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(54) **Title:** FAST MICROARRAY SCANNING USING MULTIBAND FILTERS

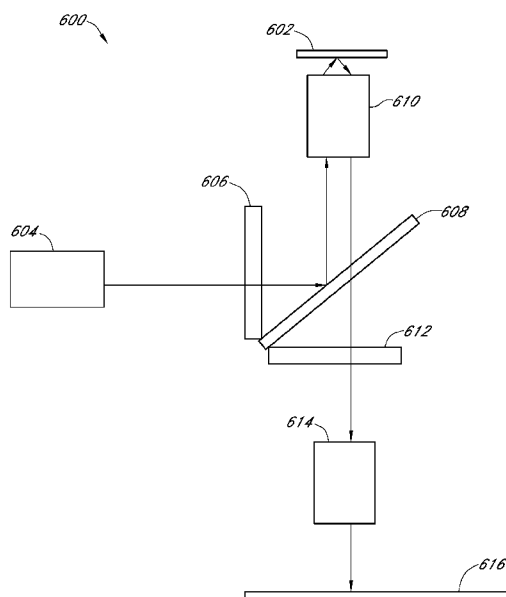


FIG. 6

(57) **Abstract:** A system for sample holder scanning is provided. The system includes a light source, a multiband excitation filter configured to select at least two excitation bands of light for fluorescent dyes used in the sample holder. The system further includes a multiband dichroic filter configured to reflect the at least two excitation bands of light, a multiband emission filter configured to transmit at least two bands of fluorescent emission light from each reaction site of the sample holder. The multiband dichroic filter is further configured to transmit the at least two bands of fluorescent emission light. The system also includes an optical sensor configured to detect the at least two bands of fluorescent emission light to generate an image of a sample holder.

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FAST MICROARRAY SCANNING USING MULTIBAND FILTERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 63/511,435, filed on June 30, 2023, which is incorporated herein in its entirety by reference.

BACKGROUND

[0002] Optical systems for biological and biochemical reactions have been used to monitor, measure, and/or analyze such reactions. Such systems are commonly used in sequencing, genotyping, polymerase chain reactions (PCR), and other biochemical reactions to monitor progress and provide quantitative data.

[0003] For example, an optical system may be used in DNA-DNA hybridization arrays to illuminate fluorescent DNA-binding dyes or fluorescent probes to produce fluorescent signals indicative of the degree of similarity between sample and probe. In a hybridization analysis, arrays are set up with selected sets of DNA sequences of interest. If a sample has complementary sequences to the target DNA in the array, a fluorescent dye can be attached. When excitation light shines on the DNA array after hybridization, array spots fluoresce if a dye is attached. An optical system is needed to determine fluorescence emission.

[0004] Previously, in these optical systems, fluorescent emission data is read by taking multiple images at each sub-array location to scan an entire sample holder. Further, for each sub-array location, two or more matched filter sets are mechanically moved to take images of two or more fluorescent dye emissions from the sample holder adding to the time required to analyze the array. Each filter set includes excitation, dichroic, and emission filters matched to a fluorescent dye used in the array. As a result, scanning an entire array with more than one fluorescent dye is a lengthy process.

[0005] Moreover, there are increasing demands to provide greater numbers of reactions per test or experiment, resulting in instruments that are able to conduct large numbers of reactions

simultaneously. Increasing the number of reactions also increases the time the optical system takes to scan an entire sample holder.

[0006] The combination of having large numbers of samples and the desire to perform experiments in a faster manner has created a need for optical systems that scan quickly and also provide high optical performance for observing, testing, and/or analyzing one or more biological samples.

SUMMARY

[0007] In one exemplary embodiment, a system for sample holder scanning is provided. The system includes a light source. The system further includes a multiband excitation filter configured to select at least two excitation bands of light. Each of the at least two excitation bands of light is an excitation band of light for a fluorescent dye used in the sample holder. The system further includes a multiband dichroic filter configured to reflect the at least two excitation bands of light, a lens configured to direct the at least two excitation bands of light toward a sample holder, and a multiband emission filter configured to transmit at least two bands of fluorescent emission light from each reaction site of the sample holder. The multiband dichroic filter is further configured to transmit the at least two bands of fluorescent emission light. The system also includes an optical sensor configured to detect the at least two bands of fluorescent emission light from the multiband emission filter to generate an image of a sample holder.

[0008] In another exemplary embodiment, a method for sample holder scanning is provided. The method includes generating light from a light source, and selecting, by a multiband excitation filter configured to transmit at least two excitation bands of light, a first excitation band of light. Each of the at least two excitation bands of light is an excitation band of light for a fluorescent dye used in the sample holder. The method further includes reflecting, by a multiband dichroic filter configured to reflect the at least two excitation bands of light, the first excitation band of light, and directing, by a lens, the first excitation band of light toward a sample holder. The method includes transmitting, by a multiband emission filter configured to transmit at least two fluorescent emission bands of light, the first band of fluorescent emission light from each reaction site of the sample holder, and detecting, by an optical sensor, the first

band of fluorescent emission light from the multiband emission filter to generate an image of a sample holder.

DESCRIPTION OF THE FIGURES

[0009] FIG. 1 illustrates a block diagram of a general fluorescent optical system configuration.

[0010] FIG. 2A illustrates an exemplary fluorescent transmission and emission data plot.

[0011] FIG. 2B illustrates an exemplary fluorescent transmission and emission data plot with single band excitation, dichroic, and emission optical filters.

[0012] FIG. 3 illustrates a block diagram of an inverted general fluorescent optical system configuration.

[0013] FIG. 4 illustrates a diagram of an inverted fluorescent optical system according to various embodiments described herein.

[0014] FIG. 5 illustrates a sample holder according to various embodiments described herein.

[0015] FIG. 6 illustrates an optical system according to various embodiments described herein.

[0016] FIG. 7 is a flowchart of a method of optical scanning according to various embodiments described herein.

[0017] FIG. 8 illustrates an exemplary fluorescent transmission and emission data plot according to various embodiments described herein.

[0018] FIG. 9 illustrates an exemplary fluorescent transmission and emission data plot according to various embodiments described herein.

[0019] FIG. 10 illustrates an exemplary fluorescent transmission and emission data plot according to various embodiments described herein.

[0020] FIG. 11 illustrates an exemplary fluorescent transmission and emission data plot according to various embodiments described herein.

[0021] FIG. 12 illustrates an exemplary fluorescent transmission and emission data plot according to various embodiments described herein.

[0022] FIG. 13 illustrates an emission plot of a Xenon light source according to various embodiments described herein.

[0023] FIG. 14 illustrates an exemplary optical system utilizing a Xenon lamp according to various embodiments described herein.

[0024] FIG. 15 illustrates an emission plot of a white LED light source according to various embodiments described herein.

[0025] FIG. 16 illustrates an exemplary optical system utilizing a LED lamp according to various embodiments described herein.

[0026] FIG. 17 illustrates an emission plot of a narrow band LED light source according to various embodiments described herein.

[0027] FIG. 18 illustrates another exemplary optical system utilizing a LED lamp according to various embodiments described herein.

DETAILED DESCRIPTION

[0028] To provide a more thorough understanding of the present invention, the following description sets forth numerous specific details, such as specific configurations, parameters, examples, and the like. It should be recognized, however, that such description is not intended as a limitation on the scope of the present invention but is intended to provide a better description of the exemplary embodiments.

[0029] As discussed above, in performing biological analyses, a large number of samples is tested and analyzed. Quickly performing the biological analysis has been previously hindered by

the large number of samples that need to be imaged by an optical system and the mechanical movement to switch filters to read different fluorescent dyes.

[0030] As such, there is a need to reduce optical scan time by reducing time-consuming mechanical motion within an optical system.

[0031] In various embodiments, the devices, instruments, systems, and methods described herein may be used to detect one or more types of biological components of interest. These biological components of interest may include, but are not limited to, DNA sequences, RNA sequences, genes, oligonucleotides, or cells (e.g., circulating tumor cells).

[0032] In various embodiments, the devices, instruments, systems, and methods described herein may be used to detect one or more types of biological components of interest. These biological components of interest may be any suitable biological target including, but are not limited to, DNA sequences (including cell-free DNA), RNA sequences, genes, oligonucleotides, molecules, proteins, biomarkers, cells (e.g., circulating tumor cells), or any other suitable target biomolecule.

[0033] Embodiments of the present disclosure are generally directed to devices, instruments, systems, and methods for monitoring or measuring a biological reaction for a large number of small volume samples. As used herein, samples may be referred to as sample volumes, or reactions volumes, for example.

[0034] Hybridization of the fluorescent dye to the specimen results in fluorescent emission light. Hybridization analyses are an inexpensive way to screen a large number of targets. As such, this method is a simple and useful in a way to screen for hereditary susceptibility to different problems, such as cancer, sickle cell disease, Alzheimer's, thyroid disease, hereditary deafness or blindness, for example. It can also be used to promote health and productivity in agriculture by using it on animals and plants as part of good breeding (e.g. corn with high yield that is not susceptible to blight or mold).

