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Apparatus for emitting and detecting beams of light

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Abstract of the disclosure

Subject of the present invention is to provide an apparatus, an instrument, and a method particularly useful in multiplex PCR applications permitting short sample measuring times of many samples combined with high sensitivity.

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**ORIGINAL COMPLETE SPECIFICATION
STANDARD PATENT**

Invention Title

Apparatus for emitting and detecting beams of light

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

P/00/011

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Apparatus for emitting and detecting beams of light

Field of the invention

5 Subject of the present invention is an apparatus for emitting and detecting beams of light, an instrument for amplifying and detecting nucleic acids comprising an apparatus for emitting and detecting beams of light and a method for detecting particular specimens (e.g., nucleic acids) in a sample.

Background of the invention

10 The invention is particularly useful in the field of health care as well as research in biological and medical science, particularly in nucleic acid analysis, gene quantification and genotyping, where reliable analysis of samples for components contained therein is needed. Monitoring chemical reactions by the use of optical systems is well known, for example from Molecular Diagnostics, where chemical reactions are detected and quantified by fluorescent dyes (e.g. propidium iodide, cybergreen, acridine orange) that
15 intercalate between the stacked bases at the centre of the DNA double helix or where specific products of the chemical reaction are detected and quantified by oligonucleotides labeled with compounds such as fluorescein, rhodamine or cyanine dyes where these labeled oligonucleotides specifically hybridize to target DNA sequences. Herein, an important aspect is monitoring the reaction by exciting the dyes
20 via beams of light of specific wavelengths and measuring the light emitted by these dyes. Precision in these steps is a prerequisite for the accuracy of such methods.

A method that uses reaction cycles including denaturation and amplification steps is the polymerase chain reaction (PCR). This technology has revolutionized the field of nucleic acid treatment, particularly the analysis of nucleic acids, by providing a tool to
25 increase the amount of nucleic acids of a particular sequence from negligible to detectable amounts. PCR is described e.g. in EP 0201184 and EP 0200362. An instrument for performing thermocycles in controlled manner on samples in tubes using heating and cooling an extended metal block is disclosed e.g. in EP 0236069.

30 More recently improved and more powerful PCR techniques have been developed. Quantitative real time PCR is a laboratory technique used to simultaneously amplify and quantify a specific part of a given DNA molecule. It is used to determine whether or

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not a specific sequence is present in the sample and if present, the number of copies in the sample can be quantified. Two common methods of quantification are the use of fluorescent dyes that intercalate with double-strand DNA and modified DNA oligonucleotide probes that fluoresce when hybridized with a complementary DNA. Such methods are described e.g. in EP 0512334.

Furthermore, multiplex PCR was developed that enables amplification of two or more products in parallel in a single reaction tube. It is widely used in genotyping applications and different areas of DNA testing in research, forensic, and diagnostic laboratories. Multiplex PCR can also be used for qualitative and semi-quantitative gene expression analysis using cDNA as a starting template originating from a variety of eukaryotic and prokaryotic sources.

Various instruments for performing, detecting, and monitoring such methods are known in the art. The Roche Cobas® TaqMan® instrument as described in EP 0953837 and the Roche Lightcycler 480 instrument make use of a white light source for providing excitation beams to a sample. Using a conventional white light source as excitation light source is disadvantageous as the lifespan of such white light sources generally is below 1000 hours of operation leading to increased maintenance and reparation efforts and costs. Furthermore, the spectral power of some white light sources (particularly halogen bulbs) is rather low in the blue range leaving only limited energy for exciting the sample leading to elongated measuring times. Furthermore, white light sources are disadvantageous as they use various spectral wavelengths necessitating the use of expensive filters because a vast fraction of light having other wavelengths is not used in the application and needs to be blocked by the filter. In addition, white light sources produce heat which needs to be deduced from the instrument.

In other instruments known in the art the excitation beams are produced by a single light-emitting diode such as the Roche Lightcycler® 1.5 and 2.0 instrument or by a laser such as the ABI Prism 7700, 7900 instrument as described in WO 2003/098278 or by using multiple light-emitting diodes of the same wavelength such as the Eppendorf Mastercycler® realplex instrument described in WO 2003/002991. Due to the limitation of these instruments to one particular excitation wavelength hydrolysis multiplex applications can hardly be performed as certain dyes may not or may barely be excited or only with complex modifications in the instrument buildup.

Again other instruments such as the Cepheid Smartcycler as disclosed in US 6369893 use several light-emitting diodes of different wavelengths for exciting one particular reaction region. Several detectors are used for detection of the emitted light from the same particular reaction region. This is disadvantageous in case that a plurality of
5 reaction regions needs to be analyzed because all the components, such as LED's and detectors, need to be multiplied according to the number of reaction regions to be analyzed. In addition, the number of filters and dichroic mirrors are multiplied similarly as well as the electronic circuits for driving the LED's and preamplify the signals of the photodiodes. In addition, the complexity is still more increased when
10 more than 4 LED types of distinct wavelengths are used. Therefore, when several reaction regions need to be detected with a detection system as described in US 6369893 the costs of the detection system becomes very high and the complexity becomes high as well.

Furthermore, spectrometer instruments as disclosed in DE 4424961 are known in the
15 art using a static light source and optical fibers, each fiber being connected on the one end to one sample area and on the other end to a rotary wheel. By turning the wheel each reaction region may be brought into optical contact with the static light source. However, an instrument with this concept has the disadvantage that the light of the single light source is applied sequentially to each reaction region. It is not possible to
20 detect several dye-markers with distinct excitation and emission spectra in several reaction regions in parallel with this instrument. This results in long measurement times especially when a lot of samples need to be measured repeatedly as in PCR applications leading to long overall PCR process times.

An object of the present invention therefore is to provide an optical system particularly
25 useful in multiplex PCR applications permitting short sample measuring times of many samples combined with high sensitivity. In addition, the number of components needed (i.e. excitation light sources, filters and detectors) to analyze several reaction regions should be kept at a minimum in order to reduce complexity and costs.

Brief description of the drawings

Preferred embodiments of the invention are described below, by way of example, with reference to the accompanying drawings, wherein:

- 5 Figure 1 shows an apparatus for emitting and detecting beams of light comprising an excitation module (11) with multi-wavelength illuminators as the light sources (12), an emission module (21) comprising a rotary filter wheel (23) and detectors (22), a control unit (41), and first (31) and second (32) light guides for routing the light emitted from one light source to one reaction region (1) and light emitted from this reaction region to the emission module (21), respectively.
- 10 Figure 2 shows the apparatus for emitting and detecting beams of light comprising an excitation module (11) with multi-wavelength illuminators as the light sources (12) using bifurcated excitation fiber bundles as first light guides (31).
- Figure 3 displays another embodiment of the apparatus for emitting and detecting beams of light comprising an excitation module (11) with light emitting diodes (LED's) of various distinct wavelengths as the light sources (12) mounted on a rotary wheel (13), an emission module (21) comprising a rotary filter wheel (23) and detectors (22), a control unit (41), and first (31) and second (32) light guides for routing the light emitted from one light source to one reaction region (1) and light emitted from this reaction region to the emission module (21), respectively.
- 15
- 20 Figure 4 shows the apparatus for emitting and detecting beams of light comprising an excitation module (11) with light emitting diodes of various distinct wavelengths as the light sources (12) mounted on a rotary wheel (13) using bifurcated excitation fiber bundles as first light guides (31).
- Figure 5 depicts a schematic representation of the distribution of the first (31) and second (32) light guides in an embodiment of the invention as shown in Figures 1 and 3, each first light guide routing the light emitted from a light source (12) at a particular position to a particular reaction region (1) and each second light guide (32) routing the light emitted from a particular reaction region to a particular position within the emission module 21).
- 25
- 30 Figure 6 displays a schematic representation of the distribution of the first (31) and second (32) light guides in an embodiment of the invention as shown in Figures 2 and

4, each bifurcated first light guide routing the light emitted from a light source (12) at a particular position to two particular reaction regions (1) and each second light guide routing the light emitted from a particular reaction region to a particular position within the emission module (21).

5 Figure 7 and 8 show the amplification curves of the fluorescence intensity of a PCR run using an instrument according to the invention and a target template concentration of 10^6 copies of HCV mRNA per μl and 40 copies of quantification standard (QS) per μl . The target was labeled with FAM-dye while the QS was labeled with HEX-dye. These two types of targets were amplified simultaneously by a multiplex PCR run. A total of 10
10 samples were processed. Figure 7 shows the amplification curves of the target FAM-fluorescence intensity levels, Figure 8 those of the QS HEX-fluorescence intensity levels.

