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(54) **OPTICAL DISCRIMINATION APPARATUS AND METHODS ADAPTED TO MONITOR REACTIONS**

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***G01N 21/64*** (2006.01)

(52) **U.S. Cl.**
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See application file for complete search history.

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(57) **ABSTRACT**

An optical discrimination apparatus adapted for use in PCR testing and the like. The apparatus includes a multi-color light emitter to emit excitation light, a sample holder configured to hold dye-marked nucleic acid fragments in a PCR solution at a position configured to receive the excitation light along a first direction, light emission collection optics configured to collect scattered excitation light and light emission (fluorescent emission) from the sample holder along a second direction that is approximately orthogonal to the first direction, a spectrally-dispersive element configured to spectrally disperse scattered light and emission light, and a spectral detector configured to receive the separated emission light and excitation light on different photosites of the spectral detector. Systems and methods are provided, as are other aspects.

**11 Claims, 4 Drawing Sheets**

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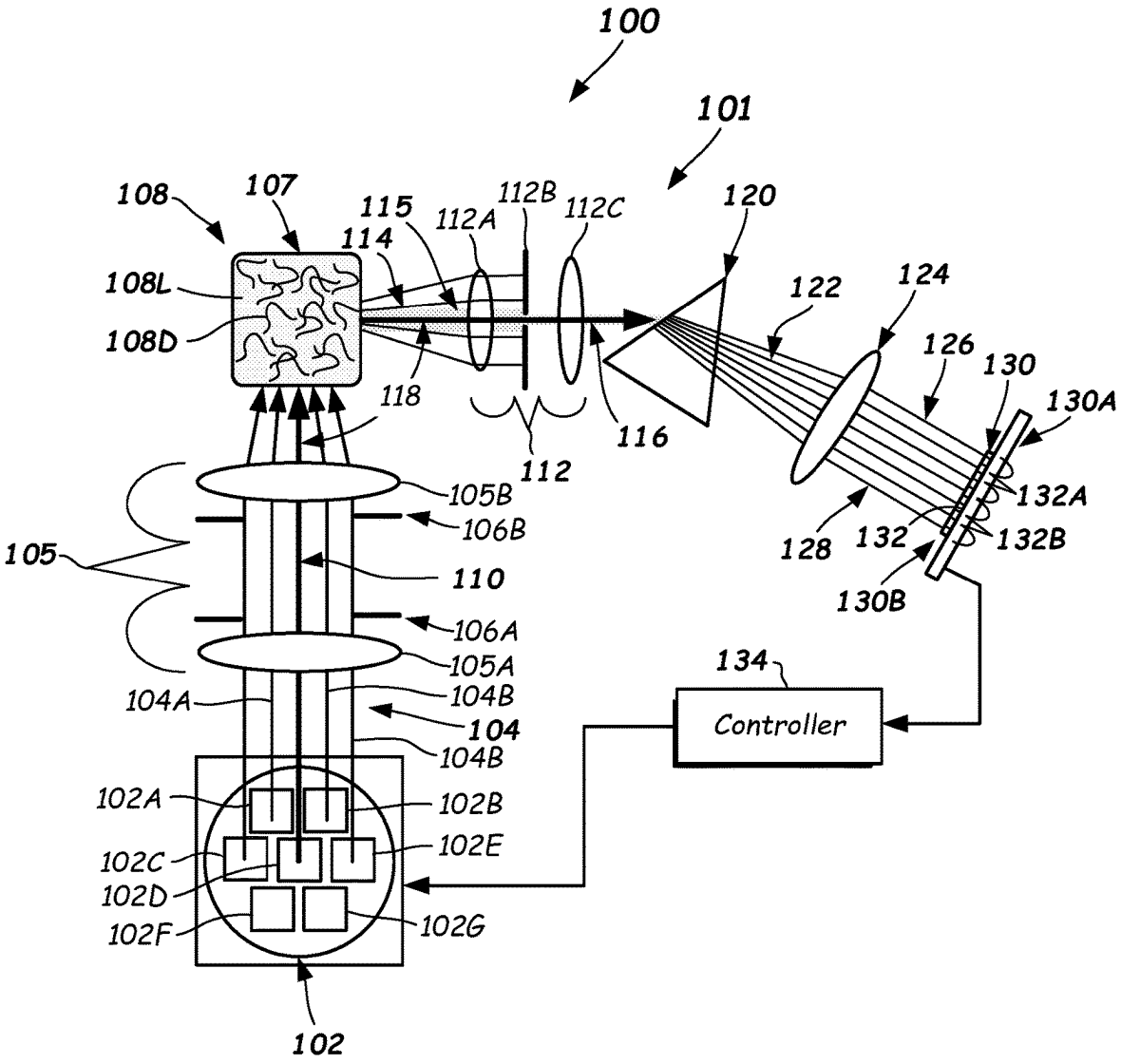
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101
108
107
115
114
112A
112B
112C
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108D
122
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116
112
105B
106B
105
110
106A
105A
132A
132B
128
132
130B
134
Controller
104A
104B
104
104B
102A
102B
102C
102E
102D
102F
102G
102