[0035] In a hybridization analysis, there are millions of array spots featuring short strands of DNA on fused silica substrates. Arrays are set up with selected sets of DNA sequences of interest. A fluid sample is prepared for a particular person, plant, or animal's DNA. The array is

then immersed in this sample and baked in a hybridization oven, where, if the subject's DNA is complementary to the DNA targets on the array, they will bond (hybridize). When they hybridize, a ligation region is formed near the bond of the array DNA and the sample DNA. Thus, a fluorescent dye can be attached to this ligation region. When excitation light shines on the DNA array after hybridization, array spots light up (fluoresce) if dye is attached. This means that location (with its known DNA array content) was complementary with the sample's DNA.

[0036] FIG. 1 illustrates a block diagram 100 of a general fluorescent optical system configuration that can be used in a hybridization analysis. In this example, specimen 102 is being imaged for fluorescent emission light. Light from light source 104 includes a plurality of wavelengths of light. Light from light source 104 passes through excitation filter 106, which is designed to transmit a certain bandwidth of light to excite the fluorescent dye used in an assay. The transmitted light is reflected by dichroic filter 108. The reflected light then passes through lens 110 to direct the reflected light to reaction site 102. Reaction site 102 includes a specimen and a fluorescent dye.

[0037] Any fluorescent emission light from reaction site 102 then passes through lens 110 and dichroic filter 108. Emission filter 112 is designed to transmit the expected fluorescent emission bandwidth to detector 114. Detector 114 generates an image of specimen 102 and fluorescent emission intensity can be determined.

[0038] Previously, excitation filter 106, dichroic filter 108, and emission filter 112 were configured to transmit, reflect, and transmit expected bandwidths for a certain fluorescent dye used in a biological analysis. If a second dye is used, excitation filter 106, dichroic filter 108, and emission filter 112 would need to be changed to a second set of filters designed for the second dye. Changing to the second set of filters for the second dye has been accomplished previously by mechanical movement to replace emission filter 102, dichroic filter 108, and excitation filter 106, resulting in a longer time to scan reaction site 102. Further, scanning one sample holder to examine every reaction site requires multiple images to be generated at each location compounding the time required to complete an analysis.

[0039] As mentioned above, fluorescent dyes used in a biological analysis provide a fluorescent signal that varies according to an amount of target nucleotide sequence contained in

various reaction sites. Fluorescent dyes absorb at a lower wavelength and emit at a longer wavelength, which is known as a Stokes shift. As such, optical filters may be used to effectively separate excitation and emission light.

[0040] An exemplary fluorescent transmission and emission intensity data plot is illustrated in FIG. 2A for fluorescent dye R-phycoerythrin (RPE). RPE excitation data curve is shown in 204. The RPE emission data curve 206 is shown and shifted from excitation data curve 204 after excitation of the RPE dye.

[0041] Optical filters are illustrated in fluorescent transmission and emission data plot 202, with reference to FIG. 2B. Excitation filter 208 includes wavelengths from the RPE excitation bandwidth. Further, emission filter 210 includes wavelengths from the RPE emission bandwidth. In other words, excitation filter 208 transmits RPE excitation light and emission filter 210 transmits RPE emission light.

[0042] In various embodiments, an inverted fluorescent optical system may be used. In an inverted system, the system is upside down with reference to FIG. 1. FIG. 3 illustrates a block diagram of an inverted general fluorescent optical system configuration. An inverted system is favorable for some sample types, such as large and thick samples. In applications for various embodiments described herein, a reaction site is imaged while immersed in a fluid. Thus, in an inverted system, the reaction site may be imaged through glass, keeping the fluid surrounding the reaction site.

[0043] Reaction site on sample holder 302 is being imaged for fluorescent emission light. Light from light source 304 includes a plurality of wavelengths of light. Light from light source 304 passes through excitation filter 306, which is designed to transmit a certain bandwidth of light for excitation of a fluorescent dye. The transmitted light is reflected by dichroic filter 308. Instead of dichroic filter 308 reflecting the light downward as in system 100, the light is reflected upwards in an inverted system. The reflected light then passes through objective lens 310 to direct the reflected light to sample holder 302. A plurality of reaction sites may be included in sample holder 302. Sample holder 302 may be immersed in a fluid (not shown).

[0044] A sample holder may be, according to various embodiments described herein, an array plate, a microarray, a flow cell, a substrate, a multi-well tray, such as a standard microtiter 96-well, a 384-well plate, or a microcard, or a substantially planar support, such as a glass or plastic slide or a silicon chip, or the like. In accordance with various embodiments described herein, reaction sites may include, but are not limited to, through-holes, wells, indentations, spots, cavities, sample retainment regions, and reaction chambers, for example.

[0045] Any fluorescent emission light from sample holder 302 then passes through objective lens 310 and dichroic filter 308. Emission filter 312 is designed to transmit the expected fluorescent emission bandwidth through tube lens 314 to optical detector 316. Optical detector 316 generates an image of a reaction site on sample holder 302 and fluorescent emission intensity can be determined.

[0046] A drawing of an inverted fluorescent optical system 400 according to various embodiments described herein is shown in FIG. 4. Reaction site 402 is being analyzed by system 400. Light from light source 404 is directed through a tube lens 406 to filter cube 408. Light source 404 may be a LED/Laser Pumped Phosphor (LPP) light phosphor. Filter cube 408 includes an excitation filter, dichroic filter, and emission filter, described with reference to FIG. 3. The excitation filter transmits the light to dichroic filter of filter cube 408. The dichroic filter reflects the light through objective lens 416 to reaction site 402. The reflected light will excite the fluorescent dye to emit light that will pass through objective lens 416 through an emission filter included in filter cube 408. The emitted light passes through tube lens 410 to mirror 412 and to a camera 414 to detect the emitted light. According to various embodiments described herein, camera 414 is a monochrome camera. Only a portion of reaction site 402 can be imaged at a time as mentioned above. Several images of reaction site 402 are taken to analyze all the reaction sites within the sample holder. According to applications of various embodiments described herein it is helpful to know the dimensions of the sample holder and the precise location of the optical sensor. Taking several images of small features in a large sample holder requires thousands of images to be stitched together to generate an image of the entire sample holder.

[0047] As an example, a reaction site may be approximately 3 μm , with centers of reaction sites 5 μm apart. In this example, we would have 8 camera pixels across the reaction site and 3 camera pixels in between, while requiring a microscope objective to get a good image, i.e. at high resolution using an optical system with high numerical aperture. However, even at the low magnification (10X, NA 0.45), the field of view is only about 1 mm x 1 mm. Thus, covering 96 pegs included in a sample holder (each with a 6 mm x 6 mm = 36 mm² area) requires taking 6,912 images per sample holder (3,456 images x 2 colors).

[0048] FIG. 5 illustrates a sample holder according to various embodiments described herein. Sample holders may have various reaction sites. According to various embodiments, each reaction site is included on a peg. Peg 502 is illustrated showing 36 different areas that need to be imaged to generate an image of an entire reaction site or peg, according to various embodiments. In other embodiments, each peg may be divided into 9-36 areas to be imaged by an optical system to get an image of the reaction for analysis.

[0049] In one example, a sample holder includes 96 pegs. According to various embodiments of the present teachings, an image may be 1mm x 1mm. In other embodiments, an image may be 2mm x 2mm. To keep the number images to a reasonable number for this type of application, the size of the features to be analyzed and image size should be considered. In other words, there is a trade-off between magnification (how much of the reaction site you see in the image) and the numerical aperture (resolution). In one embodiment, the optical system has a numerical aperture of 0.45 and a 10x magnification.

[0050] An optical system is moved to each portion, or area, of a peg to scan the entire peg. Then, the optical system is moved to all of the pegs within the sample holder to generate a complete analysis of the samples. Further, with reference back to FIG. 4, there is mechanical motion to adjust filter cube 408 if more than one fluorescent dye is used. The mechanical movement of filter cube 408 increases the time needed to complete a scan of a sample holder.

[0051] As such, various embodiments described herein reduce scan time of a large number of samples. FIG. 6 illustrates an optical system according to various embodiments described herein. Using multiband filters that can quickly analyze at least two channels can greatly reduce the time needed to analyze a sample holder. According to various embodiments, multiband

filters can also be configured for multiple channels. In some embodiments, multiband filters are configured for 3-5 channels.

[0052] Multiband filters are also more difficult to design, and manufacture compared to previously used single band filters. Extensive experimentation is needed to determine the matching bands of the filters. Further, various applications may each require a different multiband filter, each requiring a lot of time and experimentation to design and manufacture. However, using a multiband filter according to various embodiments of the present teachings simplifies performing the application and saves a great amount of time in generating a result.