Figure 9 shows a sequence diagram of the different measurement steps which are required in order to measure all dye-markers in all reaction regions. The sequence diagram refers to an embodiment with 6 different colors of light emitting diodes. The
15 sequence diagram applies to a total of twelve reaction sites which contain samples with 6 different dye markers in each of them.

The embodiments represented in the Figures are set forth to aid the understanding of the invention, but are not to be construed as limiting.

20 Summary of the invention

A first subject of the invention is an apparatus for emitting and detecting beams of light comprising

- at least two reaction regions,
- an excitation module comprising at least two excitation light sources capable of
25 providing excitation beams of distinct spectra and wavelengths,
- at least two first light guides, each light guide being capable of directing a beam of light emitted from one of said excitation light sources to at least one of said reaction regions,
- at least two second light guides, each second light guide being capable of directing
30 a beam of light emitted from one of said reaction regions to an emission module,

- an emission module capable of detecting beams emitted from each of the at least two reaction regions separately and simultaneously, said emission module at least comprising two detectors and a rotary filter wheel, said rotary filter wheel being positioned between said at least two second light guides and said detectors, and
- 5 - a control unit capable of controlling the activity of said excitation light sources and of said emission module,

wherein the activity of said excitation light sources and the rotation of said rotary filter wheel of said emission module are electronically coupled and wherein said rotary filter wheel is rotated in such a manner that for each reaction region the transmission spectrum of the filter mounted on said rotary filter wheel corresponds to the emission spectrum of said beam of light emitted from the respective reaction region when said reaction region is excited by respective excitation beams characterized in that at the same time a first excitation light source of said at least two excitation light sources emits light of a first wavelength which is transmitted via a first light guide to at least a first reaction region and a second excitation light source of said at least two excitation light sources emits light of a second wavelength different from said first wavelength which is transmitted via a second light guide to at least a second reaction region.

A second subject of the invention is an instrument for amplifying and detecting nucleic acids at least comprising

- 20 - a sample placed in one of the at least two reaction regions comprising a plurality of components and detectable markers capable of generating light emission upon excitation, which is different when a target is present from when a target is not present,
- a device for subjecting said sample to amplification and/or melting reactions, and
- 25 - an apparatus according to the invention.

A third subject of the invention is a method for detecting particular specimens in a sample using an apparatus according to the invention comprising

- providing a sample in one of the at least two reaction regions comprising at least two detectable markers, wherein each detectable marker emits a respective detectable beam of light when excited with an excitation beam including light of a respective excitation wavelength,
- 30 - illuminating said sample with excitation beams of distinct spectra and wavelengths emitted from at least two excitation light sources, and

- detecting the emission beam emitted from the sample with an emission module while the detectable marker is excited by a respective excitation beam.

Detailed description of the invention

5 The present invention relates to an apparatus for emitting and detecting beams of light and an instrument for amplifying and detecting nucleic acids comprising such an apparatus. Herein, a 'light source' according to the invention preferably is a light-emitting diode (LED), an organic light-emitting diode, a laser (e.g., gas laser, chemical laser, excimer laser, solid state laser, semiconductor laser, dye laser, micro-wire laser) or a combination thereof.

10 A light-emitting diode (LED) is a semiconductor device that emits incoherent narrow-spectrum light (electroluminescence) when electrically biased in the forward direction. The color of the emitted light depends on the chemical composition of the semiconducting material used and can be near-ultraviolet, visible or near-infrared. The wavelength of the light emitted, and therefore its color, depends on the band gap energy
15 of the materials forming the p-n junction. In silicon or germanium diodes, the electrons and holes recombine by a non-radiative transition which produces no optical emission, because these are indirect band gap materials. If the emitting layer material of an LED is an organic compound, it is known as an Organic Light Emitting Diode (OLED). To function as a semiconductor, the organic emitting material must have conjugated pi
20 bonds. The emitting material can be small organic molecules in a crystalline phase, or a polymer. Polymer materials can be flexible; such LED's are known as PLED's or FLED's. Compared with regular LED's, OLED's are lighter and polymer LED's can have the added benefit of being flexible. Recently, a new generation of LED's (e.g. type Luxeon Star from the Philips Lumiled lighting company) was developed, which have
25 considerably more optical power than conventional LED's. These LED's may be used in optical systems if the optical power of conventional LED's is not sufficient for measuring various samples with sufficient sensitivity. Furthermore, these high-power LED's are just a little bigger than conventional LED's. Thus, only small cooling blocks are required because the emitted heat is considerably smaller than with conventional
30 white light sources

Furthermore, LED's called 'multi-wavelength illuminator' are known in the art. Herein, one device provides multiple LED chips packaged into a compact, thermally conductive

ceramic substrate having individually controlled multiple wavelengths that range from ultra violet through the visible red spectrum.

The use of LED's is advantageous as LED's are hard to break and are extremely durable with a life-span of 100'000 operation hours and more. Another advantage is that LED's
5 are very small in comparison to traditional white light sources. Therefore, several LED's may be placed fix in an 'excitation module' or may be mounted on a rotary wheel in an 'excitation module' along with cooling blocks and the belonging optics such as lenses and filters.

A 'light source' may also be a laser that emits photons in a coherent beam. A laser
10 commonly contains an active laser medium or gain medium capable of generating stimulated emission on electronic or molecular transitions to a lower energy state starting from a higher energy state to which it had been previously stimulated by energy transfer from an external source. Various materials have been found to possess the required characteristics to form the laser gain medium needed to power a laser (gas,
15 liquids, dyes, solid materials). Thus, many types of lasers with different characteristics suitable for different applications have been developed. The advantage for the use of lasers in an apparatus according to the invention is that laser light typically is emitted in a narrow beam and is near-monochromatic and consists of a single wavelength or color. Furthermore, lasers may be used in applications where many samples are excited with
20 one color in parallel as lasers provide light of high power.

The 'excitation module' refers to a part of the apparatus containing one or more of the 'light sources' which emit light in order to excite particular dyes in a sample placed in the reaction regions. In certain embodiments the 'excitation module' of the apparatus
25 further comprises a 'rotary wheel' which contains or carries the light sources for excitation of the reaction regions. Furthermore, in certain embodiments filters of appropriate center wavelengths and bandwidths may be mounted on the rotary wheel along with lenses which may be necessary to collimate the light from the light sources.

The 'emission module' refers to a part of the apparatus containing at least two detectors which detect light emitted from the samples in the reaction regions. A 'detector' is a
30 component which converts light into an electrical current proportional to the incident light (photons). The 'emission module' further comprises a 'rotary filter wheel' which contains filters that filter the light emitted from the samples in the reaction regions. Filters are needed in order to block and herewith separate light emitted by the light

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sources from the light emitted by the dyes within the samples because the light from the light sources may be scattered in the reaction regions and in other components of the optical system. Lenses may be present in the emission module in order to collimate the light through the filters.

- 5 A 'light guide' is used for transporting or distributing natural or artificial light. A 'light guide' may also be an optical fiber made up of glass or plastic and designed to guide light along its length by total internal reflection. Such optical fibers are widely used in fiber-optic communication. A 'light guide' may on the one hand be a single glass or plastic fiber or on the other hand may consist of such fibers. Another example for a
10 'light guide' is a glass or a plastic rod. Another type of 'light guide' is a fluid light guide. All these types of 'light guides' can either be bifurcated or not bifurcated. Furthermore, bifurcated 'light guides' may either be dually or multiply branched.

As used herein the term 'reaction region' refers to an area or section within the apparatus of the invention that is capable of holding a sample containing dyes to be
15 measured. In certain embodiments a 'reaction region' refers to a vessel which is placed at a particular position within the apparatus and which contains a sample comprising dyes which are measured by the apparatus.

The 'control unit' contains the controller which controls the movement of the rotary filter wheel and the operation of the light sources as well as the detectors. Particularly,
20 the 'control unit' coordinates the rotation of the rotary filter wheel and the operation of light sources and detectors. In certain embodiments the 'control unit' further controls the movement of the rotary wheel carrying the light sources and coordinates the movement of the rotary wheel and the operation of the light sources with the movement of the rotary filter wheel and the operation of the detectors.

