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(54) **UNIVERSAL MULTIDETECTION SYSTEM FOR MICROPLATES**

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(58) **Field of Classification Search** 356/319, 356/301, 316, 317, 318, 333; 422/82.08; 436/172

See application file for complete search history.

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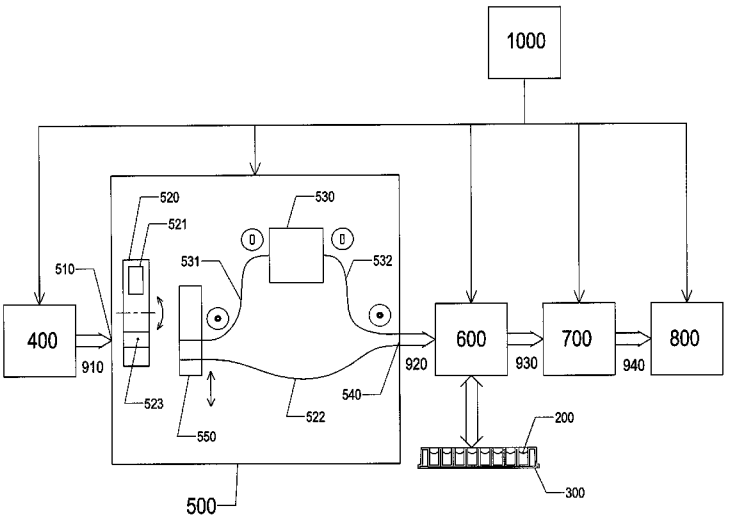
(74) *Attorney, Agent, or Firm* — Sughrue Mion, PLLC

(57) **ABSTRACT**

An apparatus and a method for optically analyzing a sample are provided. The apparatus includes a first optical device that transmits a narrow waveband of light and has a first filter and a first monochromator that provide different paths for the narrow waveband of the light. The apparatus may also include a light source that generates the light as broadband excitation light, in which case the first optical device transmits a narrow waveband of the broadband excitation light through the first filter or the first monochromator. Further, the apparatus may include a second optical device that directs the narrow waveband of the broadband excitation light onto the sample and receives emission light from the sample, a third optical device that transmits a narrow waveband of the emission light, and a detector that converts the narrow waveband of the emission light into an electrical signal.

31 Claims, 10 Drawing Sheets



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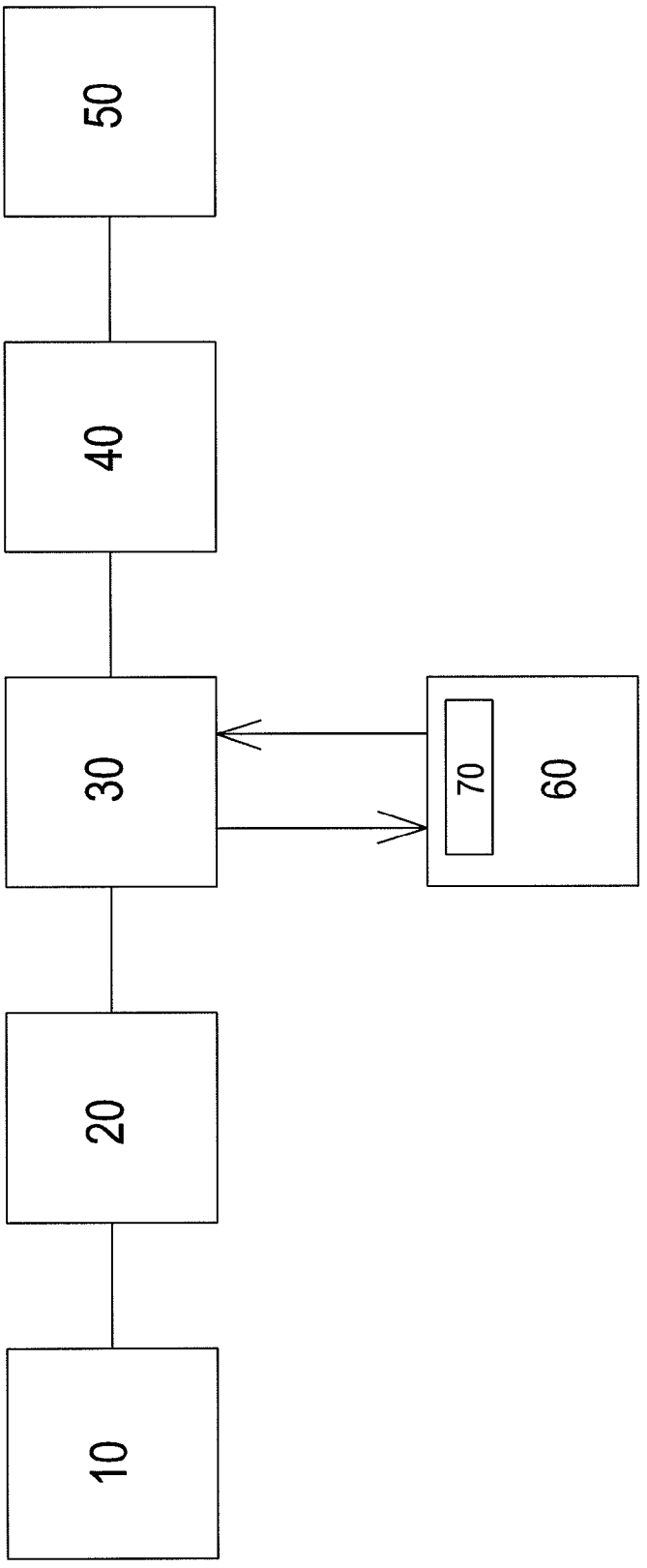

Fig. 1

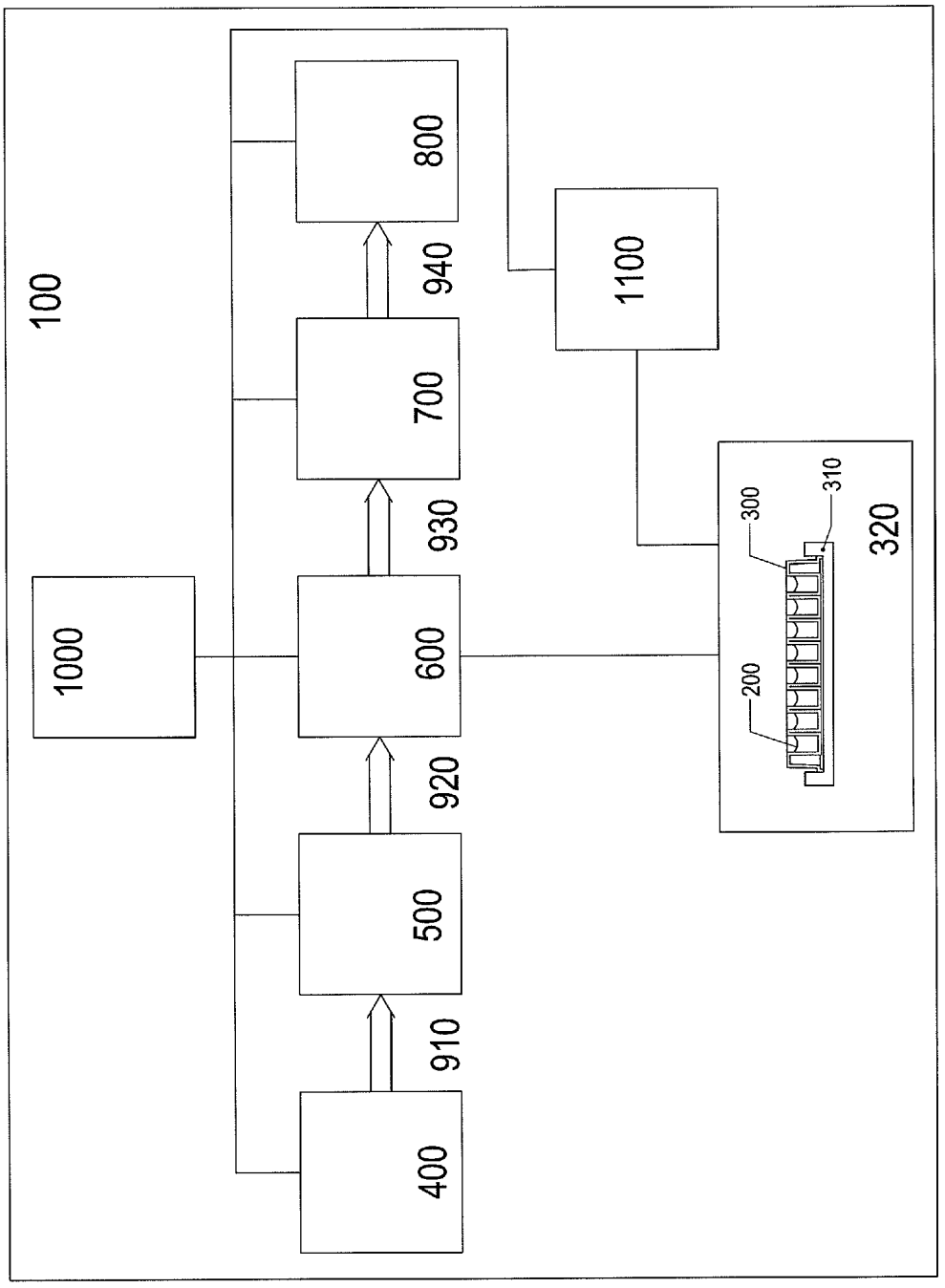
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Fig. 2