FIG. 1

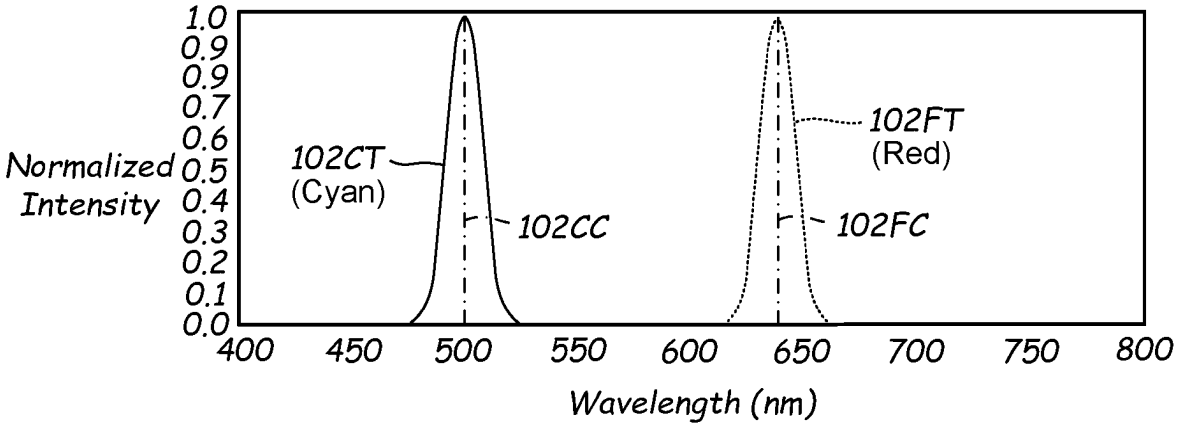
1.0
0.9
0.9
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Normalized Intensity
102CT
(Cyan)
102CC
102FT
(Red)
102FC
400
450
500
550
600
650
700
750
800
Wavelength (nm)

FIG. 2A

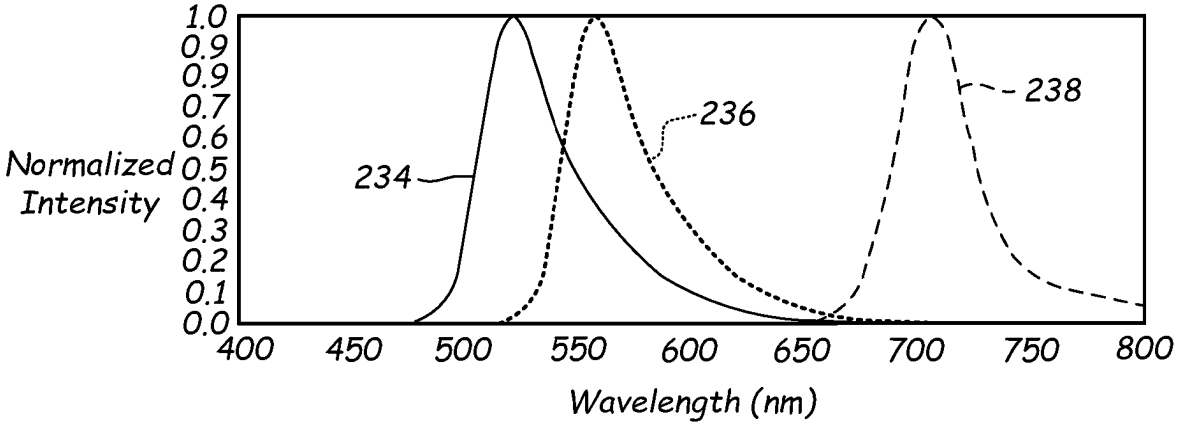
1.0
0.9
0.9
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Normalized Intensity
234
236
238
400
450
500
550
600
650
700
750
800
Wavelength (nm)

FIG. 2B

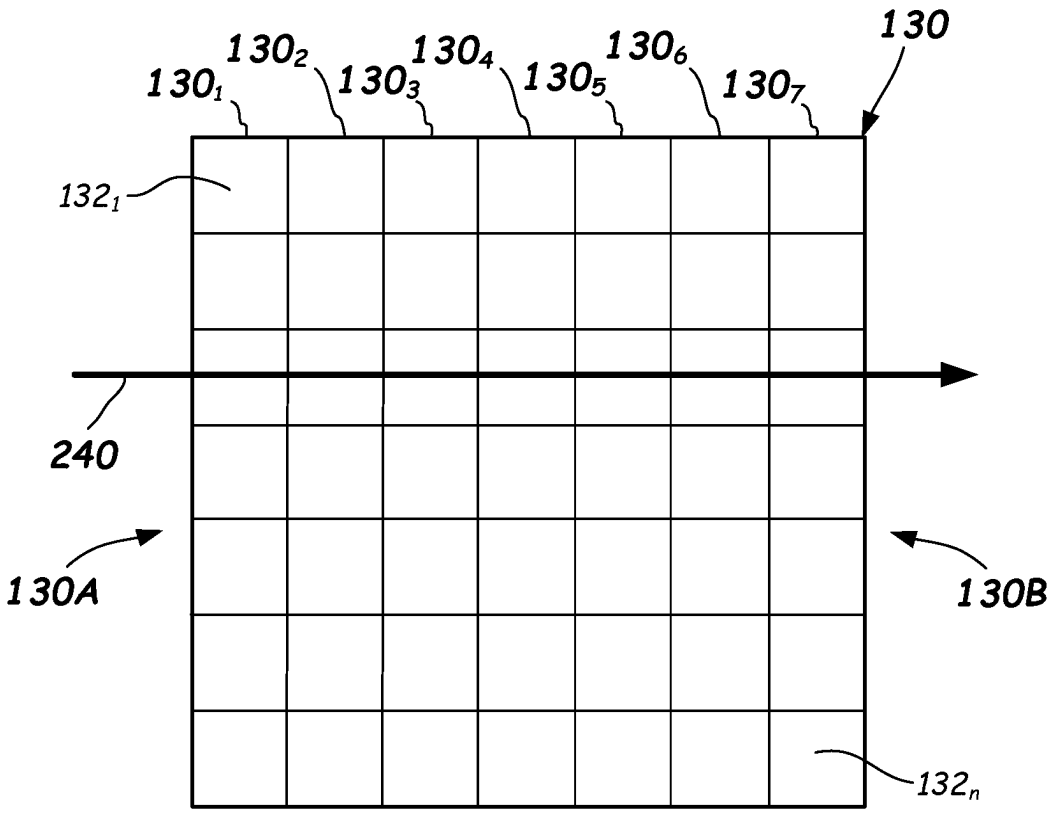
130
130₁
130₂
130₃
130₄
130₅
130₆
130₇
132₁
240
130A
130B
132ₙ

