

MULTI-COLOR FLUORESCENT EXCITATION AND DETECTION DEVICE

FIELD OF THE INVENTION

[0001] The present invention relates to a fluorescence detection device, and more particularly to a multi-color fluorescent excitation and detection device having a liquid lens module.

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, the amplified DNA segments could be detected through the help of fluorescent molecules.

[0003] To simultaneously detect and analyze the presence of targeted nucleic acids in a batch of biological samples, fluorescent detection technique is usually applied. After the light source at specific wavelength illuminates on the targeted nucleic acids, the DNA-binding dyes or fluorescein-binding probes of the nucleic acids would react, and fluorescent

signals are 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 isothermal amplification method. 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] Instead of using thermal cycling, isothermal amplification relies on proteins that use in vivo mechanisms of DNA/RNA synthesis and dominated by enzyme activity. Therefore, miniaturize isothermal system has advantages of simple design and extremely low energy consumption. Today, various isothermal based amplification methods in terms of assay complexity (multiple enzymes or primers), acceptable detection sensitivity, and specificity have been developed, including nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), helicase-dependent amplification (HDA), loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA) and nicking enzyme amplification reaction (NEAR).

[0005] 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, most of the existing fluorescent detection systems are bulky, complex and expensive. Moreover, most of the fluorescent signal emitted from the fluorescent detection has low Signal-to-Noise ratio (SNR). Besides, the development of fluorescent detection device for portable isothermal method lags behind its biochemical

technique development. Because isothermal amplification bears higher tolerance on the sample purity, most of commercial isothermal platforms focus on creating a stable temperature environment and detection methods with middle and high throughput. More importantly, as most isothermal based devices are preferred in mobile detection environment, and therefore the system is highly integrated. As a result, most prevalent optical unit designs, though being widely adopted by most instrument manufacturers, are no longer suitable for the isothermal based device.

SUMMARY OF THE INVENTION

[0006] An object of the present invention is to provide a multi-color fluorescent excitation and detection device for concentrating fluorescent signals through a liquid lens module to a detection module to achieve high signal to noise ratio, minimizing the overall size and weight and still providing superior performance for a portable isothermal PCR system with low cost, and allowing the deviation of the detection well.

[0007] Another object of the present invention is to provide a multi-color fluorescent excitation and detection device with compact optical structure for preventing the difficulty of alignment and assembly, high performance, robust design structure for preventing light transmission loss and maintaining high signal to noise ratio.

[0008] In accordance with an aspect of the present disclosure, there is provided a multi-color fluorescent excitation and detection device. The multi-color fluorescent excitation and detection device comprises at least one illumination module, a cartridge, a liquid lens module and at least one detection module. Each of the at least one illumination module provides illumination light at a specified range of wavelengths. The cartridge comprises a detection chip comprising plural detection wells arranged around the peripheral of the detection chip. Each of the detection wells is

adapted for accommodating a corresponding fluorescent sample therein. Each of the detection wells includes a first wall and a second wall. The illumination light transmits through the first wall to excite the fluorescent sample within the detection well so as to generate a fluorescent signal. The liquid lens module comprising at least one liquid lens attached on the second wall. The liquid lens module is configured to enhance the fluorescent signal while the fluorescent signal passes through the second wall and the liquid lens. The at least one detection module receives the enhanced fluorescent signal and converts the enhanced fluorescent signal to an electrical signal.

[0009] The above contents of the present invention will 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

[0010] FIG. 1 shows a schematic view of a nucleic acid analysis apparatus employing a multi-color fluorescent excitation and detection device according to the embodiment of the present invention;

[0011] FIG. 2 shows the nucleic acid analysis apparatus of FIG.1 with opened chamber;

[0012] FIG. 3 shows a schematic view of the multi-color fluorescent excitation and detection device according to the embodiment of the present invention;

[0013] FIG. 4 is an enlarged schematic view showing the multi-color fluorescent excitation and detection device of FIG. 3;

[0014] FIG. 5 is a schematic view showing the detection wells and the liquid lens module of FIG. 4;

[0015] FIG. 6 shows the intensity distribution of fluorescent dyes FAM of different concentrations; and

[0016] FIG. 7 shows the intensity distribution of fluorescent dyes Cy5 of different concentrations.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0017] 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.