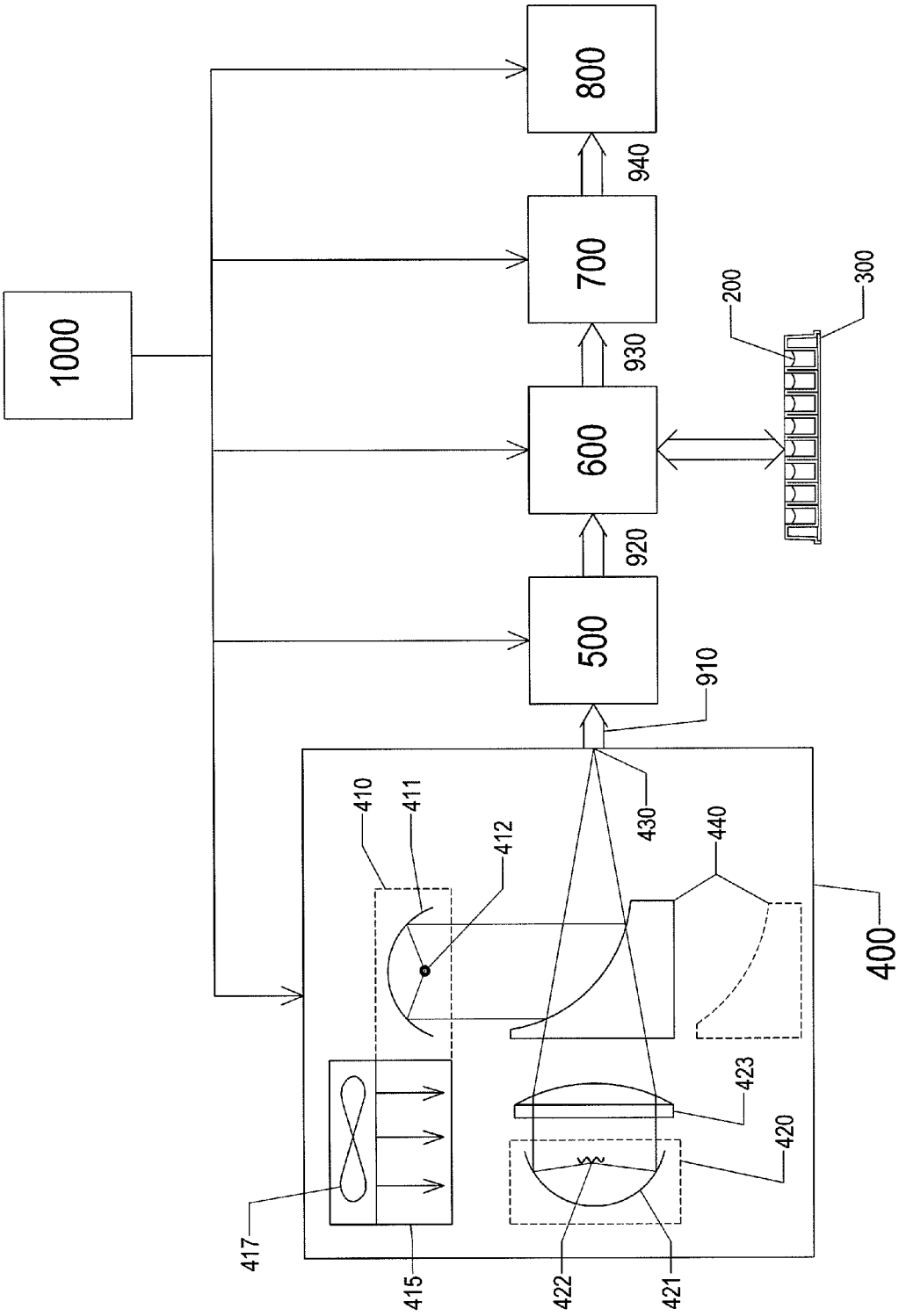
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Fig. 3

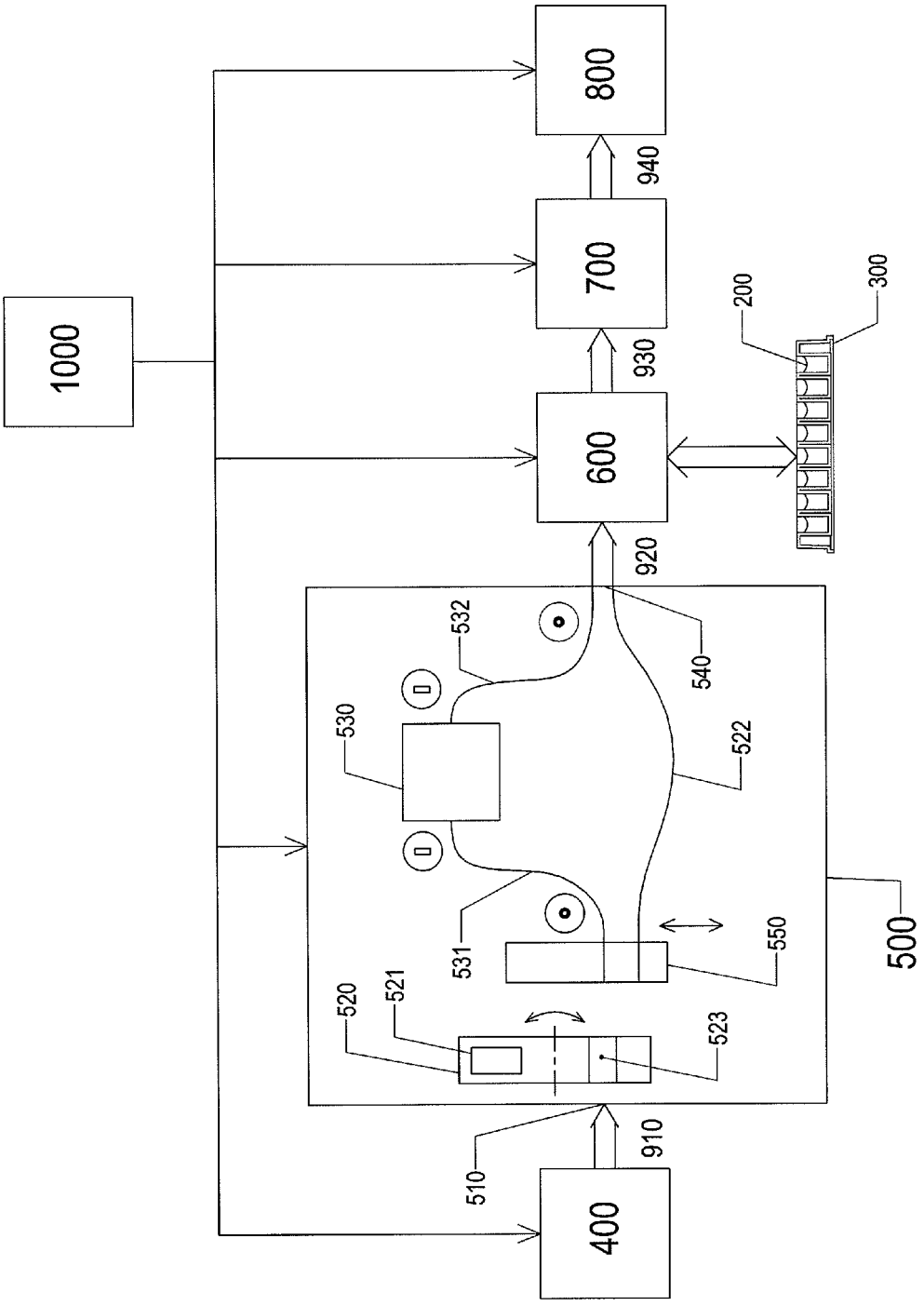
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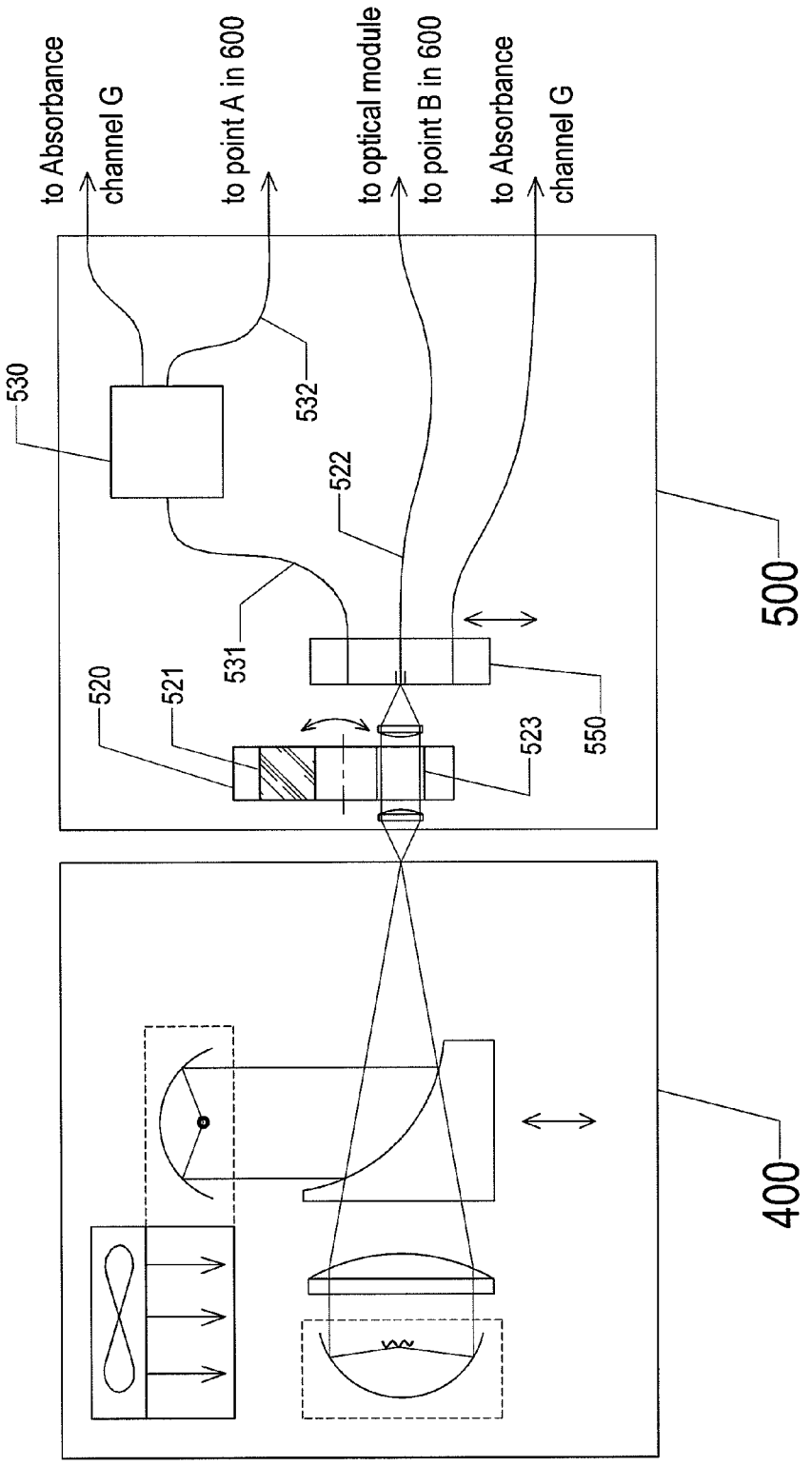
Fig. 4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to point A in 600
to optical module
to point B in 600
to Absorbance channel G
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Fig. 5