FIG. 2C

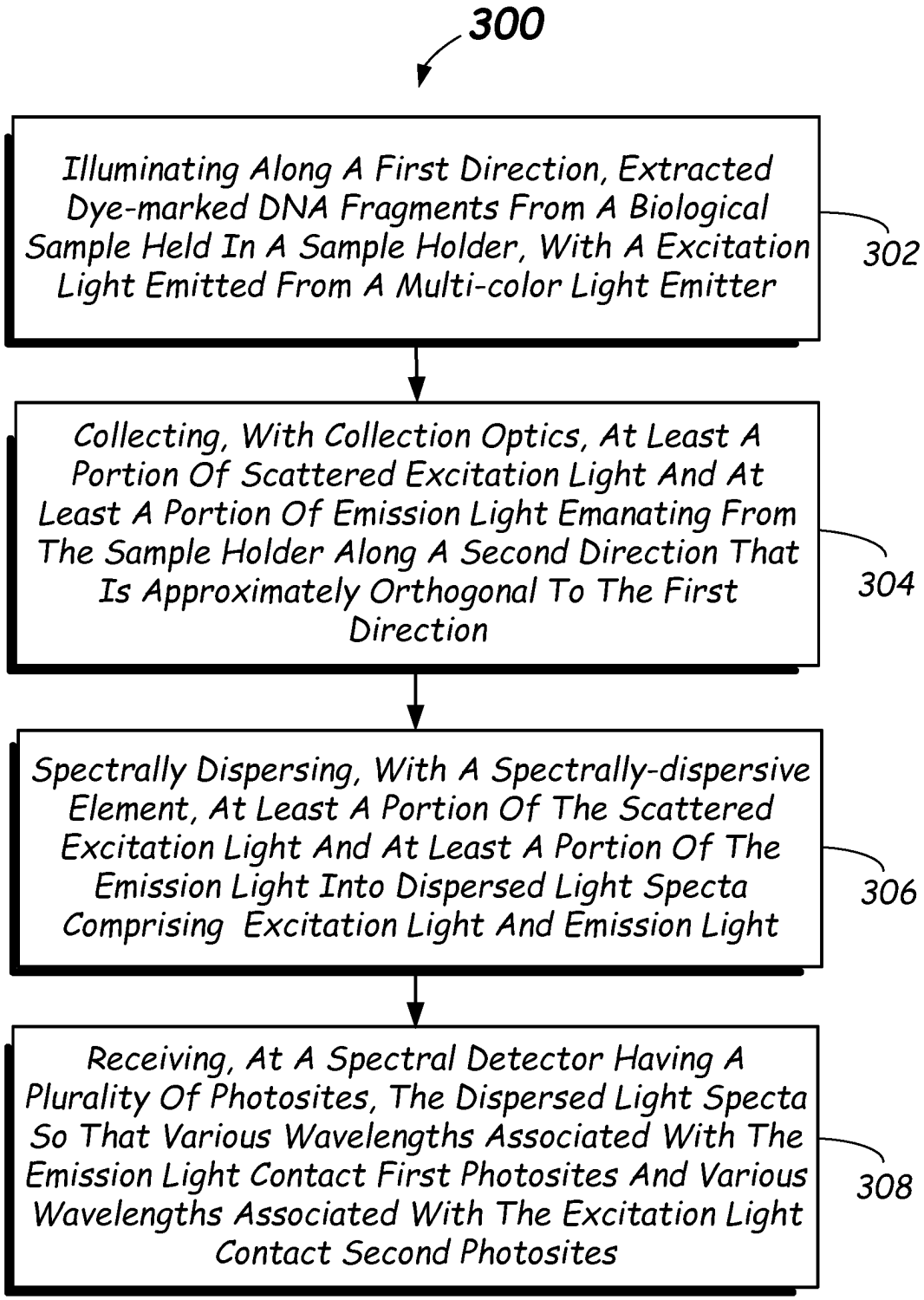
300
Illuminating Along A First Direction, Extracted Dye-marked DNA Fragments From A Biological Sample Held In A Sample Holder, With A Excitation Light Emitted From A Multi-color Light Emitter
302
Collecting, With Collection Optics, At Least A Portion Of Scattered Excitation Light And At Least A Portion Of Emission Light Emanating From The Sample Holder Along A Second Direction That Is Approximately Orthogonal To The First Direction
304
Spectrally Dispersing, With A Spectrally-dispersive Element, At Least A Portion Of The Scattered Excitation Light And At Least A Portion Of The Emission Light Into Dispersed Light Specta Comprising Excitation Light And Emission Light
306
Receiving, At A Spectral Detector Having A Plurality Of Photosites, The Dispersed Light Specta So That Various Wavelengths Associated With The Emission Light Contact First Photosites And Various Wavelengths Associated With The Excitation Light Contact Second Photosites
308

FIG. 3

# OPTICAL DISCRIMINATION APPARATUS AND METHODS ADAPTED TO MONITOR REACTIONS

## FIELD

This disclosure relates to optical discrimination apparatus and more particularly, to apparatus, systems, methods configured to carry out measurements of light emissions (e.g., fluorescent light) emanating from a reaction vessel containing dye-labeled nucleic acids.

## BACKGROUND

In some automated optical discrimination systems, a sample container (e.g., a cuvette) containing extracted and labeled components (e.g., four nucleotides of nucleic acid strands for DNA: adenine (A), guanine (G), cytosine (C), and thymine (T)) extracted from a specimen (e.g., biological specimen) and amplified via known PCR sample preparation methods can be positioned at a desired location in an optical-based system. Thereafter, readings can be obtained of light emissions (e.g., fluorescent light) emanating from the extracted and labeled components.