[0018] The present invention provides a multi-color fluorescent excitation and detection device for using in a nucleic acid analysis apparatus. More particularly, the present invention provides an all-in-one nucleic acid analysis apparatus with isothermal based amplification, which integrates a fluid delivery unit, a thermal unit, a rotational driven unit, and a multi-color fluorescent excitation and detection device on one single device, so that the processes of sample purification, nucleic acid extraction, nucleic acid amplification and nucleic acid detection can be performed on the all-in-one apparatus to realize nucleic acid analysis in real time.

[0019] FIG. 1 shows a schematic view of the nucleic acid analysis apparatus according to the embodiment of the present invention, and FIG. 2 shows the nucleic acid analysis apparatus of FIG. 1, wherein the nucleic acid analysis apparatus is opened, and the cartridge is moved out of the nucleic acid analysis apparatus. As shown in FIGS. 1 and 2, the nucleic acid analysis apparatus 100 includes a chamber 1, a fluid delivery unit 2, a thermal unit 3, a rotational driven unit 4, and a multi-color fluorescent excitation and detection device 9, wherein the multi-color fluorescent excitation and detection device 9 includes a cartridge 6, at least one illumination module 7, a liquid lens module 5 (see FIG. 3) and at least one detection module 8. The chamber 1 is able to be opened for mounting the

cartridge 6 therein. The fluid delivery unit 2 is connected with the chamber 1 and adapted to transport reagents within the cartridge 6 for sample purification and/or nucleic acid extraction. The thermal unit 3 is disposed in the chamber 1 and adapted to provide a predefined temperature for nucleic acid amplification. The rotational driven unit 4 is connected with the chamber 1 and capable of rotating the cartridge 6 within the chamber 1 with a predefined program. In an embodiment, the rotational driven unit 4 is able to clamp the cartridge 6. The at least one illumination module and at least one detection module 8 are disposed on the chamber 1. Each of the at least one illumination module includes at least one optical component for excitation, and each of the at least one detection module 8 includes at least one optical component for detection, such as nucleic acid detection or sample reaction detection.

[0020] In an embodiment, the chamber 1 includes a top chamber 11 and a bottom chamber 12. The top chamber 11 and the bottom chamber 12 are connected through a hinge 13, but not limited thereto. The bottom chamber 12 has a cavity 121 specifically designed for mounting the cartridge 6 therein. The top chamber 11 can be opened, so that the cartridge 6 is able to be placed into the cavity 121 of the bottom chamber 12. When the top chamber 11 is closed, a confined space is formed in the chamber 1. In an embodiment, the shape of the chamber 1 could be but not limited as cylindrical, spherical, cubic, conical or olivary, and the chamber 1 could be made but not limited by metal, ceramic, polymer, polymer compound, wood, glass, or other materials as long as it is able to provide good thermal insulation.

[0021] The bottom chamber 12 is connected with the fluid delivery unit 2 through tubing or channels. Once the cartridge 6 is mounted in bottom chamber 12, the cartridge 6 is locked and forced to tightly contact

the fluid delivery unit 2 without leakage. For example, the cartridge 6 is locked on the bottom chamber 12 by at least one fixing component, such as a clip but not limited thereto.

[0022] As shown in FIG. 2, the cartridge 6 includes a detection chip 62 and a reagent storing body 63, and the detection chip 62 is disposed on the top of the reagent storing body 63. The detection chip 62 is a planar fluidic chip, and includes plural detection wells 625 (see FIG. 4), at least one first channel 64 and at least one second channel 65. Each of the detection wells 625 is adapted for accommodating a corresponding fluorescent sample therein. The at least one first channel 64 is connected with the detection wells 625 through the at least one second channel 65. In an embodiment, the detection wells 625 are arranged around the peripheral of the detection chip 62 and contains samples or reagents for nucleic acid amplification and/or detection. For example, the detection wells 625 may be coated with samples or reagents for nucleic acid amplification and/or detection, such as reagents containing different fluorescent dyes. The number of the detection wells 625 is not limited, and may be 40 or even more, and the apparatus could perform multiplexing nucleic acid analysis. In an embodiment, the shape of the detection chip 62 is substantially a circular shape, so that the detection chip 62 has plural curve side surfaces to be in line with the at least one detection module 8 to facilitate light focusing.

