



- (51) International Patent Classification:
G01N 21/64 (2006.01)
- (21) International Application Number:
PCT/EP2015/062989
- (22) International Filing Date:
10 June 2015 (10.06.2015)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
10 2014 108 143.9 10 June 2014 (10.06.2014) DE
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- (81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ,
LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK,
MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA,
PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD,
SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

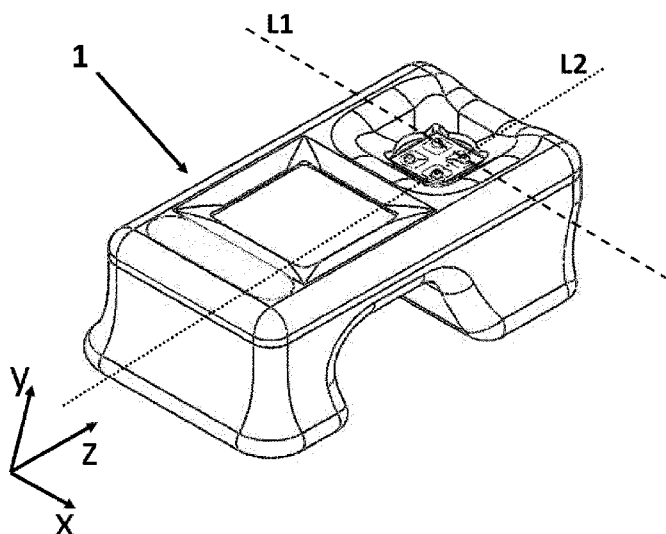
- (84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))

- (54) Title: AN OPTICAL SYSTEM FOR DETECTING FLUORESCENT OR LUMINESCENT SIGNALS OF AT LEAST TWO
SAMPLES

Fig.1



(57) Abstract: The present invention relates to an optical system, and particularly to a compact optical system, for detecting fluorescent or luminescent signals of at least two samples. It is the object of the invention to provide a compact optical system, which allows the manufacture of a small, inexpensive and portable (handheld) real-time PCR system. The object for an optical system for detecting fluorescent or luminescent signals of at least two samples, comprising for each detection system a light source for generating excitation light, an excitation filter, whereby said excitation filter transmits the excitation light from the light source to a dichroic mirror, whereby said dichroic mirror reflects said excitation light transmitted by said excitation filter to a fluorophore in a sample and whereby the emitted light from each of the samples passes through the dichroic mirror and through an emission filter to a detector that detects said emitted light, is met by placing only one excitation filter in front of the at least two next to each other situated light sources, whereby said excitation filter transmits the excitation light beams of each light source to only one dichroic mirror, whereby said dichroic mirror reflects said excitation light beams transmitted by the excitation filter to the fluoro-

phore in each sample and whereby the emitted light of each of the samples passes next to each other through the dichroic mirror and through only one shared emission filter to at least one detector.

An optical system for detecting fluorescent or luminescent signals of at least two samples.

The present invention relates to an optical system, and particularly to a compact optical system, for detecting fluorescent or luminescent signals of at least two samples.

Polymerase chain reaction (PCR) is a technology to amplify copies of specific deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) fragments in a reaction chamber. Due to simple and easy application using repeated cycles of three steps, namely denaturation, annealing and extension, the PCR technology has been proven to be highly efficient and reliable. Hence, PCR technology has been extensively used in medicine, science, agriculture, veterinary medicine, food science, environmental science as well as in molecular biology, archaeology and anthropology.

In recent years genetic analysis also became a commonly used technique for detecting the presence and potential for disease. Blood or tissue samples, taken in a minimally invasive manner from localized regions, can be used to perform DNA analysis via PCR. For quantification, generally, the end-point PCR products are separated on a gel and the approximate amount is estimated using spectrophotometers. However, an accurate measurement of the amount of DNA is not achievable with this end-point method. Thus, to overcome these problems, a real-time PCR method and a fluorescence detection method have been introduced in which at each PCR cycle the accumulated PCR products are measured. Using these two methods an amplification plot showing fluorescent intensities versus cycle numbers can be obtained.

However, most of the analysis is carried out in laboratories, which use systems that are designed for high-throughput analysis. The samples taken in the field are sent to these laboratories, where the DNA is extracted and placed in a chamber with a so called PCR cocktail, whereupon the analysis is run.

Having an easily portable real-time PCR instrument to immediately perform the DNA analysis in the field is thus extremely valuable.

Different detection techniques have been employed in these portable detection systems, including mass flow, electrochemical and optical detection methods. Optical detection methods, such as detection of fluorescent intensity or luminescent decay time, are used more frequently due to robustness, high signal to noise ratio and sensitivity.