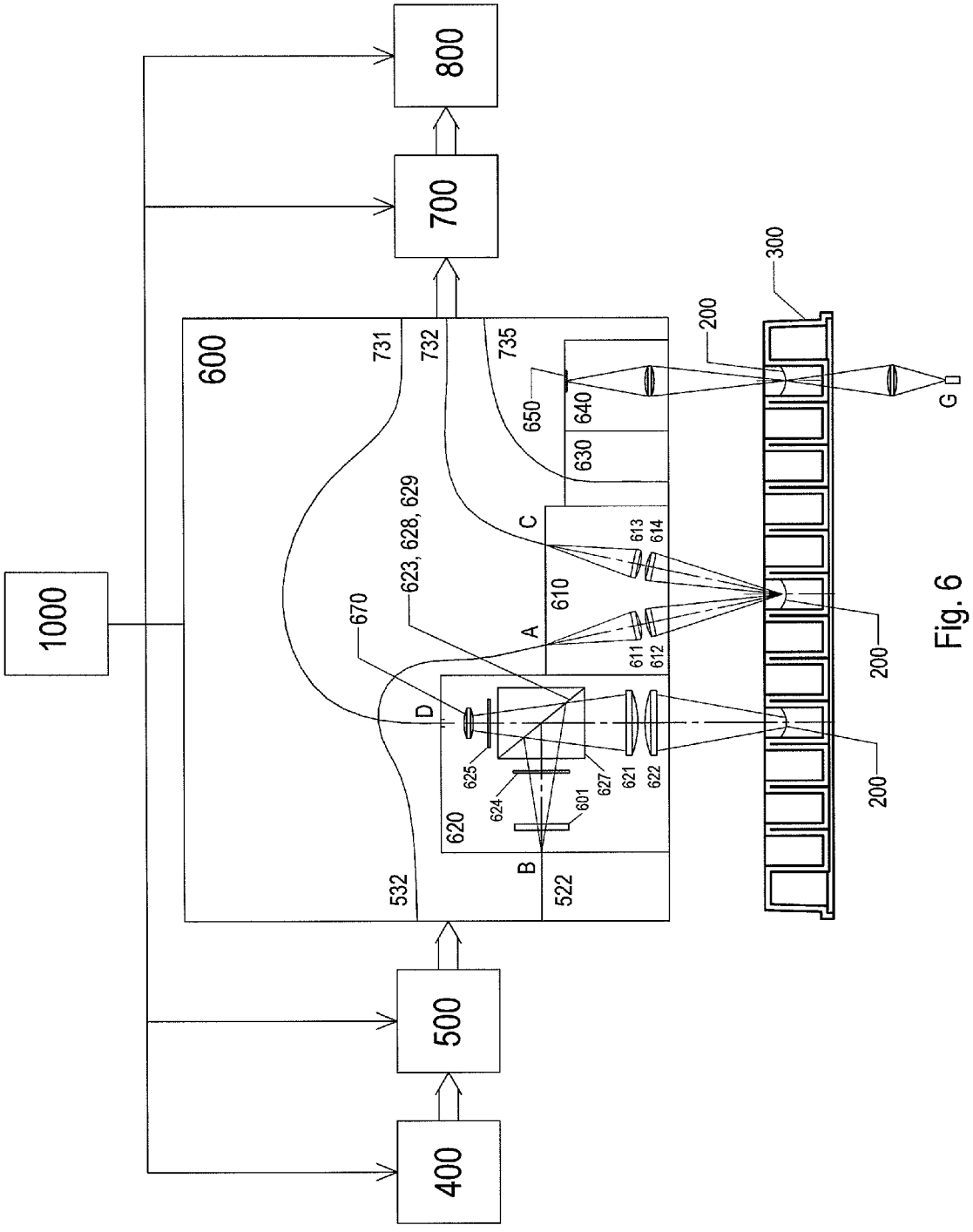
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Fig. 6

