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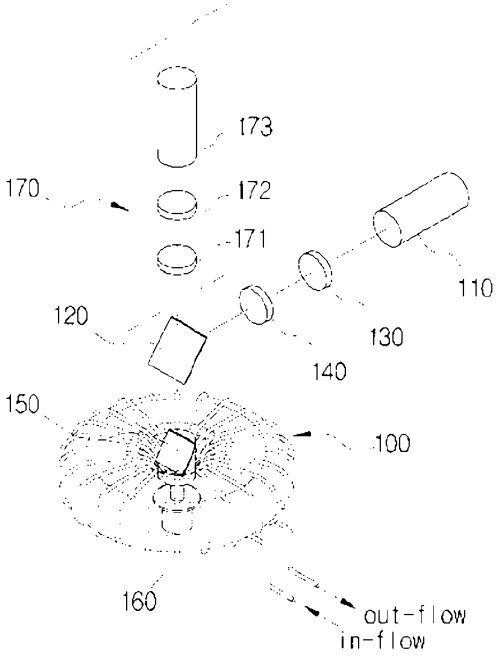
(54) Title: THERMAL CYCLING REACTION BLOCK AND CONTINUOUS REAL-TIME MONITORING APPARATUS USING THE SAME

[Fig. 5]



(57) Abstract: Provided is a real-time monitoring apparatus, including a thermal cycling reaction block having heating block which is formed of a hollow part and divided by an insulating layer, and a capillary tube through which a sample is flowed in and/or out and which is wound on the heating block so that different temperatures are transferred and thus reaction cycle is repeatedly performed a light source; a band pass filter; a condensing lens; a beam splitter a reflecting mirror which is rotatably connected with a motor so as to transfer the excitation light reflected from the beam splitter to the capillary tube and reflect the fluorescence generated from the sample in the capillary tube; and a fluorescence detecting part.

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Description

THERMAL CYCLING REACTION BLOCK AND CONTINUOUS REAL-TIME MONITORING APPARATUS USING THE SAME

Technical Field

[1] The present invention relates to a thermal cycling reaction block and a continuous real-time monitoring apparatus using the same, and more particularly, to a thermal cycling reaction block which is capable of heating or cooling samples at different temperatures so as to generate a polymerase chain reaction (PCR) and allow the PCR to be monitored in real-time, and a continuous real-time monitoring apparatus using the same.

Background Art

[2] A polymerase chain reaction (PCR) is a method of amplifying DNA by multiple synthesis of a selected region of the DNA, thereby producing a large amount of DNAby cloning a very small amount of the DNA.

[3] Through the PCR, only a desired segment of DNA may be amplified from very large DNA like genomic DNA. The PCR generally includes denaturation, primer annealing and DNA polymerization processes.

[4] Recently, real-time PCR is well known to those of ordinary skill in the art. The real-time PCR is a technology which allows monitoring of a reaction state in real-time by measuring an intensity of fluorescence showing the level of DNA amplification at every cyclein a status that a reaction product in a gel is not separated by electrophoresis. Therefore, in the real-time PCR, there are some advantages in that precise quantitative analysis is allowed, and it is possible to simply and rapidly perform the analysis without the electrophoresis, and also there is less risk of contamination.

[5] A real-time PCR apparatus includes a thermal cycler for PCR and a fluorometer for detecting fluorescence of a reaction product. A conventional real-time PCR apparatus is comprised of a thermoelectric element, a thermal block for transferring heat to a reaction tube in which a sample is received, a light source for irradiating excitation light to the sample in the tube, and a light receiving part for receiving the fluorescence generated from the sample. In the conventional real-time PCR apparatus, cooling and heating cycles are repeatedly performed by using the thermoelectric element so as to react the sample, and the excitation light is irradiated to the sample using the light source and the light receiving part at every end of each cycle, and then an amount of the fluorescence generated from the sample is measured so as to display the progress of the PCR in real-time.

[6] However, in the conventional real-time PCR apparatus, it is possible to treat a

plurality of samples, but it is impossible to successively react the samples at regular time intervals, and also it is impossible to provide other samples in the reaction tube during the reaction of the sample until the reaction is completed.

[7] To solve the above problems, there has been proposed various continuous real-time monitoring apparatuses.

[8] In U.S. Patent No. 6,033,880, there is disclosed a PCR apparatus using a capillary tube. According to the PCR apparatus, a heat transfer block includes four constant temperature blocks, and samples and reagents are supplied to or removed from the capillary tube using a solution supplying unit. The PCR is performed byrotating the heat transfer block and changing temperature transferred to the capillary tube using the above-mentioned apparatus. The problem in this type apparatus is that the heat transfer block should be rotated to perform the PCR, and also the reproducibility of the PCR is deteriorated since the PCR may be changed depend on a contacting level between the capillary tube and the heat transfer block.

[9] Further, in this type apparatus, it is impossible to perform the PCR at time intervals. Furthermore, since the above apparatus can measure the progress of the reaction only after completion of the PCR, there is another problem that a user cannot check the progress of the reaction before the completion of the PCR.

[10] To solve the above problems, there has been proposed a new PCR real-time monitoring apparatus in Korean Patent No.593263 (titled "a high throughput device for performing continuous-flow reactions"), in which a temperature circulating unit for PCR, comprised of a capillary tube and a circular heating block, is provided.