Optical systems for the detection of fluorescent signals typically consist of the following components: a light source for emitting light at a suitable wavelength range, excitation filter to eliminate unwanted light, dichroic mirror for the optical separation of excitation and emission channels, emission filter, and a detector with electronics for signal processing.

In the last few years, fluorescence systems based on light emitting diodes (LED) became popular for their low cost and long lifetime. LEDs are more than a thousand times cheaper than alternative light sources and they are also superior to lasers and mercury lamps due to their long lifetime. On the top of that, the LED's light output as well as semiconductor lasers' can be modulated. As the LEDs are only a few mm in diameter as well as in length, they can be integrated into portable systems, such as a real-time PCR system.

The most popular detectors found in fluorescence detection systems are photo multiplier tubes (PMTs), avalanche photodiodes, and photon counting modules (PCMs). Even though all these methods and/or devices allow miniaturization up to a certain point, still each device is regulated by its depending control system. This demands certain required dimensions which increases the dimensions of the system. In addition, if they allow miniaturization, often these devices are costly and/or require special operating conditions, such as operation in complete darkness and/or being operated at a certain temperature, which is often below room temperature.

It would therefore be of great benefit if a device for the direct quantification of PCR products could be built, which is simple in design, compact and which overcomes or alleviates one or more of the above problems.

It is the object of the invention to provide a compact optical system, which allows the manufacture of a small, inexpensive and portable (handheld) real-time PCR system.

The object for an optical system for detecting fluorescent or luminescent signals of at least two samples, comprising for each detection system a light source for generating excitation light, an excitation filter, whereby said excitation filter transmits the excitation light from the light source to a dichroic mirror, whereby said dichroic mirror reflects said excitation light transmitted by said excitation filter to a fluorophore in a sample and whereby the emitted light from each of the samples passes through the dichroic mirror and through an emission filter to a detector that detects said emitted light, is met by placing only one excitation filter in front of the at least two next to each other situated light sources, whereby said excitation filter transmits the excitation light beams of each light source to only one dichroic mirror, whereby said dichroic mirror reflects said excitation light beams transmitted by the excitation filter to the fluorophore in each sample and whereby the emitted light of each of the samples passes next to each other through the dichroic mirror and through only one shared emission filter to at least one detector.

It is within the scope of the invented method that two optical systems can be placed opposite to each other, whereby there is only one excitation filter placed in front of the at least two next to each other situated light sources of each optical system and whereby said excitation filter transmits the excitation light beams of each light source to only one dichroic mirror for each optical system, whereby said dichroic mirrors reflect said excitation light beams transmitted by the excitation filter to the fluorophore in each sample and whereby the emitted light of each of the samples passes next to each other through the dichroic mirrors of both optical systems and through only one, by both optical systems shared, emission filter to at least one detector.

This design of the opposite placed optical systems allows space saving grouping of the sample wells. Thus, the dimensions of the system can be minimized. However, it is also intended that more than two sample wells are present per optical system. Thus, multiple samples can be measured simultaneous. Even though one could extend each optical system separately, due to sharing of filters it is preferable to extend the opposite placed optical systems simultaneous.

The detector could be a charge-coupled device (CCD) camera or sensor with optical fibers, an avalanche photodiode (APD), a photodiode or a photomultiplier tube (PMT). However, for

the preferred embodiment the detection unit may be a single-channeled or multi-channeled fluorescent detector that detects the emitted fluorescent intensity or luminescence decay time.

In a preferred embodiment it is money-wise expedient and space-saving that the PCR system utilizes a photodiode, in addition or preferentially instead of a CCD camera. The preferred photodiode for the embodiment may hereby be a silicon photodiode as e.g. BPW21 from Siemens, Inc.

It is further advantageous that the measurement is performed by means of multiple detectors, the detectors being controlled individually during the measurements.

It is required that the light source that generates the excitation light has a wavelength sufficient to excite a fluorophore in a sample.

Typical real-time quantitative PCR systems comprise a laser to induce fluorescence and in order to read from all samples in a plate, the plate is moved, whereby the laser individually addresses each and every well in the plate. However, a system such as this is bulky and expensive.

To avoid above mentioned drawbacks it is thus advantageous that the measurement is performed by means of affordable light sources that generate the excitation light with a wavelength sufficient to excite a fluorophore in a sample.

Hereby the light source that generates excitation light can be a laser, a photodiode, a laser diode or a lamp, such as blue or turquoise light emitting diodes (LEDs).

However, it is advantageous to have a light source which is small, reliable and able to excite the dye, such as SYBR Green I, generally used for the real-time PCR method. It is further advantageous that the transmitters are inexpensive. Thus blue light emitting diodes (LEDs) or laser diodes are a preferred choice for the light source. For example, the turquoise colored LED model ETG-5CE490-15 (ETG. Corp.) can be used for this invention. The LED has a peak emission wavelength of 490 nm with a luminous intensity of 6 cd (candela) and a

viewing angle of 15°. However, also LED's with a peak emission wavelength of 470 nm are a preferred choice.