25 In one embodiment of the invention the apparatus for emitting and detecting beams of light comprises at least two reaction regions, an excitation module comprising at least two excitation light sources capable of providing excitation beams of distinct spectra and wavelengths. It further comprises at least two first light guides, each first light guide being capable of directing a beam of light emitted from one of the excitation light
30 sources to at least one of the reaction regions and at least two second light guides, each second light guide being capable of directing a beam of light emitted from one of the reaction regions to an emission module. The apparatus further comprises an emission module capable of detecting beams emitted from each of the at least two reaction

regions separately and simultaneously. The emission module at least comprises two detectors and a rotary filter wheel, wherein the filter wheel is positioned between the at least two second light guides and the detectors. Furthermore, the apparatus comprises a control unit capable of controlling the excitation module (i.e., the activation of the light sources mounted thereon) and the emission module (i.e., the rotation of the rotary filter wheel). Herein the activity of the excitation light sources and the rotation of the rotary filter wheel of the emission module are electronically coupled. The rotary filter wheel is rotated in such a manner that for each reaction region the transmission spectrum of the filter mounted on the rotary filter wheel corresponds to the emission spectrum of the beam of light emitted from the respective reaction region when the reaction region is excited by respective excitation beams. Moreover, the excitation light sources are capable of providing excitation beams of various distinct spectra and wavelength. Such excitation light sources are LED's, laser diodes or any other light sources with distinctive color. Such an apparatus allows that at the same time a first excitation light source of said at least two excitation light sources emits light of a first wavelength which is transmitted via a first light guide to at least a first reaction region and a second excitation light source of said at least two excitation light sources emits light of a second wavelength different from said first wavelength which is transmitted via a second light guide to at least a second reaction region. This enables the parallel excitation and measurement of the at least two reaction regions with different wavelengths in one measurement step. This is advantageous as particular measurement sequences may be performed in a fast and efficient manner allowing in a first step the simultaneous measurement of the at least two reaction regions excited with beams of light of a different wavelength and in a successive second step allowing the simultaneous measurement of the at least two reaction regions excited with beams of light of different wavelength e.g. inversely to the wavelengths used in the first step by altering the excitation wavelengths of the light sources within the excitation module and the concurrent rotation of the rotary filter wheel in the emission module. In applications such as multiplex PCR more than one dye marker is present in each sample. Using such a sequence of measurement steps therefore leads to a much shorter overall sequence measurement time compared to successive applications of measurement steps of just one dye specific excitation and measurement wavelength not to all reaction sites at a time. An exemplary embodiment of such a measurement sequence is described below for Fig. 9.

Another advantage is that by guiding the emitted optical power from one particular light source to just one or a few reaction regions the optical power at each reaction region is high. This enables to shorten each particular measurement step which leads to a short sequence measurement time.

5 In certain embodiments LED's are used as the 'light source'. This is advantageous as LED's are comparatively inexpensive and as LED's in general have long life times leading to low maintenance costs. In certain aspects multi-wavelength illuminators capable of providing several wavelengths are used.

10 In a particular embodiment the first light guide may be bifurcated. In such an embodiment more than one reaction region may be excited at the same time by one particular light source. Such an embodiment provides an even higher throughput of samples to be measured.

15 In another embodiment each one of said at least two first light guides is on the one end affixed to a particular position on said excitation module allowing optical contact of one excitation light source with said one first light guide and on the other end said at least one first light guide is affixed to at least one reaction region mediating the beam of light emitted from said excitation light source to a defined position at said at least one reaction region. The first and the second light guides are locally fixed in a particular position within the apparatus in such a way, that one particular first light guide guides the light from one particular position next to one light source (e.g., a multi-wavelength illuminator) to one particular reaction region and that one particular second light guide guides the light emitted from this reaction region to one particular position next to the rotary filter wheel. Instead of one light source multiple light sources of the same wavelength may be present at a particular position on the excitation module in order to increase the intensity of light. Alternatively, light emitted from multiple light sources of the same wavelength may be conveyed to a particular position on the excitation module using a third light guide or a bundle of third light guides. By emitting light of desired wavelength from the light sources and by rotating the rotary filter wheel in a coordinated manner one sample in one reaction region containing several dyes may be excited with beams of light of different wavelengths correlating to the excitation spectra of the various dyes in a short period of time, while during each excitation beam acts on the sample the emission spectrum of the sample for this excitation beam can be measured simultaneously.

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In certain aspects the excitation light sources are switched off by the control unit while the rotary wheel rotates. This is advantageous because it allows recording a dark value and correcting the measured signals emitted from the reaction regions which besides the signal contain the dark value that is caused by the offset of the signal processing electronics. Furthermore, switching off the light sources is advantageous with certain types of light sources, such as LED's, as it reduces the spectral changes of the emitted light. Spectral changes of the light emitted from LED's may carry weight because of the higher warm-up of the LED's or semiconductor laser diodes during continuous operation.

10 In certain embodiments each of said at least two excitation light sources are capable of providing excitation beams of various distinct spectra and wavelength. Two exemplary embodiments of such an apparatus are shown in Figures 1 and 2. Herein, the excitation module (11) comprising multi-wavelength illuminators as the light sources (12), an emission module (21) comprising a rotary filter wheel (23) and detectors (22), a control unit (41), and first (31) and second (32) light guides for routing the light emitted from one light source (12) to one reaction region (1) and light emitted from this reaction region to the emission module (21), respectively. In the exemplary embodiment in Figure 2 the apparatus comprising an excitation module (11) with multi-wavelength illuminators as the light sources (12) using bifurcated excitation fiber bundles as first light guides (31).

Figure 1 shows a certain embodiment of the invention having fixed multi-wavelength illuminators as light sources (12) and not bifurcated first light guides (31) as well as twelve reactions regions (1). The light sources (12) are contained in the excitation module (11). All twelve light sources (12) are from the same type and each of these multi-wavelength illuminator light sources may provide six distinct wavelengths which may sequentially be switched on and off. In a particular embodiment the activation of the multi-wavelength illuminators is controlled in such a manner that always pairs of two adjacent multi-wavelength illuminators (12) emit light of the same color and wavelength. During each measurement step within a measuring sequence all light sources (12) are switched on in such a way that the six multi-wavelength illuminator pairs have distinct colors. Thereby, always two reaction regions (1) are illuminated with light of the same wavelength and up to six different wavelengths may be used in parallel in each measurement step. By applying to each reaction region (1) a measurement sequence of six consecutive measurement steps, wherein the color and wavelength of the

excitation beam is altered for each measurement step, every reaction region (1) may be excited with light of six different wavelengths in a fast and efficient way. The excitation light beams are guided from each of the multi-wavelength illuminators (12) to one of the reaction regions (1) by a first light guide (31). The emission beams from each of the twelve reaction regions (1) are guided to the emission module (21) by twelve second light guides (32). In Figure 1 only two exemplary first light guides and two exemplary second light guides for two reaction regions are shown, whereas Figure 5 displays a detailed schematic view of the arrangement of the first (31) and second light guides (32) showing all light guides. In the emission module (21) each emission beam from one reaction region (1) is lead to one of the twelve detectors (22). Thereby, each emission beam is lead through one filter which is mounted on a rotary filter wheel (23). The filters on this rotary filter wheel are selected and assembled in such a manner that the center wavelengths fit to the spectra of the respective emission beams. In this particular embodiment filters of the same type regarding the spectral specifications are arranged pair wise and are mounted adjacent to each other on the rotary filter wheel (23). However, the arrangement of the filters on the rotary filter wheel (23) depends on the arrangement of the first (31) and second light guides (32) with respect to the reaction regions (1) and may be adapted as suitable. Within a measurement sequence it is necessary that each pair of multi-wavelength illuminators (12) sequentially emits beams of light of all suitable wavelengths to be measured. The rotary filter wheel (23) is rotated at the end of each measurement step within the measurement sequence in such a way that the filters are positioned to provide the appropriate spectral specifications in order to detect the emission spectra of the dye-markers excited by the appropriate excitation beams. The change of the wavelengths of the excitation beam emitted from the multi-wavelength illuminators (12) and the rotation of the rotary filter wheel (23) is coordinated by a control unit (41).

Figure 2 shows another embodiment of the invention. In contrast to the embodiment of Figure 1 the number of multi-wavelength illuminators (12) is reduced to six instead of twelve. The first light guides (31) are bifurcated with two branches in order to enable that two reaction regions (1) are illuminated with excitation beams of the same spectra from the same single multi-wavelength illuminator. In Figure 2 only two exemplary bifurcated first light guides having two branches and four exemplary second light guides for four reaction regions are shown, whereas Figure 6 displays a detailed schematic view of the arrangement of the first (31) and second light guides (32) showing all light guides.

In further embodiments the first light guides (31) may be bifurcated having more than two branches in order to increase the number of reaction regions (1) which are illuminated by excitation beams of the same spectra from the same single multi-wavelength illuminator (12) at a time. Moreover, in most applications it is reasonable to correlate the number of detectors (22) in the emission module (21) to the number of reaction regions (1) which need to be analyzed in order to allow for a defined measurement signal allocation as well as a fast and parallel measurement of all reaction regions. The filters on the rotary filter wheel need to be selected regarding their spectral specifications in such a manner that the number of filters with the same spectral specifications correlates to the number of reaction regions which are excited by excitation light of the same spectra.