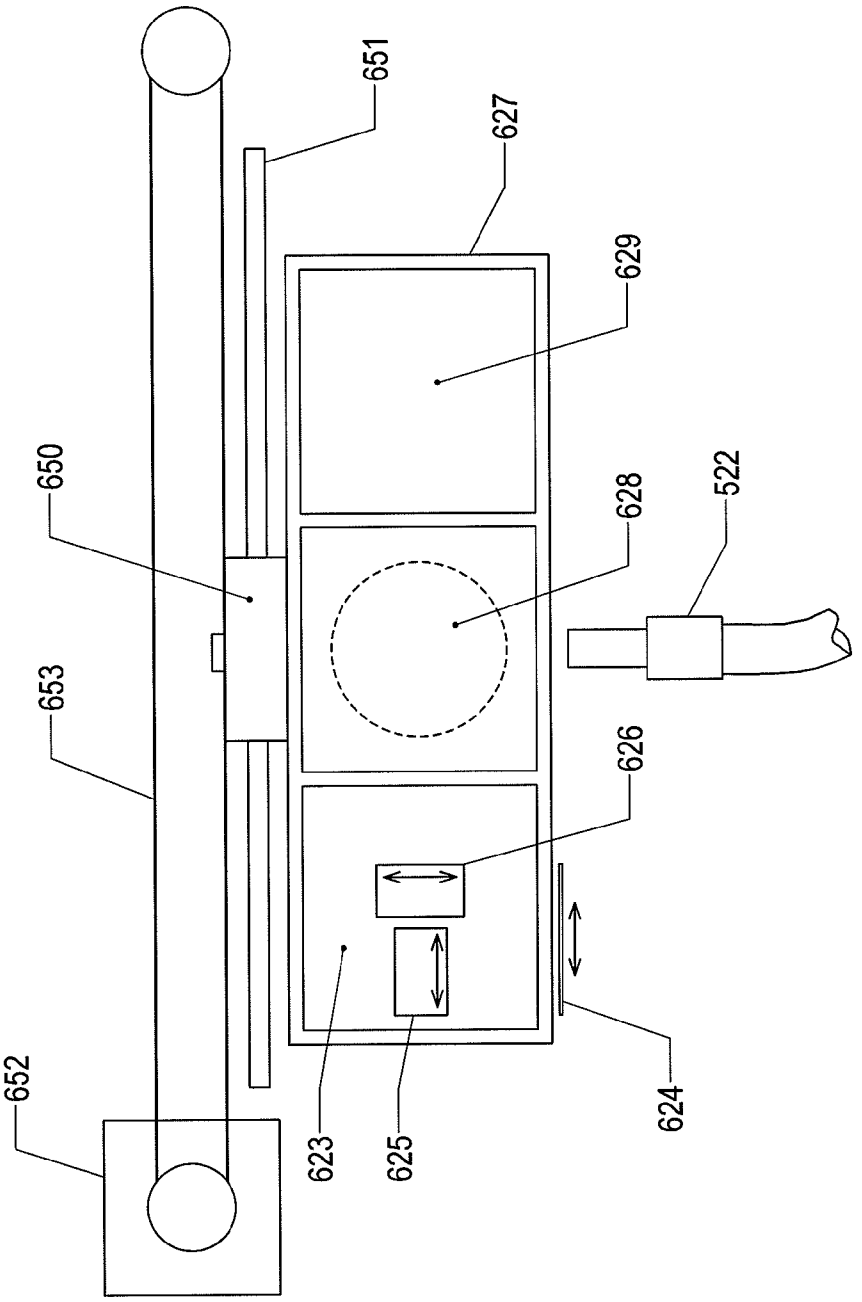

Fig. 7

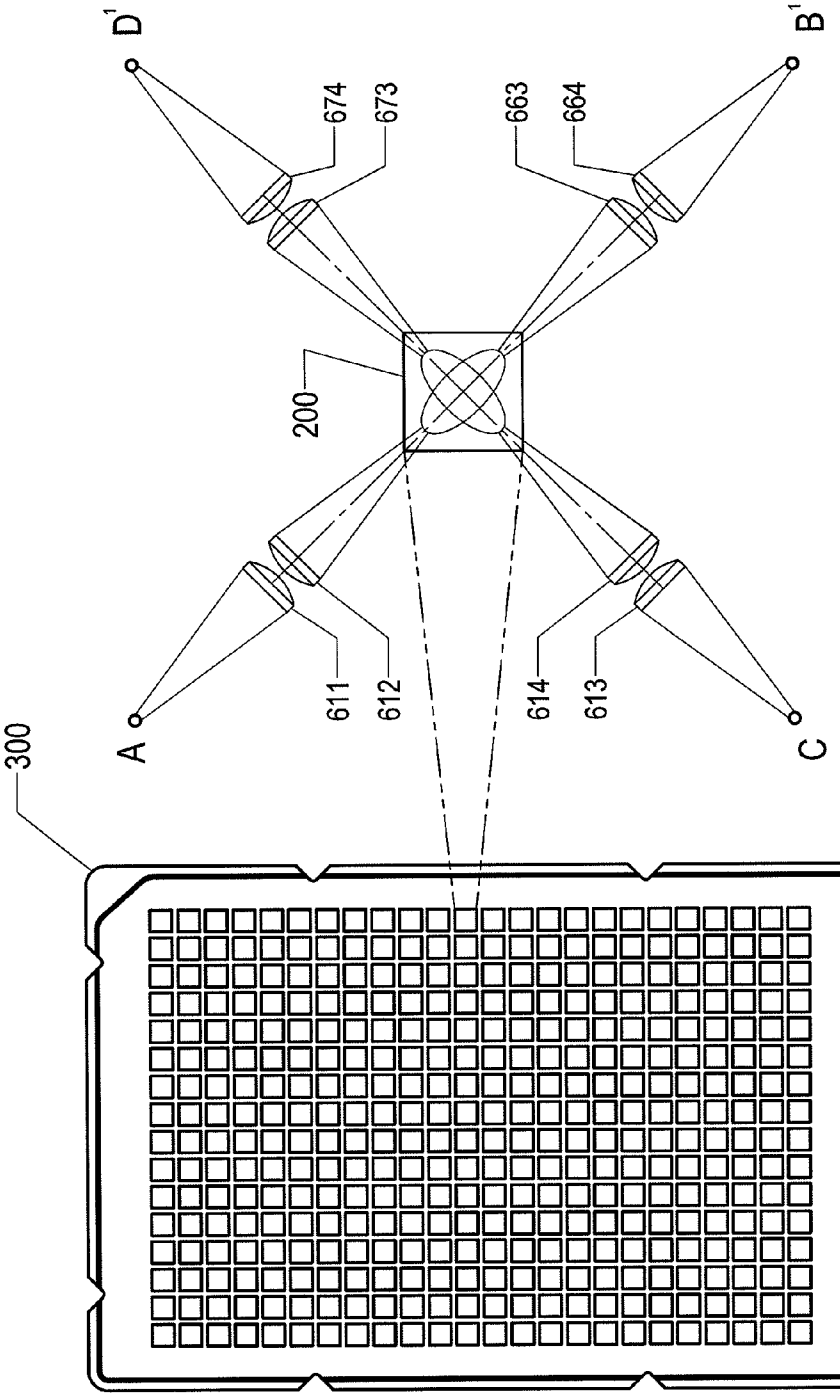

Fig. 8

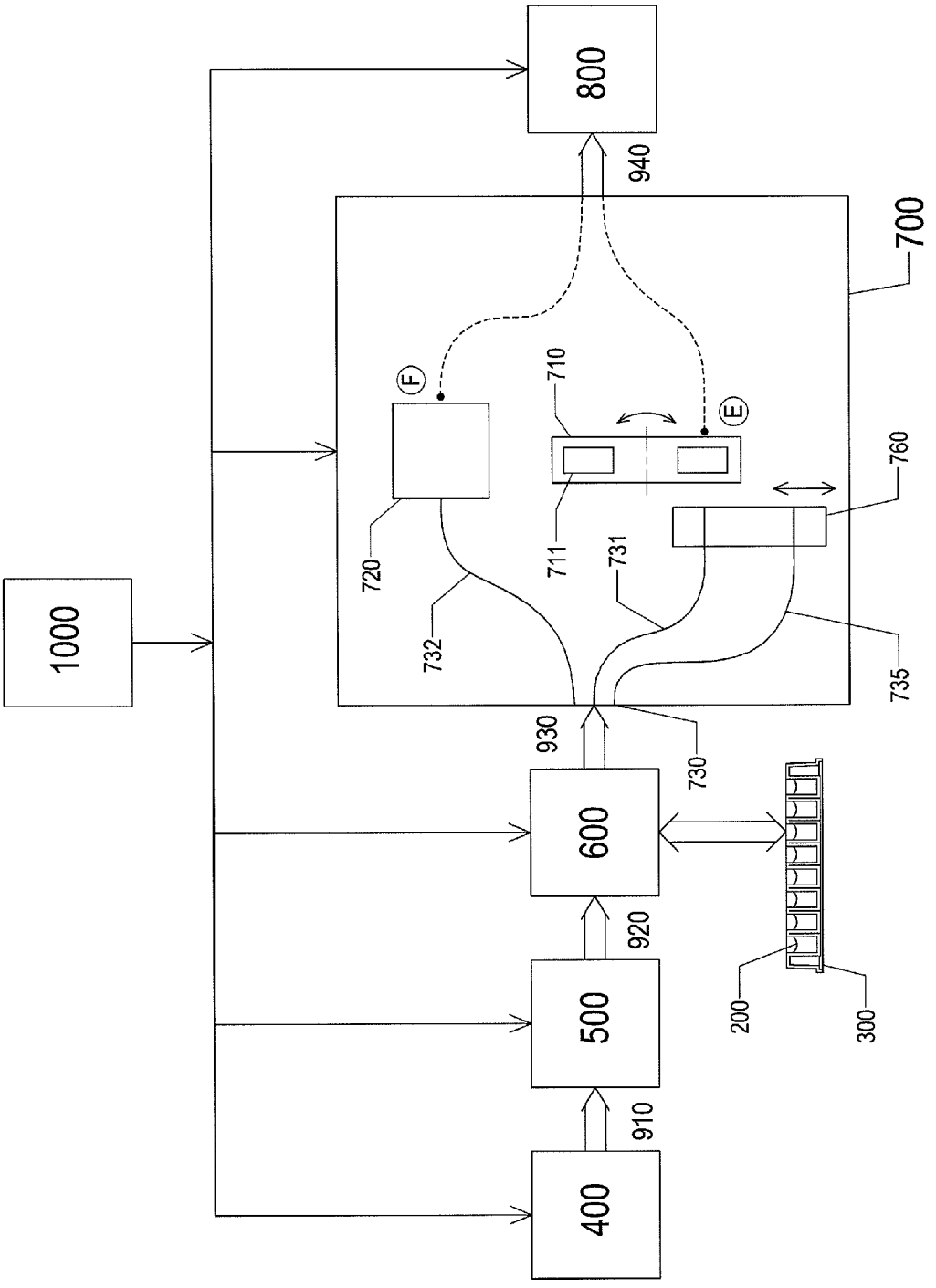
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Fig. 9