[11] In this apparatus, the capillary tube of 3.5 meters in length is wound 33 times on a copper block of 30mm in diameter, which is divided into melting, annealing and extension temperature regions. When a reaction mixture flowed in the capillary tube is circulated once around the heating block formed of copper, each cycle of the PCR is performed. In this method, the capillary tube through which the PCR sample is flowed is wound on the heating block, and the capillary tube is scanned by a scanning unit having a fluorescence detector. Thus, the scanning unit is a means for irradiating light to the capillary tube wound on the heating block using a light irradiating unit and measuring an amount of fluorescence generated in the capillary tube.

[12] Accordingto the above-mentioned method, the light irradiating unit for irradiating light to the capillary tube wound on the heating block and a sensor for measuring the fluorescence generated from the capillary tube are installed at a moving stage, so that the scanning unit is linearly driven and the light is irradiated, in turn, to the capillary tube according to movement of the scanning unit. Then, the fluorescence generated from the capillary tube is measured, in turn, according to the movement of the scanning unit.

[13] In the above-mentioned technology, there is disclosed the scanning unit in which a fluorescence detecting sensor and a light source for generating a light beam having a desired wavelength are moved at a constant speed above the heating block on which the capillary tube is wound. The light source and the fluorescence detecting sensor installed at the scanning are moved in an axial direction that is parallel with a central axis of the heating block on which the capillary is wound or that is cross the central axis thereof, so as to irradiate the light to the capillary tube or measure the fluorescence. Whenever perform the scanning, monitoring of the PCR is performed once, and the multiple capillary tubes are scanned upon the scanning. In order to scan the fluorescence generated from the sample in the capillary tube while the light source and the fluorescence detecting sensor installed at the moving stage are moved at a constant speed, it is necessary to provide a motor, a conveying unit like liner conveying means, a conveying guide unit, driving means for providing power the conveying unit and so on. However, since the light source including a plurality of optical lenses uses an expensive lens like an object lens and it is also necessary to precisely arrange the light source and the fluorescence detecting sensor in order to precisely control an optical path, there is a problem that a manufacturing cost of the PCR apparatus is remarkably increased. In addition, since the PCR apparatus includes the plurality of lenses, the conveying unit, the power transferring unit, the driving means and the like, this may cause increase of its size and malfunction thereof.

[14] Therefore, there is necessity of providing a new continuous PCR real-time monitoring apparatus which solves the above-mentioned problems and has an excellent and economical real-time monitoring effect.

[14a] A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission or a suggestion that the document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

Disclosure of the Invention

Technical Problem

[15] It would be desirable that the present invention provides a thermal cycling reaction block which provides a simple monitoring apparatus so as to continuously monitor a polymerase chain reaction (PCR) in real-time, and facilely detects a PCR, and enhances detecting accuracy of the apparatus.

2009263158 15 May 2014

Technical Solution

[16] A first aspect of the present invention provides a real-time monitoring apparatus comprising: a thermal cycling reaction block 100, including: a doughnut-shaped heating blocks 10a and 10b which are formed of a hollow part 11 at a central portion thereof and divided by an insulating layer so as to respectively provide different temperatures; and a capillary tube 20 through which a sample is flowed in and/or out and which is wound on the heating blocks 10a and 10b at regular intervals to pass through the hollow part 11, so that the different temperatures are transferred and thus reaction cycle is repeatedly performed; a light source 110 for irradiating excitation light; a band pass filter 130 for passing the excitation light having only a desired wavelength irradiated from the light source 110; a first condensing lens 140 for condensing the excitation light; a beam splitter 120 which reflects the excitation light and passes fluorescence generated from a sample in a capillary tube 20; a reflecting mirror 150 which is rotatably connected with a motor 160 so as to transfer the excitation light reflected from the beam splitter 120 to the capillary tube 20 and reflect the fluorescence generated from the sample in the capillary tube 20, wherein the reflecting mirror 150 is disposed at the hollow part 11 formed at the central portion of the thermal cycling rotation block 100; and a fluorescence detecting part 170 for detecting the fluorescence that is reflected by the reflecting mirror 150 and then passes through the beam splitter 120.

[17] Preferably, the heating blocks 10a and 10b further include an additional heating block 13 which surrounds the outer heating block 10b, on which the capillary tube 20 is wound, so as to be coupled with an outer side of the outer heating block 10b.

[18] Preferably, an inserting groove 12, which has a regular size and a regular interval for partial insertion of the capillary tube 20, is formed in an outer surface of the heating blocks 10a and 10b so as to increase a contacting surface area between the heating blocks 10a and 10b and the capillary tube 20.