In one embodiment of the invention it is possible that each light source generates the same excitation wavelength and said excitation and emission filter is each a single band pass filter.

It is envisaged that for each sample at least one light source is assigned, which generates at least one excitation wavelength.

Because the footprint of an LED or laser diode is very small, it is further conceivable that multiple LEDs or lasers of different wavelength could be integrated into a single package or several packaged LEDs/laser, which can be very closely spaced to excite one well/sample.

Hence it is within the scope of the invention that for each sample at least one light source is assigned, which generates at least one excitation wavelengths, wherein the said excitation filter comprises at least two filters for filtering at least two lights with different wavelengths in order to excite fluorophores in the samples and that said emission filter comprises at least two filters for filtering at least two wavelengths of the emitted light from the samples. Thus, the excitation wavelength from each light source can be individually modulated and demodulated. In an embodiment of the invention, an e.g. turquoise LED can hence be replaced by a triple color LED and the single bandpass filter set by a triple bandpass filter set. Each color of the LED is modulated at a unique frequency and has its own demodulator. This set-up allows the detection of up to three different wavelengths independently from each other. It further can be used in real-time PCR applications allowing internal negative and positive controls (multiplexing).

Furthermore it is feasible within the scope of the invention that the excitation light can be pulsed.

This is favorable, since by having the light source off when the detector is not detecting the fluorescence from a sample allows the light source to cool and reduces the total power required for the light source if run continuously. The timing of when the pulsed light source is on and off provides an opportunity for optimizing the performance of the optical system under

various circumstances including, but not limiting to, row pulsing, sample pulsing and high frequency pulsing. In addition, due to the pulsed excitation light, scattering of the at least two light sources of one optical system can be avoided. Further, when multiple optical systems are used for multiplexing applications (detection of different fluorogenic probes from the same sample), scattered light from one system can reach another opposite system and thereby increase its background and reduce its sensitivity. Pulsing provides an opportunity to temporally stagger the light from different colored sources that are tuned to different fluorophores. Timing the pulses in such a way that only one light source is on and thus only one signal is detected at a time eliminates the problem from light scattering and increases the combination of fluorophores that can attain optical performance, including pairs of fluorophores, one of which has an excitation wavelength close to or the same as the emission wavelength of the other.

Furthermore, pulsing of light sources is also beneficial for real-time PCR applications because pulsing allows for the possibility of lock-in detection. Lock-in detection enhances sensitivity by amplifying signals only at the pulse frequency; noise and/or signals at other frequencies are not amplified. That allows operating the system in an ambient environment, thus not having to place it in a black chamber or box.

It is further a preferred embodiment of the invention that a first lens is positioned between said light source and said excitation filter. This has the advantage that the excitation light can be collimated.

In another preferred embodiment of the invention it is envisaged that a second lens is positioned to focus the excitation light reflected by the dichroic mirror toward the sample.

For further miniaturization purpose, it is envisaged that the filters and lenses are mounted on the light source and/or the detector. This would be advantageous, since no holder or attaching device would have to be separately installed in the optical system, and thus miniaturization is feasible.

It is within the scope of the invention that the optical performance of the system can be further improved by forming filters (made by evaporation of multilayers) directly onto the

surface of the lenses. This technique, together with mounting the lenses directly onto the LED and the photodiode, reduces the number of optical interfaces. It further enables to design the optical system even more compact, thus increasing the transmission along the optical path.

Further it is within the scope of the invention to fill the empty space of the optical system with optically matching materials, as e.g. refractive index matching epoxy, to eliminate all surface reflections and thus eliminating the necessity of antireflective coatings at all elements.

The present optical detection system may be used in portable devices where miniaturization is desirable, including devices used for quantitative real-time PCR or reverse transcription (RT)-PCR, real-time bioluminescence and chemiluminescence assays, or any other fluorescence-based assays for detection.

The above and other features, details and advantages of the present invention will become more apparent by describing in detail exemplary embodiments thereof with reference to the attached drawings in which:

Fig. 1 shows a portable device for operating a real-time polymerase chain reaction in a perspective view,

Fig. 2 shows a section through the portable device, depicting the optical system,

Fig. 3 shows a section through the portable device in perspective view, depicting the optical system and an enlarged view of the section,

Fig. 4 shows a cross-section through the portable device in perspective view, depicting the optical system and an enlarged view of the cross-section.

