

MULTI-COLOR FLUORESCENCE DETECTION DEVICE

FIELD OF THE INVENTION

[0001] The present invention relates to a fluorescence detection device, and more particularly to a multi-color fluorescence detection device.

BACKGROUND OF THE INVENTION

[0002] The demand of acquiring large amounts of a specific segment of DNA efficiently for different purposes is booming in recent years. Among the entire existing DNA sequencing techniques, Polymerase Chain Reactions (PCR) is one of the most economical and straightforward techniques amplifying billion copies of targeted DNA segments in short period of time. The applications of PCR technique are broadly adopted, such as selective DNA isolation for genetic identification, forensic analysis for analyzing ancient DNA in archeology, medical applications for genetic testing and tissue typing, fast and specific diagnosis of infectious diseases for hospitals and research institutes, inspection of environmental hazards for food safety, genetic fingerprint for investigating criminals, and so on. For PCR technique, only small amount of DNA samples are required from blood or tissues. By utilizing fluorescent dye into the nucleic acids solutions, fluorescent molecules help to detect the amplified DNA segments.

[0003] To simultaneously detect and analyze the presence of targeted nucleic acids in a batch of biological samples, fluorescent dyes detection technique is usually adopted. After the light source at specific wavelength illuminated on the targeted nucleic acids, the DNA-binding dyes or fluorescein-binding probes of the nucleic acids would react, and fluorescent signals emitted. The fluorescent signal is an indication of the existence of the targeted nucleic acids. This technique has been employed for the novel PCR technique, which is called real time quantitative

PCR or qPCR. qPCR is the early-phase PCR detection with higher sensitivity and better precision than the conventional PCR technique which is an end-point PCR detection. An optical device is essential to detect the fluorescent light emitted from the specific nucleic acids segments for qPCR technique. The optical device has to provide a light source to excite fluorescent probes at their specific wavelengths, and in the meanwhile, it detects the fluorescent signals emitted from the probes.

[0004] The fluorescent detection systems have been well developed in many fields, such as the application of fluorescence spectroscopy and fluorescence microscopy. An array of single color light source with a set of filters and optical components could easily apply on particular fluorescent probe. However, the difficulties of developing a multi-color fluorescent detection device for portable qPCR system have not been solved in the market because of its cost and size with high signal-to-noise ratio (SNR). Users are restricted to use few kinds of fluorophores because of the limited selection of filter sets in a conventional multi-color qPCR system.

[0005] In light of the requirements and the issues addressed above, there is a need of providing an improved fluorescence detection device for multi-color qPCR application.

SUMMARY OF THE INVENTION

[0006] An object of the present invention is to provide a multi-color fluorescence detection device for minimizing the size and the weight of the device, and still providing superior performance and broad selection of fluorophores for a portable qPCR system with affordable cost.

[0007] According to an aspect of the present invention, there is provided a multi-color fluorescence detection device including an illumination unit, a

fluorescence chamber, a detection unit and an actuation unit. The fluorescence chamber is adapted for accommodating a fluorescent mixture. The illumination unit includes at least one light source and at least one first tunable filter, wherein the light source provides a broad band illumination, and the first tunable filter passes light at different pass bands to excite the fluorescent mixture based on locations or angles of the first tunable filter. The detection unit includes at least one second tunable filter and at least one photo-detector, wherein the second tunable filter passes light emitted from the fluorescent mixture at different pass bands based on locations or angles of the second tunable filter, and the photo-detector receives fluorescent signals and converts the fluorescent signals to electrical signals. The actuation unit is adapted for driving the first tunable filter and the second tunable filter to different locations or angles to control variations of pass bands for excitation and emission, respectively.

[0008] In an embodiment, the first tunable filter of the illumination unit and the second tunable filter of the detection unit both include an angle variable filter or a linear variable filter.

[0009] In an embodiment, the maximum pass band of the first tunable filter of the illumination unit and the second tunable filter of the detection unit is 60 nm.

[0010] In an embodiment, the light source is an LED.

[0011] In an embodiment, a pinhole is provided in front of the first tunable filter of the illumination unit.

[0012] In an embodiment, the pinhole has an aperture of 2 ± 0.5 mm in diameter.

[0013] In an embodiment, the illumination unit further comprises a focusing optics positioned between the first tunable filter of the illumination unit and the fluorescence chamber.