[19] A second aspect of the present invention provides a real-time monitoring apparatus including a thermal cycling reaction block 100, comprising: a doughnut-shaped heating blocks 10a and 10b which are formed of a hollow part 11 at a central portion thereof and divided by an insulating layer so as to respectively provide different temperatures; and a capillary tube 20 through which a sample is flowed in and/or out and which is wound on the heating blocks 10a and 10b at regular intervals to pass through the hollow part 11, so that the different temperatures are transferred and thus reaction cycle is repeatedly performed; a light source 110 for irradiating excitation light; a band pass filter 130 for passing only the excitation light having a desired wavelength irradiated from the light source 110; a first condensing lens 140 for condensing the excitation

2009263158 15 May 2014

light; a beam splitter 120 which reflects the excitation light and passes fluorescence generated from a sample in a capillary tube 20; a reflecting mirror 150 which is rotatably connected with a first motor 160a so as to transfer the excitation light reflected from the beam splitter 120 to the capillary tube 20 and reflect the fluorescence generated from the sample in the capillary tube 20; a second condensing lens 141 which is positioned between the reflecting mirror 150 and the thermal cycling reaction block 100 so as to condense the excitation light reflected from the reflecting mirror 150 and the fluorescence generated from a sample in a capillary tube 20, wherein the reflecting mirror 150 is disposed at the hollow part 11 formed at the central portion of the thermal cycling reaction block 100; and a fluorescence detecting part 170 for detecting the fluorescence that is reflected by the reflecting mirror 150 and then passed through the beam splitter 120.

[20] Preferably, the fluorescence detecting part 170 includes a fluorescence condensing lens 171 for condensing the fluorescence passing through the beam splitter 120; a fluorescence band pass filter 172 for passing the condensed fluorescence having only a desired wavelength; and a fluorescence detecting sensor 173 for detecting the fluorescence having the desired wavelength passing through the fluorescence band pass filter 172. Further, the fluorescence detecting part 170 further includes one or more fluorescence condensing lenses 171, fluorescence band pass filters 172 and fluorescence beam splitters 174 according to a wavelength region of the fluorescence.

[21] Also described is a real-time monitoring apparatus including a thermal cycling reaction block 100 a light source 110 for irradiating excitation light; a band pass filter 130 for passing the excitation light having only a desired wavelength irradiated from the light source 110; a first condensing lens 140 for condensing the excitation light; a beam splitter 120 which reflects the excitation light and passes fluorescence generated from a sample in a capillary tube 20; a reflecting mirror 150 which is rotatably connected with a first motor 160a so as to transfer the excitation light reflected from the beam splitter 120 to the capillary tube 20 and reflect the fluorescence generated from the sample in the capillary tube 20; a second condensing lens 141 which is positioned between the reflecting mirror 150 and the thermal cycling reaction block 100 so as to condense the excitation light reflected from the reflecting mirror 150 and the fluorescence generated from a sample in a capillary tube 20; and a fluorescence detecting part 170 for detecting the fluorescence that is reflected by the reflecting mirror 150 and then passed through the beam splitter 120.

[22] Preferably, the fluorescence detecting part 170 includes a fluorescence condensing lens 171 for condensing the fluorescence passing through the beam splitter 120; a fluorescence band pass filter fixing part 175 which has one or more fluorescence band

20092631158 15 May 2014

pass filters 172 for passing the fluorescence having different desired wavelengths from the condensed fluorescence; a second motor 160b for rotating the fluorescence band pass filter fixing part 175; and a fluorescence detecting sensor 173 for detecting the fluorescence having the desired wavelength passing through the fluorescence band pass filters 172.

[23] Preferably, the real-time monitoring apparatus further includes a polarizer or a polarizer film 131 between the light source 110 and the first condensing lens 140 and at a fluorescence measuring part.

[24] Preferably, the first and second motors are a constant rotation motor for rotating at a constant speed.

Advantageous Effects

[25] Unlike the conventional apparatus using the scanning unit in which the movable light source, the movable fluorescence detecting part and the plurality of expensive object lenses are precisely arranged, the continuous real-time monitoring apparatus of the present invention uses the fixed light source and fixed fluorescence detecting part so as to be controlled by only the motor without the movement of the light source and the fluorescence detecting part, so that the real-time monitoring is performed at a fixed position. Therefore, it is facile to detect the amplification of the sample, and it is possible to enhance the detecting accuracy and reduce the manufacturing cost and effort, and it is also possible to reduce the malfunction and size thereof.

Brief Description of Drawings

[26] The above and other features and advantages of the present invention will become apparent from the following description of preferred embodiments given in conjunction with the accompanying drawings, in which:

[27] Fig. 1 is a perspective view of a heating block in accordance with an embodiment of the present invention.

[28] Fig. 2 is a cross-sectional perspective view of the heating block in accordance with the embodiment of the present invention.

[29] Fig. 3 is a cross-sectional view of the heating block in accordance with another embodiment of the present invention.

[30] Fig. 4 is a perspective view of a thermal cycling reaction block in accordance with the embodiment of the present invention.

[31] Figs. 5 and 6 are perspective views showing a schematic structure of a real-time monitoring apparatus using the thermal cycling reaction block in accordance with an embodiment of the present invention.

[32] Figs. 7 and 8 are schematic views showing the real-time monitoring apparatus using the thermal cycling reaction block in accordance with another embodiment of the present invention.

[33] [Detailed Description of Main Elements]

[34] 10: heating block 11: hollow part

[35] 12: inserting groove 13: additional heating block

[36] 20: capillary tube 30: insulating layer

[37] 100: thermal cycling reaction block

[38] 110: light source

[39] 120: beam splitter 130: band pass filter

[40] 131: polarizer or polarizer film

[41] 132: ND filter 140, 141: first and second condensing lenses

[42] 150: reflecting mirror 160: motor

[43] 170: fluorescence detecting part

[44] 171: fluorescence condensing lens

[45] 172: fluorescence band pass filter

[46] 173: fluorescence detecting sensor

[47] 174: fluorescence beam splitter

[48] 175: fluorescence band pass filter fixing part

Best Mode for Carrying out the Invention

[49] Hereinafter, the embodiments of the present invention will be described in detail with reference to accompanying drawings.