Fig. 1 shows a portable PCR device (1), which can be assembled due to the small miniaturized optical system. The device (1) has dimensions of maximum 3.5 cm x 6.0 cm x 9.5 cm (width (x) · height (y) · length (z)) and weighs less than 0.2 kg. It is thus feasible to carry the pocket-sized device (1) in one hand in the field, as e.g. needed for field research studies, studies in remote areas or for temporary laboratories. It is further within the scope of

the invention that the solution volume (reaction volume) of the sample (6), which is situated in a reaction chamber, is less than 1.5 μL . However, the features of the miniaturized optical system further allow for a reaction volume in the range of 0.1 μL to 1.0 μL . Further depicted in Fig. 1 are the vertical cuts for the sections, which are depicted in the Figures 2 to 4. The first cut (L1) is a vertical cut along the z-axis, which results in a parallel sectional plane of the given x-y plane. The second cut (L2) is a vertical cut along the x-axis, which results in a parallel sectional plane of the given y-z plane.

Fig. 2 depicts a section through the portable PCR device (1), which is sectioned along the first cut (L1). Depicted are two optical systems, which are placed opposite to each other. The optical system for detecting fluorescent or luminescent signals of at least two samples (6) comprises for each detection system a light source (2) for generating excitation light (9). An excitation filter (3) is placed before the light source (2), whereby said excitation filter (3) transmits the excitation light (9) from the light source (2) to a dichroic mirror (4) and said dichroic mirror (4) reflects said excitation light (9), which is transmitted by the excitation filter (3) to a fluorophore in a sample (6). The emitted light (10) from each of the samples (6) passes through the dichroic mirror (4) and through an emission filter (7) to a detector (8) that detects said emitted light (10). The emitted light (10) of each of the samples (6) passes next to each other through the dichroic mirrors (4) of both optical systems and through only one, by both optical systems shared, emission filter (7) to at least one detector (8).

Fig. 3 A and B depict a section through the portable PCR device (1), which is sectioned along the second cut (L1). Fig. 3 B is an enlarged view of Fig. 3 A, indicated by the dashed frame in Fig. 3 A. Depicted is one optical system for detecting fluorescent or luminescent signals of at least two samples (6). As can be seen in the enlarged view (Fig. 3 B) there is only one excitation filter (3) placed in front of the at least two next to each other situated light sources, whereby said excitation filter (3) transmits the excitation light beams of each light source to only one dichroic mirror (4), whereby said dichroic mirror (4) reflects said excitation light beams transmitted by the excitation filter (3) to the fluorophore in each sample (6) and whereby the emitted light of each of the samples (6) passes next to each other through the dichroic mirror (4) and through only one shared emission filter (7) to at least one detector (8).

Fig. 4 A and 4 B depict a cross-section through the portable PCR device (1), which is sectioned along the first and the second cut (L1, L2). Fig. 4 B is an enlarged view of the optical system, shown in Fig. 4 A, indicated by the dashed frame in Fig. 4 A. Depicted is one optical system for detecting fluorescent or luminescent signals of at least two samples (6). As can be seen in the enlarged view (Fig. 4 B) there is only one excitation filter (3) placed in front of the at least two next to each other situated light sources (2), whereby said excitation filter (3) transmits the excitation light beams of each light source to only one dichroic mirror (4), whereby said dichroic mirror (4) reflects said excitation light beams transmitted by the excitation filter (3) to the fluorophore in each sample (6) and whereby the emitted light of each of the samples (6) passes next to each other through the dichroic mirror (4) and through only one shared emission filter (7) to at least one detector (8).

For assembly of the optical system many materials (lenses, detectors, filter...) can be used. However, one assembly used comprised blue or turquoise LEDs (preferred peak emission wavelength between 470 nm and 490 nm) as a light source (2), which excited light (9) was filtered by an exciter (3) (ET470/40x, Chroma, Inc.), reflected by the dichroic mirror (4) (T495LP, Chroma, Inc.) and focused on the sample of interest (6) by a Geltech™ molded glass aspheric lense (5) with a diameter of 6.35 mm, focal length of 3.1 mm, and numerical aperture (N.A.) of 0.68 (Thorlabs, Inc.), thereby forming a circular shape of excitation light (9). The dichroic mirror has preferably a cut off at 505 nm. Also a filter set consisting of three filters, i.e. excitation filter, dichroic mirror and detection filter, was successfully used (Chroma Optical, Model 49002). The emitted light (10) was collected by a second lens. The emitted light (10) passed through the dichroic mirror, was filtered by an emitter (7) (ET525/50 m), and collected by a silicon photodiode (8) (BPW21, Siemens, Inc.). The silicon photodiodes radiant sensitive area was 7.34 mm² with a quantum yield of 0.8, resulting in an optical sensitivity of 10 nA lx⁻¹ (nanoamperes per lux).