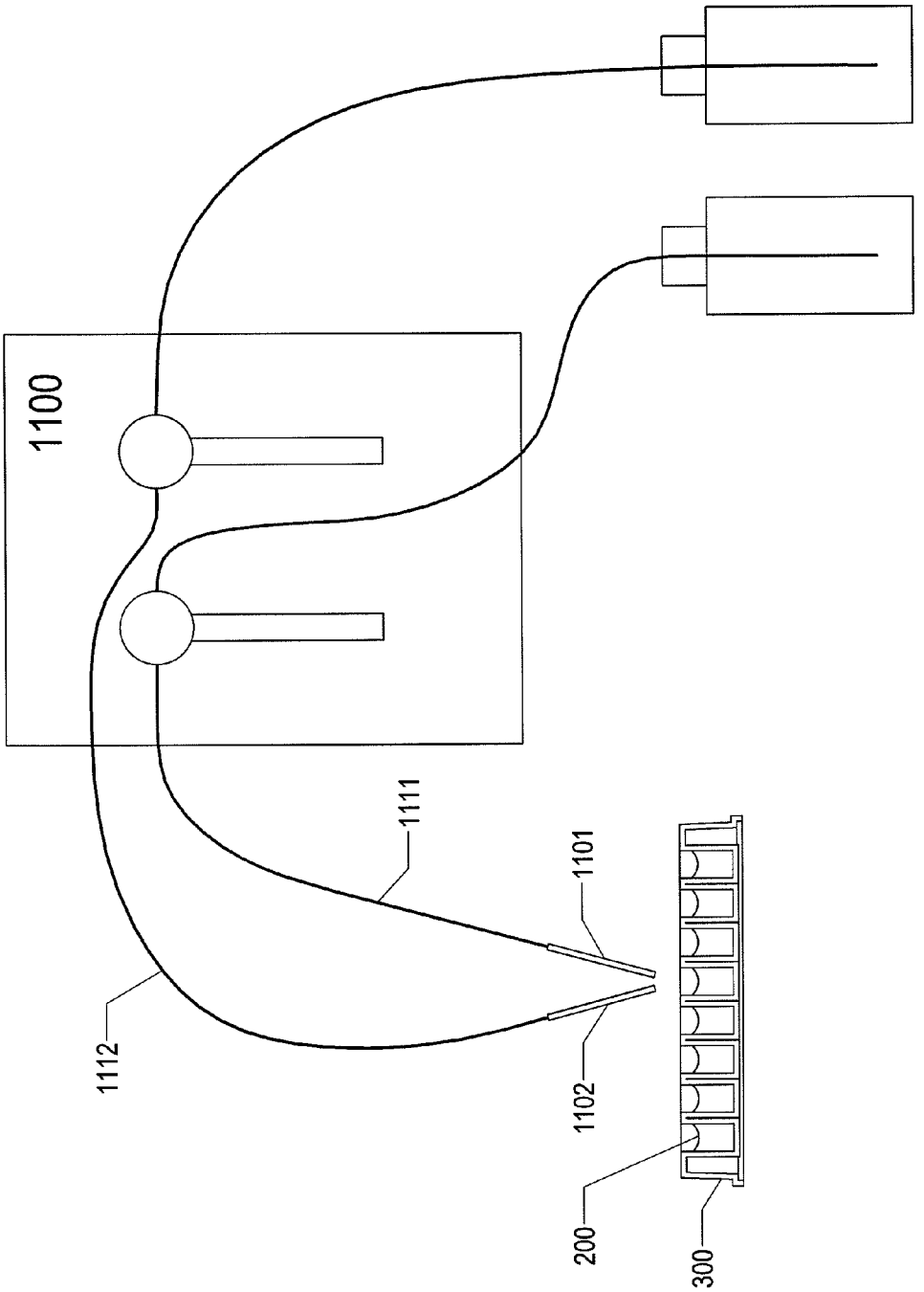

Fig. 10

UNIVERSAL MULTIDETECTION SYSTEM FOR MICROPLATES

CROSS-REFERENCE TO RELATED APPLICATION

This is a divisional of application Ser. No. 11/802,831 filed May 25, 2007. The entire disclosure of the prior application, application Ser. No. 11/802,831, is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field

Apparatuses and methods consistent with the present invention relate to detection systems, including the detection of fluorescence, absorbance, and chemiluminescence in samples placed in the wells of microplates.

2. Description of the Related Work

Multiple analytical instruments are used in laboratories to evaluate samples under test that are placed into vessels of various shapes. In the past twenty years, a microplate format has become very popular, as it lends itself to testing many samples on a single matrix-style receptacle. The first detection systems for microplates were absorbance readers. Later dedicated fluorometers were developed, followed by instruments to measure chemiluminescence.

The range of assay chemistries and labeling technologies continues to grow. Currently employed detection methods include absorbance, multiplexed fluorescence and chemiluminesece, fluorescence polarization (FP), time-resolved fluorescence (TRF), fluorescence resonance energy transfer (FRET), quenching methods, and specially designed labels with intensity and spectral responsiveness to environmental conditions. Along with this range of detection methods, users are conjugating an ever-growing array of organic and inorganic labels for targets, ranging from small-molecule drug candidates to proteins and nucleic acids, and to subcellular structures and cells.

FIG. **1** illustrates the general structure of a related art multimode detection system. As shown in FIG. **1**, a typical system comprises a light source **10**, an excitation spectral device **20**, an optical module **30**, a measurement chamber **60** with samples **70**, an emission spectral device **40**, and a detector **50**. There are two distinct types of related art multimode detection systems: filter-based units and monochromator-based units.

Filter-based units, when offered with high quality filters in combination with dichroic mirrors, allow for measurements with very low detection limits. This is mainly due to a high signal level, which is achieved with the filters, in combination with a high signal-to-noise ratio, which is achieved by a high level of blocking of the unwanted radiation around the desired waveband. The transmittance of filters is routinely over 50%, and this high level of transmittance can be achieved independent of the wavelength. Therefore, a very broad spectral range can be covered equally well from the deep ultraviolet (UV) to the infrared (IR), and the bandpass of the filter can be tailored to the specific application.

However, the filter-based unit cannot obtain a spectral scan for excitation or emission of the substance under investigation. A user must know upfront what substance he or she is working with and order an appropriate filter set. In addition, when working in the deep UV, filters tend to degrade when exposed to the UV radiation of the light source, due to solarization. Also, maintaining libraries of filters for the full range of labels is prohibitively expensive, and appropriate combinations are often not readily available for a given label, conjugation chemistry, target molecule, and assay condition. Further, the effects of these conditions are not always predictable based on the nominal spectra of the basic label.

Monochromator-based instruments offer a high level of flexibility in terms of choosing the wavelengths and obtaining scans of excitation and emission spectra, thus allowing the user to work with unknown substances. This also permits optimization of the measurements for perturbations to the spectra of labels due to assay conditions, conjugation chemistries, and target molecules. Additionally, when working with real biological or biochemical samples, interfering signals from other sample components may require optimization of excitation and emission wavelengths for the exact assay conditions.

The monochromators used in modern instruments are usually based on diffraction gratings, and use a flat grating for dispersion and concave mirrors for focusing light, or concave gratings that combine dispersive and focusing functions. Monochromators require order sorting filters to separate high spectral orders, but in the range from 200 nm to about 380 nm, no order sorting filters are needed. Therefore, there is no need for filters that withstand UV radiation, and the solarization problem is avoided.

However, the response of the monochromator is not constant across the wavelength range. One can obtain a system with a good signal in the UV, the visible, or the IR; however, one cannot obtain a system with a good signal in all of the wavelength ranges in the same monochromator-based unit. A usual compromise is to optimize the excitation monochromator in the UV and to optimize the emission monochromator in the visible or IR, because the wavelength of the emission light shifts to the right with respect to the wavelength of the excitation light.

In order to obtain low detection limits, the monochromator must have very low stray light. A traditional way to achieve this in the monochromator-based system is to employ two stage monochromators. These are called double monochromators, and contain two single monochromators placed in series. While this does result in very low stray light, the penalty is a dramatic decrease in signal, especially in spectral regions where the response of the single stage monochromator is already low. There are several instruments in the field based on this method, such as the Tecan INFINITE and the Thermo VARIOSKAN.

In terms of performance, the filter-based units achieve significantly lower detection limits in fluorescence intensity applications across the full spectral range, and work significantly better with techniques such as TRF, FP, and Homogeneous Time-Resolved Fluorescence (HTRF), all of which require the strong signal provided by the filter-based units. On the other hand, the monochromator-based units provide the flexibility of choosing any wavelength and the ability to obtain a spectral scan, at the expense of lower sensitivity.

U.S. Pat. No. 6,313,471 describes a method that combines bandpass filters and monochromators in series in a detection system. In this method, the bandpass filter acts as a crude first stage monochromator. The instrument splits the full spectral range of interest into several regions corresponding to the number of filters employed, and blocks radiation from adjacent regions by using additional filters. The single stage monochromator that follows the bandpass filters then selects the wavelength of interest from this prefiltered range.

However, with a limited number of prefiltered regions, this method is limited in flexibility. If both the excitation and emission wavelengths fall into one region, the method is not effective in achieving low stray light or high performance.

True spectral scanning is not readily accomplished with this method. This limits its utility for spectral measurement and optimization under conditions of fine spectral perturbation.

There is currently no related art instrument that combines the best of filter-based measurements and monochromator-based measurements in a single unit, and allows a user to choose between the two measurement types. A need exists for a single multidetection system that combines the low detection limits and assay methodology flexibility of filter-based units with the wavelength flexibility and superior UV performance of the monochromator-based units. This instrument could provide a user with a full flexibility in terms of assay type and wavelength selection.

SUMMARY

Exemplary embodiments of the present invention overcome the above disadvantages and other disadvantages not described above. Also, the present invention is not required to overcome the disadvantages described above, and an exemplary embodiment of the present invention may not overcome any of the problems described above.

According to an aspect of the present invention, there is provided an apparatus for optically analyzing a sample, which includes a first optical device that transmits a narrow waveband of light and includes a first filter and a first monochromator that provide different paths for the narrow waveband of the light. The apparatus may also include a light source that generates the light as broadband excitation light, wherein the first optical device transmits a narrow waveband of the broadband excitation light and blocks other wavebands of the broadband excitation light through the first filter or the first monochromator; a second optical device that directs the narrow waveband of the broadband excitation light onto the sample and receives emission light from the sample; a third optical device that transmits a narrow waveband of the emission light; and a detector that converts the narrow waveband of the emission light into an electrical signal; wherein the third optical device includes a second filter and a second monochromator that provide alternative paths for the narrow waveband of the emission light.