[50] Fig. 1 is a perspective view of a heating block in accordance with an embodiment of the present invention, Fig. 2 is a cross-sectional perspective view of the heating block in accordance with the embodiment of the present invention, Fig. 3 is a cross-sectional view of the heat block in accordance with another embodiment of the present invention, and Fig. 4 is a perspective view of a thermal cycling reaction block in accordance with the embodiment of the present invention.

[51] As shown in the drawings, a thermal cycling reaction block 100 has a hollow part 11 at a central portion thereof, and also includes heating blocks 10a and 10bwhich are divided by an insulating layer so as to respectively provide different temperatures and a capillary tube 20 through which a sample is flowed in and/or out and which is wound on the heating blocks 10a and 10b at regular intervals to pass through the hollow part 11, so that the different temperatures are transferred and thus reaction cycle is re-

peatedly performed.

[52] Atypical polymerase chain reaction (PCR) includes a denaturation process at 94℃ an annealing process at 45 ~ 67℃ and a polymerization process at 72℃ However, even though the polymerization process is omitted, it has no problem in the PCR. Thus, a real-time PCR has a tendency to remove the reaction time of the polymerization process in order to reduce time. Although the two-divided heating blocks 10a and 10b are described in the drawings of the present invention, the heating block may be further divided.

[53] At this time, since temperature interference between partitions of the heating blocks 10a and 10b are interrupted by the insulating layer 30, it is facile to control the temperature. The insulting layer 30 is formed of a material having a very low heat transferring rate to facilely maintain the different temperature in each partition of the heating blocks 10a and 10b.

[54] In the drawings of the present invention, although the heating blocks 10a and 10b are formed into a long hole shape, it may have various shapes such as a circular shape, an elliptical shape, a polygonal shape and a rectangular shape.

[55] Further, the thermal cycling reaction block 100 of the present invention may have an additional heating block 13 which surrounds the outer heating block 10b, on which the capillary tube 20 is wound, so as to be coupled with an outer side of the outer heating block 10b. This is a heat transfer method from an outer side of the outer heating block 10b to an inside direction thereof when transferring the heat to the capillary tube 20 through the additional heating block 13. Thus, the heat is further efficiently transferred to the capillary tube 20.

[56] The sample is flowed in and/or out through the capillary tube 20. Preferably, the capillary tube 20 is passed through the hollow part 11 and spirally wound on the heating blocks 10a and 10b at regular intervals so that the different temperatures are transferred to each of the heating blocks 10a and 10b and thus the reaction cycle is repeatedly performed. Therefore, while the capillary tube 20 is serially and repeatedly contacted with the heating blocks 10a and 10b having the different temperature, the PCR is performed so as to amplify gene(DNA etc). The reason why the capillary tube 20 is wound at regular intervals is to uniformly maintain the PCR and thus to facilely rotate at a constant angle a reflecting mirror to be described later.

[57] An inserting groove 12 in which a part of the capillary tube 20 is inserted may be formed in an outer surface of the heating blocks 10a and 10bso as to increase a contacting surface area between the heating blocks 10a and 10b and the capillary tube 20.

[58] As shown in Fig. 4, by the spiral inserting groove 12 in which a part of the capillary tube 20 is fixedly inserted and which has a constant size and a constant interval, the

contacting surface area between the heating blocks 10a and 10b and the capillary tube 20 is increased and thus the heat is further efficiently transferred from the heating blocks 10a and 10b.

[59] Moreover, since the contacting surface area between the heating blocks 10a and 10b and the capillary tube 20 is associated with the reaction time of the PCR, the contacting surface area may be changed according to the reaction time condition. The reaction time may be controlled by changing a radial width of the partition of the heating blocks 10a and 10b. And a position of the insulating layer 30 in the heating blocks 10a and 10b may be also changed according to the radial width of the heating blocks 10a and 10b.

[60] The thermal cycling reaction block 100 as described above is used in an real-time monitoring apparatus for measuring DNA amplification in real-time.

[61] Figs. 5 and 6 are perspective views showing a schematic structure of a real-time monitoring apparatus using the thermal cycling reaction block in accordance with an embodiment of the present invention.

[62] As shown in the drawings, the real-time monitoring apparatus using the thermal cycling reaction block100 in accordance with an embodiment of the present invention includes a light source 110 for irradiating excitation light; a band pass filter 130 for passing the excitation light having only a desired wavelength irradiated from the light source 110; a first condensing lens 140 for condensing the excitation light; a beam splitter 120 which reflects the excitation light and passes fluorescence generated from a sample in a capillary tube 20; a reflecting mirror 150 which is rotatably connected with a motor 160 so as to transfer the excitation light reflected from the beam splitter 120 to the capillary tube 20 and reflect the fluorescence generated from the sample in the capillary tube 20; and a fluorescence detecting part 170 for detecting the fluorescence that is reflected by the reflecting mirror 150 and then passed through the beam splitter 120.