Claims

1. An optical system for detecting fluorescent or luminescent signals of at least two samples (6), comprising for each detection system a light source (2) for generating excitation light (9), an excitation filter (3), whereby said excitation filter (3) transmits the excitation light (9) from the light source (2) to a dichroic mirror (4), whereby said dichroic mirror (4) reflects said excitation light (9) transmitted by said excitation filter (3) to a fluorophore in a sample (6) and whereby the emitted light (10) from each of the samples (6) passes through the dichroic mirror (4) and through an emission filter (7) to a detector (8) that detects said emitted light (10), **characterized in that** there is only one excitation filter (3) placed in front of the at least two next to each other situated light sources (2), whereby said excitation filter (3) transmits the excitation light beams (9) of each light source (2) to only one dichroic mirror (4), whereby said dichroic mirror (4) reflects said excitation light beams (9) transmitted by the excitation filter (3) to the fluorophore in each sample (6) and whereby the emitted light (10) of each of the samples (6) passes next to each other through the dichroic mirror (4) and through only one shared emission filter (7) to at least one detector (8).
2. The optical system of claim 1,
characterized in that two optical systems can be placed opposite to each other, whereby there is only one excitation filter (3) placed in front of the at least two next to each other situated light sources (2) of each optical system and whereby said excitation filter (3) transmits the excitation light beams (9) of each light source (2) to only one dichroic mirror (4) for each optical system, whereby said dichroic mirrors (4) reflect said excitation light beams (9) transmitted by the excitation filter (3) to the fluorophore in each sample (6) and whereby the emitted light (10) of each of the samples (6) passes next to each other through the dichroic mirrors (4) of both optical systems and through only one, by both optical systems shared, emission filter (7) to at least one detector (8).
3. The optical system of claim 1,
characterized in that said detector (8) is a single-channeled or multi-channeled fluorescent detector that detects the emitted fluorescent intensity or luminescence decay time.

4. The optical system of claim 1,
characterized in that said detector (8) is a photodiode detector.
5. The optical system of claim 1,
characterized in that the measurement is performed by means of multiple detectors (8),
the detectors being controlled individually during the measurements.
6. The optical system of claim 1,
characterized in that the light source (2) that generates the excitation light has a
wavelength sufficient to excite a fluorophore in a sample.
7. The optical system of claim 1,
characterized in that the light source (2) that generates excitation light is a laser, a
photodiode, a laser diode or a lamp, such as blue light emitting diodes (LEDs).
8. The optical system of claim 1,
characterized in that each light source (2) generates the same excitation wavelength and
said excitation filter (3) and emission filter (7) is each a single band pass filter.
9. The optical system of claim 1,
characterized in that for each sample (6) at least one light source (2) is assigned, which
generates at least one excitation wavelength.
10. The optical system of claim 9,
characterized in that for each sample (6) at least one light source (2) is assigned, which
generates at least one excitation wavelengths, wherein the said excitation filter (3)
comprises at least two filters for filtering at least two lights with different wavelengths in
order to excite fluorophores in the samples and that said emission filter (7) comprises at
least two filters for filtering at least two wavelengths of the emitted light (10) from the
samples (6).

11. The optical system of claim 1,
characterized in that the excitation light (9) is pulsed.
12. The optical system of claim 1,
characterized in that a first lens is positioned between said light source (2) and said excitation filter (3).
13. The optical system of claim 1,
characterized in that a second lens is positioned to focus the excitation light (3) reflected by the dichroic mirror (4) toward the sample (6).
14. The optical system of claim 1,
characterized in that the filters and lenses are mounted on the light source (2) and/or the detector (8).