[0023] The reagent storing body 63 includes plural reagent cells (not shown) used to store reagents for sample purification and/or nucleic acid extraction. The reagent storing body 63 also includes plural channels connected with the reagent cells for fluid delivery. In an embodiment, the reagent storing body 63 is but not limited to a cylindrical body. The detection chip 62 further includes at least one opening 66 at the top surface

of the detection chip 62, and the opening 66 aligns and communicates with at least one reagent cell of the reagent storing body 63 for adding sample to the cartridge 6.

[0024] FIG. 3 shows a schematic view of the multi-color fluorescent excitation and detection device according to the embodiment of the present invention. FIG. 4 is an enlarged schematic view showing the multi-color fluorescent excitation and detection device of FIG. 5. As shown in FIGS. 1, 2, 3 and 4, the multi-color fluorescent excitation and detection device 9 comprises a cartridge 6, at least one illumination module 7, a liquid lens module 5 and at least one detection module 8. Preferably but not exclusively, the multi-color fluorescent excitation and detection device 9 includes four illumination modules 7 and four detection modules 8. Each of the illumination modules 7 is disposed in a corresponding accommodation space 14 of the bottom chamber 12 (see FIG. 2) and provides illumination light at a specified range of wavelengths.

[0025] Please refer to FIG. 4. Each of the detection wells 625 includes a first wall 623, a second wall 621, a third wall 622, a fourth wall 624, a fifth wall and a sixth wall (not shown). The first wall 623 is opposite to the third wall 622. The second wall 621 is opposite to the fourth wall 624. The fifth wall is opposite to the sixth wall. The second wall 621, the fourth wall 624, the fifth wall and the sixth wall are connected with and located between the first wall 623 and the third wall 622. In this embodiment, the first wall 623 is a lower wall, the second wall 621 is a front wall, the third wall 622 is an upper wall, the fourth wall 624 is a rear wall, the fifth wall is a first lateral wall, and the sixth wall is a second lateral wall.

[0026] FIG. 5 is a schematic view showing the detection wells and the liquid lens module of FIG. 4. As shown in FIGS. 4 and 5, in this

embodiment, the liquid lens module 5 comprises a liquid lens 51 and a first lens 52, and the liquid lens module 5 is configured to enhance the fluorescent signal while the fluorescent signal passes through the second wall 921 and the liquid lens 51. The first lens 52 is a condensing lens and attached on the first wall 623 (i.e. lower wall) of the detection wells 625. Accordingly, when the illumination light emitted from the illumination module 7 transmits through the first lens 52 attaching on the first wall 623, the illumination light can be concentrated upward to excite the fluorescent sample within the detection well 625 so as to generate a fluorescent signal.

[0027] The liquid lens 51 attached on the second wall 623 (i.e. front wall) of the detection wells 625 and comprises a second lens 511, a liquid layer 512 and a third lens 513. The liquid layer 512 is sandwiched between the second lens and the third lens. In this embodiment, the liquid layer 512 is filled with oil (i.e. mineral oil), but not limited thereto, which can be filled with water, gel or other liquid materials. In some embodiments, the nominal refractive index of the oil filled in the liquid layer 512 is 1.46, and it has at least 0.05 differences with the index of cartridge material.

[0028] In some embodiments, the second lens 511 is used for concentrating light signal in X direction. In other embodiments, the third lens 513 is used for concentrating light signal in Y direction. In this embodiment, the surface of the second lens 511 and the third lens 513 could be a deformable membrane (e.g. thin polymer or soft elastomer). By changing the pressure or volume of the liquid layer 512 between surface the second lens 511 and inner wall of the third lens 513, the curvature of the second lens 511 and the third lens 513 will change. The two optical curvature of the second lens 511 and the third lens 513 with biconic surface design techniques are utilized to fully maximize the optical energy

transmission and enhance the system performance. Variable focus optics could enhance the fluorescent detection for different wavelengths. Therefore, the fluorescent signal emitted from the fluorescent sample transmitting through the second wall 621 and the liquid lens 51 is enhanced by the liquid lens 51, and the detection module 8 receives the enhanced fluorescent signal transmitted from the liquid lens 51 and converts the enhanced fluorescent signal to an electrical signal.