[63] The light source 110 functions to generate the excitation light and includes a white light source such as a tungsten halogen lamp and a xenon discharge lamp, and a single-colored light source such as LED and laser. But the light source 110 is not limited to them.

[64] The band pass filter 130 functions to pass the excitation light having only a desired wavelength irradiated from the light source 110.

[65] The first condensing lens 140 functions to condense the excitation light irradiated from the light source 110. The first condensing lens 140 includes any lens which condenses the excitation light, preferably, a double convex lens.

[66] The beam splitter 120 functions to reflect the excitation light irradiated from the light source 110 and pass fluorescence generated from the sample in the capillary tube 20

Preferably, the beam splitter 120 is a dichroic beam splitter.

[67] The excitation light reflected by the beam splitter 120 is transferred to the reflecting mirror 150, and the fluorescence passing through the beam splitter 120 is transferred to the fluorescence detecting part 170.

[68] The reflecting mirror 150 that the excitation light reflected by the beam splitter 120 is transferred is disposed at the hollow part 11 of the thermal cycling reaction block 100. The reflecting mirror 150 functions to transfer the excitation light reflected from the beam splitter 120 to the capillary tube 20 that is spirally wound in the thermal cycling reaction block 100 and also functions toreflect the fluorescence generated from the sample in the capillary tube 20 to the beam splitter 120. The fluorescence reflected from the reflecting mirror 150 is passed through the beam splitter 120 and then transferred to the fluorescence detecting part 170. The reflecting mirror 150 is connected with the motor 160 for rotating the reflecting mirror 150. The motor 160 functions to rotate the reflecting mirror 150 so that the excitation light is reflected to the sample in the capillary tube 20 by the reflecting mirror 150 and the fluorescence generated from the sample is reflected to the fluorescence detecting part 170. Preferably, the motor 160 is a constant rotation motor for rotating the reflecting mirror 150 at a constant speed.

[69] The fluorescence detecting part 170 functions to detect the fluorescence that is reflected by the reflecting mirror 150 and then passed through the beam splitter 120, thereby estimating the DNA amplification.

[70] The fluorescencedetecting part 170 may include a fluorescence condensing lens 171 for condensing the fluorescence passing through the beam splitter 120, a fluorescence band pass filter 172 for passing the condensed fluorescence having only a desired wavelength, and a fluorescence detecting sensor 173 for detecting the fluorescence having the desired wavelength passing through the fluorescence band pass filter 172. Fig. 5 shows a status of detecting the fluorescence having one wavelength.

[71] As shown in Fig. 6, in order to detect the fluorescence having various kinds of wavelengths, the fluorescencedetecting part 170 may further include one or more fluorescence condensing lenses 171, fluorescence band pass filters 172 and fluorescence beam splitters 174 according to a wavelength region of the fluorescence. At this time, the fluorescence beam splitters 174a and 174b are equipped differently from each other according to a wavelength of the fluorescence to be detected. The fluorescence condensing lenses 171a, 171b and 171c are equipped differently from each other according to a distance between the capillary tube 20 and the fluorescence detecting sensors 173a, 173b and 173c. The fluorescence band pass filters 172a, 172b and 172c are also equipped differently from each other according to a wavelength of the fluorescence to be detected.

2009263158 15 May 2014

[72] Preferably, the fluorescence beam splitters 174a and 174b are the dichroic beam splitters by which a long wavelength is passed and a short wavelength is reflected on the basis of a desired wavelength. The desired wavelength is changed according to fluorescent dyes.

[73] Figs. 7 and 8 are schematic views showing the real-time monitoring apparatus using the thermal cycling reaction block in accordance with another embodiment of the present invention.

[74] The real-time monitoring apparatus using the thermal cycling reaction block 100 in accordance with another embodiment of the present invention includes a light source 110 for irradiating excitation light; a band pass filter 130 for passing the excitation light having only a desired wavelength irradiated from the light source 110; a first condensing lens 140 for condensing the excitation light; a beam splitter 120 which reflects the excitation light and passes fluorescence generated from a sample in a capillary tube 20; a reflecting mirror 150 which is rotatably connected with a first motor 160a so as to transfer the excitation light reflected from the beam splitter 120 to the capillary tube 20 and reflect the fluorescence generated from the sample in the capillary tube 20; a second condensing lens 141 which is positioned between the reflecting mirror 150 and the thermal cycling reaction block 100 so as to condense the excitation light reflected from the reflecting mirror 150 and the fluorescence generated from a sample in a capillary tube 20; and a fluorescence detecting part 170 for detecting the fluorescence that is reflected by the reflecting mirror 150 and then passed through the beam splitter 120.

[75] The light source 110 may include a white light source such as a tungsten halogen lamp and a xenon discharge lamp, and a single-colored light source such as LED and laser, but the light source 110 is not limited to them. In case that the light source 110 is laser, a neutral density (ND) filter 132 may be further provided to control an intensity of the laser.

[76] Preferably, the second condensing lens 141 which is positioned between the reflecting mirror 150 and the thermal cycling reaction block 100 so as to condense the excitation light reflected from the reflecting mirror 150 and the fluorescence generated from a sample in a capillary tube 20 is an aspheric lens.