Such optical discrimination systems can utilize optical components such as a white light emitting diode (LED) as a light source, one or more filters, one or more dichroic mirrors, various focusing optics, and a detection sensor. In particular, in nucleic acid sequencing to determine the nucleotide sequence of particular nucleic acid molecules, the nucleotides (AGCT) in DNA fragments may be labeled with four separate fluorescent markers in current sequencing methods. The fluorescent markers (fluorophores) are molecules that are capable of absorbing the filtered excitation light from the LED and emitting it at one or more well-defined wavelengths. The fluorescent dye markers are incorporated into the DNA strands by PCR processing and provide the extracted and labeled DNA. For example, ddATP can be labeled with a green dye; ddGTP can be labeled with a yellow dye; ddCTP can be labeled with blue; and ddTTP can be labeled with red dye. Then the sequence of the DNA can be determined by automated techniques using measured fluorescence intensity and wavelength data.

In current fluorescence-based optical discrimination systems, for each wavelength of excitation light, one or more filters and one or more dichroic mirrors are employed that pass one wavelength of light from the white spectrum, but cuts out all others. Such optical filters and dichroic mirrors tend to be expensive and quite complicated. A different filter and dichroic mirror can be used, such as provided on a rotating filter wheel or translation stage for each separate wavelength of emitted excitation light from the white light LED, so as to enable excitation of different colors of fluorescent dyes. For example, to discriminate four different dyes, generally four different filters and/or dichroic mirrors are used. Hence, the complication and expense of these prior art optical interrogation systems is relatively high.

## SUMMARY

In some embodiments, an optical discrimination apparatus is provided. The optical discrimination apparatus includes a multi-color light emitter configured to emit excitation light, a sample holder configured to hold extracted dye-marked nucleic acid fragments from a biological sample, located at a position configured to receive the excitation light emitted from the multi-color light emitter along a first direction, light emission collection optics configured to collect scattered light and emission light from the sample holder along a second direction that is approximately orthogonal to the first direction, a spectrally-dispersive element configured to spectrally disperse the scattered light and the emission light into dispersed light spectra, and a spectral detector configured to receive at least some of the dispersed light spectra wherein the spectral detector is configured to receive at least some of the emission light and some of the excitation light on different photosites of the spectral detector.

In some embodiments, a multiplexed optical discrimination system for fluorescence detection in polymerase chain reaction testing is provided. The multiplexed optical discrimination system includes a multi-color light emitter configured to individually emit multiple wavelengths of excitation light at multiple central wavelengths, a sample holder configured to hold extracted dye-marked nucleic acid fragments from a biological sample, the sample holder located at a position configured to receive the excitation light emitted from the multi-color light emitter along a first direction, light emission collection optics configured to collect scattered light and emission light from the sample holder along a second direction that is approximately orthogonal to the first direction, a spectrally-dispersive element configured to spectrally disperse at least a portion of the scattered excitation light and the emissions light into the spectral detector, and a controller comprising a memory storing executable instructions, the executable instructions including instructions to: cause the multi-color light emitter to emit the excitation light at single central wavelength, receive signals representative of the emission light on first photosites of the spectral detector, and receive signals representative of the scattered light on second photosites of the spectral detector. The scattered light is scattered excitation light and the emission light comprises fluorescent light emissions.

In some embodiments, a method of multiplexed optical discrimination for fluorescence detection in polymerase chain reaction testing is provided. The method includes illuminating along a first direction, extracted dye-marked nucleic acid fragments from a biological sample held in a sample holder, with a excitation light emitted from a multi-color light emitter, collecting, with collection optics, excitation light and emission light emanating from the sample holder along a second direction that is approximately orthogonal to the first direction, spectrally dispersing, with a spectrally-dispersive element, at least a portion of the scattered light and at least a portion of the emission light into dispersed light spectra comprising emission light and excitation light, and receiving, at a spectral detector having a plurality of photosites, the dispersed light spectra so that various wavelengths associated with the emission light contact first photosites and various wavelengths associated with the excitation light contact second photosites.

Numerous other aspects are provided in accordance with these and other aspects of the disclosure. Other features and aspects of the present disclosure will become more fully apparent from the following detailed description, the claims, and the accompanying drawings.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic diagram of an optical discrimination apparatus according to embodiments of the present disclosure.

FIG. **2**A is a spectral plot of two example colors of excitation light (Cyan and Red) from a multi-color light emitter according to embodiments of the present disclosure.

FIG. **2**B is a spectral plot of emission light spectra for three different dyes that are tagged to the nucleic acid fragments according to embodiments of the present disclosure.

FIG. **2**C is a plan view of a spectral detector configured to collect scattered excitation light and emission light that have been spectrally-separated according to embodiments of the present disclosure.

FIG. **3** is a flowchart depicting an example method of multiplexed optical discrimination for fluorescence detection in polymerase chain reaction (PCR) testing according to embodiments of the present disclosure.

## DETAILED DESCRIPTION

In view of the above expressed issues and concerns, systems, methods, and apparatus that have relatively lower cost and/or complexity are desired. Further, such above-described systems are difficult to adapt to new dye colors that may fluoresce at different wavelengths, generally requiring different filters therefor. Thus, systems and apparatus that are readily adaptable to use of a new fluorescent dye that are configured to fluoresce at any new wavelength would be desirable.

In a first aspect, systems and apparatus are provided that can be implemented without any filters or dichroic mirrors. Thus, filter-less optical detection methods and apparatus adapted to detect fluorescence of multiple fluorophores is provided. Thus, advantageously, fewer components and complexity can be used resulting in relatively lower cost and lower complexity. Further, systems and apparatus with no moving parts can be implemented. In particular, systems and apparatus enabling use of a multitude of desired dyes, as well as apparatus and systems that are future-proofed are provided. Moreover, very low limits of detection can be obtained.