[0053] Reaction site on sample holder 602 is being imaged for fluorescent emission light. Light from light source 604 includes a plurality of wavelengths of light. Light from light source 604 passes through multiband excitation filter 606, which is designed to transmit at least two bandwidths of light. Multiband excitation filter 606 is configured to transmit predetermined bandwidths of light for the types of fluorescent dyes used in the system. The predetermined bandwidths of light are expected excitation light for the at least two fluorescent dyes utilized in the assay. In some embodiments, a lens or series of collection lenses (not shown) is used to collimate and direct the light towards a multiband filter cube. A multiband filter cube includes multiband excitation filter 606, multiband dichroic filter 608 and multiband emission filter 612. The at least two transmitted bands are reflected by multiband dichroic filter 608. Multiband dichroic filter 608 is at 45 degree in this embodiment. The at least two reflected bands of light then passes through objective lens 610 to focus the at least two bands of reflected light to sample holder 602. A plurality of reaction sites may be included in sample holder 602. In accordance with various embodiments described herein, reaction sites may include, but are not limited to, through-holes, wells, indentations, spots, cavities, sample retainment regions, and reaction chambers, for example.

[0054] Any fluorescent emission light from sample holder 602 then passes through objective lens 610 and multiband dichroic filter 608. Multiband dichroic filter 608 is configured to transmit at least two predetermined bands of emission light from the fluorescent dyes.

[0055] Multiband emission filter 612 is designed to transmit the at least two emission bands of expected fluorescent emission bandwidth through tube lens 614 to optical detector 616.

Further, multiband emission filter 612 transmits expected fluorescent bands of light while eliminating any unwanted wavelengths. The combination of multiband excitation filter 606, multiband dichroic filter 608, and multiband emission filter 612 is configured to analyze at least two different fluorescent dye emissions without changing filters to image each fluorescent dye. Thus, no movement of the multiband filters is needed to image the different fluorescent dye emissions. In this way, time to scan an array is greatly reduced.

[0056] According to various embodiments of the present teachings, multiband excitation filter 606, multiband dichroic filter 608, and multiband emission filter 612 can be configured for two, three, four, or more fluorescent dyes.

[0057] Tube lens 614 focuses the light onto optical detector 616. Optical detector 616 generates an image of a reaction site on sample holder 602 and fluorescent emission intensity can be determined. According to various embodiments, optical detector 616 may be a CCD, CMOS, or sCMOS sensor, for example. Optical detector 616 collects the light and ADC electronics convert the light into digital signals and relay them to a computer for useful analysis.

[0058] In this example, optical detector 616 is stationary and sample plate 602 may be moved with respect to objective lens 610 during imaging.

[0059] The chart below compares previous optical systems to multiband optical systems according to various embodiments of the present teachings.

	Excitation Filter (0 deg)	Dichroic Filter (45 deg)	Emission Filter (0 deg)
Previous	<u>Green Filter</u> Transmits excitation light for shorter wavelength dye (RPE), Reflects all other light (T is < 1PPM) <u>Red Filter</u> Transmits excitation	<u>Green Filter</u> Reflects excitation light for shorter wavelength dye (RPE), Transmits all other light (In particular, the emission light created	<u>Green Filter</u> Transmits emission light for shorter wavelength dye (RPE), Reflects all other light (T is < 1PPM) <u>Red Filter</u> Transmits excitation

	light for longer wavelength dye (APC) Reflects all other light (T is < 1PPM)	by the green). <u>Red Filter</u> Transmits excitation light for longer wavelength dye (APC) Transmits all other light (In particular, the fluorescent light created by the red).	light for longer wavelength dye (APC) Reflects all other light (T is < 1PPM)
Multiband	Green-Red Dual Band Filter Transmits both green and red (the filter has two transmission bands) Reflects all other light (T is < 1PPM)	Red-Green Dual Band Filter Reflects both green and red excitation (the filter has two reflection bands) Transmits all other light	Green-Red Dual Band Filter Transmits emission light for both RPE and APC Both the shorter and longer wavelength bands (the filter has two transmission bands) Reflects all other light (T is < 1PPM)

[0060] In various embodiments of the present teachings, a mechanical apparatus is included to move the sample holder in x and y directions so that the optical system can raster scan images of the sample holder. The mechanical apparatus may also have movement in the z-direction to focus the sample holder with respect to the objective lens.

[0061] According to various embodiments described herein, a method of optical scanning is also provided. A flowchart 700 of method of optical scanning with multiband filters is illustrated in FIG. 7. Step 702 includes generating light from a light source. Step 704 includes selecting,

by a multiband excitation filter configured to transmit at least two excitation bands of light, a first excitation band of light. Each of the at least two excitation bands of light is an excitation band of light for a fluorescent dye used in the sample holder. In step 706, a multiband dichroic filter configured to reflect the at least two excitation bands of light, reflects the first excitation band of light. Next, in step 708, a lens directs the first excitation band of light toward a sample holder and collects the resulting fluorescent light. A multiband emission filter configured to transmit at least two fluorescent emission bands of light, in step 710, transmits the first band of fluorescent emission light from each reaction site of the sample holder. Step 712 includes detecting, by an optical sensor, the at least two bands of fluorescent emission light from the multiband emission filter to generate an image of a sample holder.

[0062] To image emissions from a second fluorescent dye, the multiband excitation filter, the multiband dichroic filter, and the multiband emission filter do not need to be switched according to various embodiments described herein. Next, the multiband excitation filter can transmit the second excitation band of light, where the second excitation band of light is selected based on the use of a second fluorescent dye. The multiband dichroic filter then reflects the second excitation band of light toward the sample holder. The multiband emission filter may then transmit the second fluorescent emission band of light from the reaction sites. The second fluorescent emission band of light is also transmitted by the multiband dichroic filter to the optical sensor to generate an image of the sample holder.

[0063] According to various embodiments of the present teachings, the multiband excitation filter, the multiband dichroic filter, and the multiband emission filter may be configured for three, four, and five fluorescent dyes, for example. In this way, the multiband excitation filter, the multiband dichroic filter, and the multiband emission filter do not need to be switched, or physically moved in the system to image all fluorescent emissions included in the sample holder, reducing the time needed to complete a scan of the sample holder.

[0064] Several examples of multiband filters are illustrated in FIGS. 8-12 according to various embodiments described herein. In FIGS. 8-12, the excitation and emission curves of fluorescent dyes are illustrated. The excitation light is shifted to a longer wavelength when it is emitted, known as a “Stokes Shift”. As such, different filters are configured to transmit either

excitation light or emission light. Moreover, dichroic filters are configured to reflect excitation wavelengths of light while also transmitting emission wavelengths of light. According to various embodiments described herein, a multiband excitation filter is configured to include filters for excitation light for the fluorescent dyes used in a hybridization array. Similarly, according to embodiments described herein, a multiband dichroic filter is configured to include filters for expected emission light for the fluorescent dyes used, while reflecting expected excitation light, according to various embodiments described herein. Furthermore, according to embodiments described herein, a multiband emission filter is configured to include filters for expected emission light for the fluorescent dyes used. In this way, filters do not need to be changed to image each fluorescent dye.

[0065] FIG. 8 illustrates an exemplary fluorescent transmission and emission data plot illustrating a dual-band filter configuration for RPE and APC dyes according to various embodiments described herein. The x-axis shows the wavelength of light and the y-axis shows the percent of transmission. In this example, Multiband excitation filter 814 is configured to include RPE excitation filter 802 and APC excitation filter 1306. Multiband excitation filter 814 is configured to permit transmission of both RPE and APC excitation bands of light.

[0066] Further, multiband dichroic filter 816 includes RPE dichroic filter 810 and APC dichroic filter 812. Multiband dichroic filter 816 is configured to reflect RPE and APC excitation bands of light. Multiband dichroic filter 816 is further configured to transmit RPE and APC emission bands of light.

[0067] Multiband emission filter 818 includes RPE emission filter 804 and APC emission filter 808. Multiband emission filter 818 is configured to transmit expected RPE and APC emission bands of light.

[0068] In various embodiments of the present teachings, multiband excitation filter 814, multiband dichroic filter 816, and multiband emission filter 818 do not need to be moved or switched to image all the fluorescent emissions used in this example. All fluorescent dyes used are imaged sequentially, without the need to physically move filters. According to various embodiments, multiband excitation filter 814, multiband dichroic filter 816, and multiband emission filter 818 are included in a filter cube.

[0069] In another example, according to various embodiments of the present teachings, FIG. 9 illustrates a fluorescent transmission and emission data plot using two fluorescent dyes. In this example, multiband excitation filter 910 is configured to include dye 1 excitation filter 902 and dye 2 excitation filter 906. In this way, multiband excitation filter 910 permits the transmission of expected excitation light of dye 1 and dye 2.