[77] The fluorescence detecting part 170 may include a fluorescence condensing lens 171 for condensing the fluorescence passing through the beam splitter 120 a fluorescence band pass filter fixing part 175 which has one or more fluorescence band pass filters

172for passing the fluorescence having different desired wavelengths from the condensed fluorescence; a second motor 160b for rotating the fluorescence band pass filter fixing part 175; and a fluorescence detecting sensor 173 for detecting the fluorescence having the desired wavelength passing through the fluorescence band pass filters 172.

[78] The fluorescence detecting part170 has one or more fluorescence band pass filters 172 provided on the fluorescence band pass filter fixing part 175. The fluorescence band pass filter fixing part 175 is connected with the second motor 160b to be rotated, so that the fluorescence is passed through each fluorescence band pass filter 172 provided on the fluorescence band pass filter fixing part 175 and then detected, thereby enhancing fluorescence detection and space efficiency.

[79] In other words, as shown in Fig. 6, if multiple fluorescence band pass filters are used in the real-time monitoring apparatus, it is necessary to provide multiple fluorescence condensing lenses 171, fluorescence band pass filters 172 and fluorescence beam splitters 174 according to the desired wavelength region of the fluorescence. However, as shown in Figs. 7 and 8, since the real-time monitoring apparatus in accordance to another embodiment of the present invention has the multiple fluorescence band pass filters 172 provided on the fluorescence band pass filter fixing part 175, the fluorescence beam splitters 174 of Fig. 6 are not needed. Further, since only one fluorescence condensing lens 171 is needed, it is possible to reduce a manufacturing cost and a space occupation.

[80] Preferably, the second motor 160b is a constant rotation motor for rotating the fluorescence band pass filter fixing part 175 including the fluorescence band pass filters 172 at a constant speed.

[81] Preferably, the fluorescence detecting sensor 173 is a photo multiplier tube, and the fluorescence condensing lens 171 is an aspheric lens, but the fluorescence detecting sensor 173 and the fluorescence condensing lens 171 are not limited to them.

[82] Further, in order to enhance an efficiency of separating the excitation light and the fluorescence, as shown in Fig.8, the real-time monitoring apparatus of the present invention may further include a polarizer or a polarizer film 131 between the light source 110 and the first condensing lens 140 and at the fluorescence measuring part.

[83] While the present invention has been described with respect to the specific embodiments, it will be apparent to those skilled in the art that various changes and

modifications may be made without departing from the spirit and scope of the invention as defined in the following claims.

[83a] Where the terms "comprise", "comprises", "comprised" or "comprising" are used in this specification (including the claims) they are to be interpreted as specifying the presence of the stated features, integers, steps or components, but not precluding the presence of one or more other features, integers, steps or components, or group thereto.

Industrial Applicability

[84] According to the present invention, since the real-time monitoring apparatus uses the fixed light source and fixed fluorescence detecting sensor so as to be fixed at a positioned controlled by rotation of the motor, it is facile to detect the amplification of the sample, and it is possible to enhance the detecting accuracy and reduce the manufacturing cost and effort, and it is also possible to reduce the malfunction and size thereof.

2009263158 15 May 2014

The claims defining the invention are as follows:

1. A real-time monitoring apparatus comprising:

a thermal cycling reaction block, comprising:

a doughnut-shaped heating blocks which are formed of a hollow part at a central portion thereof and divided by an insulating layer so as to respectively provide different temperatures; and

a capillary tube through which a sample is flowed in and/or out and which is wound on the heating blocks at regular intervals to pass through the hollow part, so that the different temperatures are transferred and thus reaction cycle is repeatedly performed;

a light source for irradiating excitation light;

a band pass filter for passing the excitation light having only a desired wavelength irradiated from the light source;

a first condensing lens for condensing the excitation light;

a beam splitter which reflects the excitation light and passes fluorescence generated from a sample in a capillary tube;

a reflecting mirror which is rotatably connected with a motor so as to transfer the excitation light reflected from the beam splitter to the capillary tube and reflect the fluorescence generated from the sample in the capillary tube, wherein the reflecting mirror is disposed at the hollow part formed at the central portion of the thermal cycling reaction block; and

a fluorescence detecting part for detecting the fluorescence that is reflected by the reflecting mirror and then passes through the beam splitter.

2. A real-time monitoring apparatus comprising:

a thermal cycling reaction block, comprising:

a doughnut-shaped heating blocks which are formed of a hollow part at a central portion thereof and divided by an insulating layer so as to respectively provide different temperatures; and

a capillary tube through which a sample is flowed in and/or out and which is wound on the heating blocks at regular intervals to pass through the hollow part, so that the different temperatures are transferred and thus reaction cycle is repeatedly performed;

2009263158 15 May 2014

a light source for irradiating excitation light;

a band pass filter for passing only the excitation light having a desired wavelength irradiated from the light source;

a first condensing lens for condensing the excitation light;

a beam splitter which reflects the excitation light and passes fluorescence generated from a sample in a capillary tube;

a reflecting mirror which is rotatably connected with a first motor so as to transfer the excitation light reflected from the beam splitter to the capillary tube and reflect the fluorescence generated from the sample in the capillary tube;

a second condensing lens which is positioned between the reflecting mirror and the thermal cycling reaction block so as to condense the excitation light reflected from the reflecting mirror and the fluorescence generated from a sample in a capillary tube, wherein the reflecting mirror is disposed at the hollow part formed at the central portion of the thermal cycling reaction block; and

a fluorescence detecting part for detecting the fluorescence that is reflected by the reflecting mirror and then passes through the beam splitter.