A measurement sequence diagram for the measurement of all reaction regions as described above is depicted in Figure 9. It shows the measurement sequence of an embodiment of the apparatus according to the invention having twelve reaction regions and six or twelve multi-wavelength illuminators respectively. The necessary measurement steps within a measurement sequence in order to analyze all samples in the reaction regions in case six different dye-markers are present in each reaction region are displayed. A channel in this sequence diagram refers to a specific set of filters which allows to measure one particular dye-marker which is present in the twelve reaction regions. In an embodiment having six multi-wavelength illuminators a channel encompasses one excitation filter and two emission filters of correlating spectral specifications. In an embodiment having twelve multi-wavelength illuminators preferably two excitation filters for one channel are used. The excitation filters are positioned after the collimation lenses on the rotary wheel. In both embodiments channel 1 preferably is applied to the first and second reaction region when measurement step 1 within a six-step measuring sequence is performed. At the same time the other channels 2 - 6 appropriately equipped for measuring the other types of dye-markers present in the samples are applied to the remaining reaction regions 3 - 12. This is advantageous because suitable dye-markers in reaction regions 3 - 12 are measured in parallel to that in the reaction regions 1 and 2. Before the initiation of measurement step 2 all multi-wavelength illuminators switch their colors and wavelengths of the light emitted and the rotary filter wheel is rotated in such a way that channel 1 now is applied to the samples in the reaction regions 3 and 4. Thus, in measurement step 2 the same type of dye-marker as measured in reaction regions 1 and 2 during measurement step 1 is now being measured in reaction region 3 and 4. At the

same time all other channels 2 - 6 are positioned towards a new pair of reaction regions in comparison to measurement step 1 allowing the measurement of another type of suitable dye-marker present in the reaction regions. After applying all six measurement steps to all samples the six dye-markers in the twelve reaction regions were measured
5 with all six channels. Therefore, using this measurement sequence with an apparatus according to the invention is particularly useful in multiplex PCR applications permitting short sample measuring times of many samples combined with high sensitivity.

10 In other embodiments the number of reaction regions, excitation light sources, and excitation wavelengths may vary. There is also no necessity that the number of reaction regions needs to be a multiple of the number of dye-markers to be measured or a multiple of the number of different multi-wavelength illuminator colors respectively. Furthermore, neither the number of multi-wavelength illuminators which emit the same color at a time nor the number of filter types having the same spectral
15 specifications is restricted.

In another embodiment of the invention an apparatus for emitting and detecting beams of light comprises at least two reaction regions, an excitation module comprising at least two excitation light sources mounted on a rotary wheel capable of providing excitation beams of distinct spectra and wavelengths. It further comprises at least two first light
20 guides, each first light guide being capable of directing a beam of light emitted from one of the excitation light sources to at least one of the reaction regions and at least two second light guides, each second light guide being capable of directing a beam of light emitted from one of the reaction regions to an emission module. The apparatus further comprises an emission module capable of detecting beams emitted from each of the at
25 least two reaction regions separately and simultaneously. The emission module at least comprises two detectors and a rotary filter wheel, wherein the filter wheel is positioned between the at least two second light guides and the detectors. Furthermore, the apparatus comprises a control unit capable of controlling the excitation module (i.e., the rotation of the rotary wheel to a predetermined position and the activation of the
30 light sources mounted thereon) and the emission module (i.e., the rotation of the rotary filter wheel). Herein, the rotary wheel carrying the excitation light sources may be rotated relative to the position of the at least one first light guide at least allowing one of the excitation light sources to emit light beams to one defined first light guide. Moreover, the rotation of the rotary wheel carrying the light sources, the activity of the

excitation light sources and the rotation of the filter wheel of the emission module are coupled electronically via the controller within the control unit allowing the rotary filter wheel to be rotated in such a manner that for each reaction region the transmission spectrum of the filter mounted on the filter wheel corresponds to the emission spectrum of the beam of light emitted from the respective reaction region. Thus, the rotary wheel carrying the excitation light sources may be rotated relative to the position of the first light guide at least allowing one of the excitation light sources to emit beams of light to one defined first light guide. The rotation of the rotary wheel to a predetermined position preferably is controlled by the control unit. Such an apparatus allows that at the same time a first excitation light source of said at least two excitation light sources mounted on said rotary wheel emits light of a first wavelength which is transmitted via a first light guide to at least a first reaction region and a second excitation light source of said at least two excitation light sources mounted on said rotary wheel emits light of a second wavelength different from said first wavelength which is transmitted via a second light guide to at least a second reaction region. This enables the parallel excitation and measurement of the at least two reaction regions with different wavelengths in one measurement step. This is advantageous as particular sequences of measurement steps may be performed in a fast and efficient manner allowing in a first step the simultaneous measurement of the at least two reaction regions excited with beams of light of a different wavelength and in a successive second step allowing the simultaneous measurement of the at least two reaction regions excited with beams of light of different wavelength e.g. inversely to the wavelengths used in the first step by the coupled rotation of said rotary wheel carrying the light sources and the concurrent rotation of the rotary filter wheel in the emission module. An exemplary embodiment of such a measurement sequence is described below for Fig. 9.

In a certain embodiment the first light guide may be bifurcated or a light guide bundle may be used. In such an embodiment more than one reaction region may be excited at the same time by one light source mounted on the rotary wheel. Such an embodiment provides an even higher throughput of samples to be measured.

In certain aspect LED's are used as the 'light source' in the apparatus according to the invention. This is advantageous as LED's are comparatively inexpensive and as LED's in general have long life times leading to low maintenance costs. Furthermore, the use of LED's is less demanding on the blocking requirements of the filters to be used because only smaller spectral ranges require high blocking which leads to much less expensive

filters. Furthermore, a rotary wheel carrying LED's is less space consuming and allows the construction of more compact analytical instruments.

In certain embodiments each one of said at least two first light guides is on the one end affixed to a particular position on said excitation module allowing optical contact of one excitation light source with said one first light guide and on the other end said at least one first light guide is affixed to at least one reaction region mediating the beam of light emitted from said excitation light source to a defined position at said at least one reaction region.

In certain aspects the excitation light sources are switched off by the control unit while the rotary wheel rotates. This is advantageous because it allows recording a dark value and correcting the measured signals emitted from the reaction regions which besides the signal contain the dark value that is caused by the offset of the signal processing electronics. Furthermore, switching off the light sources is advantageous as it reduces the amount of heat emitted by the light sources and, thus, decreases the risk that the spectral changes of the light sources caused by their higher inside temperatures have an undesired impact on the accuracy of the measurements.

In certain embodiments the excitation light sources each represent different excitation wavelengths. In other embodiments the apparatus according to the invention comprises light sources of different colors with several light sources of the same color being present at each particular position on the rotary wheel in such a manner that at each position on the rotary wheel the several light sources of the same color conjointly form one light source. Herein, at each particular light source position on the rotary wheel the light emitted by multiple light sources of the same color can be guided to the reaction regions by bifurcated or not bifurcated light guides. The advantage of this embodiment is to provide enough optical power to the reaction regions especially when a lot of reaction regions are present.

During one measurement step light of a defined wavelength is emitted from one excitation light source mounted on a rotary wheel and is guided to a reaction region via a first light guide. Within the reaction region the light may interact with at least one particular dye contained in a sample leading to the emission of light from the reaction region. The emitted light is guided to a filter mounted on the rotary filter wheel of the emission module via a second light guide. The transmissibility of the filter is chosen to correlate to the wavelength of the light emitted from the reaction region. Light that has

passed through the filter wheel is then detected by a detector of the emission module. The signal of the detector is then evaluated and may be edited. The first and the second light guides are locally fixed in a particular position within the apparatus in such a way, that one particular first light guide guides the light from one particular position next to the rotary wheel carrying the light sources to one particular reaction region and that one particular second light guide guides the light emitted from this reaction region to one particular position next to the rotary filter wheel. By rotating the rotary wheel carrying the light sources and by rotating the rotary filter wheel in a coordinated manner one sample in one reaction region containing several dyes may be excited with beams of light of different wavelengths correlating to the excitation spectra of the various dyes in a short period of time, while during each excitation beam acts on the sample the emission spectrum of the sample for this excitation beam can be measured simultaneously. In principal every position of the rotary wheel carrying the light sources may be correlated to a user-defined position of the rotary filter wheel leading to a high flexibility of combining excitation and emission wavelengths. Thus, with a limited amount of coordinated rotary movements of the two rotary wheels one sample can be excited with light of different wavelengths in a short period of time, which is advantageous particularly when evaluating a sample in a multiplex PCR assay. Thus, it becomes possible to illuminate all samples provided in various reaction regions with light of different wavelengths by just a few rotations. Because of the arrangement of the different light sources on the rotary wheel the different samples are illuminated by different colors during each single measurement step. This illumination pattern is changed at the next following measurement step of a measurement sequence in this way that at the end of the sequence all samples were combined with all colors. The total time of a measurement sequence is minimized because of the parallelization of many measurements at every measurement time point.