The systems and apparatus described herein can be filter-less, thus eliminating expensive components. Further, the systems can include multiple different wavelength light sources that can be individually excited to produce different colors of excitation light. Further, the excitation of different fluorescent dyes can be accomplished with no change to the structure of the apparatus, (e.g., no changing of filters and/or dichroic mirrors) as in the prior art, other than the addition of the light emitter adapted to emit the new color of excitation light. In particular also, the relative ratio of excitation intensity Ee to fluorescence emission intensity Ef at the detector tends to be quite low, whereas the ratio Ee/Ef in the prior art can be as high as 1,000,000:1. Thus, signal-to-error ratio can be dramatically improved.

Further details and examples of apparatus, systems, and methods of the disclosure are provided in FIGS. **1-3** herein.

FIG. **1** depicts an example embodiment of an optical discrimination system **100** adapted to measure intensities and wavelengths of fluorescence (fluorescent emissions) of multiple dyes tagged to nucleic acid strands **108**D of a sample contained in a sample holder **107** at a sample location **108**. The optical discrimination system **100** includes and optical discrimination apparatus **101** controlled by a suitable controller **134**.

In more detail, the optical discrimination apparatus **101** comprises a multi-color light emitter **102**, a sample holder **107**, light emission collection optics **112**, a spectrally-dispersive element **120**, and spectral detector **130**. Other optical components may be present in the optical discrimination apparatus **101**, as will be apparent from the following.

Optical discrimination apparatus **101** comprises a multi-color light emitter **102** that can be configured to emit excitation light **104** at more than one color at more than one distinct wavelength ranges. For example, as shown, multi-color light emitter **102** can comprise seven individual light sources **102**A-**102**G, six of which are configured to emit light at an individual predominant peak wavelength $\lambda_0$. The individual predominant wavelength (peak) for each is designated as a central wavelength $\lambda_0$, but it should be understood that each of the individual colored light sources **102**A-**102**F will produce a distinct narrow spectral range about their central wavelength $\lambda_0$. White light may have multiple peaks and a quite broad range.

For example, as shown below in FIG. **2**A, an individual light source **102**C (Cyan) may emit a particular color signature or trace **102**CT of normalized intensity versus wavelength (nm). The trace (e.g., **102**CT) will include the central wavelength $\lambda_0$ (peak) **102**CC, in this case located at approximately 500 nm, yet the spectral distribution of normalized intensity will comprise a distribution about the central wavelength **102**CC such as is shown, for example, such as a normal or slightly non-normal distribution. The other light sources **102**A-**102**B and **102**D-**102**F) may emit light as shown in Table 1. Each has a central wavelength $\lambda_0$ (peak) and a narrow spectral range about the central wavelength $\lambda_0$. Thus, each color can be emitted from the light emitter **102**, independently. Red colored light source trace **102**FT is shown with its corresponding central wavelength $\lambda_0$ **102**FC.

TABLE 1

Example light sources

| Trace | Color | Approx. Central Wavelength (nm) | Approx. Wavelength Range (nm) |
|---|---|---|---|
| 102AT | Violet | 405 | 370-450 |
| 102BT | Blue | 450 | 420-490 |
| 102CT | Cyan | 500 | 450-550 |
| 102DT | Green | 543 | 475-575 |
| 102ET | Amber | 576 | 550-620 |
| 102FT | Red | 633 | 590-660 |
| 102GT | Cool White | 430 and 570 | 400-800 |

Although seven individual light sources are shown in Table 1, more or less number of individual light sources can be used, and different central wavelengths $\lambda_0$ may be used depending upon the particular dye being used and its excitation characteristics. Individual light sources may be paired with particular dyes being used for excitation thereof. Individual light sources can be light emitting diodes (LEDs), for example. The multi-color light emitter **102** may include multiple different colored LEDs and may also include a flat lens in some embodiments.

In some embodiments, the multi-color light emitter **102** can output red, amber, green, cyan, blue, and violet. The the multi-color light emitter **102** can also emit white light, which may be used for system calibration or other purposes. However, any suitable combination of colors can be used, depending on the particular series of dyes that are tagged to the nucleic acid strands **108**D. DNA as used herein is used to denote deoxyribonucleic acid. However_, the present invention is equally applicable to ribonucleic acid (RNA).

The term nucleic acid denotes DNA and RNA, and, thus, analysis of either DNA or RNA may be undertaken by using the present disclosure.

In some embodiments, the multi-color light emitter **102** may be a LZ7 series LUXIGEN™ available from LED ENGIN of San Jose, CA Other suitable types of the multi-color light emitter **102** can be used.

Optical discrimination apparatus **101** further includes a sample holder **107**, configured to hold extracted dye-marked nucleic acid fragments **108**D in a PCR solution that have been obtained (extracted in eluate) from a biological sample, such as bodily fluid (e.g., serum, plasma, urine, and the like). The sample holder **107** can be a cuvette or other vessel that is optically transparent or translucent, such as a plastic or glass. The walls of the sample holder **107** may be planar or can be curved or combinations thereof.

The dye-marked nucleic acid fragments **108**D are provided in a suitable liquid **108**L, such as a PCR liquid including, for example, a solution of eluate, PCR master mix, and primer (or probe), and possibly a reagent and/or deionized water. The sample holder **107** is located at a position **108** configured to receive the excitation light **104** emitted from the multi-color light emitter **102** along a first direction **110**. The excitation light **104** can be provided from any one of the multiple individual light sources (e.g., light sources **102**A-**102**G). The multi-color light emitter **102** can be a multi-color emitter configured to emit multiple colors of light having central wavelengths $\lambda_0$ ranging from 350 nm to 700 nm. Multi-color light emitter **102** may optionally include the capability of emitting white light.