[0029] Please refer to FIGS. 3 and 4 again, the illumination module 7 is located beside the first wall 623 of the detection well 625, and the optical axis of the illumination module 7 is aligned with the first wall 623 of the detection well 625, so that the first lens attached on the first wall 623 of the detection well 625 receives the illumination light at the specified range of wavelengths. The detection module 8 is located beside the second wall 621 of the detection well 625, and the optical axis of the detection module 8 is aligned with the second wall 621 of the detection well 625, so that the detection module 8 receives the fluorescent signal transmitted through the second wall 621 of the detection well 625 and the liquid lens 51.

[0030] In an embodiment, the illumination module 7 comprises a light source 71 and a first filter 72. The light source 71, such as a LED or a laser diode, is configured to emit the illumination light at wide bandwidth of wavelengths. The first filter 72 is arranged between the light source 71 and the first wall 623. The first filter 72 allows the illumination light at the specified range of wavelengths emitted from the light source 71 to pass through and forbids the unwanted range of wavelengths emitted from the light source 71 to pass through.

[0031] The illumination module 7 further comprises a first pinhole 73. The first pinhole 73 is arranged between the light source 71 and the first filter 72. The first pinhole 73 of the illumination module 7 guides the

illumination light generated from the light source 71 to be aligned on the first filter 72 and the first wall 623 of the detection well 625. An aperture of the first pinhole 73 is ranged from 2.0mm to 3.0mm, but not limited thereto.

[0032] In an embodiment, the first channel 64 is in communication with the detection wells 625 through the corresponding second channels 65. The first channel 64 is used to dispense the sample to the detection wells 625. Preferably, a cross-section area of the second channel 65 is smaller than a cross-section area of the first channel 64. Therefore, the second channel 65 has a capillary value for passive flow controlling.

[0033] In an embodiment, the third wall 622 and the first wall 623 of the detection well 625 are optical membranes respectively. A thickness of the optical membrane of the third wall 622 and a thickness of the optical membrane of the first wall 623 are ranged from 0.1mm to 0.2 mm, respectively, but not limited thereto. A refractive index of the optical membrane of the third wall 622 and a refractive index of the optical membrane of the first wall 623 are ranged from 1.3 to 1.6, respectively, but not limited thereto.

[0034] In an embodiment, the volume of the detection chip 62 is ranged from 10 μ L to 50 μ L, but not limited thereto. The detection chip 62 is made of polycarbonate (PC), polymethyl methacrylate (PMMA) or cyclic olefin copolymer (COC). A refractive index of the detection chip 62 is ranged from 1.3 to 1.6, but not limited thereto.

[0035] The detection module 8 comprises a second filter 81 and a detector 82. The second filter 81 is configured to receive the fluorescent signal transmitted from the liquid lens 51 and allow the fluorescent signal at a specific range of wavelengths to pass through and forbid the unwanted range of wavelengths to pass through. The detector 82 is configured to

receive the fluorescent signal at the specified range of wavelengths passed through the second filter 81 and convert the fluorescent signal to the electrical signal. In an embodiment, the detector 82 is but not limited to a photodiode (PD), avalanche photodiode (APD), charge coupled device (CCD) or complementary metal-oxide semiconductor (CMOS).

[0036] The detection module 8 further comprises a second pinhole 83. The second pinhole 83 is arranged between the second wall 621 of the detection well 625 and the second filter 81. The second pinhole 83 of the detection module 8 guides the fluorescent signal generated from the fluorescent sample to be aligned on the detection module 8. An aperture of the second pinhole 83 is ranged from 2.0 mm to 3.0 mm, but not limited thereto.

[0037] In some embodiments, the multi-color fluorescent excitation and detection device 9 comprises plural illumination modules 7 and plural detection modules 8, for example but not limited to four illumination modules 7 and four detection modules 8. Each of the plural illumination modules 7 provides different color illumination light for fluorescent detection to the respective detection wells 625. The plural detection modules 8 receive the corresponding fluorescent signals, and thus the plural detection modules 8 can detect multiple targets simultaneously and realize multiplexing detection.