Two exemplary embodiments of such embodiments of the apparatus are depicted in Figures 3 and 4. Figure 3 shows a certain embodiment of the invention having twelve LED's as light sources (12) mounted on a rotary wheel (13), twelve reaction regions (1) and not bifurcated first light guides (31). The LED's (12) mounted on the rotary wheel (13) are contained in the excitation module (11). In this particular embodiment always two adjacent LED's (12) of the twelve LED's mounted on the rotary wheel (13) pair wise have distinct colors. The excitation light beams are guided from the LED's (12) to the reaction regions (1) by first light guides (31). In this setup always two reaction regions are illuminated with the same color. As the position of the light guides (31) within the

excitation module (11) is fixed every reaction region (1) can be brought into optical contact with every light source by rotating the rotary wheel (13) to a predetermined position. At the beginning of each measurement step within a measuring sequence all LED's are switched on. Thereby, always two reaction regions are illuminated with light
5 of the same wavelength and up to six different wavelengths may be used in parallel in each measurement step. After each measurement step the rotary wheel (13) carrying the LED's (12) is rotated by two positions in order to allow the LED's that were in optical contact with the first light guides (31) leading to reaction regions 1 and 2 in measurement step 1 to be in optical contact with the first light guides leading to reaction
10 regions 3 and 4 in measurement step 2 and so forth. By applying a measurement sequence of six measurement steps, wherein the color and wavelength of the excitation beam is altered for each measurement step by rotating the rotary wheel (13) carrying the LED's accordingly, every reaction region (1) may be excited with light of six different wavelengths in a fast and efficient way. The emission beams emitted from the twelve
15 reaction regions (1) are guided to the emission module (21) by twelve second light guides (32). In Figure 3 only one exemplary first (31) and second light guide (32) for one reaction region is depicted, whereas Figure 5 displays a detailed schematic view of the arrangement of the first (31) and second light guides (32) showing all light guides. In the emission module (21) each emission beam is lead to one of the twelve detectors
20 (22) by a second light guide (32). Hereby, each emission beam is lead through one filter which is mounted on a rotary filter wheel (23). The filters on this rotary filter wheel are selected and assembled in such a manner that their spectral specifications fit to the spectra of the respective emission beams. In this particular embodiment filters of the same type regarding the spectral specifications are arranged pair wise and are mounted
25 adjacent to each other on the rotary filter wheel (23). However, the arrangement of the filters on the rotary filter wheel (23) depends on the arrangement of the first (31) and second light guides (32) with respect to the reaction regions (1) and may be adapted as suitable. The color pattern of the six LED pairs and the filters on the rotary filter wheel (23) should be selected in such a way that all pairs of reaction regions (1) are
30 illuminated by excitation beams of distinct spectra. Before the beginning of each measurement step within a measurement sequence each pair of LED's is guided to the ends of the first light guides (31) fixed in the excitation module (11) by rotation of the rotary wheel (13). Thereby, within one measurement step all pairs of reaction regions (1) are illuminated with distinct excitation beams. In parallel the rotary filter wheel (23)
35 is rotated at the beginning of each measurement step in such a way that the filters are

positioned to provide the appropriate spectral specifications in order to detect the emission spectra of the dye-markers excited by the appropriate excitation beams. After measurement at the end of the measurement step and before the initiation of the next measurement step the rotary wheel (13) carrying the light sources (12) and the rotary filter wheel (23) are rotated to the next measurement position. The rotation of the rotary wheel (13) and the rotation of the filter rotary wheel (23) in a coordinated manner are controlled by a control unit (41).

Figure 4 shows another embodiment of the invention. In contrast to the embodiment of Figure 3 the number of LED's (12) is reduced to six instead of twelve, wherein these six LED's have distinct colors. The first light guides (31) are bifurcated with two branches in order to enable that two reaction regions (1) are illuminated with excitation beams of the same spectra from the same single LED (12). In Figure 4 only two exemplary bifurcated first light guides (31) having two branches and four exemplary second light guides (32) for four reaction regions are shown, whereas Figure 6 displays a detailed schematic view of the arrangement of the first (31) and second light guides (32) showing all light guides. In further embodiments the first light guides may be bifurcated having more than two branches in order to increase the number of reaction regions (1) which are illuminated by excitation beams of the same spectra from the same LED at a time. Moreover, in most applications it is reasonable to correlate the number of detectors (22) in the emission module (21) to the number of reaction regions (1) which need to be analyzed in order to allow for a defined measurement signal allocation as well as a fast and parallel measurement of all reaction regions. The filters on the rotary filter wheel (23) need to be selected regarding their spectral specifications in such a manner that the number of filters with the same spectral specifications correlates to the number of reaction regions (1) which are excited by excitation light of the same spectra.

The measurement sequence diagram for the measurement of twelve reaction regions and each reaction region comprising a sample containing six different dye-markers as described above and as depicted in Figure 9 may also be applied to embodiments as displayed in Figures 3 and 4. In each measurement step all six LED's or six pairs of LED's with the same color are guided to the next adjacent pair of first light guide ends in the excitation module by rotation of the rotary wheel. In parallel the rotary filter wheel is rotated in the same manner accordingly. This ensures that the channel in optical contact with reaction regions 1 and 2 in a first measurement step is applied to the samples in the reaction regions 3 and 4 in the next measurement step. Thus, in this

measurement step the same dye-marker as measured in the previous measurement step in reaction regions 1 and 2 may now be measured in reaction regions 3 and 4. At the same time all other channels 2 - 6 are in position to a new pair of reaction regions in comparison to the previous measurement step allowing the measurement of another
5 type of suitable dye-marker present in the reaction regions. After applying all six measurement steps to all samples the six dye-markers in the twelve reaction regions were measured with all six channels. Therefore, using this measurement sequence with an apparatus according to the invention is particularly useful in multiplex PCR applications permitting short sample measuring times of many samples combined with
10 high sensitivity.

In other embodiments the number of reaction regions, excitation light sources, and excitation wavelengths may vary. There is also no necessity that the number of reaction regions needs to be a multiple of the number of dye-markers to be measured or a multiple of the number of different light source colors respectively. Furthermore,
15 neither the number of light sources which emit the same color at a time nor the number of filter types having the same spectral specifications is restricted.

In certain embodiments of the invention the excitation light sources may be fixed in the excitation module while the ends of the first light guides next to the excitation light sources may be rotated relative to the excitation light sources. The other ends of the
20 light guides stay affixed to the reaction regions in this embodiment. In this way the same measurement sequence as displayed in Figure 9 can be applied to the reaction regions.

In certain embodiments of the invention the mono color light sources (e.g., the LED's) can be fixed within the excitation module instead of being mounted on a rotary wheel. In such embodiments the light is transferred to the first light guides by an optical device
25 (e.g. prisms, optical Microelectromechanical systems (MEMS), mirrors). This device enables to route the light of each light source to each first light guide in such a way that the same advantageous measurement sequence as shown in Figure 9 may be applied. Different embodiments of such an optical device are conceivable. The whole device may be rotated in order to route the light from the light sources to the first light guides or the
30 device itself may contain optical switches (e.g. switchable mirrors) which route the light from the different light sources to the first light guides.

Apparatuses according to the invention may in certain embodiments further comprise at least one control light guide for monitoring the power of the light sources. Such a

light guide is combined with one of the first light guides which is affixed to a certain site in the excitation module and leads to a separate detector. In doing so, the power of the light source can be monitored simultaneously with the excitation of the respective reaction region. In certain embodiments more than one control light guide may be present each of them leading to separate detectors. Hereby, each light source can be monitored simultaneously during the excitation of each reaction region.

The apparatus as described above may be used in an instrument for amplifying and detecting nucleic acids. Besides the apparatus for emitting and detecting beams of light such an instrument at least comprises a sample placed in one of the at least two reaction regions comprising a plurality of components and detectable markers capable of generating light emission upon excitation, which is different when a target is present from when a target is not present and a device for subjecting the at least one sample to amplification and/or melting reactions. In a certain embodiment the sample may be provided in a vessel that is placed in the reaction region. The apparatus can be combined with a thermal cycler which applies specific temperature cycles to the reaction regions in order to amplify the samples contained in the reaction regions. Besides a thermal cycler and an apparatus according to the invention the instrument may contain means to extract nucleotide acids from blood plasma (e.g., a pipetting device, incubators, wash and separation stations as well as means for loading reagent bottles, reagent racks and sample tubes). Furthermore, such an instrument may be an overall process solution ranging from the nucleotide acid extraction to the quantification of the initial target concentration and encompassing all electronic printed circuit boards (PCB's) along with personal computer and specifically designed software for controlling the process.