Drive signals from controller **134** to a particular one of the light sources **102**A-**102**G can be provided to cause the illumination and emission of the excitation light **104** therefrom. The multi-color light emitter is a capable of emitting at least three individual colors of light. For example, red (R), Green (G) and Blue (B) can be used. Other numbers of light sources can be used, such as 4 or more individual light sources, such as 5 or more individual light sources, such as 6 or more individual light sources, or even such as 6 or more individual light sources coupled with a white light source.

Optical discrimination apparatus **101** may further include collimating components **105**. Collimating components **105** can include collimating lens **105**A, focusing lens **105**B to concentrate light intensity into the sample location **108**, and apertures **106**A, **106**B. The apertures **106**A, **106**B prevent the divergent light from the various individual sources of the multi-color light emitter **102** from being exposed to the sample location **108**, and thereby allowing the central collimated light beam in the first direction **110** through to the into the sample location **108**.

Optical discrimination apparatus **101** may further include light emission collection optics **112** configured to collect scattered light **114** and emission light **115** emanating from the sample holder **107** along a second direction **116** that is approximately orthogonal (collection angle **118**) to the first direction **110**. By approximately orthogonal it is meant that the second direction **116** is oriented at a collection angle **118** of from 70 degrees to 110 degrees, or even from 80 degrees to 100 degrees, in relationship to the first direction **110**.

Optical discrimination apparatus **101** may further include a spectrally-dispersive element **120** that may be configured to spectrally disperse at least a portion of the scattered light **114** and emission light **115** into dispersed light spectra **122** comprising emission light and excitation light. Emission light **115** may be confined predominantly to one end of the spectrum, while excitation light may be confined predominantly to the other end of the spectrum. The spectrally-dispersive element **120** can be a prism (e.g., a triangular prism), or optionally a diffraction grating, although a diffraction grating may have more optical losses.

Optical discrimination apparatus **101** may further include a focusing lens **124** that may be configured to focus the dispersed light spectra **122** onto the spectral detector **130**. Any suitable focusing lens or combination of lenses may be used.

Optical discrimination apparatus **101** may further include the spectral detector **130** configured to receive at least some of the dispersed light spectra **122** wherein the spectral detector **130** is configured to receive at least some of the emission light **126** and at least some of the excitation light **128** on different photosites $\mathbf{132}_1$ through $\mathbf{131}_n$ of the spectral detector **130**. The different photosites $\mathbf{132}_1$ through $\mathbf{131}_n$ of the spectral detector **130** can be arranged as shown in FIG. **2**C, wherein columns $\mathbf{130}_1$ through $\mathbf{130}_7$ are arranged from a first side **130**A of the spectral detector **130** to a second side **130**B of the spectral detector **130**. The spectral detector **130** is an optical sensor (e.g., a sensor array) and may be a CCD device (e.g., monochrome CCD camera), CMOS device, or the like.

Spectral detector **130** can comprise a plurality of different photosites $\mathbf{132}_1$ through $\mathbf{132}_n$ and the spectrally-dispersive element **120** separates various wavelengths of light so that the wavelengths of light that are emission light **126** contact different ones of the plurality of photosites as compared to the scattered excitation light **128**. For example, emission light **126** can contact a first group **132**A of the photosites, that are arranged in columns $\mathbf{130}_1$ through $\mathbf{130}_7$ and the scattered light **128** can contact a second group **132**B of the photosites **132** arranged in columns $\mathbf{130}_1$ through $\mathbf{130}_7$.

Data signals from the spectral detector **130** may be communicated to the controller **134**, which processes them to detect at least the portions of the signals that relate to the emission light **126**. Thus, for any particular excitation input from the multi-color light emitter **102**, the scattered light **128** can be discriminated from the emission light **126** and respective data can be obtained. FIG. **2**B, for example, illustrates the emissions plots **234**, **236**, **238** of normalized intensity versus wavelength (in nm) for each of three different example dyes. For example, the trace of a first dye **234** can be for FAM dye; trace of a second dye **236** can be for CAL Fluor Orange 560 dye; and trace of a third dye **238** can be for Quasar 705 dye. Pixel intensities across one or more rows of the spectral detector **130** can be recorded. Row is signified by arrow **240**. Each column $\mathbf{130}_1$-$\mathbf{130}_7$ may include hundreds of pixels. Each pixel along the row corresponds to a discreet wavelength or small wavelength range. Thus, by recording the emission responses to particular color excitation inputs, the particular dyes present can be determined, as well as magnitudes thereof. For example, excitation with **102**CT (cyan) as shown in FIG. **2**A causes emission light **126** to be received at the detector **130** and fluorescence traces **234** and **236** can be reconstructed therefrom via multiplexing method described herein based upon measured magnitude of light intensity as a function of wavelength. Likewise, excitation with red source **102**FT in FIG. **2**A causes emission light **126** to be received at the detector **130** and fluorescence trace **238** can be reconstructed from the detector readings via the multiplexing method herein.

In one aspect, the optical discrimination apparatus **101** is devoid of any optical filter, i.e., the optical discrimination apparatus **101** does not contain any filters therein. As such, the system **101** is much more adaptable and multiple input wavelengths can be used as individual excitation light inputs in rapid succession, without having to change out a filter or

dichroic mirror as in the prior art. Thus, not only can the optical discrimination apparatus **101** be manufactured for relatively less cost, because the emission light **126** is captured orthogonally to the first direction **110** of the exciting light **104**, the overall level of intensity of the scattered excitation light **128** is less, so that on a relative basis, the ratio of emissions light **126** to the excitation light **128** is made larger as compared to prior art systems, thus the signal-to-noise ratio is advantageously made greater.

In another embodiment, the present disclosure is directed at a multiplexed optical discrimination system **100** for fluorescence detection in polymerase chain reaction testing. The multiplexed optical discrimination system **100** comprises the multi-color light emitter **102** configured to individually emit multiple wavelengths of excitation light **104** at multiple emission wavelengths (e.g., at individual central wavelengths $\lambda_0$ from 350 nm to 700 nm), and possibly also white light.