[0070] Further, a multiband dichroic filter (not shown) is configured to reflect expected excitation light of dye 1 and dye 2. The multiband dichroic filter is further configured to transmit expected fluorescent emission light of dye 1 and dye 2.

[0071] Multiband emission filter 912 includes dye 1 emission filter 904 and dye 2 emission filter 908. Multiband emission filter 912 is configured to transmit expected fluorescent emission wavelengths of dye 1 and dye 2.

[0072] In yet another example, according to various embodiments of the present teachings, FIG. 10 illustrates a fluorescent transmission and emission data plot using three fluorescent dyes. The x-axis shows the wavelength of light and the y-axis shows the percent of transmission. In this example, multiband excitation filter 1014 is configured to include a dye 1 excitation filter 1002, dye 2 excitation filter 1006, and dye 3 excitation filter 1010 to permit transmission of expected excitation wavelengths of light of dye 1, dye 2, and dye 3.

[0073] Further, a multiband dichroic filter (not shown) is configured to reflect expected wavelengths of light for dye 1, dye 2, and dye 3. The multiband dichroic filter (not shown) is also configured to transmit expected fluorescent emission light of dye 1, dye 2, and dye 3.

[0074] Multiband emission filter 1016 includes dye 1 emission filter 1004, dye 2 emission filter 1008, and dye 3 emission filter 1012. Multiband emission filter 1016 is configured to transmit expected fluorescent emission light of dye 1, dye 2, and dye 3.

[0075] In yet another example, according to various embodiments of the present teachings, FIG. 11 illustrates a fluorescent transmission and emission data plot using four fluorescent dyes. The x-axis shows the wavelength of light and the y-axis shows the percent of transmission. In this example, multiband excitation filter 1118 includes dye 1 excitation filter 1102, dye 2 excitation filter 1106, dye 3 excitation filter 1110, and dye 4 excitation filter 1114 to permit

transmission of those wavelengths within dye 1 excitation band of light, dye 2 excitation band of light, dye 3 excitation band of light, and dye 4 excitation band of light.

[0076] Further, a multiband dichroic filter (not shown) is configured to reflect expected excitation wavelengths of dye 1, dye 2, dye 3, and dye 4. A multiband dichroic filter (not shown) is further configured to transmit expected fluorescent emission wavelengths of dye 1, dye 2, dye 3, and dye 4.

[0077] Multiband emission filter 1120 includes dye 1 emission filter 1104, dye 2 emission filter 1108, dye 3 emission filter 1112, and dye 4 emission filter 1116. Multiband emission filter 1120 transmits expected fluorescent emission wavelengths of dye 1, dye 2, dye 3, and dye 4.

[0078] In yet another example, according to various embodiments of the present teachings, FIG. 12 illustrates a fluorescent transmission and emission data plot using two fluorescent dyes. The x-axis shows the wavelength of light and the y-axis shows the percent of transmission. In this example, a multiband excitation filter 1214 is configured to include dye 1 excitation filter 1202 and dye 2 excitation filter 1206 to permit transmission of dye 1 expected excitation bands of light and dye 2 expected excitation band of light.

[0079] Further, multiband dichroic filter 1216 includes dye 1 dichroic filter 1210 and dye 2 dichroic filter 1212. Multiband dichroic filter 1216 is configured to reflect wavelengths within dye 1 excitation filter 1202 and dye 2 excitation filter 1206. Multiband dichroic filter 1216 is further configured to transmit expected emission wavelengths of light of dye 1 and dye 2.

[0080] Multiband emission filter 1218 is composed of dye 1 emission filter 1204 and dye 2 emission filter 1208. Multiband emission filter 1218 is configured to transmit expected emission wavelengths of dye 1 and dye 2.

[0081] According to various embodiments described herein, the light source is configured for use with the multiband filters. With reference back to FIG 8, light source 804 may be configured so that light source 804 is restricted and switch on and off independently. Typically, a Xenon light source may be used. However, a LED or a halogen light source may also be used according to various embodiments described herein.

[0082] FIG. 13 illustrates an emission plot 1300 of a Xe light source according to various embodiments described herein. To work with multiband band filters, a Xe lamp would have its emission light restricted (from all of the visible range) to narrow bands matched to the multiband emission filter bands.

[0083] FIG. 14 illustrates an exemplary optical system utilizing Xe lamp 1402 according to various embodiments described herein utilizing filter wheel 1408 containing a multiplicity of single bandpass filters, being switched on one at a time. Each single bandpass filter is configured to allow wavelengths corresponding to the excitation wavelengths of the fluorescent dyes used in a particular experiment. Xe lamp 1402 emits light through lens 1406 and then is restricted in wavelength by one of the several excitation filters in the filter wheel so that only one band of the multiband filter allows light through. In addition, the light from continuous light source 1402 is switched off and on with a shutter 1410 for controlled short camera exposures.

[0084] As described above, the light then passes through multiband excitation filter 1412, to multiband dichroic filter 1414, through lens 1424, to a reaction site included on sample holder 1404. Fluorescent emission passes back through lens 1424, through multiband dichroic filter 1414, through multiband emission filter 1416, through lens 1420, and to optical sensor 1422 to generate an image. Multiband excitation filter 1412, multiband dichroic filter 1414, and multiband emission filter 1416 are included in multiband filter cube 1418. After an image of the first fluorescent dye emissions are imaged, filter wheel 1408 changes the bandpass filter on Xe lamp 1402 to emit wavelengths of light corresponding to the second fluorescent dye used to capture the next image by optical sensor 1422. Other filters may be used for Xe lamp 1402 to capture all the fluorescent dye images needed. After the fluorescent dye images are captured for one reaction site included on sample holder 1404, then the next reaction site included on sample holder 1404 is imaged, and so on until all the images are generated. Multiband filter cube 1418 does not need to be changed according to various embodiments of the present teachings, reducing the time needed to image sample holder 1404.

[0085] In other various embodiments of the present teachings, at least two narrowband visible light sources with bands corresponding to the transmission bands of the multiband excitation filter, are either quickly switched off and on so that only one band of the multiband

filter allows light through, or a fast mechanical shutter is used to turn the light off and on for controlled short periods.

[0086] FIG. 15 illustrates an emission plot 1500 of a white LED light source according to various embodiments described herein. A white LED has broad spectral emissions and would need at least two changeable bandpass filters. Each of the bandpass filters match a single band of the multiband excitation filter.

[0087] Similar to using bandpass filters for the Xe lamp, bandpass filters with a filter wheel may be used with a white LED light source according to various embodiments of the present teachings. To work with multiband band filters, a LED light source would have its emission light restricted (from all of the visible range) to narrow bands matched to the multiband emission filter bands.

[0088] FIG. 16 illustrates an exemplary optical system utilizing a white LED lamp 1602 according to various embodiments described herein utilizing filter wheel 1608 containing a multiplicity of single bandpass filters, being switched on one at a time. Each single bandpass filter is configured to allow wavelengths corresponding to the excitation wavelengths of the fluorescent dyes used in a particular experiment. LED lamp 1602 emits white light through lens 1606 and is filtered by a bandpass filter in filter wheel 1608 so that only one band of the multiband filter allows through light. Shutter 1610 allows the light to quickly switched on and off for short camera exposures. As described above, the light then passes through multiband excitation filter 1612, to multiband dichroic filter 1614, through lens 1624, to a reaction site included on sample holder 1604. Fluorescent emissions pass back through lens 1624, through multiband dichroic filter 1614, through multiband emission filter 1616, through lens 1620, and to optical sensor 1622 to generate an image. Multiband excitation filter 1612, multiband dichroic filter 1614, and multiband emission filter 1616 are included in multiband filter cube 1618. After an image of the first fluorescent dye emissions are imaged, filter wheel 1608 changes the bandpass filter on LED lamp 1602 to emit wavelengths of light corresponding to the second fluorescent dye used to capture the next image by optical sensor 1622. Other filters may be used for LED lamp 1602 to capture all the fluorescent dye images needed. After the fluorescent dye images are captured for one reaction site included on sample holder 1604, then the next reaction

site included on sample holder 1604 is imaged, and so on until all the images are generated. Multiband filter cube 1618 does not need to be changed according to various embodiments of the present teachings, reducing the time needed to image sample holder 1604.

[0089] In another embodiment according to the present teachings, shutter 1610 can be eliminated and white LED 1602 may be turned on and off to control camera exposure time.

[0090] FIG. 17 illustrates an emission plot of a narrow band LED light source according to other various embodiments described herein. In this embodiment, different narrowband LED are used as the light source.

[0091] FIG. 18 illustrates an exemplary optical system utilizing first narrowband LED lamp 1802 and second narrowband LED lamp 1806 according to various embodiments described herein. Each narrowband LED corresponds to the excitation wavelengths of the fluorescent dyes used in the sample holder. After emissions from one fluorescent dye are imaged, another narrowband LED is turned on to capture an image of second fluorescent dye emissions. Other narrowband LED are turned on one at a time to capture images of corresponding fluorescent dyes. The next reaction site is imaged in a similar way and so forth to image the sample holder for analysis. Each narrowband LED lamp can be independently turned on and off, which can be much faster than moving a filter wheel or dichroic filter cube, reducing the scanning time.