- [0014] In an embodiment, a position offset of the focusing optics from an optical axis of the light source is 1.3 ± 0.2 mm.
- [0015] In an embodiment, the fluorescence chamber has a front aperture and a rear aperture.
- [0016] In an embodiment, the front aperture locates 7 degrees tilted clockwise from a direct optical axis.
- [0017] In an embodiment, the multi-color fluorescence detection device further includes a heater for providing thermal control for the fluorescence mixture.
- [0018] In an embodiment, the heater includes a thermoelectric cooling (TEC) heater.
- [0019] In an embodiment, the detection unit further includes a condensing optics positioned between the fluorescence chamber and the second tunable filter of the detection unit.
- [0020] In an embodiment, the detection unit further includes an imaging optics positioned between the second tunable filter and the photo-detector of the detection unit.
- [0021] In an embodiment, the photo-detector includes a silicon photodiode.
- [0022] In an embodiment, the actuation unit includes a servo motor, a rotary actuator driven by fluid or vacuum pressure, a piezoelectric (PZT) actuator, an electro-actuated polymer (EAP) actuator, or an electromagnetic motor.
- [0023] In an embodiment, the multi-color fluorescence detection device includes plural channels positioned linearly, each of which has its own light source, fluorescence chamber, photo-detector and a pair of first and second tunable filters.
- [0024] In an embodiment, the multi-color fluorescence detection device includes plural channels positioned linearly, each of which has its own light source,

fluorescence chamber and photo-detector, wherein a single pair of first and second tunable filters is moved to scan through the plural channels in turn.

[0025] In an embodiment, the first tunable filter of the illumination unit and the second tunable filter of the detection unit both comprise an angle variable filter.

[0026] In an embodiment, the actuation unit has plural configurations for detecting plural fluorescent dyes respectively.

[0027] The above objects and advantages of the present invention become more readily apparent to those ordinarily skilled in the art after reviewing the following detailed description and accompanying drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] FIG. 1 shows a schematic view of the multi-color fluorescence detection device according to a preferred embodiment of the present invention;

[0029] FIG. 2 shows a cross-sectional view of the multi-color fluorescence detection device of FIG. 1;

[0030] FIGS. 3A, 3B and 3C show the optical paths of the illumination unit when the first tunable filter is rotated at 0, 40 and 75 degrees, respectively;

[0031] FIG. 4 shows the superimposed optical paths of FIGS. 3A, 3B and 3C;

[0032] FIG. 5 shows the spectra of the first tunable filter of the illumination unit;

[0033] FIGS. 6A, 6B and 6C show the optical paths of the detection unit when the second tunable filter is rotated at 0, 40 and 75 degrees, respectively;

[0034] FIG. 7 shows the superimposed optical paths of FIGS. 6A, 6B and 6C;

[0035] FIG. 8 shows the examples of fluorescent dyes applied;

[0036] FIGS. 9A, 9B and 9C show the optical performance of the tricolor combination of FAM, Alexa 514 and HEX; and

[0037] FIGS. 10A, 10B and 10C show the crosstalk effect of the tricolor combination of FAM, Alexa 514 and HEX.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0038] The present invention will now be described more specifically with reference to the following embodiments. It is to be noted that the following descriptions of preferred embodiments of this invention are presented herein for purpose of illustration and description only; it is not intended to be exhaustive or to be limited to the precise form disclosed.

[0039] The present invention provides a multi-color fluorescence detection device which is an optical module sequentially illuminating multiple fluorescent samples arranged in linear position and sequentially detect specific fluorescent signal emitted from the probes. During the qPCR amplification process, this device provides light with different colors to excite different fluorescent probes, and detect fluorescent signals emitted from the probes by changing the orientation of the filters, and thus this invention could work on a fluorescent mixture with multiple colors.

[0040] Please refer to FIGS. 1 and 2, wherein FIG. 1 shows a schematic view of the multi-color fluorescence detection device according to a preferred embodiment of the present invention, FIG. 2 shows a cross-sectional view of the multi-color fluorescence detection device of FIG. 1. As shown in FIGS. 1 and 2, the multi-color fluorescence detection device mainly includes an illumination unit 1, a heating unit 2, a detection unit 3 and an actuation unit 4. The illumination unit 1 is located in front of the heating unit 2, and the detection unit 3 is located behind the heating unit 2, and the actuation unit 4 is connected with the illumination unit 1 and the detection unit 3, respectively.