According to another aspect of the present invention, there is provided a method of optically analyzing a sample, including the step of transmitting a narrow waveband of light through a first filter or a first monochromator, wherein the first filter and the first monochromator provide alternative paths for the narrow waveband of the light. The method may further include the steps of generating the light as broadband excitation light; transmitting a narrow waveband of the broadband excitation light through the first filter or the first monochromator; directing the narrow waveband of the broadband excitation light onto the sample; receiving emission light from the sample; transmitting a narrow waveband of the emission light; and converting the narrow waveband of the emission light into an electrical signal; wherein the step of transmitting the narrow waveband of the emission light includes transmitting the narrow waveband of the emission light through a second filter or a second monochromator; the first filter and the first monochromator provide alternative paths for the narrow waveband of the broadband excitation light; and the second filter and the second monochromator provide alternative paths for the narrow waveband of the emission light.

According to another aspect of the present invention, there is provided an optical apparatus for separating emission light from excitation light, which includes a holder that moves in a direction perpendicular to a plane defined by optical axes of the excitation light and the emission light; a first linear polarizer with a polarization axis perpendicular to the plane defined by the optical axes of the excitation light and the emission light; a second linear polarizer with a polarization axis parallel to the plane defined by the optical axes of the excitation light and the emission light; a third linear polarizer with a polarization axis parallel to the polarization axis of the first linear polarizer; and a partially reflective and partially transparent mirror that directs the excitation light toward a sample and receives the emission light from the sample; wherein the holder can be moved to position at least one of the first linear polarizer, the second linear polarizer, the third linear polarizer, and the mirror in a path of at least one of the excitation light and the emission light.

According to another aspect of the present invention, there is provided a light source for optically analyzing a sample, which includes a first parabolic reflector that collimates light from a first lamp; and an off-axis parabolic reflector that focuses the collimated light from the first lamp onto a focal point. The light source may also include a second parabolic reflector that collimates light from a second lamp; and a lens that focuses the collimated light from the second lamp onto the focal point; wherein the off-axis parabolic reflector moves out of an optical path of the collimated light from the second lamp if the second lamp is emitting light.

According to another aspect of the present invention, there is provided an optical apparatus for separating emission light from excitation light, which comprises a first lens that directs a first narrow waveband of excitation light onto a sample and a second lens that receives a first emission light from the sample, wherein optical axes of the first lens and the second lens form a V shape in which the sample is positioned at a bottom of the V shape. The optical apparatus may further include a third lens that directs a second narrow waveband of excitation light onto the sample and a fourth lens that receives a second emission light from the sample; wherein optical axes of the third lens and the fourth lens form a V shape in which the sample is positioned at a bottom of the V shape; and none of the optical axes of the first lens, the second lens, the third lens, and the fourth lens are parallel to each other.

BRIEF DESCRIPTION OF THE DRAWINGS

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

FIG. **1** illustrates a general structure of a multimode detection system;

FIG. **2** illustrates certain components of a Universal Multidetection System (UMS) according to an exemplary embodiment of the present invention;

FIG. **3** illustrates a light source according to an exemplary embodiment of the present invention;

FIG. **4** illustrates an excitation spectral device according to an exemplary embodiment of the present invention;

FIG. **5** shows optical connections between the light source, the excitation spectral device, and the excitation-emission separation device according to an exemplary embodiment of the present invention;

FIG. **6** illustrates an excitation-emission separation device according to an exemplary embodiment of the present invention;

FIG. **7** illustrates a holder according to an exemplary embodiment of the present invention;

FIG. **8** shows a view of the excitation-emission separation device along vertical axes toward the microplate according to an exemplary embodiment of the present invention;

FIG. **9** illustrates an emission spectral device according to an exemplary embodiment of the present invention; and

FIG. **10** shows a fluid dispenser according to an exemplary embodiment of the present invention.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS OF THE INVENTION

FIG. **2** illustrates certain components of a Universal Multidetection System (UMS) **100** according to an exemplary embodiment of the present invention. As shown in FIG. **2**, samples are dispensed into the array of microwells **200** in the microplate **300**. The microplate **300** is transported by the carriage **310** into the measurement chamber **320**, which may be incubated, and is positioned sequentially for measurements. The light source **400** generates excitation light. The excitation spectral device **500** selects and transmits a narrow band of the excitation light. The waveband is typically between 5 and 40 nm wide. The excitation-emission separation device **600** directs the excitation light to the microwells **200**, and then separates the emission light generated in the sample within the microwells **200** from the excitation light. The excitation-emission separation device **600** transmits the emission light to the emission spectral device **700**, which transmits a narrow band of the emission light. The emission spectral device **700** should be configured to transmit as much emission light as possible, while blocking as much excitation light as possible and maximizing the signal-to-noise ratio. The detector **800** converts the emission light into an electrical signal. Although the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, and the detector **800** are shown as separate modules, they can also be combined in a variety of ways.

As shown in FIG. **2**, relay devices **910**, **920**, **930**, and **940** provide optical connections between the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, and the detector **800**. The controller **1000** stores emission signals from samples in the microplate **300**, analyzes the emission signals, computes parameters categorizing the optical measurements, and sends commands to the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, or the detector **800**. The commands can instruct the light source **400**, the excitation spectral device **500**, the excitation-emission separation device **600**, the emission spectral device **700**, or the detector **800** to change an internal parameter. For example, the commands can instruct the excitation spectral device **500** or the emission spectral device **700** to use one internal device instead of another internal device. Further, an optional dispenser **1100** delivers reagent to the microwells **200**.

FIG. **3** illustrates the structure of the light source **400** according to an exemplary embodiment of the present invention. In a preferred embodiment, the light source **400** comprises only two light generating devices: a Xenon flash lamp **410** and a Tungsten lamp **420**. In other embodiments the light source **400** may comprise a Xenon continuous wave lamp, a light emitting diode (LED), a laser, or any other light-generating device.

Tungsten sources are very stable, and their radiation extends from blue in the visible spectrum to the far IR, and peaks around 1 μm. They are most suitable for measurements in the visible and IR regions of the spectrum. In contrast, Xenon flash sources deliver most of their radiation in the deep UV, UV, and short visible spectral ranges. In addition, Xenon flash sources provide a very fast burst of light, lasting for several microseconds with a fast decay, and are therefore suitable for time resolved measurements in modern multidetection systems.

The Xenon flash lamp **410** has a parabolic reflector **411** positioned such that the arc **412** of the lamp **410** is located near the focal point of the reflector **411**, providing an essentially collimated beam from the reflector **411**. The Tungsten lamp **420** has a parabolic reflector **421** positioned such that the filament **422** of the lamp **420** is located near the focal point of the reflector **421**, providing an essentially collimated beam from the reflector **421**. FIG. **3** shows that a lens **423** may be used to focus the beam from the reflector **421** onto the exit portal **430** of the light source **400**. As shown in FIG. **5**, relay optics may be used to focus the beam onto the entrance of an optical fiber. Alternatively, the lens **423** may focus the beam from the reflector **421** directly onto the entrance of an optical fiber within the excitation spectral device **500**.

The movable off-axis parabolic reflector **440** has two working locations. In the first location, depicted by a solid line in FIG. **3**, the reflector **440** reflects and focuses light from the reflector **411**. In the second location, depicted by a dashed line in FIG. **3**, the reflector **440** stays out of the way of light from the reflector **421**. This arrangement allows light from either lamp to be focused at the same location. Further, the fan **417** directs air across the fins **415** of a cooling extrusion for the Xenon source **410** and onto the Tungsten source **420**. This arrangement allows both sources to share a single cooling system.

The arrangement of two light sources in close proximity to each other, with their optical axes offset, and preferably at an angle of approximately 90 degrees to each other, allows for a very compact illumination system with a shared cooling system. The use of parabolic reflectors around the light sources, in combination with off-axis parabolic reflectors, results in very highly efficient coupling of light from the arc and filament into the system. Here the final focusing point of both light sources is the same. This system allows a more compact arrangement than a system which utilizes separate light source compartments with separate exit light points for each compartment, thus requiring a mechanical movement of the optical relay system to switch between sources.

FIG. **4** illustrates the structure of the excitation spectral device **500** according to an exemplary embodiment of the present invention. The exit portal **430** of the light source **400** is in close proximity to the input portal **510** of the excitation spectral device **500**. The relay device **910** is unfilled space inside the UMS **100**.

In a preferred embodiment, the excitation spectral device **500** has two spectral selection devices, which differ by the physical technology by which they separate light with different wavelengths. The first device is a filter selection device **520**, which has a variety of user-replaceable filters **521**. The second device is a double monochromator **530**.

As shown in FIG. **4**, the light exiting the light source **400** via the exit port **430** is directed to the entry point **510** of the excitation spectral device **500**. Light entering the excitation spectral device **500** is then directed to the exit port **540** of the excitation spectral device **500** along one of two paths.