[0092] First narrowband LED lamp 1802 emits light, which is transmitted through dichroic filter 1826 to lens 1808. Light transmitted through lens 1808 then passes through multiband excitation filter 1812, to multiband dichroic filter 1814, through lens 1810, to a reaction site included on sample holder 1804. Fluorescent emissions pass back through lens 1810, through multiband dichroic filter 1814, through multiband emission filter 1816, through lens 1820, and to optical sensor 1822 to generate an image. Multiband excitation filter 1812, multiband dichroic filter 1814, and multiband emission filter 1816 are included in multiband filter cube 1818. After an image of the first fluorescent dye emissions are imaged, first narrowband LED lamp 1802 is switched off and second narrowband LED lamp 1806 is switched on.

[0093] Narrowband LED light from LED lamp 1806 is emitted in much the same way through the optical system as described above to image the fluorescent emissions from the

second fluorescent dye. However, mirror 1824 reflects light from LED lamp 1806. Further, dichroic filter 1826 reflects the light from LED lamp 1806 to lens 1808. Mirror 1824 and dichroic filter 1826 work to combine light from the two different wavelength bands along a common optical path for use in the balance of the system shown in FIG 18. In embodiments where three or more LED lamps are used, a series of dichroic filters may be used so that LED light from different LED lamps have a common optical path for use with multiband filters with 3 or more bands.

[0094] As just described, according to various embodiments, 2, 3, 4, or more narrowband LED lamps may be used. In this way, the LED light sources are separated in wavelength, so no optical filters are needed to separate the light source lights other than the multiband filters in multiband filter cube 1818.

[0095] Multiband filter cube 1818 does not need to be changed to image the different fluorescent dyes according to embodiments of the present teachings, reducing the time needed to image sample holder 1804.

[0096] Examples

[0097] The following numbered examples are embodiments:

1. A system for sample holder scanning, the system comprising:

a light source;

a multiband excitation filter configured to select at least two excitation bands of light, wherein each of the at least two excitation bands of light is an excitation band of light for a fluorescent dye used in the sample holder;

a multiband dichroic filter configured to reflect the at least two excitation bands of light;

a lens configured to direct the at least two excitation bands of light toward a sample holder;

a multiband emission filter configured to transmit at least two bands of fluorescent emission light from each reaction site of the sample holder, wherein the multiband dichroic filter is further configured to transmit the at least two bands of fluorescent emission light; and

an optical sensor configured to detect the at least two bands of fluorescent emission light from the multiband emission filter to generate an image of a sample holder.

2. The system of example 1, further comprising:

a mechanical apparatus configured to move the sample holder in a x and a y direction such that the optical sensor raster scans each reaction site in the sample holder.

3. The system of any of the examples 1 to 2, wherein the mechanical apparatus moves the sample holder such that the optical sensor detects emission light from a plurality of areas of each reaction site in the sample holder.

4. The system of example 3, wherein the plurality of areas is 9-36 areas.

5. The system of any of the examples 1 to 4, wherein the optical sensor is selected from the group consisting of: a CCD sensor, CMOS sensor, and a sCMOS sensor.

6. The system of any of the examples 1 to 5, wherein the light source is a xenon lamp.

7. The system of any of the examples 1 to 6, further comprising:

a light control apparatus configured to:

restrict the output of the light source to a narrow wavelength band selected based on a desired band of light to pass through the multiband excitation filter, and

switch the light off and on for a predetermined time period to set an exposure time for the optical sensor.

8. The system of any of the examples 1 to 7, wherein the light control apparatus includes an optical filter.

9. The system of any of the examples 1 to 7, wherein the light control apparatus includes a mechanical shutter.

10. The system of any of the examples 1 to 5, wherein the light source is a white LED.

11. The system of example 10, further comprising:

at least two bandpass filters.

12. The system of any of the examples 1 to 5, wherein the light source comprises at least two LED sources, wherein each of the two at least LED sources is matched to one of the at least two expected excitation bands of dyes selected for use in the sample holder, wherein the at least two LED sources have minimal spectral overlap.

13. The system of example 12, wherein the light source comprises at least one a laser pumped phosphor device which is configured to match one of the at least two expected excitation bands of dyes selected for use in the sample holder.

14. A method for sample holder scanning, the method comprising:

generating light from a light source;

selecting, by a multiband excitation filter configured to transmit at least two excitation bands of light, a first excitation band of light, wherein each of the at least two excitation bands of light is an excitation band of light for a fluorescent dye used in the sample holder;

reflecting, by a multiband dichroic filter configured to reflect the at least two excitation bands of light, the first excitation band of light;

directing, by a lens, the first excitation band of light toward a sample holder;

transmitting, by a multiband emission filter configured to transmit at least two fluorescent emission bands of light, the first band of fluorescent emission light from each reaction site of the sample holder; and

detecting, by an optical sensor, the first band of fluorescent emission light from the multiband emission filter to generate an image of a sample holder.

15. The method of example 14, further comprising

moving, by a mechanical apparatus, the sample holder in a x and a y direction such that the optical sensor raster scans each reaction site in the sample holder.

16. The method of example 15, wherein moving the sample holder includes moving the sample holder so that the optical sensor detects emission light from a plurality of areas of each reaction site in the sample holder.

17. The method of any of the example 14 to 16, wherein the plurality of areas is 9-36 areas.

18. The method of any of the examples 14 to 17, further comprising:

detecting the at least two bands of fluorescent emission light from the plurality of areas of each reaction site in the sample holder to generate the image of the sample holder.

19. The method of any of the examples 14 to 18, further comprising:

designing the multiband excitation filter, multiband dichroic filter, and multiband emission filter to minimize crosstalk between fluorescent dyes used in the sample holder.

20. The method of any of the examples 14 to 19, wherein the optical sensor is selected from the group consisting of: a CCD sensor, CMOS sensor, and a sCMOS sensor.

21. The method of any of the examples 14 to 20, wherein the light source is a xenon lamp.

22. The method of any of the examples 14 to 21, further comprising:

turning on, by a light control apparatus, the xenon lamp on for a predetermined time period;
and

restricting the output of the light source to a narrow wavelength band selected based on a desired band of light to pass through the multiband excitation filter.

23. The method of any of the examples 14 to 22, wherein the light control apparatus includes an optical filter.

24. The method of any of the examples 14 to 22, wherein the light control apparatus includes a mechanical shutter.

25. The method of any of the examples 14 to 20, wherein the light source is a white LED.

26. The method of any of examples 14 to 20, wherein the light source comprises two LED sources, wherein each of the two LED sources is matched to one of the at least two expected excitation bands of dyes selected for use in the sample holder.

[0098] Although the present invention has been described with respect to certain exemplary embodiments, examples, and applications, it will be apparent to those skilled in the art that various modifications and changes may be made without departing from the invention.

CLAIMS

What is claimed is:

1. A system for sample holder scanning, the system comprising:
 - a light source;
 - a multiband excitation filter configured to select at least two excitation bands of light, wherein each of the at least two excitation bands of light is an excitation band of light for a fluorescent dye used in the sample holder;
 - a multiband dichroic filter configured to reflect the at least two excitation bands of light;
 - a lens configured to direct the at least two excitation bands of light toward a sample holder;
 - a multiband emission filter configured to transmit at least two bands of fluorescent emission light from each reaction site of the sample holder, wherein the multiband dichroic filter is further configured to transmit the at least two bands of fluorescent emission light; and
 - an optical sensor configured to detect the at least two bands of fluorescent emission light from the multiband emission filter to generate an image of a sample holder.
2. The system of claim 1, further comprising:
 - a mechanical apparatus configured to move the sample holder in a x and a y direction such that the optical sensor raster scans each reaction site in the sample holder.
3. The system of any of the claims 1 to 2, wherein the mechanical apparatus moves the sample holder such that the optical sensor detects emission light from a plurality of areas of each reaction site in the sample holder.
4. The system of claim 3, wherein the plurality of areas is 9-36 areas.
5. The system of any of the claims 1 to 4, wherein the optical sensor is selected from the group consisting of: a CCD sensor, CMOS sensor, and a sCMOS sensor.