[0041] The illumination unit 1 includes at least one light source 11 and at least one first tunable filter 12. The light source 11 provides a broad band illumination. The first tunable filter 12 includes an optical filter which passes light at different pass bands while the light enters the first tunable filter 12 at different locations or angles. Thus, different fluorescent dyes in the fluorescent mixture are selectively excited by driving the first tunable filter 12 at different locations or angles. The heating unit 2 includes a heater 21 and at least one fluorescence chamber 22, wherein the heater 21 provides thermal control for qPCR amplification, and the fluorescence chamber 22 is adapted for accommodating a liquid container for the fluorescent mixture. The detection unit 3 includes at least one second tunable filter 31 and at least one photo-detector 32. In the same manner, the second tunable filter 31 passes light emitted from the different fluorescent dyes in the fluorescent mixture at different pass bands when the second tunable filter 31 is rotated at different angles or moved at different locations. The first tunable filter 12 and the second tunable filter 31 are configured to be driven correspondingly for detecting a specific fluorescent dye. The photo-detector 32 receives fluorescent signals and converts the fluorescent signals to electrical signals. The actuation unit 4 drives the first tunable filter 12 of the illumination unit 1 and the second tunable filter 31 of the detection unit 3 to different locations or angles to control variations of pass bands for excitation and emission, respectively, so as to match specific wavelengths of the targeted fluorescent probes. In an embodiment, the actuation unit 4 has plural configurations for detecting different fluorescent dyes respectively. When the actuation unit 4 is switched among different configurations, the first tunable filter 12 and second tunable filter 31 are both driven correspondingly to control variations of pass bands for excitation and emission, so as to conduct detections for different fluorescent dyes.

[0042] The light source 11 emits light within a broad band in visible wavelengths, which could cover targeted excitation wavelengths. In an embodiment, the light source 11 is an LED having a viewing angle of 10 degrees. However, the light source 11 is not limited to the LED, and other kinds of light source, such as mercury lamps and halogen lamps covering visible wavelength are feasible.

[0043] In an embodiment, the illumination unit 1 includes a housing 15, and plural channels are formed in the housing 15 and are positioned linearly. Each channel has its own light source 11, and a pinhole 14 with an aperture of 2 ± 0.5 mm in diameter is provided in front of the coating surface of the first tunable filter 12. For example, as shown in FIG. 1, there are three channels positioned linearly. Certainly, the number of the chambers is not limited to three, and may be varied for different requirement.

[0044] In an embodiment, the material of the housing 15 of the illumination unit 1 is black ABS (acrylonitrile butadiene styrene) for its low thermal conductivity, high thermal resistivity, and reduction of internal light scattering. Other kinds of black plastic materials with low reflectivity, or aluminum coated by black anodized coating possessing low reflectance and high absorptance are feasible.

[0045] In an embodiment, the illumination unit 1 further includes a focusing optics 13 positioned between the first tunable filter 12 and the fluorescence chamber 22. The focusing optics 13 converges the filtered light to the liquid container held in the fluorescence chamber 22. The position of optics offset downward from the optical axis of the light source 11 is around 1.3 ± 0.2 mm, which is optimized to reduce the noise signal. The focusing optics 13 is made of BK7 glass, and other kinds of optical grade polymer are feasible.

[0046] FIGS. 3A, 3B and 3C show the optical paths of the illumination unit when the first tunable filter is rotated at 0, 40 and 75 degrees, respectively, and FIG. 4 shows the superimposed optical paths of FIGS. 3A, 3B and 3C. As shown in FIGS. 2-4, the light provided by the light source 11 passes the pinhole 14 first, and then filtered by the first tunable filter 12. The filtered light is converged by the focusing optics 13 to the liquid container held in the fluorescence chamber 22 to excite the fluorescent dye of the targeted probe.

[0047] The first tunable filter 12 of the illumination unit 1 works as an excitation filter, and the orientation of angular rotation of the first tunable filter 12 faces towards the focusing optics 13. Besides, the pinhole 14 with the aperture of 2 ± 0.5 mm in diameter is required in front of the coating surface of the first tunable filter 12. In an embodiment, the size of the first tunable filter 12 is 6 x 9 x 2 mm for each channel, but its size might change for different applications. The pinhole 14 is required to prevent the excitation light beam at large angle causing scattered light, which is one of the sources of background noise.