3. The real-time monitoring apparatus of claim 1 or claim 2, further comprising an additional heating block which surrounds the outer heating block, which surrounds the outer heating block, on which the capillary tube is wound, so as to be coupled with an outer side of the outer heating block.

4. The real-time monitoring apparatus of claim 1 or claim 2, wherein an inserting groove in which a part of the capillary tube is inserted is formed in an outer surface of the heating block so as to increase a contacting surface area between the heating block and the capillary tube.

5. The apparatus of claim 1, wherein the fluorescence detecting part comprises:

a fluorescence condensing lens for condensing the fluorescence passing through the beam splitter;

a fluorescence band pass filter for passing only the condensed fluorescence having a desired wavelength; and

a fluorescence detecting sensor for detecting the fluorescence having the desired wavelength passing through the fluorescence band pass filter.

2009263158 15 May 2014

6. The apparatus of claim 5, wherein the fluorescence detecting part further comprises one or more fluorescence condensing lenses, fluorescence band pass filters and fluorescence beam splitters according to a wavelength region of the fluorescence.

7. The apparatus of claim 1, wherein the motor is a constant rotation motor for rotation at a constant speed.

8. The apparatus of claim 2, wherein the fluorescence detecting part comprises:

a fluorescence condensing lens for condensing the fluorescence passing through the beam splitter;

a fluorescence band pass filter fixing part which has one or more fluorescence band pass filters for passing the fluorescence having different desired wavelengths from the condensed fluorescence;

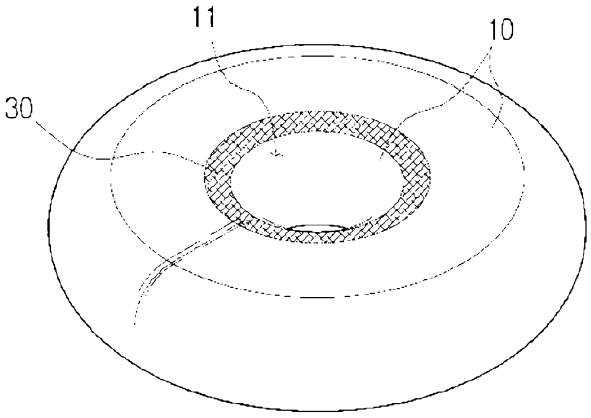
a second motor for rotating the fluorescence band pass filter fixing part; and

a fluorescence detecting sensor for detecting the fluorescence having the desired wavelength passing through the fluorescence band pass filters.

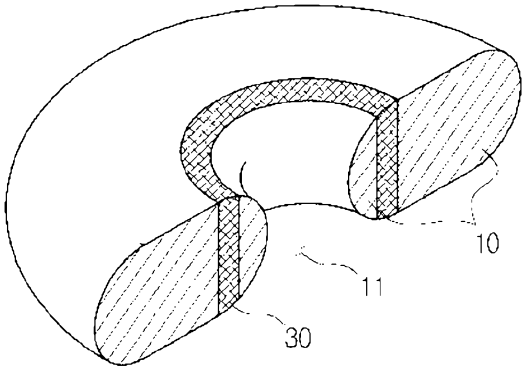
9. The apparatus of claim 2, further comprising a polarizer or a polarizer film between the light source and the first condensing lens and at a fluorescence measuring part.

10. The apparatus of claim 8, wherein the first and second motors are constant rotation motors for rotation at a constant speed.

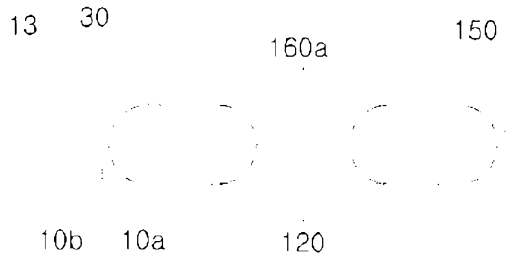
[Fig. 1]



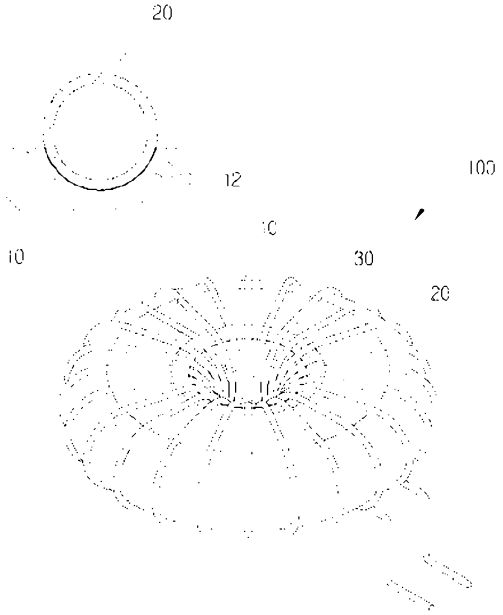
[Fig. 2]



[Fig. 3]