6. The system of any of the claims 1 to 5, wherein the light source is a xenon lamp.
7. The system of any of the claims 1 to 6, further comprising:
a light control apparatus configured to:
restrict the output of the light source to a narrow wavelength band selected based on a desired band of light to pass through the multiband excitation filter, and
switch the light off and on for a predetermined time period to set an exposure time for the optical sensor.
8. The system of any of the claims 1 to 7, wherein the light control apparatus includes an optical filter.
9. The system of any of the claims 1 to 7, wherein the light control apparatus includes a mechanical shutter.
10. The system of any of the claims 1 to 5, wherein the light source is a white LED.
11. The system of claim 10, further comprising:
at least two bandpass filters.
12. The system of any of the claims 1 to 5, wherein the light source comprises at least two LED sources, wherein each of the two at least LED sources is matched to one of the at least two expected excitation bands of dyes selected for use in the sample holder, wherein the at least two LED sources have minimal spectral overlap.
13. The system of claim 12, wherein the light source comprises at least one a laser pumped phosphor device which is configured to match one of the at least two expected excitation bands of dyes selected for use in the sample holder.
14. A method for sample holder scanning, the method comprising:
generating light from a light source;

selecting, by a multiband excitation filter configured to transmit at least two excitation bands of light, a first excitation band of light, wherein each of the at least two excitation bands of light is an excitation band of light for a fluorescent dye used in the sample holder;

reflecting, by a multiband dichroic filter configured to reflect the at least two excitation bands of light, the first excitation band of light;

directing, by a lens, the first excitation band of light toward a sample holder;

transmitting, by a multiband emission filter configured to transmit at least two fluorescent emission bands of light, the first band of fluorescent emission light from each reaction site of the sample holder; and

detecting, by an optical sensor, the first band of fluorescent emission light from the multiband emission filter to generate an image of a sample holder.

15. The method of claim 14, further comprising
moving, by a mechanical apparatus, the sample holder in a x and a y direction such that the optical sensor raster scans each reaction site in the sample holder.

16. The method of claim 15, wherein moving the sample holder includes moving the sample holder so that the optical sensor detects emission light from a plurality of areas of each reaction site in the sample holder.

17. The method of any of the claim 14 to 16, wherein the plurality of areas is 9-36 areas.

18. The method of any of the claims 14 to 17, further comprising:
detecting the at least two bands of fluorescent emission light from the plurality of areas of each reaction site in the sample holder to generate the image of the sample holder.

19. The method of any of the claims 14 to 18, further comprising:
designing the multiband excitation filter, multiband dichroic filter, and multiband emission filter to minimize crosstalk between fluorescent dyes used in the sample holder.

20. The method of any of the claims 14 to 19, wherein the optical sensor is selected from the group consisting of: a CCD sensor, CMOS sensor, and a sCMOS sensor.

21. The method of any of the claims 14 to 20, wherein the light source is a xenon lamp.

22. The method of any of the claims 14 to 21, further comprising:
turning on, by a light control apparatus, the xenon lamp on for a predetermined time period; and
restricting the output of the light source to a narrow wavelength band selected based on a desired band of light to pass through the multiband excitation filter.

23. The method of any of the claims 14 to 22, wherein the light control apparatus includes an optical filter.

24. The method of any of the claims 14 to 22, wherein the light control apparatus includes a mechanical shutter.

25. The method of any of the claims 14 to 20, wherein the light source is a white LED.

26. The method of any of claims 14 to 20, wherein the light source comprises two LED sources, wherein each of the two LED sources is matched to one of the at least two expected excitation bands of dyes selected for use in the sample holder.