[0048] FIG. 5 shows the spectra of the first tunable filter of the illumination unit. As shown in FIG. 5, the spectrum of the first tunable filter 12 is gradually shifted to shorter wavelength areas when the rotation angle of the first tunable filter 12 increases. The maximum pass band is around 60 nm. By selecting the proper fluorescent dyes, the illumination unit 1 could excite a series of fluorescent mixtures falling within the spectra range. It provides higher flexibility of dye selection for potential customers.

[0049] In an embodiment, the first tunable filter 12 includes an angle variable filter (AVF). The peak wavelength of the first tunable filter 12 shifts up to 60 nm for its rotation. However, other types of tunable filter might be applied, such as linear variable filter. Linear shift of this kind of filter in front of the optical

channel could selectively allow photo signal of particular wavelength to pass through.

[0050] Referring to FIGS. 1-2 again. The liquid container held in the fluorescence chamber 22 of the heating unit 2 is a PCR tube for containing a bio-sample and fluorescent probes during qPCR amplification and detection. Each front and rear side of the fluorescence chamber 22 has one circular aperture for the transmission of excitation and emission light signal. The front aperture 221 works as an entrance of the excitation light. The diameter of the front aperture 221 is about 2.5 mm, and the front aperture 221 locates 7 degrees tilted clockwise from the direct optical axis to achieve maximum optical intensity. The diameter of the rear aperture 222 is about 3 mm, and the rear aperture 222 locates right on the axis of the system.

[0051] The material of the fluorescence chamber 22 is copper for its superior thermal conductivity, but other thermally conductive material such as aluminum is feasible. Each chamber is positioned linearly for the batch process.

[0052] The fluorescence chambers 22 are mounted on the top of the heater 21. In an embodiment, the heater 21 includes but not limited to a thermoelectric cooling (TEC) heater. The TEC heater changes temperature in cycle followed by the variation of input current. The temperature control of the TEC heater could be within a fraction of degree, so the requirement of cyclic PCR amplification could be fulfilled. Comparing to other thermal cycler, the compactness of the TEC heater contributes the miniaturization of the system. The TEC heater has long life time, and is easy to maintain. Certainly, other conventional thermal cycling methods through air or liquid are also applicable.

[0053] In an embodiment, the detection unit 3 further includes a condensing optics 33 positioned between the fluorescence chamber 22 and the second tunable

filter 31 of the detection unit 3. The condensing optics 33 collects the fluorescent light emitted from the fluorescent mixture, and refracts to a uniformly distributed parallel beam on the second tunable filter 31 of the detection unit 3. The radius of curvature of the condensing optics 33 is 4 mm, and the plano surface is faced to the fluorescent mixture. The material of the condensing optics 33 is BK7, but other types of glass, optical grade polymers, or ceramics are feasible.

[0054] In an embodiment, the detection unit 3 further includes an imaging optics 34 positioned between the second tunable filter 31 and the photo-detector 32 of the detection unit 3. The imaging optics 34 focuses the filtered fluorescent light on the imaging plane of the photo-detector 32. The photo-detector 32 is made of BK7 glass whose radius of curvature is around 11 mm, however, other types of optical grade material, such as polymers, glass, ceramics are feasible as well.

[0055] In an embodiment, the photo-detector 32 includes a silicon photodiode, however, other kinds of detector such as photomultiplier tube (PMT), charged-couple device (CCD), complementary metal-oxide semiconductor (CMOS), or avalanche photodiode (APD) are considered.

[0056] In an embodiment, the detection unit 3 further includes a photodiode amplifier to convert electrical current into voltage, and amplifies the electrical signal up to 10 to the 8 power for further data analysis and utilization.

[0057] FIGS. 6A, 6B and 6C show the optical paths of the detection unit when the second tunable filter is rotated at 0, 40 and 75 degrees, respectively, and FIG. 7 shows the superimposed optical paths of FIGS. 6A, 6B and 6C. As shown in FIGS. 2 and 6A-7, the fluorescent light emitted from the fluorescent mixtures in the fluorescence chamber 22 is condensed by the condensing optics 33 to the second tunable filter 31 of the detection unit 3. Then the filtered fluorescent light is focused to the imaging plane of the photo-detector 32 by the imaging optics 34.