The first path directs the light through one of the filters **521** in the filter selection device **520**, which transmits a narrow band of the light. The light then propagates through optical fiber **522** to the exit port **540**. The second path bypasses the filters **521** by directing the light through hole **523** in the filter selection device **520**. The light then continues via optical fiber **531**, which accepts a circular image of the arc or filament spot from the light source **400** formed at the entry port **510**, and

shapes the light spot into a slit shape to match it to the input slit of the double monochromator **530**. The monochromator **530** selects a narrow band of the light, and then the optical fiber **532** changes the shape of the light from the exit slit shape of the monochromator **530** into a circular shape that resembles the shape of a microwell **200**.

The light path selector **550** can move relative to the filter selection device **520**, providing the ability to guide light to the exit port **540** that was spectrally selected by the filters **521** or the monochromator **530**. FIG. **5** shows the optical connections between the light source **400**, the excitation spectral device **500**, and the excitation-emission separation device **600** in greater detail.

FIG. **6** illustrates the structure of the excitation-emission separation device **600** according to an exemplary embodiment of the present invention. The general purpose of the excitation-emission separation device **600** is to irradiate the sample with excitation light and/or gather emission light from the sample. The excitation-emission separation device **600** can be positioned above the microwell **200** as shown in FIG. **6**, or below the microwell **200**. Also, the Universal Multidetection System **100** can include two excitation-emission separation devices **600**, one of which is positioned above the microwell **200**, and the other of which is positioned below the microwell **200**. This arrangement enables measurements of the same microwell **200** with a filter-based system and a monochromator-based system from both the top and the bottom.

In a preferred embodiment, several light paths may be used, based on the measurement technique. For absorbance measurements, the excitation and emission light are preferably collinear. As shown in FIG. **6**, the absorbance measurements are conducted in block **640**, in which the microwell **200** is illuminated with excitation light from below at point G. This excitation light may come from the monochromator **530** or the filter selection device **520**, as shown in FIG. **5**. A detector **650** is placed on the opposite side of the microwell **200** to capture emission light that passes through the sample.

For luminescence measurements, no excitation light is required, and only emission light is gathered from the sample by the excitation-emission separation device **600**. In block **630**, a single fiber optic bundle **735** is used to maximize the light gathering capability of the system and thus improve the signal.

For fluorescence measurements, two optical paths are available to irradiate the sample with excitation light and to gather emission light from the sample. These paths can be optimized to further enhance the overall system performance.

Block **620** depicts a first optical path for fluorescence measurements, which can use a partially reflective mirror or a dichroic mirror so that excitation light and emission light are collinear when entering and exiting the sample, respectively. Light is delivered to Block **620** by the optical fiber **522**. The movable aperture **601** has several openings with diameters preferably ranging from approximately 1.5 mm to 4 mm, and is placed in front of the guide fiber **522**. An image of the opening placed in front of the optical fiber **522** is formed in the microwell **200** by lenses **621** and **622**. The size of the opening of the movable aperture **601** is selected to fill the microwell **200** as completely as possible with light, while preventing light from entering adjacent microwells and causing cross-talk.

The light is reflected by a partially transmitting mirror **623** on a movable holder **627**. More than one mirror can be placed onto the holder **627**. Some minors can be dichroic mirrors to improve the signal, as all excitation light is reflected towards the microwell **200**, and all emission light is transmitted towards exit fiber. The dichroic mirrors can also improve the signal-to-noise ratio of the measurement system, as residual excitation light that reaches the microwell **200** and is reflected by the meniscus lens is blocked from reaching the exit fiber. The emission light from the microwell **200** is gathered onto the fiber optic bundle **731** by lenses **621**, **622**, and **670**. A collective lens **670** in front of the fiber optic bundle **731** assures that emission light from the full depth of the microwell **200** is collected, thus maximizing the system signal.

The high energy collection characteristics of the system assure low detection limits and allow for various levels of fluid to produce acceptable results without the need to refocus the optical system based on the fluid volume. This is in contrast with, for example, the confocal style measurements described in U.S. Pat. No. 6,097,025, which uses a confocal optical system that collects light only from the small portion of the microwell.

In a preferred embodiment, linear polarizers **624** and **625** are added to the holder **627**, and the same motion that positions appropriate mirrors in the light path also can be used to select polarizers for fluorescence polarization measurements. This eliminates the need for a separate mechanism to switch the polarizers, and thus improves the reliability of the system.

Block **610** depicts a second optical path for fluorescence measurements, which uses a tilted V arrangement of optics for direct well illumination and light gathering. This allows the system to channel the full amount of light from the fiber optic **532** into the microwell **200**. The numerical aperture of the optics **611** and **612** is matched to the fiber optic **532** for this purpose. The cone of excitation light enters the microwell **200** and excites the contents of the microwell **200** via the first leg of the V. The emission light is collected by the second leg of the V. The numerical aperture of lenses **614** and **613** matches the exit fiber optic **732**. The V is tilted with respect to the vertical plane to direct excitation light that is specularly reflected from the surface of the microwell **200** away from the light collecting leg of the V. Therefore, this arrangement introduces a spatial separation of emission and excitation light in addition to the spectral separation, and significantly improves the signal-to-noise ratio. This tilted V arrangement can also be used to conduct fluorescence polarization measurements.

The entry ports A and B of the excitation-emission separation device **600** accept fiber bundles from the excitation spectral device **500**. Fibers can be positioned to direct light that is spectrally separated by filters in the excitation spectral device **500** into input B of Block **620**. Fibers can also be positioned to direct light spectrally separated by monochromators in the excitation spectral device **500** into input A of Block **610**. Alternatively the inputs can be reconfigured by switching fibers **522** and **532**. This switching may be accomplished manually. The emission light is gathered by fibers **731** and **732** from ports C and D. The placement of fibers **731** and **732** in the exit ports C and D determines the origin of the emission light in the fibers.

FIG. **7** illustrates the holder **627** with associated dichroic mirrors **623**, **628**, and **629** and linear polarizers **624**, **625**, and **626** according to an exemplary embodiment of the present invention. The holder **627** is affixed to the slider **650**, which slides along rail **651** due to the applied force from the motor **652** through the belt **653**. The holder **627** moves in a direction perpendicular to the plane defined by the optical axes of the excitation and emission light. Although two different fibers **522** and **532** could occupy the fiber position depicted in FIG. **7**, for the sake of clarity only fiber **522** is shown.

In the depicted design there are five possible positions for the holder **627** relative to the fiber **522**, which delivers the

excitation light. The first position, which is depicted in FIG. **7**, represents a situation where the center of mirror **628** is aligned with the optical axis of the fiber **522**. In this position fluorescence polarization based assays cannot be conducted. If the holder **627** is moved to the left for a distance equal to the distance between the centers of mirror **628** and **629**, the holder **627** will be in the second position. In the second position, the mirror **629** plays an active role, and fluorescence polarization based assays cannot be conducted.

The three other positions of the holder **627** correspond to three different situations. First, when the right third of the mirror **623** is positioned in front of the fiber **522**, fluorescence polarization based assays cannot be conducted. Second, when the middle third of the mirror **623** is positioned in front of the fiber **522**, the linear polarizer **624** is in the optical path of the excitation light, and the linear polarizer **626** is in the optical path of the emission light. In this case the polarization vectors of the excitation and emission light are crossed. Third, when the left third of the mirror **623** is positioned in front of the fiber **522**, the linear polarizer **624** is still in the optical path of excitation light, and another linear polarizer **625** is in the optical path of the emission light. In this case the polarization vectors of the excitation and emission light are parallel. Thus the linear motion of the holder **627** not only selects which mirror is placed in the optical path, but also allows for fluorescence polarization measurements.

As shown in FIG. **7**, the linear polarizers **625** and **626** have parallel surface orientations and perpendicular polarization axis orientations. They have active areas of equal sizes, and each size is comparable to the size of the cross-section of the emission light. The polarization axis of the linear polarizer **624** is parallel to the polarization axis of the linear polarizer **625**, and perpendicular to the polarization axis of the linear polarizer **626**. The area of the linear polarizer **624** is at least twice the area of the linear polarizer **625**. The area of the mirror **623** is at least three time the area of the linear polarizer **625**. The mirror **623** is partially reflective and partially transparent.

FIG. **8** shows a view from above the microplate **300**, along vertical axes toward the microplate **300** of the block **610** of the excitation-emission separation device **600**. Points A and B' are input portals of the excitation-emission separation device **600**. Lenses **611**, **612**, **663**, and **664** focus excitation light onto the microwell **200** in the microplate **300**. Lenses **613**, **614**, **673**, and **674** collect emission light and focus it into points C and D', which are exit portals of the excitation-emission separation device **600**. Standard 384 well microplates have an upper edge with a nearly square shape. The optical axes of lenses **611**, **612**, **663**, **664**, **613**, **614**, **673**, and **674** are oriented along the diagonals of microwells **200**. Using this arrangement a reading may be taken on the same microwell **200** simultaneously via filter-based or monochromator-based spectral systems. Because the excitation light from point A is reflected toward point B' and vice versa, very little excitation light is reflected toward exit portals C and D'. Therefore, the emission light is spatially separated from the excitation light.