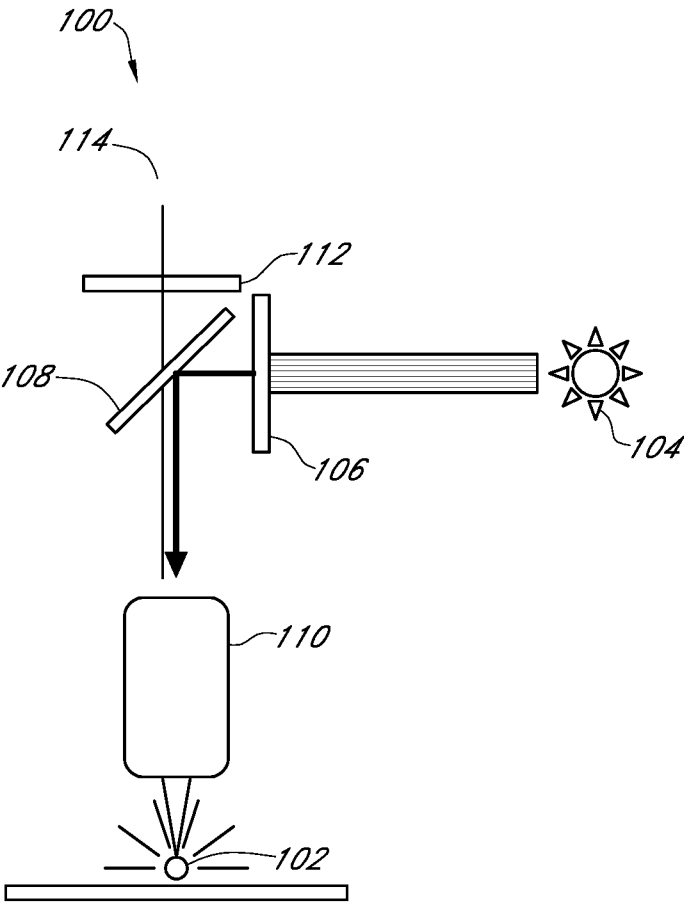


FIG. 1

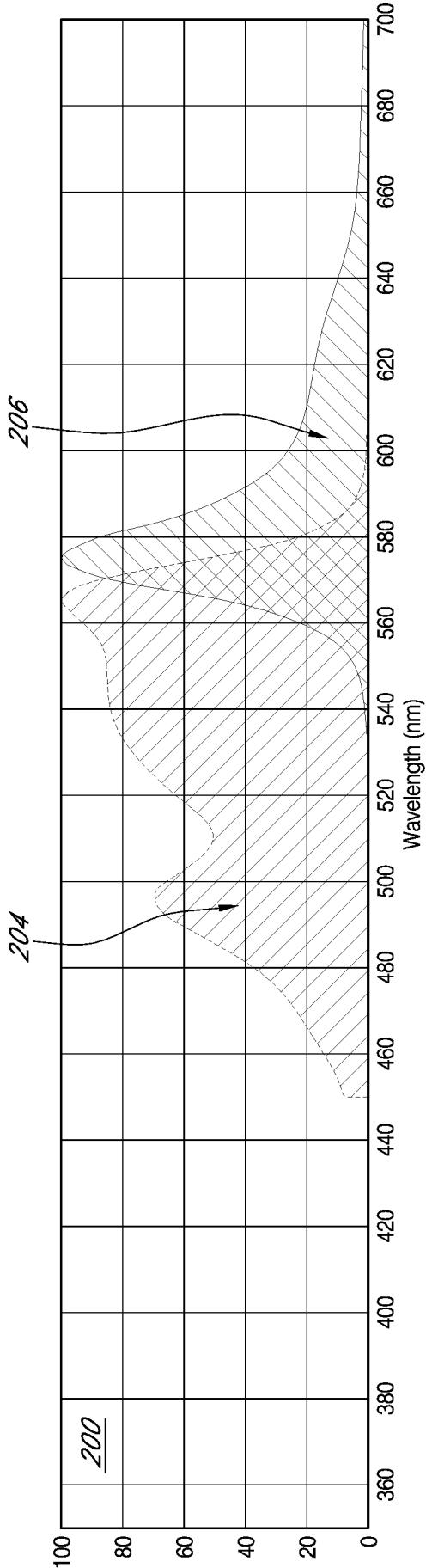


FIG. 2A

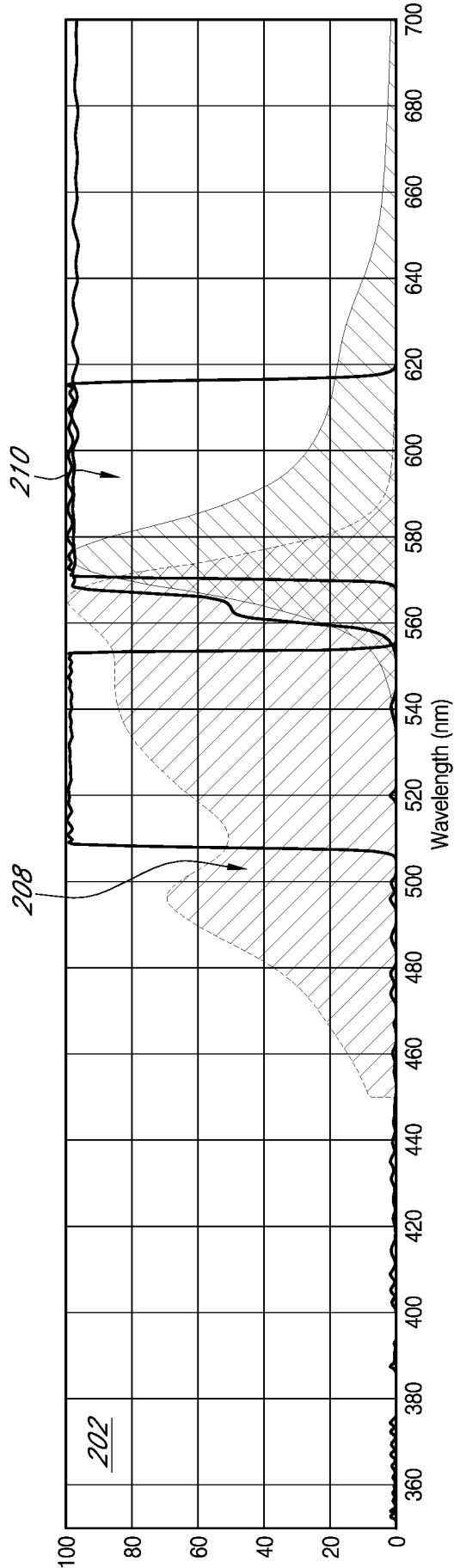


FIG. 2B

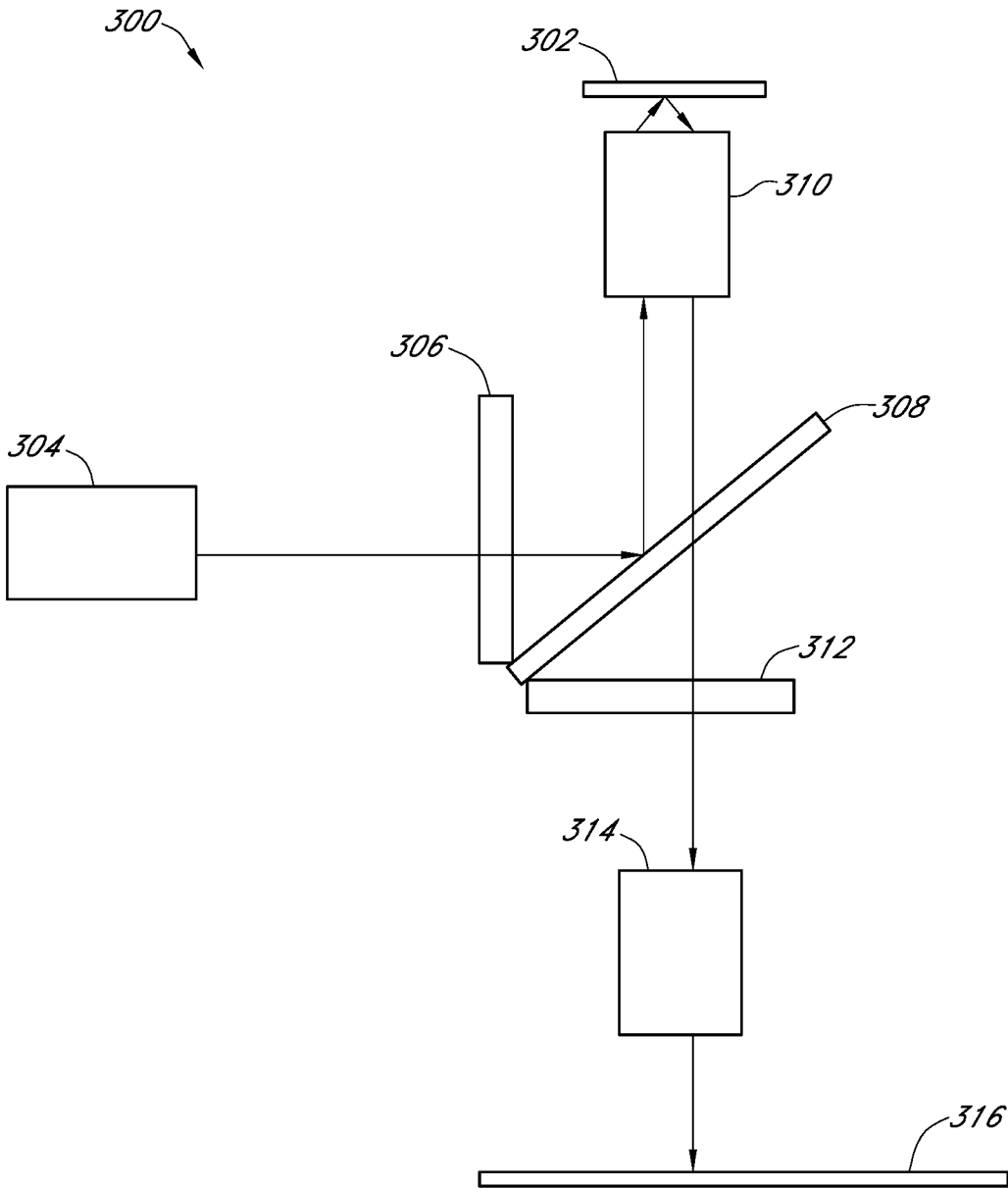


FIG. 3

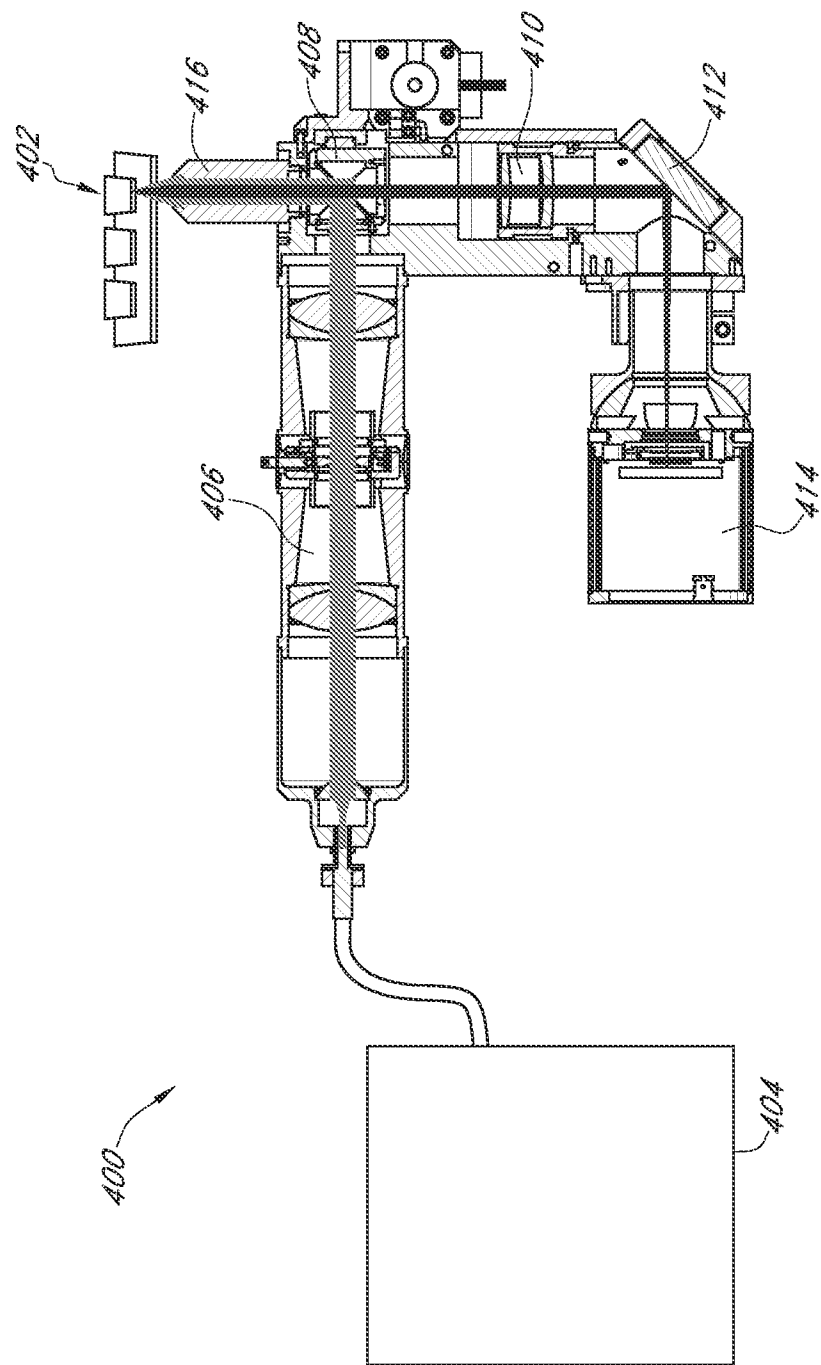