[0058] The second tunable filter 31 of the detection unit 3 works as an emission filter, and the orientation of angular rotation of the second tunable filter 31 faces towards the imaging optics 34. The coating surface of the second tunable filter 31 faces the condensing optics 33. In an embodiment, the size of the second tunable filter 31 is 6 x 9 x 2 mm for each channel, but its size might change for different applications.

[0059] In an embodiment, the second tunable filter 31 of the detection unit 3 includes an angle variable filter (AVF). The peak wavelength of the second tunable filter 31 shifts up to 60 nm for its rotation. However, other types of tunable filter might be applied, such as linear variable filter. Linear shift of this kind of filter in front of the optical channel could selectively allow photo signal of particular wavelength to pass through.

[0060] Similar to the first tunable filter 12 of illumination unit 1, the second tunable filter 31 of the detection unit 3 also has a spectrum which is gradually shifted to shorter wavelength areas when the rotation angle of the second tunable filter 31 increases. The maximum pass band is around 60 nm. By selecting the proper fluorescent dyes, the detection unit 3 could detect the fluorescent light falling within the spectra range. It provides higher flexibility of dye selection for potential customers.

[0061] In the present invention, the fluorescent dyes whose excitation (Ex) and emission (Em) wavelength falls within the range of the AVF sets, which include the excitation filter (first tunable filter 12 of illumination unit 1) and the emission filter (second tunable filter 31 of detection unit 3), are all applicable. One of the AVF sets chosen could excite the fluorescent dyes whose excitation wavelength is from 487 nm to 547 nm, and the emission wavelength is from 500 nm to 560 nm. FIG. 8 shows the examples of fluorescent dyes applied. For

example, FAM is applicable for one of the filter sets when the excitation filter rotates 60 degrees, and the emission filter rotates 50 degrees. Certainly, the rotation angles are not limited to the exactly 60 degrees and the 50 degrees, and may be 60 ± 5 degrees and 50 ± 5 degrees.

[0062] In an embodiment, the number of the fluorescent dyes added in the PCR tube and detected by the fluorescence detection device of the present invention is at least one. For utilizing single fluorescence, the excitation and emission wavelengths of the non-specific fluorescent dyes should fall within the pass band of the designated AVF sets.

[0063] Particularly, the fluorescence detection device of the present invention is designed for multicolor or multiplexing. The PCR tube may contain plural different fluorescent probes for detecting different targets. By selecting the proper fluorescent dyes and rotating the AVF sets at proper angles, the different targets could be detected sequentially in one single sample. In this situation, the combination of the AVF sets and the mixture of fluorescent dyes are specified, and the excitation and emission wavelength of the specific fluorescent dyes should fall within the pass band of designated AVF sets.

[0064] For example, one of the AVF sets could be utilized for detecting tricolor fluorescent dyes combination of FAM, Alexa 514 and HEX. Table 1 shows this example of fluorescent dyes pairs and the rotation angles of the AVFs. FIGS. 9A, 9B and 9C show the optical performance of the tricolor combination of FAM, Alexa 514 and HEX, and Table 2 shows the SNR (signal to noise ratio) of the tricolor combination of FAM, Alexa 514 and HEX. As shown in FIGS. 9A to 9C and Table 2, the fluorescence detection device of the present invention provides superior performance and the SNR of FAM, Alexa 514 and HEX could be up to 60.

Table 1

Dye	Angle of Filter (°)
FAM	Ex (60) & Em (50)
Alexa 514	Ex (40) & Em (30)
HEX	Ex (20) & Em (0)

Table 2

Dye	SNR@320nM	SNR@20nM
FAM	21.75	2.24
Alexa 514	58.23	4.34
HEX	60.15	4.45

[0065] FIGS. 10A, 10B and 10C show the crosstalk effect of the tricolor combination of FAM, Alexa 514 and HEX. As shown in FIGS. 10A to 10C, the fluorescent signals of low concentrated target dyes are even stronger than high concentrated adjacent dyes, so the fluorescence detection device of the present invention presents low crosstalk effect for the tricolor combination of FAM, Alexa 514 and HEX. Further, the signal to background ratio (SBR) could be derived by the following formula: $SBR = \text{PD voltage_Target Dye} / \text{PD voltage_Target Dye}$. As long as the signal to background ratio (SBR) is greater than or equal to 2, crosstalk effect among fluorescent mixtures is negligible.