[0038] Please refer to FIGS. 6 and 7, wherein FIG. 6 shows the intensity distribution of fluorescent dyes FAM of different concentrations, FIG. 7 shows the intensity distribution of fluorescent dyes Cy5 of different concentrations. In the embodiment, the fluorescent dyes, FAM or Cy5 are utilized. The fluorescent dyes are commercially available fluorescent dyes. Although the preferred embodiment of this invention is described

with these fluorescent dyes, the system of this invention is not limited to these types of fluorescent dyes. As shown in FIGS. 6 and 7, in comparisons with prior art, the intensity of detected signal and signal to noise of the present disclosure are higher.

[0039] Table 1 shows signal to noise ratio (SNR) of two types of the fluorescent dyes applied to the multi-color fluorescent excitation and detection device 9 and the prior art, wherein the concentration of two types of the fluorescent dyes are 320 nM respectively. It clearly presents that signal to noise ratio of two types of the fluorescent dyes applied to the multi-color fluorescent excitation and detection device 9 are high, and could be up to 77 and 30 for FAM and CY5. That means sensitivity of the multi-color fluorescent excitation and detection device 9 is great, and the signal from target fluorescent dyes is highly distinguishable.

Table 1

	FAM	Cy5
Prior art	19.9	17.7
Present disclosure	77.10	30.77

[0040] In an embodiment, the illumination module 7 is mounted in the accommodation space 14 of the bottom chamber 12. During the operation, each illumination module 7 aligns to one of the detection wells 625 of the cartridge 6 in order to offer effective illumination for detection. The detection module 8 is mounted in the edge of the top chamber 11 to realize the optical detection so that the sample could be detected in real time during the nucleic acid amplification. Once the cartridge 6 is clamped, the detection module 8 is in line with one of the detection wells 625 on the cartridge 6 and therefore the results of nucleic acid analysis are interpreted. The rotation of the cartridge 6 allows each detection well 625 pass through different illumination module 7 and detection module 8

sequentially. In an embodiment, each illumination module 7 and detection module 8 could offer unique color of illumination and detection so as to provide different colors for fluorescent based detection, and thus the nucleic acid analysis apparatus 100 can detect multiple targets simultaneously and realize multiplexing detection.

[0041] In realistic operation, there probably has some deviation when the optical axis of the illumination module 7 or the optical axis of the detection module 8 is aligned with the detection well 625. From an experiment result, when the fluorescent sample well deviates within ± 2 degree, the signal to noise ratio of the system at targeting fluorescent dyes still maintains good performance. That means the multi-color fluorescent excitation and detection device 9 allows some deviation when the optical axis of the illumination module 7 or the optical axis of the detection module 8 is aligned with the detection well 625. In some embodiments, the acceptance angle is within ± 3.5 degree.

[0042] In conclusion, the embodiment of the present invention provides a multi-color fluorescent excitation and detection device. The multi-color fluorescent excitation and detection device could be applied in many fields which utilized fluorescent dyes as a medium, such as qPCR, isothermal PCR, fluorescent microscopy, fluorescent spectroscopy. The multi-color fluorescent excitation and detection device which integrates the illumination module, the cartridge and the detection module on one single device, so that the multi-color fluorescent excitation and detection device has compact structure, smaller volume and lighter weight. Besides, the multi-color fluorescent excitation and detection device has a liquid lens module for enhancing the fluorescent detection for different wavelengths so as to maximize the optical energy transmission and enhance the system

performance. Accordingly, the multi-color fluorescent excitation and detection device does not need expensive optical components so that the multi-color fluorescent excitation and detection device has lower cost. Further, due to the arrangements of multiple illumination modules, multiple detection wells and multiple detection modules, both multiplexing nucleic acid analysis and multiple color multiplexing detections are achieved. Moreover, signal to noise of the multi-color fluorescent excitation and detection device of the present invention is high. In addition, the deviation of the rotating of the cartridge is allowed.

[0043] 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.