FIG. **9** illustrates the structure of the emission spectral device **700** according to an exemplary embodiment of the present invention. In a preferred embodiment, the emission spectral device **700** has two spectral selection devices, which differ by the physical technology by which they separate light with different wavelengths. The first device is a filter selection device **710**, which has a variety of filters **711**. The second device is a double monochromator **720**. As shown in FIGS. **6** and **9**, fibers **731**, **732**, and **735** extend from their respective locations within the excitation-emission separation device **600** into the emission spectral device **700**. The selector switch **760** is used to direct light from fibers to the filter selection device **710**. FIG. **9** shows that the fiber **731** from fluorescent measurement block **620** and the fiber **735** from the luminescence measurement block **630** are connected to the selector switch **760**. FIG. **9** also shows that the fiber **732** from the fluorescent measurement block **610** is connected to the monochromator **720**. However, the arrangement in FIG. **9** is merely exemplary, and a user can change the connections by physically switching the fiber connections within the excitation-emission separation device **600**, or within the emission spectral device **700**.

In a preferred embodiment, the exit portals of the excitation-emission separation device **600** are in close proximity to the input portal **730** of the emission spectral device **700**. Points E and F may represent the exit portals of the emission spectral device **700**. The detector **800** may comprise two photomultiplier tubes (PMTs) positioned at points E and F (not shown).

FIG. **10** shows a fluid dispenser **1100** according to an exemplary embodiment of the present invention. The purpose of the fluid dispenser **1100** is to inject fluid into microwells **200** to initiate the reaction under investigation. Often the time from initiation to the time measurements have to take place is very short. Therefore, the injection ports **1101** and **1102** may be placed in close proximity to the optical reading system. Further, two separate fluid lines **1111** and **1112** may be used. Each fluid line connects to the stepper motor driven syringe drive for positive displacement fluid delivery. A three-way valve alternately connects a syringe to supply bottles on a suction stroke or to an injector line for dispensing.

As discussed above, exemplary embodiments of the present invention provide a Universal Multidetection System for determining fluorescence, chemiluminescence, and/or light absorbance of a sample in a microplate that allows the user to reconfigure and optimize the measurement system for a particular modality. The Universal Multidetection System allows the user to choose the best measurement method for his assay. The user can select interference filters for their low detection limits and ability to run FP, TRF, and HTRF measurements with state of the art results, dual monochromators for their wavelength flexibility and spectral scanning, or a combination of both filters and monochromators. Both the excitation spectral device **500** and the emission spectral device **700** may contain interchangeable filter systems and monochromators.

In addition to providing unmatched flexibility, the Universal Multidetection System also opens an avenue to run experiments that were not previously possible. For example, in the fluorescence mode, when only a small amount of an unknown fluorofore is available and it is not possible to increase the signal in the monochromator-based system by increasing the sample concentration, the user can obtain rough excitation and emission scans by using monochromators **530** and **720** in the excitation spectral device **500** and the emission spectral device **700**, respectively. Based on these initial excitation and emission scans, the user can then select an appropriate filter **521** to excite the sample with light that causes much stronger emission from the sample. The stronger emission spectrum can then be re-recorded. The user can also select a filter **711** to replace the emission monochromator **720** and re-record the excitation spectrum. It is important to have high-quality measurements of both the excitation spectrum and the emission spectrum. This process increases the emission signal and the signal-to-noise ratio, resulting in improved excitation and emission spectra, as compared with spectra obtained with just

a monochromator-based system. It also allows a user to work with an unknown sample, and optimize the measurement conditions for that sample.

This approach can also be used to achieve maximum sensitivity in end-point reads. The user can select particular excitation wavelengths by using a monochromator **530** in the excitation spectral device **500**, and transmit the emission light through a filter **711** in the emission spectral device **700**. A similar benefit can be obtained by using a filter **521** in the excitation spectral device **500** with a monochromator **720** in the emission spectral device **700** during an end-point read. These methods are particularly suited to enhancing performance of environmentally sensitive labels, where variations in conditions give rise to perturbations of excitation or emission spectra, such as ion sensitive probes, pH sensitive probes, spectral shifts in polar dyes with conjugation, and binding and membrane probes.

Exemplary embodiments of the present invention have been described for illustrative purposes, and those skilled in the art will appreciate that various modifications, additions and substitutions are possible without departing from the scope and spirit of the invention as disclosed in the accompanying claims. Therefore, the scope of the present invention should be defined by the appended claims and their legal equivalents.

What is claimed is:

1. A method of optically analyzing a sample, comprising the step of:

transmitting a selected waveband of light;

wherein the step of transmitting the selected waveband of the light comprises transmitting the selected waveband of the light through an apparatus comprising alternative paths for the transmission of light including one path including a first filter and a second path including a first monochromator, where the selected waveband of light is selectively passed through one of the first filter and the first monochromator, and

the first filter and the first monochromator provide alternative paths for the selected waveband of the light.

2. The method of claim **1**, further comprising the steps of:

generating the light as broadband excitation light;

transmitting a selected waveband of the broadband excitation light through a selected one of the first filter and the first monochromator;

directing the selected waveband of the broadband excitation light onto the sample;

receiving emission light from the sample;

transmitting a selected waveband of the emission light; and

converting the selected waveband of the emission light into an electrical signal;

wherein the step of transmitting the selected waveband of the emission light comprises transmitting the selected waveband of the emission light through a second filter or a second monochromator;

the first filter and the first monochromator provide alternative paths for the selected waveband of the broadband excitation light; and

the second filter and the second monochromator provide alternative paths for the selected waveband of the emission light.

3. The method of claim **2**, further comprising the step of separating the emission light from the broadband excitation light.

4. The method of claim **2**, further comprising the steps of:

storing and analyzing the electrical signal;

computing a parameter which characterizes an efficiency of the analysis;

sending a command to at least one of a light source, an optical device, and a detector to change an internal parameter.

5. The method of claim **2**, further comprising the steps of:

obtaining a broadband scan of the broadband excitation light with the first monochromator;

obtaining a broadband scan of the emission light with the second monochromator; and

selecting the first filter based on the broadband scans.

6. The method of claim **5**, further comprising the step of selecting the second filter based on the broadband scans.

7. The method of claim **2**, wherein the step of transmitting the selected waveband of the broadband excitation light is performed by the first monochromator, and the step of transmitting the selected waveband of the emission light is performed by the second filter.

8. The method of claim **2**, wherein the step of transmitting the selected waveband of the broadband excitation light is performed by the first filter, and the step of transmitting the selected waveband of the emission light is performed by the second monochromator.

9. The method of claim **2**, wherein a microplate holds the sample.

10. The method of claim **9**, further comprising the step of injecting fluid into the microplate.

11. An apparatus for optically analyzing a sample, including an optical device that transmits a selected waveband of light;

the optical device includes a first filter and first monochromator that provide different paths, each within a separate module, for the selected waveband of the light, and

wherein the apparatus performs the method of claim **1**.

12. The apparatus of claim **11**, wherein the first monochromator is a single monochromator.

13. The apparatus of claim **11**, wherein the first monochromator is a double monochromator.

14. An apparatus for optically analyzing a sample, comprising: a device which performs the method of claim **1**.

15. The method of claim **1**, wherein the first monochromator is a single monochromator.

16. The method of claim **1**, wherein the first monochromator is a double monochromator.

17. The method of claim **1**, wherein the selected waveband of light is a narrow waveband of light.

18. The method of claim **1**, wherein the wherein the first monochromator comprises multiple stages.

19. A method of optically analyzing a sample, comprising the steps of:

transmitting a selected waveband of light;

wherein the step of transmitting the selected waveband of the light comprises transmitting the selected waveband of the light through one of a first filter and a first monochromator, each within a separate module, and

the first filter and the first monochromator provide alternative paths for the selected waveband of the light.

20. The method of claim **19**, further comprising the steps of:

generating the light as broadband excitation light;

transmitting a selected waveband of the broadband excitation light through a selected one of the first filter and the first monochromator;

directing the selected waveband of the broadband excitation light onto the sample;

receiving emission light from the sample;

transmitting a selected waveband of the emission light; and

converting the selected waveband of the emission light into an electrical signal;

wherein the step of transmitting the selected waveband of the emission light comprises transmitting the selected waveband of the emission light through a second filter or a second monochromator;

the first filter and the first monochromator provide alternative paths for the selected waveband of the broadband excitation light; and

the second filter and the second monochromator provide alternative paths for the selected waveband of the emission light.

21. The method of claim **20**, further comprising the step of separating the emission light from the broadband excitation light.

22. The method of claim **20**, further comprising the steps of:

storing and analyzing the electrical signal;

computing a parameter which characterizes an efficiency of the analysis;

sending a command to at least one of a light source, an optical device, and a detector to change an internal parameter.

23. The method of claim **20**, further comprising the steps of:

obtaining a broadband scan of the broadband excitation light with the first monochromator;

obtaining a broadband scan of the emission light with the second monochromator; and

selecting the first filter based on the broadband scans.

24. The method of claim **23**, further comprising the step of selecting the second filter based on the broadband scans.

25. The method of claim **20**, wherein the step of transmitting the selected waveband of the broadband excitation light is performed by the first monochromator, and the step of transmitting the selected waveband of the emission light is performed by the second filter.

26. The method of claim **20**, wherein the step of transmitting the selected waveband of the broadband excitation light is performed by the first filter, and the step of transmitting the selected waveband of the emission light is performed by the second monochromator.

27. The method of claim **20**, wherein a microplate holds the sample.

28. The method of claim **27**, further comprising the step of injecting fluid into the microplate.

29. The method of claim **19**, wherein the first monochromator is a single monochromator.

30. The method of claim **19**, wherein the first monochromator is a double monochromator.

31. The method of claim **19**, wherein the selected waveband of light is a narrow waveband of light.

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