The apparatus and the instrument according to the invention may particularly be used in real-time PCR especially multiplex-PCR applications which comprise more than one target sequence to be processed and the target sequence or the probes specifically hybridizing to the target sequences are labeled with different dye-markers. However, besides fluorescent markers also phosphorescent, chemiluminescent and electro-chemiluminescent markers may be used in an apparatus and/or instrument according to the invention. Besides multiplex PCR applications the apparatus and the instrument according to the invention may also be used in other applications such as hybridization assays, fluorescence in situ hybridization assays using peptide nucleic acid probes, RNA-RNA hybridization assays, protein multiplex assays or other amplification-detection

applications such as quantitative PCR, quantitative real-time PCR, ligation-mediated PCR (e.g., multiplex ligation-dependent probe amplification (MLPA), ligase chain reaction), RACE-PCR, asymmetric PCR, etc..

5 In a method for detecting particular specimens in a sample the sample to be examined is provided in a reaction region and comprises at least two detectable markers, wherein each detectable marker emits a respective detectable beam of light when excited with an excitation beam including light of a respective excitation wavelength. The sample is then illuminated with excitation beams of distinct spectra emitted from at least one excitation light source. The emission beam emitted from the sample is detected with an
10 emission module while the detectable marker is excited by a respective excitation beam. In particular embodiments said particular specimens are nucleic acids. In certain aspects at least one target nucleic acid within said sample is amplified and/or melted.

Such methods using an apparatus and/or an instrument according to the invention are further described by the way of example in the example section.

15

Example

A real time multiplex PCR run with an Hepatitis C Virus (HCV) clean target with known concentration and a quantification standard (QS) was performed using two
20 apparatuses according to the invention. In each reaction region 50 μ l PCR reagent mixtures (44.6 μ l Master Mix containing the RT primer ST778AA and additionally upstream primer ST280A along with 5.4 μ l Manganese²⁺-buffer per sample) were provided along with 25 μ l of the HCV target having a concentration of 1x10⁶ copies/ μ l and 25 μ l of a quantification standard (QS) at a concentration of 40 copies/ μ l. The master mix, the Manganese²⁺-buffer and the quantification standard are commercially
25 available reagent kits (Roche HCV CTM Master Mix: Kit no. 58004181, Roche HCV CTM Manganese: Kit no. 52004183, Roche Amplicor[®] Monitor QS HCV V2.0: Kit no. 58002560). The clean targets were marked with the FAM marker dye, while the quantification standard (QS) were marked with the HEX marker dye. 62 temperature cycles along with one pre cycle and one reverse transcriptase step as shown below were
30 applied to each reaction region using two thermal cyclers. The following table shows the thermal cycler profile that was used for the amplification.

	nr	Temperature/°C	Time / sec
Pre cycle	1	50	300
Reverse Transcriptase	1	59	1800
Denaturation	2	95	15
Annealing/Extension		58	40
Denaturation	60	91	15
Annealing/Extension		58	40
Post cycle	1	40	120

The heating and cooling ramps for changing of the applied temperatures were selected as 7°C/min. for heating and 5°C/min. for cooling.

The resulting amplification curves are displayed in Figures 7 and 8.

5 Results

Figure 7 shows the amplification curves of the real time multiplex HCV-PCR run mentioned above. 10 samples containing PCR mixtures were amplified and the signals were measured real-time in the FAM-channel. The data are corrected for the crosstalk caused from the quantification standard labeled with HEX marker dye which is partly excited and detected in the FAM-channel as well. A significant signal increase is an indicator that the target sequence is present. The cycle number where a significant signal increase occurs at is a measure of the initial target concentration. The smaller the cycle number the higher the initial target concentration is. This characteristic cycle number is called elbow number. The determination of this elbow number is a specific property of the type of algorithm which is used to analyze the amplification curves. Different algorithms can determine different elbow numbers of the same amplification curve. The following table shows the elbow numbers of the amplification curves shown in Figure 7:

Thermal Cycler nr	Reaction Region nr	Target Elbow nr
1	2	23.3
1	3	23.5
1	4	23.6
1	8	23.3
1	9	23.5
2	2	23.3
2	3	23.4
2	4	23.6
2	8	23.4
2	9	23.6

Figure 8 shows the amplification curves of the quantification standard (QS) of the same real time multiplex HCV-PCR run. All 10 samples contained besides the PCR mixtures 25µl of quantification standard as well. The signal was measured real-time in the HEX-channel. The data are corrected for the crosstalk caused from the clean target labeled with FAM marker dye which is partly excited and detected in the HEX-channel as well.
 5 A significant signal increase is an indicator that the quantification standard is present. Also for the quantification standard the characteristic cycle number called elbow number was determined using an algorithm analyzing the amplification curves of QS. The following table shows the elbow numbers of the amplification curves shown in
 10 Figure 8:

Thermal Cycler nr	Reaction Region nr	QS Elbow nr
1	2	32.1
1	3	32.6
1	4	32.3
1	8	32.5
1	9	32.6
2	2	31.2
2	3	31.5
2	4	31.4
2	8	31.6
2	9	31.6

The initial target concentration can be calculated with the help of a calibration curve of the elbow number differences between the target and the quantification standard. The following table shows these calculated titers in copies/µl:

Thermal Cycler nr	Reaction Region nr	Result Target copy/µl
1	2	8.55E+05
1	3	1.42E+06
1	4	7.40E+05
1	8	1.45E+06
1	9	1.38E+06
2	2	2.66E+05
2	3	3.13E+05
2	4	2.34E+05
2	8	3.88E+05
2	9	3.14E+05

15

Reference numerals

- 1 reaction region
- 11 excitation module
- 12 excitation light source
- 13 rotary wheel
- 21 emission module
- 22 detector
- 23 rotary filter wheel
- 31 first light guide
- 32 second light guide
- 41 control unit

The claims defining the invention are as follows:

1. An apparatus for emitting and detecting beams of light comprising
 - at least two reaction regions,
 - an excitation module comprising at least two excitation light sources capable
5 of providing excitation beams of distinct spectra and wavelengths,
 - at least two first light guides, each first light guide being capable of directing a
beam of light emitted from one of said excitation light sources to at least one
of said reaction regions,
 - at least two second light guides, each second light guide being capable of
10 directing a beam of light emitted from one of said reaction regions to an
emission module,
 - an emission module capable of detecting beams emitted from each of the at
least two reaction regions separately and simultaneously, said emission
module at least comprising two detectors and a rotary filter wheel, said rotary
15 filter wheel being positioned between said at least two second light guides and
said detectors, and
 - a control unit capable of controlling the activity of said excitation light
sources and of said emission module,

wherein the activity of said excitation light sources and the rotation of said rotary
20 filter wheel of said emission module are electronically coupled and wherein said
rotary filter wheel is rotated in such a manner that for each reaction region the
transmission spectrum of the filter mounted on said rotary filter wheel
corresponds to the emission spectrum of said beam of light emitted from the
respective reaction region when said reaction region is excited by respective
25 excitation beams characterized in that at the same time a first excitation light
source of said at least two excitation light sources emits light of a first wavelength
which is transmitted via a first light guide to at least a first reaction region and a
second excitation light source of said at least two excitation light sources emits
light of a second wavelength different from said first wavelength which is
30 transmitted via a second light guide to at least a second reaction region.

2. The apparatus according to claim 1, wherein said at least two excitation light
sources are mounted on a rotary wheel and wherein said rotary wheel carrying
said excitation light sources may be rotated relative to the position of said first

light guide at least allowing one of said excitation light sources to emit light beams to one defined first light guide and wherein the rotation of said rotary wheel to a predetermined position is controlled by the control unit.