WHAT IS CLAIMED IS:

1. A multi-color fluorescent excitation and detection device, comprising:
 - at least one illumination module configured to provide illumination light at a specified range of wavelengths;
 - a cartridge comprising a detection chip comprising plural detection wells arranged around the peripheral of the detection chip, each of the detection wells being adapted for accommodating a corresponding fluorescent sample therein and includes a first wall and a second wall, the illumination light transmitting through the first wall to excite the fluorescent sample within the detection well so as to generate a fluorescent signal;
 - a liquid lens module comprising at least one liquid lens attached on the second wall, the liquid lens module being configured to enhance the fluorescent signal while the fluorescent signal passes through the second wall and the liquid lens; and
 - at least one detection module configured to receive the enhanced fluorescent signal and convert the enhanced fluorescent signal to an electrical signal.
2. The multi-color fluorescent excitation and detection device according to claim 1, wherein the illumination module is located beside the first wall and the optical axis of the illumination module is aligned with the first wall, and wherein the detection module is located beside the second wall and the optical axis of the detection module is aligned with the second wall.
3. The multi-color fluorescent excitation and detection device according to claim 1, wherein the liquid lens module further comprises a first lens attached on the first wall, and the first lens is a condensing lens.
4. The multi-color fluorescent excitation and detection device according to claim 1, wherein the liquid lens further comprises a second lens, a liquid

layer and a third lens, and the liquid layer is sandwiched between the second lens and the third lens.

5. The multi-color fluorescent excitation and detection device according to claim 4, wherein the liquid layer is filled with oil, and the surface of the second lens and the third lens are made of a deformable membrane.

6. The multi-color fluorescent excitation and detection device according to claim 7, wherein the nominal refractive index of the oil filled in the liquid layer is 1.46, and it has at least 0.05 differences with the index of cartridge material.

7. The multi-color fluorescent excitation and detection device according to claim 1, wherein the first wall is a lower wall and the second wall is a front wall.

8. The multi-color fluorescent excitation and detection device according to claim 7, wherein each of the detection wells further comprises a third wall, a fourth wall, a fifth wall and a sixth wall, the third wall is opposite to the first wall, the second wall is opposite to the fourth wall, the fifth wall is opposite to the sixth wall, wherein the third wall is an upper wall, the fourth wall is a rear wall, the fifth wall is a first lateral wall, and the sixth wall is a second later wall.

9. The multi-color fluorescent excitation and detection device according to claim 8, wherein the third wall and the first wall are optical membranes respectively, wherein a thickness of the optical membrane of the third wall and a thickness of the optical membrane of the first wall are ranged from 0.1 mm to 0.2 mm, respectively, wherein a refractive index of the optical membrane of the third wall and a refractive index of the optical membrane of the first wall are ranged from 1.3 to 1.6, respectively.

10. The multi-color fluorescent excitation and detection device according to claim 1, wherein the illumination module comprises:

a light source configured to emit the illumination light at wide bandwidth of wavelengths; and

a first filter arranged between the light source and the first wall and allowing the illumination light at the specified range of wavelengths to pass through.

11. The multi-color fluorescent excitation and detection device according to claim 10, wherein the illumination module further comprises a first pinhole arranged between the light source and the first filter, wherein an aperture of the first pinhole is ranged from 2.0 mm to 3.0 mm.

12. The multi-color fluorescent excitation and detection device according to claim 1, wherein the volume of the detection well is ranged from 10 μ L to 50 μ L.

13. The multi-color fluorescent excitation and detection device according to claim 1, wherein the detection chip are made of polycarbonate, polymethyl methacrylate or cyclic olefin copolymer, wherein a refractive index of the detection well is ranged from 1.3 to 1.6.

14. The multi-color fluorescent excitation and detection device according to claim 1, wherein the detection module comprises:

a second filter configured to receive the fluorescent signal and allow the fluorescent signal at a specific range of wavelengths to pass through; and

a detector configured to receive the fluorescent signal at the specified range of wavelengths and convert the fluorescent signal to the electrical signal.

15. The multi-color fluorescent excitation and detection device according to claim 14, wherein the detection module further comprises a second pinhole arranged between the second wall and the second filter, wherein an aperture of the second pinhole is ranged from 2.0 mm to 3.0 mm.

16. The multi-color fluorescent excitation and detection device according to claim 12, wherein the detector is a photodiode, an avalanche photodiode, a charge coupled device or a complementary metal-oxide semiconductor.

17. The multi-color fluorescent excitation and detection device according to claim 1, wherein the multi-color fluorescent excitation and detection device is applied in the fields of qPCR, isothermal PCR, fluorescent microscopy or fluorescent spectroscopy.