The multiplexed optical discrimination system **100** also includes a sample holder **107** configured to hold extracted dye-marked nucleic acid fragments **108**D from a biological sample, the sample holder **107** located at a position configured to receive the excitation light **104** emitted from the multi-color light emitter **102** along a first direction **110**.

Additionally, the multiplexed optical discrimination system **100** includes light emission collection optics **112** that are configured to collect scattered excitation light **114** and fluorescent emissions **115** emanating from the sample holder **107** along the second direction **116** that is approximately orthogonal to the first direction **110**.

A spectrally-dispersive element **120** of the multiplexed optical discrimination system **100** is configured to spectrally disperse at least a portion of the scattered excitation light **114** and of the emissions light **115** (e.g., florescent emissions) into dispersed light spectra **122** comprising emission light **126** and excitation light **128**, which are separated.

The spectral detector **130** of the multiplexed optical discrimination system **100** is configured to receive at least some of the dispersed light spectra **122** wherein the spectral detector **130** is configured to receive at least some of the emission light **122** and some of the excitation light **126** on a plurality of different photosites $\mathbf{132}_{1\text{-}n}$ of the spectral detector **130**.

The controller **134** of the multiplexed optical discrimination system **100** can comprise a memory storing executable instructions, and the executable instructions can include instructions to: cause the multi-color light emitter **102** to emit the excitation light **104** at single central wavelength $\lambda_0$ of one of the non-white light sources from multi-color light emitter **102**, and receive signals representative of the emission light **126** on a first group of photosites **132**A of the spectral detector **130**, and receive signals representative of the excitation light **128** on second group of photosites **132**B of the spectral detector **130**.

The multiplexed optical discrimination system **100** can be calibrated by using white light. Pixels of the spectral detector **130** can be calibrated to their corresponding spectral wavelength. A milky scattering medium (e.g., intralipid 20%) can be used in the sample holder **107** in order to facilitate scattering of the white light emitted from the multi-color light emitter **102**. The spectrally-dispersive element **120** (e.g., prism) then projects the "rainbow" (e.g., the spectrally-dispersed white light) onto different pixels on the group of photosites **132**A, **132**B of the spectral detector **130**. The locations of the individual colored LEDS (e.g., 405 nm (violet), 450 nm (blue), 500 nm (cyan), 543 nm (green), 576 nm (amber), and 633 nm (red) respectively) can be individually lighted to note the different colors are resolved at spatially-separated pixels on the groups of photosites **132**A, **132**B of the spectral detector **130**. This calibration can be used to assign pixels on the photosites **132**A, **132**B to their corresponding wavelengths by using the excitation LEDs or by white light LED with narrow band filters (about 10 nm) at various wavelengths between 400-800 nm. Pixel intensity averages for each pixel of the photosites **132**A, **132**B along the row **240** corresponding to the emission wavelengths can be used in calibrating the system **100** with various concentrations of dyes to obtain the emission coefficient matrix for the multiplexing method.

FIG. **3** illustrates a flowchart depicting a method **300** of multiplexed optical discrimination for fluorescence detection in polymerase chain reaction (PCR) testing. The method **300** comprises, in block **302**, illuminating along a first direction **110**, dye-marked nucleic acid fragments **108**D (in the PCR fluid) held in a sample holder **107** that were extracted from a biological sample, with a excitation light **104** (e.g., a single central wavelength $\lambda_0$ of light) emitted from a multi-color light emitter **102**. The excitation light **104** emitted may be an individual single color (e.g., Red), which may be followed in rapid sucession by excitation light **104** emitted at other colors (e.g., in sucession red, amber, green, cyan, blue, and violet, for example). Other colors may be used to excite other dyes in addition or in substitution for the foregoing. Several dyes and their corresponding excitation color are shown below in Table 2.

TABLE 2

Dyes and associated excitation colors

| Dye | Color |
|---|---|
| Quasar 705 | Red |
| Quasar 670 | Red |
| Pulsar 650 | Red |
| Cy5 | red |
| Texas Red | Amber |
| FAM | Blue |
| VIC | Cyan |
| JOE | Cyan |
| CAL Fluor Orange 560 | Cyan |

The method **300** further includes, in **304**, collecting, with collection optics **112**, scattered excitation light **114** and emission light **115** (e.g., fluorescent emissions) emanating from the sample holder **107** along a second direction **116** that is approximately orthogonal to the first direction **110**. The term "approximately" is defined the same as above.

In block **306**, the method **300** includes spectrally dispersing, with a spectrally-dispersive element **120** (e.g., prism or grating), at least a portion of the scattered light **114** and at least a portion of the emission light **115** into dispersed light spectra **122** comprising emission light **126** and excitation light **128**.

In block **306**, the method **300** includes receiving, at a spectral detector **130** having a plurality of photosites $\mathbf{132}_{1\text{-}n}$, the dispersed light spectra **122** so that various wavelengths associated with the emission light **126** contact a first group of photosites **132**A and various wavelengths associated with the excitation light **128** contact a second group of photosites **132**B. The photosites **132**A, **132**B may contain hundreds of pixels.

Following provision of the dispersed emission light **126** to the first group of photosites **132**A and various wavelengths of dispersed light associated with the excitation light **128** to the second group of photosites **132**B, the corresponding

intensity values of pixels at photosites **132**A, **132**B of at least the emissions light **126** at each excitation wavelength is collected by the controller **134**. This data may be analyzed through matrix calculations to obtain responses to the applied excitation light **104**, which may comprise one or more emission peaks.