[Fig. 4]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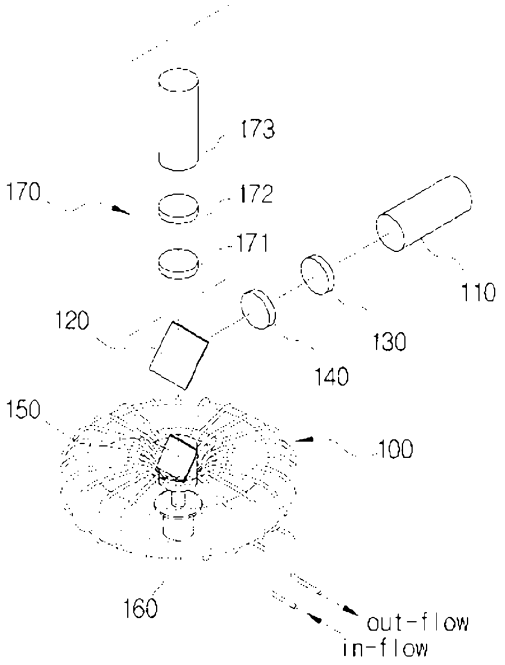
20

[Fig. 5]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171
110
120
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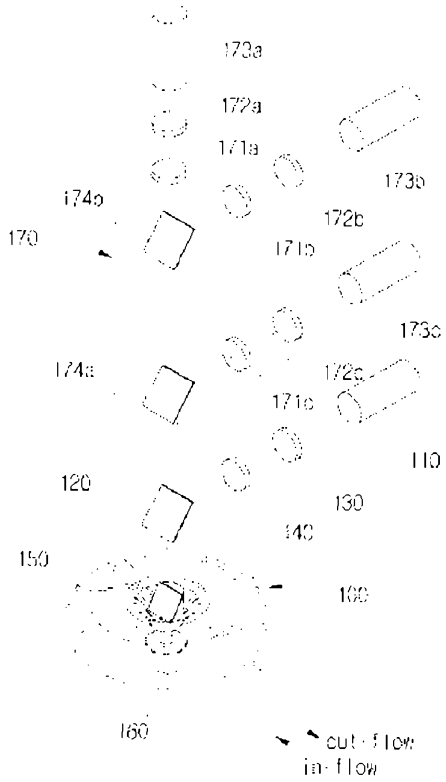
140
150
100
160
out-flow
in-flow

[Fig. 6]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173b
174b
172b
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171b
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174c
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171c
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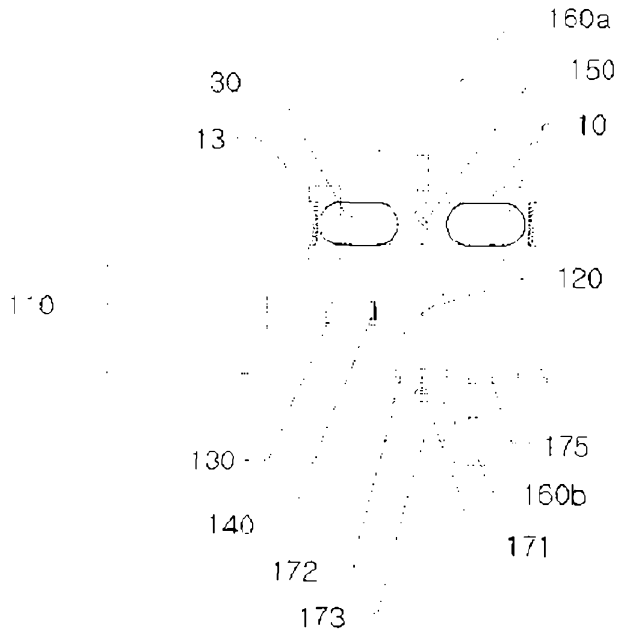
120
130
140
150
160
out-flow
in-flow

[Fig. 7]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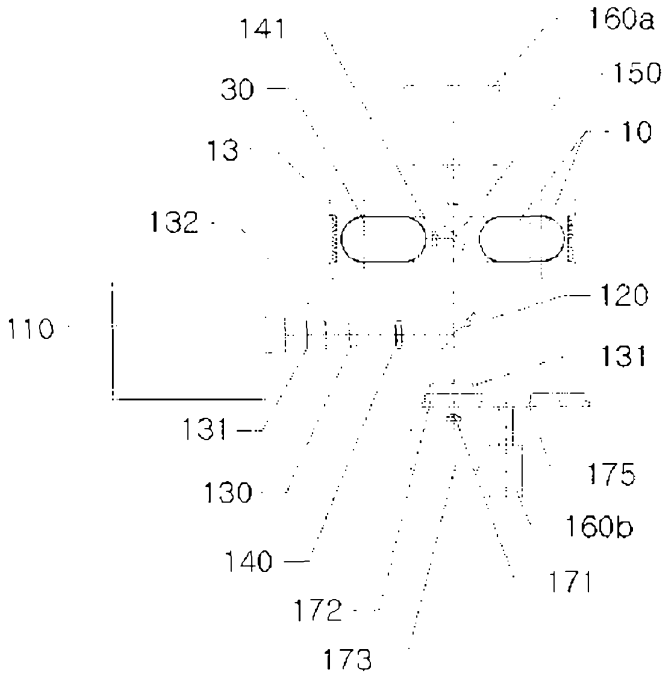
[Fig. 8]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