[0066] Certainly, the fluorescent dyes combination of the present invention is not limited to FAM, Alexa 514 and HEX. Other combinations of the designated AVF set and the fluorescent dyes mixture in some embodiments are listed in Tables 3 to 5.

Table 3

Dye	Angle of Filter (°)
FAM	Ex (60) & Em (50)
YFP	Ex (40) & Em (30)
HEX	Ex (20) & Em (0)

Table 4

Dye	Angle of Filter (°)
HEX	Ex (75) & Em (55)
ATTO 565	Ex (55) & Em (40)
ROX	Ex (35) & Em (0)

Table 5

Dye	Angle of Filter (°)
HEX	Ex (75) & Em (55)
ATTO 565	Ex (50) & Em (35)
Texas Red	Ex (30) & Em (0)

[0067] Referring to FIGS. 1-2 again. The actuation unit 4 includes a first actuator 41 and a second actuator 42, which are connected with the illumination unit 1 and the detection unit 3 for driving the excitation filter (first tunable filter 12 of illumination unit 1) and the emission filter (second tunable filter 31 of detection unit 3) to control variations of pass bands for excitation and emission, respectively, so as to match specific wavelengths of the targeted fluorescent probes.

[0068] In an embodiment, the actuation unit 4 may be but not limited to servo motors, rotary actuators driven by fluid or vacuum pressure, piezoelectric (PZT)

actuators, electro-actuated polymer (EAP) actuators, or electromagnetic motors. Further, the actuation unit 4 could be driven digitally for specific angle or analogously if continuous rotation is required.

[0069] In an embodiment, the number of the channel of the multi-color fluorescence detection device is at least one. As shown in FIG. 1, the multi-color fluorescence detection device is a three-channel fluorescence detection device. In other words, there are three illumination channels, three fluorescence chambers 22 and three detection channels, and each illumination channel maps to corresponding fluorescence chamber and detection channel. The three channels are positioned linearly for batch process, and each channel has its own light source 11, fluorescence chamber 22, photo-detector 32 and a pair of first and second tunable filters 12 and 31. Therefore, the multi-color fluorescence detection device of the present invention could also work as multi-channel fluorescence detection device. Certainly, the number of the optical channels is certainly not limited to three, and may be varied for different requirements.

[0070] In an embodiment, the quantity of the excitation and emission filter set could be one. In other words, the pair of first and second tunable filters 12 and 31 may not be equipped in each channel, and only one single pair of first and second tunable filters 12 and 31 is commonly used by the plural channels. By moving the single pair of first and second tunable filters 12 and 31 to scan through the plural channels in turn, this device could still operate for multi-color application, and the assembly of the multi-color fluorescence detection device could be further simplified.

[0071] In an embodiment, the configuration of the channels is not limited to linear, and could be any pattern for different applications.

[0072] In conclusion, the present invention provides the multi-color fluorescence detection device including the illumination unit, the heating unit, the detection unit and the actuation unit. Particularly, the illumination unit includes a tunable excitation filter and the detection unit includes a tunable emission filter. By rotating angles of the filters, namely, changing the angle of incidence (AOI), the device could excite and detect a series of fluorescent mixtures falling within the pass band of filters, and thus work on a fluorescent mixture with multiple colors. The multi-color fluorescence detection device may include plural channels positioned linearly for batch process, and thus could also work as multi-channel fluorescence detection device. The multi-color fluorescent detection device further integrates with heating unit for thermal cycling in qPCR application. The present design miniaturizes the size and reduces the cost of the device. The device has a compact optical structure, and the overall dimension of the system is about 85 x 30 x 23 mm. The miniaturized structure still provides promising performance for multi-color applications, and has high signal-to-noise ratio and presents low crosstalk effect. The device further has broad detection bandwidth, and provides higher flexibility of dye selection for potential customers. Therefore, the present invention could minimize the size and weight of the multi-color fluorescence detection, and still provides superior performance and broad selection of fluorophores for a portable qPCR system with affordable cost.

