be produced in different versions for many other applications such as analysis by microscope, scanning of biochips, reading of plates by fluorescence, cytometry, transilluminators, PCR real-time reading, the geometrical configuration of the device for each of these applications then being in a version specific to the application in question. The frame of the device to which the bases 21 are fixed is not necessarily a casing like the casing 2 illustrated; it may for example be a hoop surrounding the optics of a microscope.

The present invention is not limited to the embodiments described and represented, but encompasses any variant form thereof.

CLAIMS

1. A device for the microbiological analysis of a support (41) adapted to contain microorganisms marked by a fluorophore agent comprising illuminating means (3) adapted to illuminate said support (41) to detect, by fluorescence, the presence of said marked microorganisms as well as a window (7 ; 107) for viewing the light emitted by said support (41) in response to the excitation light produced by said illuminating means (3); characterized in that said illuminating means (3) comprise two bases (21) on each of which is mounted at least one group (27) of light sources (22), with said sources (22) of that group (27) being regularly spaced from each other to form an array for illuminating said support (41), said bases (21) being fixed to a frame (2 ; 102) of said device, one on each side of an opening (9 ; 109) formed in said frame (2 ; 102) to allow the light emitted by said support (41) to pass through said viewing window (7 ; 107), with said bases (21) being inclined towards each other in the direction of a predetermined location for reception of said support (41) in said device.

2. A device according to claim 1, characterized in that said light sources (22) each have a same light spectrum (55) centered on a predetermined wavelength (λ_1).

3. A device according to claim 2, characterized in that said light sources are light emitting diodes (22).

4. A device according to any one of claims 1 to 3, characterized in that each said base (21) is inclined by an angle (A) between 40° and 50° relative to said predetermined location for reception of said support (41).

5. A device according to any one of claims 1 to 4, characterized in that the height (H) between the center (29) of each array (27) and said predetermined location for reception of said support (41) in said device is a function of said support (41).

6. A device according to claim 5, characterized in that the center (29) of each said array (27, 28) is situated at a height (H) between 39.75 mm

and 69.75 mm from said predetermined location for reception of said support (41).

7. A device according to any one of claims 1 to 6, characterized in that on each base (21) there is also mounted, in addition to said group (27) of light sources (22), designated first group (27), another group (28) of light sources (23), designated second group, with said sources of said second group (23) also being regularly spaced from each other to form a second array for illuminating said support (41).

8. A device according to claim 7, characterized in that for each base (21), the centers (29) of said first and second arrays (27, 28) coincide.

9. A device according to claim 8, characterized in that said first and second arrays (27, 28) are interlaced within each other.

10. A device according to any one of claims 1 to 9, characterized in that said illuminating means (3) comprise, for each base (21), a diffuser (24) for the light emitted by said sources (22, 23).

11. A device according to claim 10, characterized in that each diffuser (24) is a plate of glass on which one of the faces is sandblasted.

12. A device according to any one of claims 1 to 11, characterized in that said viewing window (7 ; 107) comprises a filter (6 ; 106) adapted allow the longest wavelengths to pass.

13. A device according to claim 12, characterized in that said viewing window (107) comprises a plurality of filters (106, 106', 106'', 106''').

14. A device according to claim 13, characterized in that said filters (106, 106', 106'', 106''') belong to a slide (108) slidingly mounted on said frame (102).

15. A device according to any one of claims 1 to 14, characterized in that said illuminating means (3) are adapted to illuminate the whole of said predetermined location for reception of said support (41).

16. A device according to claim 15, characterized in that said support to analyze is a microporous membrane (41).