- 5 3. The apparatus according to claim 1, wherein each of said at least two excitation light sources are capable of providing excitation beams of various distinct wavelength spectra.
4. The apparatus according to any one of claims 1 to 3, wherein said excitation light sources are light-emitting diodes (LED's).
- 10 5. The apparatus according to any one of claims 1 to 4, wherein said first light guide is a single or a bifurcated light guide bundle capable of directing a beam of light emitted from one of said excitation light sources to at least two of said reaction regions.
6. The apparatus according to any one of claims 1, 2, 4 and 5, wherein said excitation light sources each represent different excitation wavelengths.
- 15 7. The apparatus according to any one of claims 1 to 6, wherein said excitation light sources are switched off by the control unit while the rotary filter wheel rotates.
8. The apparatus according to any one of claims 1 to 7, wherein said at least one first light guide is on the one end affixed to a position on said excitation module allowing optical contact of one excitation light source with said one first light guide and on the other end said at least one first light guide is affixed to at least 20 one reaction region mediating the beam of light emitted from said excitation light source to a defined position at said at least one reaction region.
9. The apparatus according to any one of claims 2 and 4 to 8, wherein the rotation of said rotary wheel carrying said excitation light sources and the rotation of said rotary filter wheel of said emission module are electronically coupled. 25
10. The apparatus according to any one of claims 1 to 9 further comprising at least one control light guide for monitoring the power of the light sources.
11. An instrument for amplifying and detecting nucleic acids at least comprising

- a sample placed in one of the at least two reaction regions comprising a plurality of components and detectable markers capable of generating light emission upon excitation, which is different when a target is present from when a target is not present,
 - 5 - a device for subjecting said sample to amplification and/or melting reactions, and
 - an apparatus according to any one of claims 1 to 10.
12. A method for detecting particular specimens in a sample using an apparatus according to any one of claims 1 to 10 comprising
- 10 - providing a sample in one of the at least two reaction regions comprising at least two detectable markers, wherein each detectable marker emits a respective detectable beam of light when excited with an excitation beam including light of a respective excitation wavelength,
 - illuminating said sample with excitation beams of distinct wavelength spectra emitted from at least two excitation light sources, and
 - 15 - detecting the emission beam emitted from the sample with an emission module while the detectable marker is excited by a respective excitation beam.
13. The method according to claim 12, wherein said particular specimens are nucleic acids.
- 20 14. The method according to claim 13 further comprising the step
- amplifying and/or melting at least one target nucleic acid within said sample.