[0073] While the invention has been described in terms of what is presently considered to be the most practical and preferred embodiments, it is to be understood that the invention needs not be limited to the disclosed embodiment. On the contrary, it is intended to cover various modifications and similar arrangements included within the spirit and scope of the appended claims which

are to be accorded with the broadest interpretation so as to encompass all such modifications and similar structures.

WHAT IS CLAIMED IS:

1. A multi-color fluorescence detection device, comprising:

a fluorescence chamber adapted for accommodating a fluorescent mixture;

an illumination unit comprising at least one light source and at least one first tunable filter, wherein the light source provides a broad band illumination, and each single of the at least one first tunable filter passes light at different pass bands by being rotated at different angles or moved at different locations to excite the fluorescent mixture;

a detection unit comprising at least one second tunable filter and at least one photo-detector, wherein each single of the at least one second tunable filter passes light emitted from the fluorescent mixture at different pass bands by being rotated at different angles or moved at different locations, and the photo-detector receives fluorescent signals and converts the fluorescent signals to electrical signals; and

an actuation unit adapted for driving the first tunable filter and the second tunable filter to different locations or angles to control variations of pass bands for excitation and emission, respectively.

2. The multi-color fluorescence detection device according to claim 1 wherein the first tunable filter of the illumination unit and the second tunable filter of the detection unit both comprise an angle variable filter or a linear variable filter.

3. The multi-color fluorescence detection device according to claim 1 wherein the maximum pass band of the first tunable filter of the illumination unit and the second tunable filter of the detection unit is 60 nm.

4. The multi-color fluorescence detection device according to claim 1 wherein the light source is an LED.

5. The multi-color fluorescence detection device according to claim 1 wherein a pinhole is provided in front of the first tunable filter in an optical path of the

illumination unit.

6. The multi-color fluorescence detection device according to claim 5 wherein the pinhole has an aperture of 2 ± 0.5 mm in diameter.

7. The multi-color fluorescence detection device according to claim 1 wherein the illumination unit further comprises a focusing optics positioned between the first tunable filter of the illumination unit and the fluorescence chamber.

8. The multi-color fluorescence detection device according to claim 7 wherein a position offset of the focusing optics downward from an optical axis of the light source is 1.3 ± 0.2 mm.

9. The multi-color fluorescence detection device according to claim 1 wherein the fluorescence chamber has a front aperture and a rear aperture.

10. The multi-color fluorescence detection device according to claim 9 wherein the front aperture locates 7 degrees tilted clockwise from a direct optical axis.

11. The multi-color fluorescence detection device according to claim 1 further comprising a heater for providing thermal control for the fluorescence mixture.

12. The multi-color fluorescence detection device according to claim 11 wherein the heater comprises a thermoelectric cooling (TEC) heater.

13. The multi-color fluorescence detection device according to claim 1 wherein the detection unit further comprises a condensing optics positioned between the fluorescence chamber and the second tunable filter of the detection unit.

14. The multi-color fluorescence detection device according to claim 1 wherein the detection unit further comprises an imaging optics positioned between the second tunable filter and the photo-detector of the detection unit.

15. The multi-color fluorescence detection device according to claim 1 wherein the photo-detector comprises a silicon photodiode.

16. The multi-color fluorescence detection device according to claim 1 wherein the

actuation unit comprises a servo motor, a rotary actuator driven by fluid or vacuum pressure, a piezoelectric (PZT) actuator, an electro-actuated polymer (EAP) actuator, or an electromagnetic motor.

17. The multi-color fluorescence detection device according to claim 1 further comprising plural channels positioned linearly, each of which has its own light source, fluorescence chamber, photo-detector and a pair of first and second tunable filters.

18. The multi-color fluorescence detection device according to claim 1 further comprising plural channels positioned linearly, each of which has its own light source, fluorescence chamber and photo-detector, wherein a single pair of first and second tunable filters is moved to scan through the plural channels in turn.

19. The multi-color fluorescence detection device according to claim 1, wherein the first tunable filter of the illumination unit and the second tunable filter of the detection unit both comprise an angle variable filter.

20. The multi-color fluorescence detection device according to claim 1, wherein the actuation unit has plural configurations for detecting plural fluorescent dyes respectively.