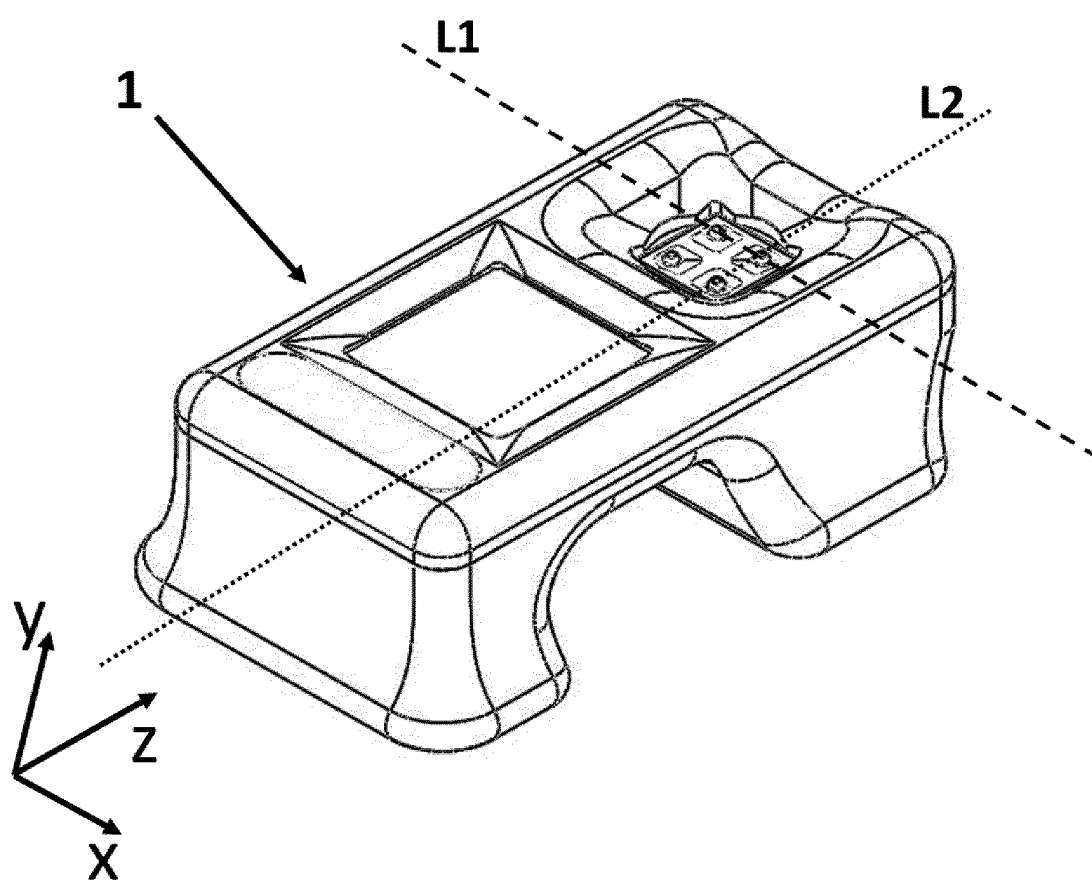
Fig.1

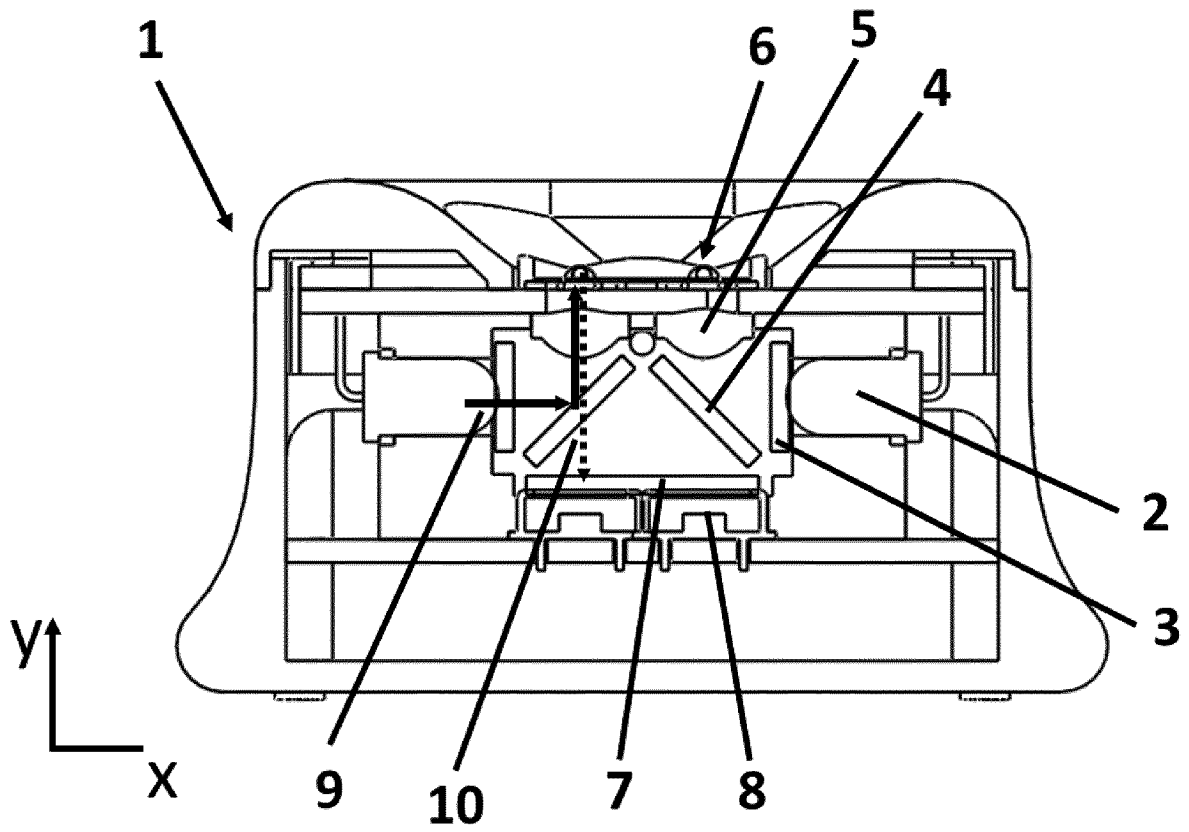
Fig. 2

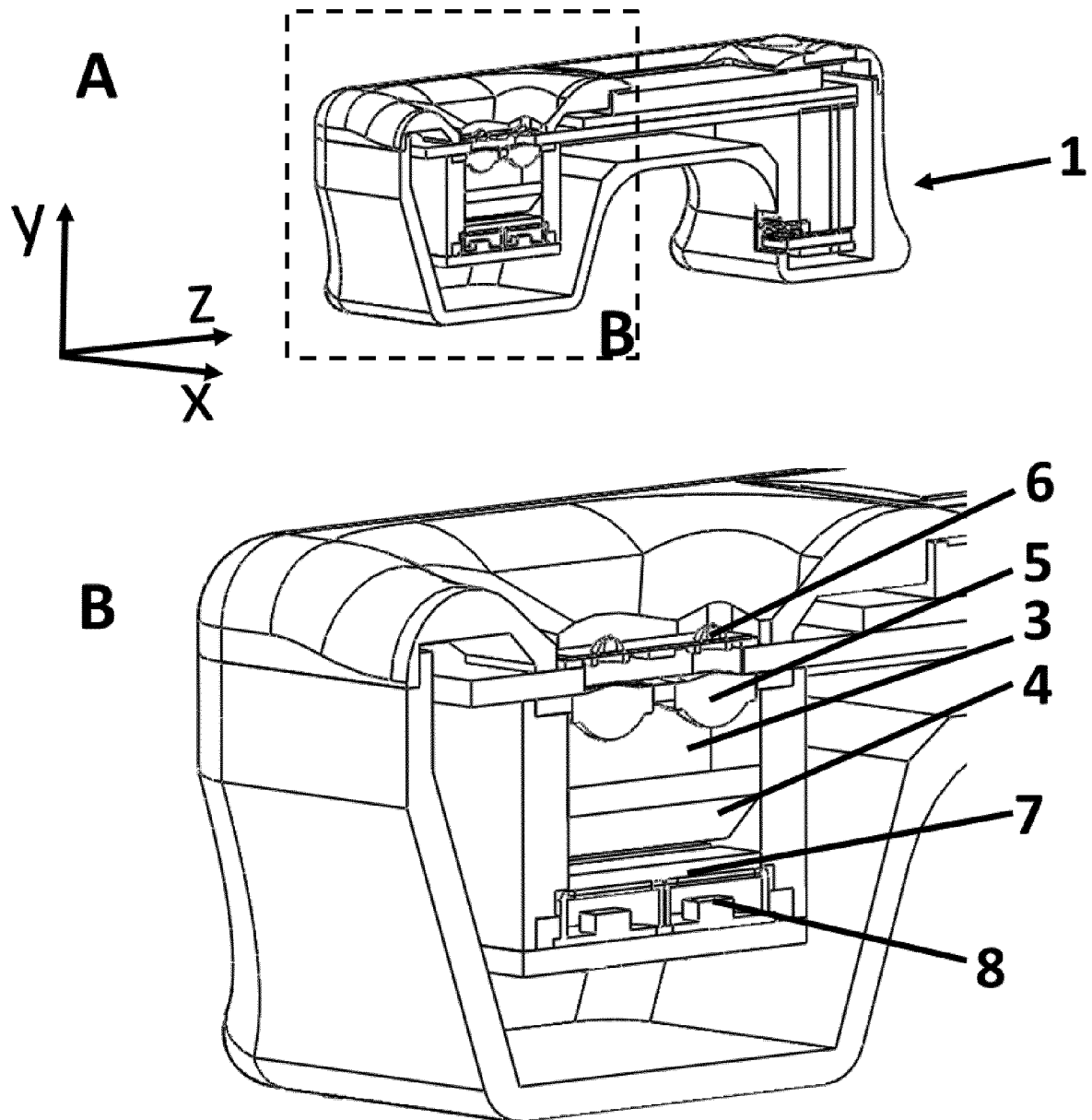
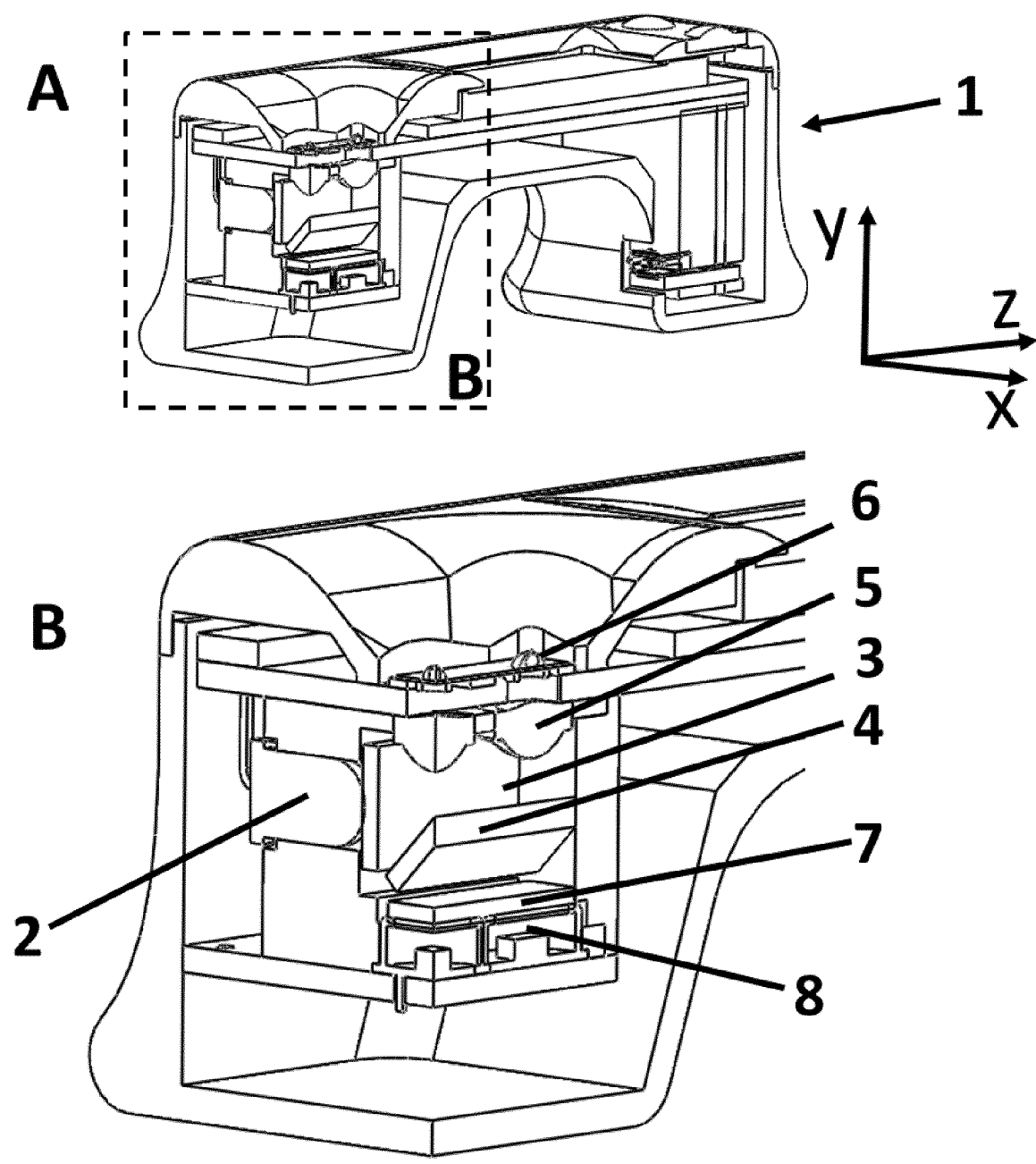
Fig. 3

Fig. 4



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/062989

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N21/64
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/313008 A1 (JO SUNG-HO [KR] ET AL) 13 December 2012 (2012-12-13)	1-3, 6-10, 12-14 3-5,11
Y	abstract; figures 2,3,5,8-10 paragraphs [0039] - [0065], [0083] -----	
Y	GB 2 351 556 A (CAMBRIDGE IMAGING LTD [GB]) 3 January 2001 (2001-01-03) abstract; figure 2 page 2, line 8 page 6, lines 23-28 page 9, lines 18-22 page 10, lines 25-26 page 11, lines 19-31 page 12, lines 23-31 page 20, lines 26-29 ----- -/-	3-5,11



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

22 September 2015

Date of mailing of the international search report

02/10/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Meacher, David

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/062989

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2013/011848 A1 (BOEGE STEVEN J [US] ET AL) 10 January 2013 (2013-01-10) figure 1 -----	1-14
A	EP 2 315 005 A2 (LIFE TECHNOLOGIES CORP.) 27 April 2011 (2011-04-27) abstract; figure 6 -----	1-14
A	US 2012/090996 A1 (TROST PETER KARL [US] ET AL) 19 April 2012 (2012-04-19) abstract -----	1-14
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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