FIG. 1

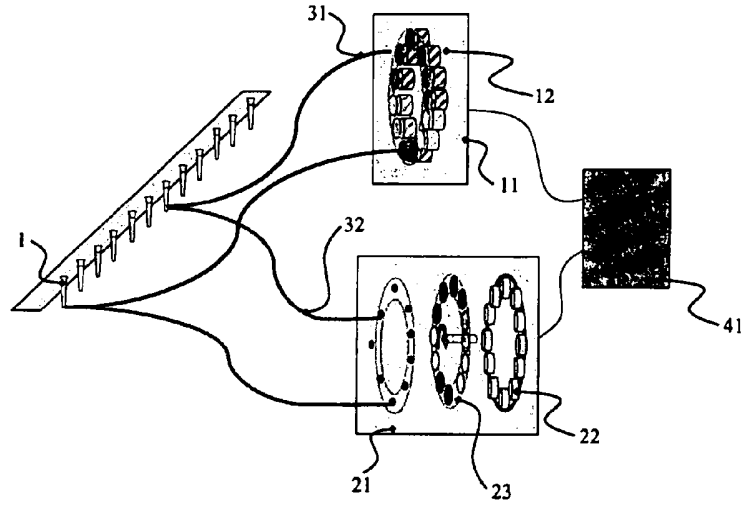


FIG. 2

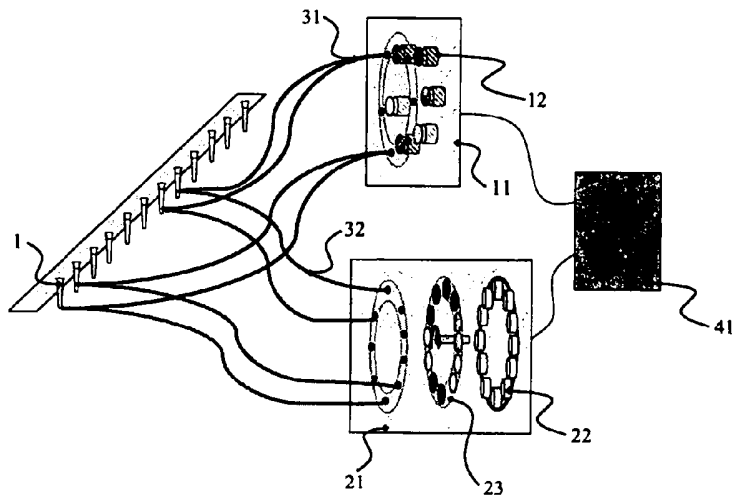


FIG. 3

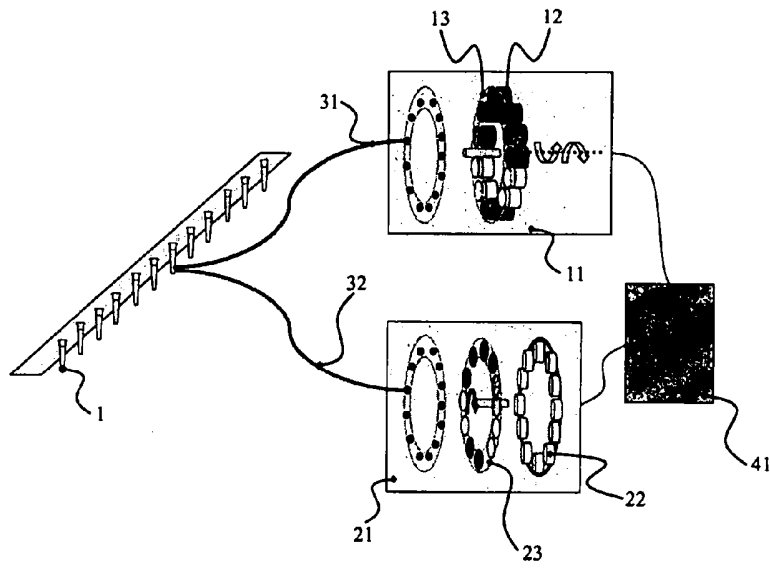


FIG. 4

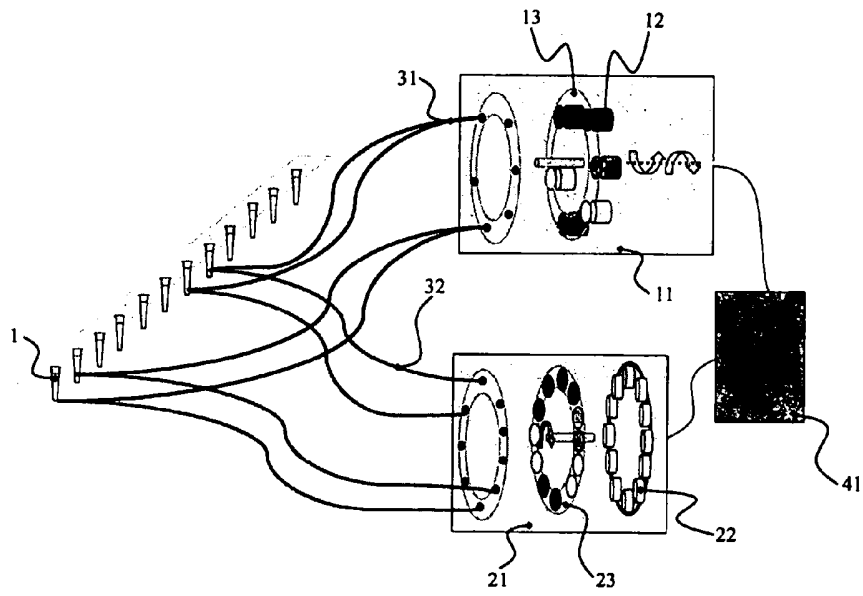


FIG. 5

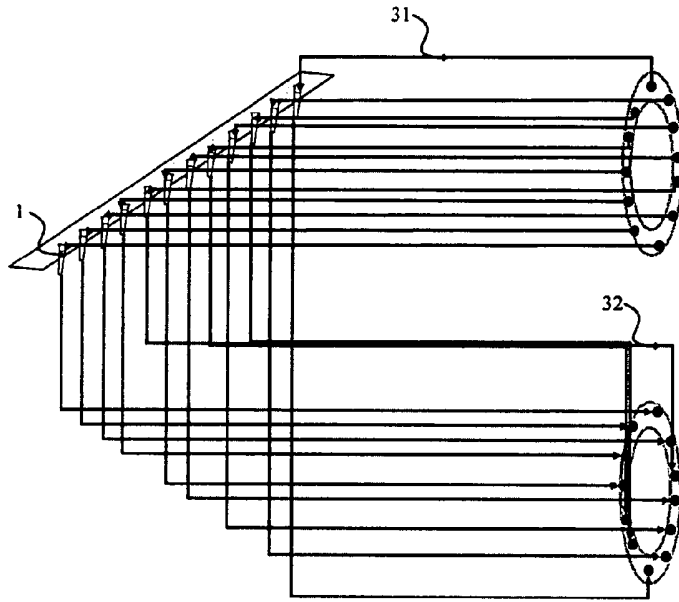
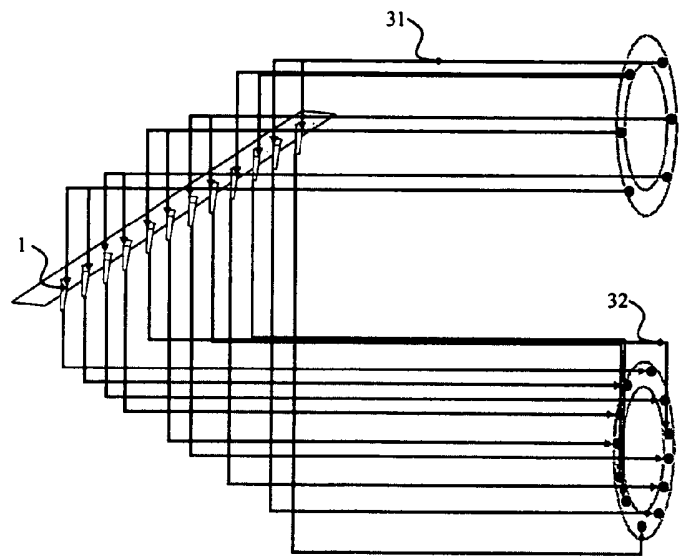


FIG. 6



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FIG. 7

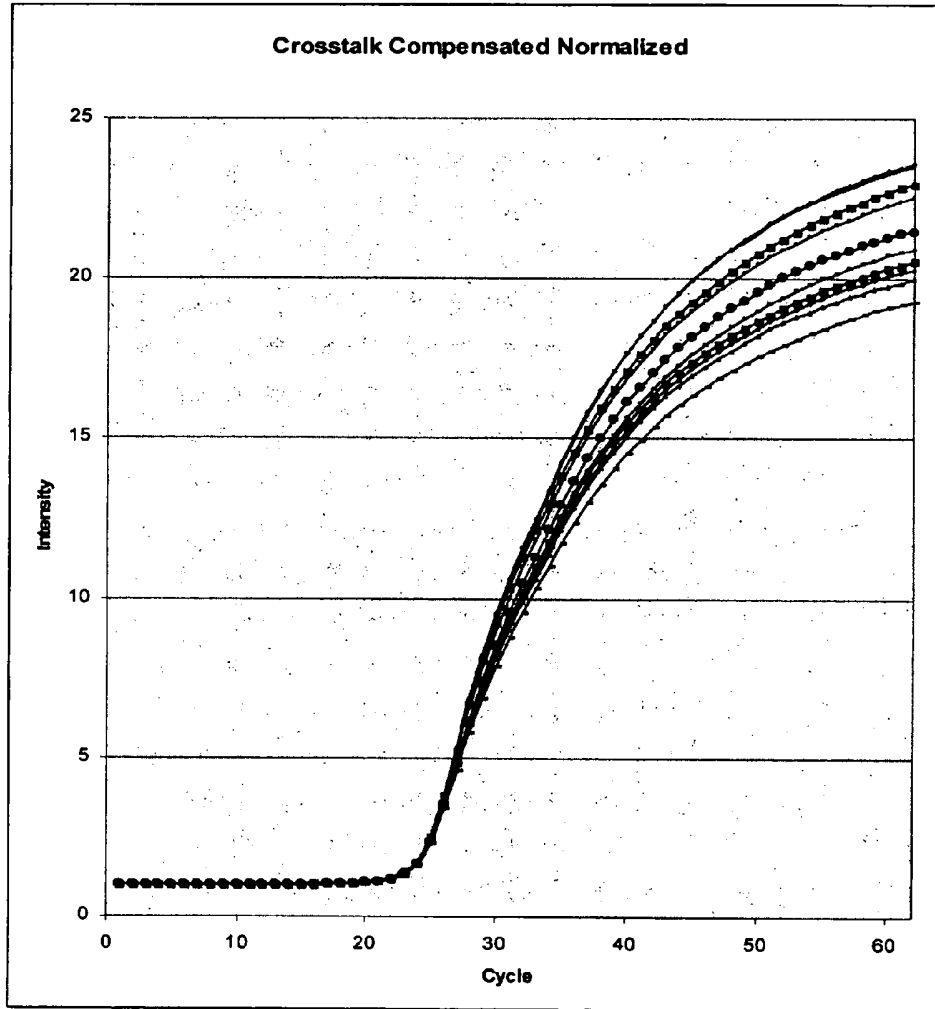


FIG. 8

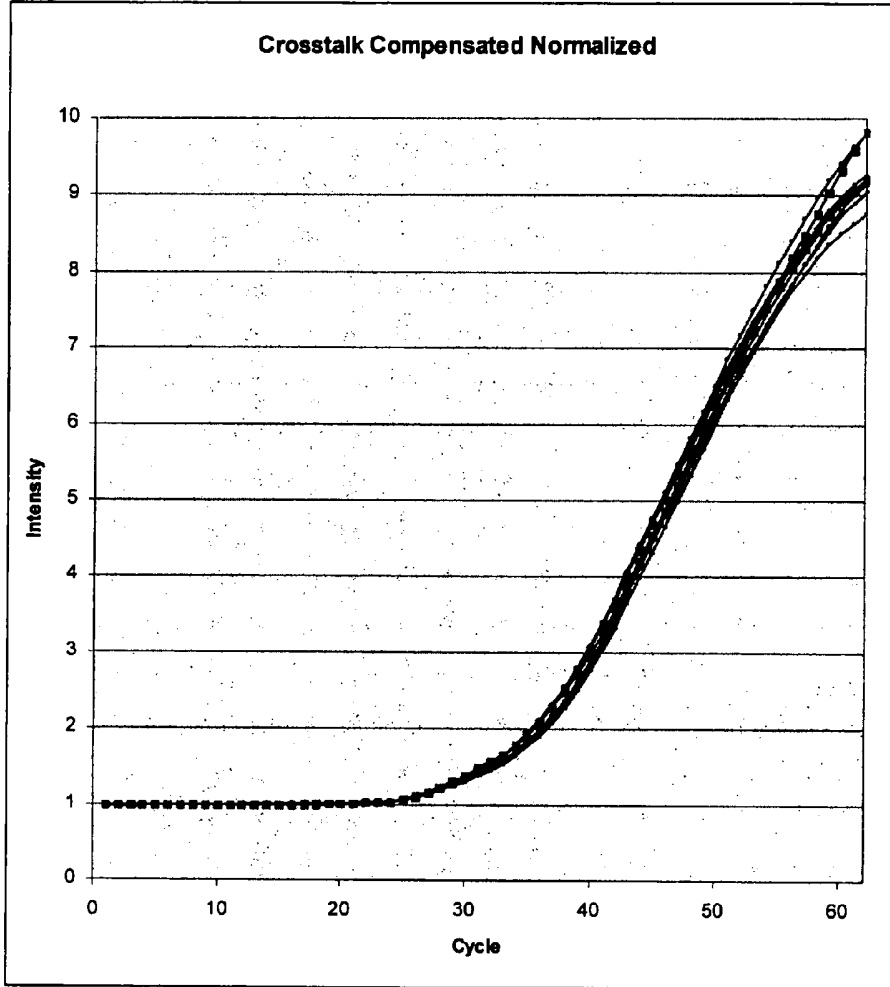


FIG. 9

Measurement Step	Reaction Region 1	Reaction Region 2	Reaction Region 3	Reaction Region 4	Reaction Region 5	Reaction Region 6	Reaction Region 7	Reaction Region 8	Reaction Region 9	Reaction Region 10	Reaction Region 11	Reaction Region 12
1	Channel1	Channel1	Channel6	Channel6	Channel6	Channel6	Channel4	Channel4	Channel4	Channel3	Channel2	Channel2
2	Channel2	Channel2	Channel1	Channel1	Channel6	Channel6	Channel5	Channel5	Channel4	Channel4	Channel3	Channel3
3	Channel3	Channel3	Channel2	Channel2	Channel1	Channel1	Channel6	Channel6	Channel5	Channel5	Channel4	Channel4
4	Channel4	Channel4	Channel3	Channel3	Channel2	Channel2	Channel1	Channel1	Channel6	Channel6	Channel5	Channel5
5	Channel5	Channel5	Channel4	Channel4	Channel3	Channel3	Channel2	Channel2	Channel1	Channel1	Channel6	Channel6
6	Channel6	Channel6	Channel5	Channel5	Channel4	Channel4	Channel3	Channel3	Channel2	Channel2	Channel1	Channel1