In particular, the data can be analyzed using a multiplexing method that has been trained using calibration training sets for each dye to be used with different concentrations, such as 1.25 nM, 2.5 nM, 5 nM, 10 nM, 50 nM, and 100 nM, for example. Other suitable increasing nanomolar concentrations may be used. Pixel intensity averages for each pixel or photosites **132**A, **132**B across a pixel row from the spatial detector **130** can be obtained for each known concentration of each dye (Dye Concentration). From this data an emission coefficient matrix can be obtained as shown below using matrix algebra.

Pixel Intensity Average Emission Coeffient Matrix Dye Concentration

$$\begin{bmatrix} Intensity_{pixel1} \\ Intensity_{pixel2} \\ Intensity_{pixel3} \end{bmatrix} \begin{bmatrix} EM_{FAM1} & EM_{CAL1} & EM_{QUS1} \\ EM_{FAM2} & EM_{CAL2} & EM_{QUS2} \\ EM_{FAM3} & EM_{CAL3} & EM_{QUS3} \end{bmatrix} \begin{bmatrix} Conc_{FAM} \\ Conc_{CAL} \\ Conc_{QUS} \end{bmatrix}$$

Fluorescence emissions (emission light **126**) and excitation residue from scattered excitation light **128** can easily be discriminated as they occur at different locations on the photosites **132**A, **132**B of the spatial detector **130**, as determined by calibration with known dye concentrations. After sequentially exciting the sample holder **107** with the different colored lights and recording the emissions intensity responses thereto per pixel, the known emissions coefficient matrix constructed from the calibration can be used to determine the concentrations for each of the dyes present. Thus, the present disclosure allows multiplexing and determination of the concentrations of the particular dye-tagged nucleic acid components when multiple dyes are present.

Although the embodiments are described herein with reference to specific examples, the scope of the disclosure is not intended to be limited to the details and specific examples described herein. Rather, various modifications may be made to the embodiments and details within the scope and range of equivalents of the claims.

What is claimed is:

**1**. An optical discrimination apparatus, comprising:

a multi-color light emitter configured to emit excitation light;

a sample holder, configured to hold extracted dye-marked nucleic acid fragments from a biological sample, located at a position configured to receive the excitation light emitted from the multi-color light emitter along a first direction;

light emission collection optics configured to collect scattered light and emission light from the sample holder along a second direction that is approximately orthogonal to the first direction;

a spectrally-dispersive element configured to spectrally disperse the scattered light and the emission light into dispersed light spectra; and

a spectral detector configured to receive at least some of the dispersed light spectra wherein the spectral detector is configured to receive at least some of the excitation light and at least some of the emission light on different photosites of the spectral detector.

**2**. The optical discrimination apparatus of claim **1**, wherein the multi-color light emitter is configured to emit multiple colors of light each having a dominant wavelength $\lambda_0$ ranging from 350 nm to 700 nm.

**3**. The optical discrimination apparatus of claim **1**, wherein the multi-color light emitter is configured to emit six colors of light wherein the dominant wavelength $\lambda_0$ of individual emitters is approximately 405 nm, 450 nm, 500 nm, 543 nm, 576 nm, and 633 nm.

**4**. The optical discrimination apparatus of claim **1**, wherein the multi-color light emitter is a capable of emitting at least three individual colors of light.

**5**. The optical discrimination apparatus of claim **1**, wherein the spectral detector is an optical sensor comprising a plurality of photosites and the spectrally-dispersive element separates various wavelengths of light so that the various wavelengths of light contact different ones of the plurality of photosites.

**6**. The optical discrimination apparatus of claim **1**, wherein the spectrally-dispersive element is a prism.

**7**. The optical discrimination apparatus of claim **1**, wherein the spectrally-dispersive element is a grating.

**8**. The optical discrimination apparatus of claim **1**, wherein the optical discrimination apparatus is devoid of any optical filter.

**9**. The optical discrimination apparatus of claim **1**, wherein the second direction is oriented at a collection angle of from 70 degrees to 110 degrees in relationship to the first direction.

**10**. A multiplexed optical discrimination system for fluorescence detection in polymerase chain reaction testing, comprising:

a multi-color light emitter configured to individually emit multiple wavelengths of excitation light at multiple emission wavelengths;

a sample holder configured to hold extracted dye-marked nucleic acid fragments from a biological sample, the sample holder located at a position configured to receive the excitation light emitted from the multi-color light emitter along a first direction;

light emission collection optics configured to collect scattered excitation light and fluorescent emissions emanating from the sample holder along a second direction that is approximately orthogonal to the first direction;

a spectrally-dispersive element configured to spectrally disperse at least a portion of the scattered excitation light and the fluorescent emissions into dispersed light spectra comprising emission light and excitation light;

a spectral detector configured to receive at least some of the dispersed light spectra wherein the spectral detector is configured to receive at least some of the emission light and some of the excitation light on different photosites of the spectral detector; and

a controller comprising a memory storing executable instructions, the executable instructions including instructions to:

cause the multi-color light emitter to emit the excitation light at a single emission wavelength, and

receive signals representative of the emission light on first group of photosites of the spectral detector, and

receive signals representative of the excitation light on second group of photosites of the spectral detector.

**11**. A method of multiplexed optical discrimination for fluorescence detection in polymerase chain reaction testing, comprising:

illuminating along a first direction, extracted dye-marked nucleic acid fragments from a biological sample held in a sample holder, with a excitation light emitted from a multi-color light emitter;

collecting, with collection optics, scattered excitation light and emissions light emanating from the sample holder along a second direction that is approximately orthogonal to the first direction;

spectrally dispersing, with a spectrally-dispersive element, at least a portion of the scattered excitation light and at least a portion of the emissions light **115** into dispersed light spectra comprising emission light **126** and excitation light; and

receiving, at a spectral detector having a plurality of photosites, the dispersed light spectra so that various wavelengths associated with the emission light contact a first group of photosites and various wavelengths associated with the excitation light contact a second group of photosites.

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