FIG. 4

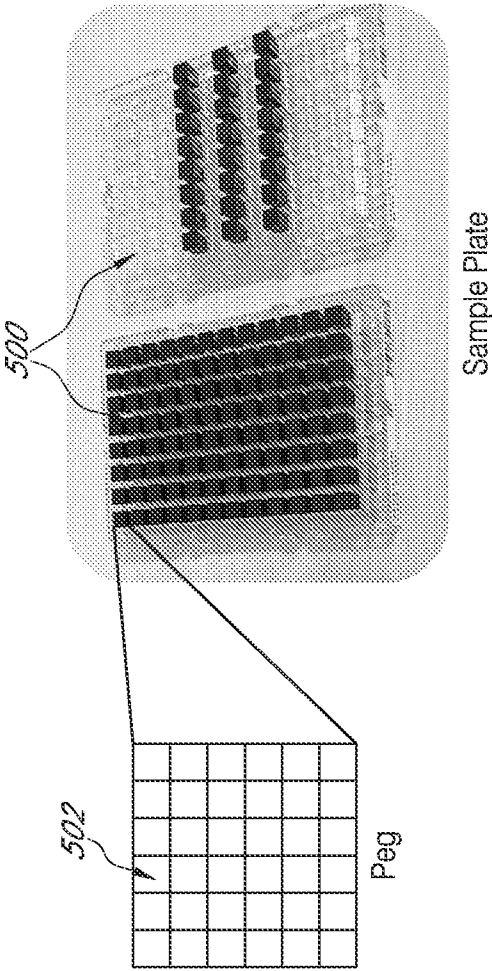


FIG. 5

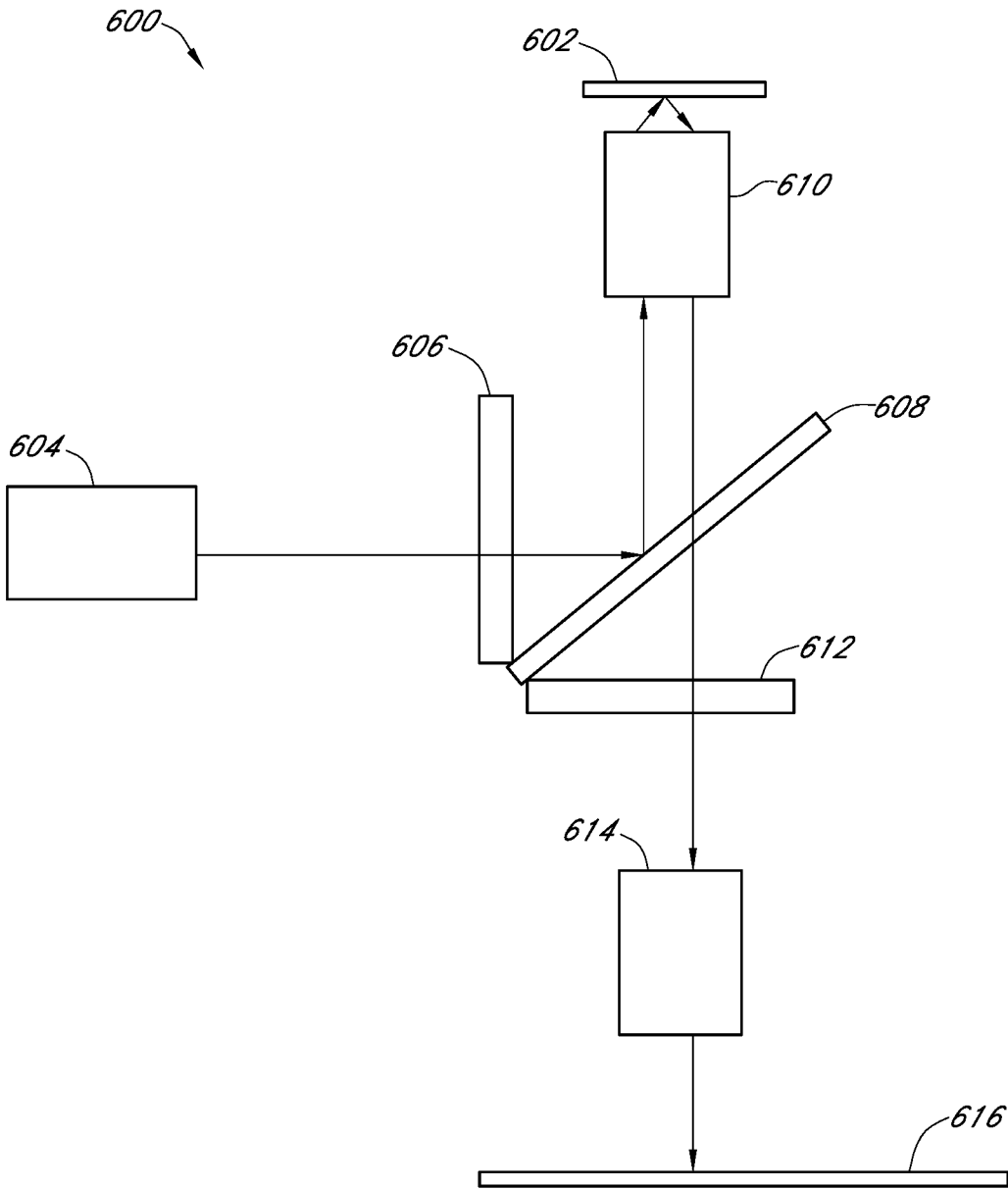


FIG. 6

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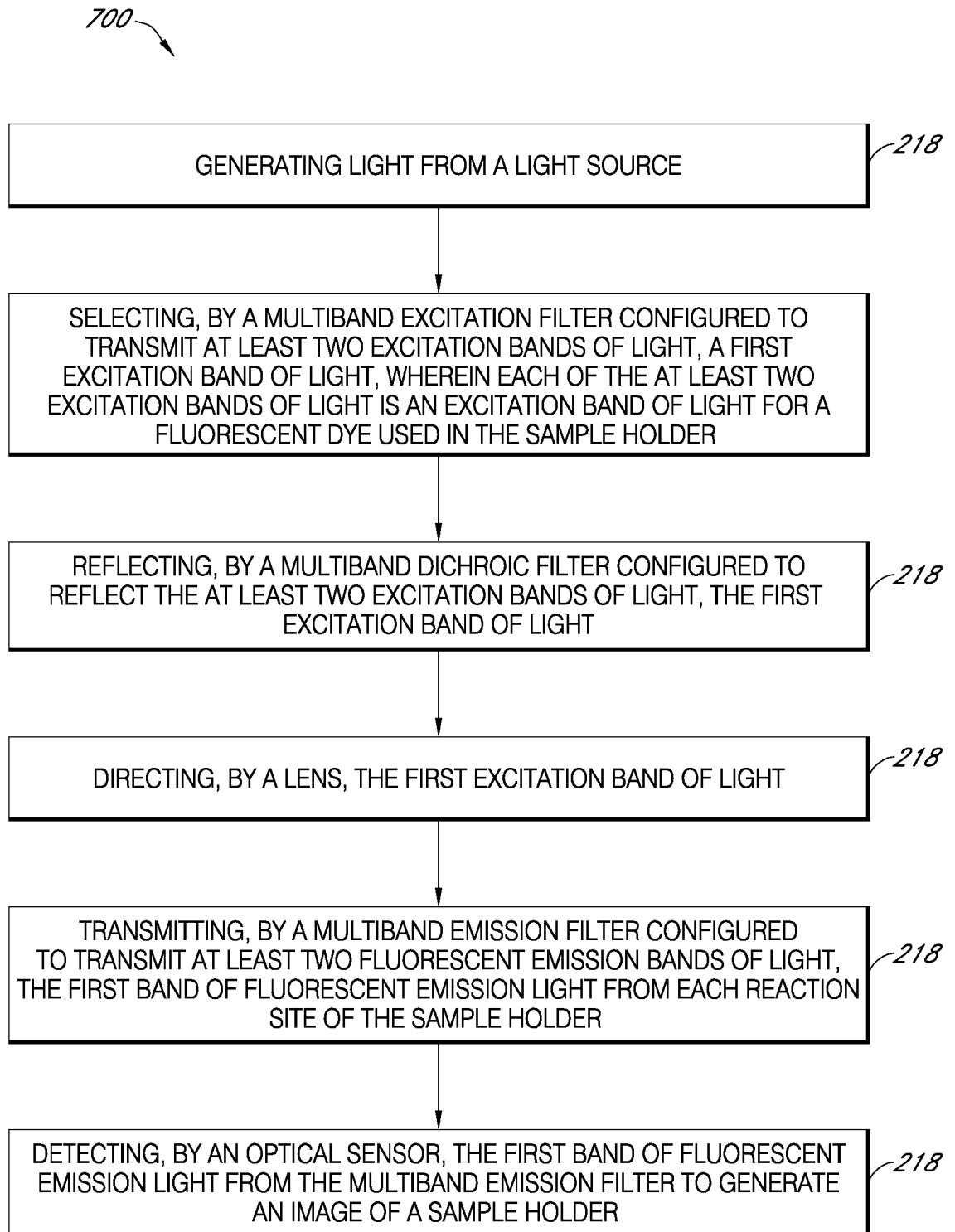


FIG. 7

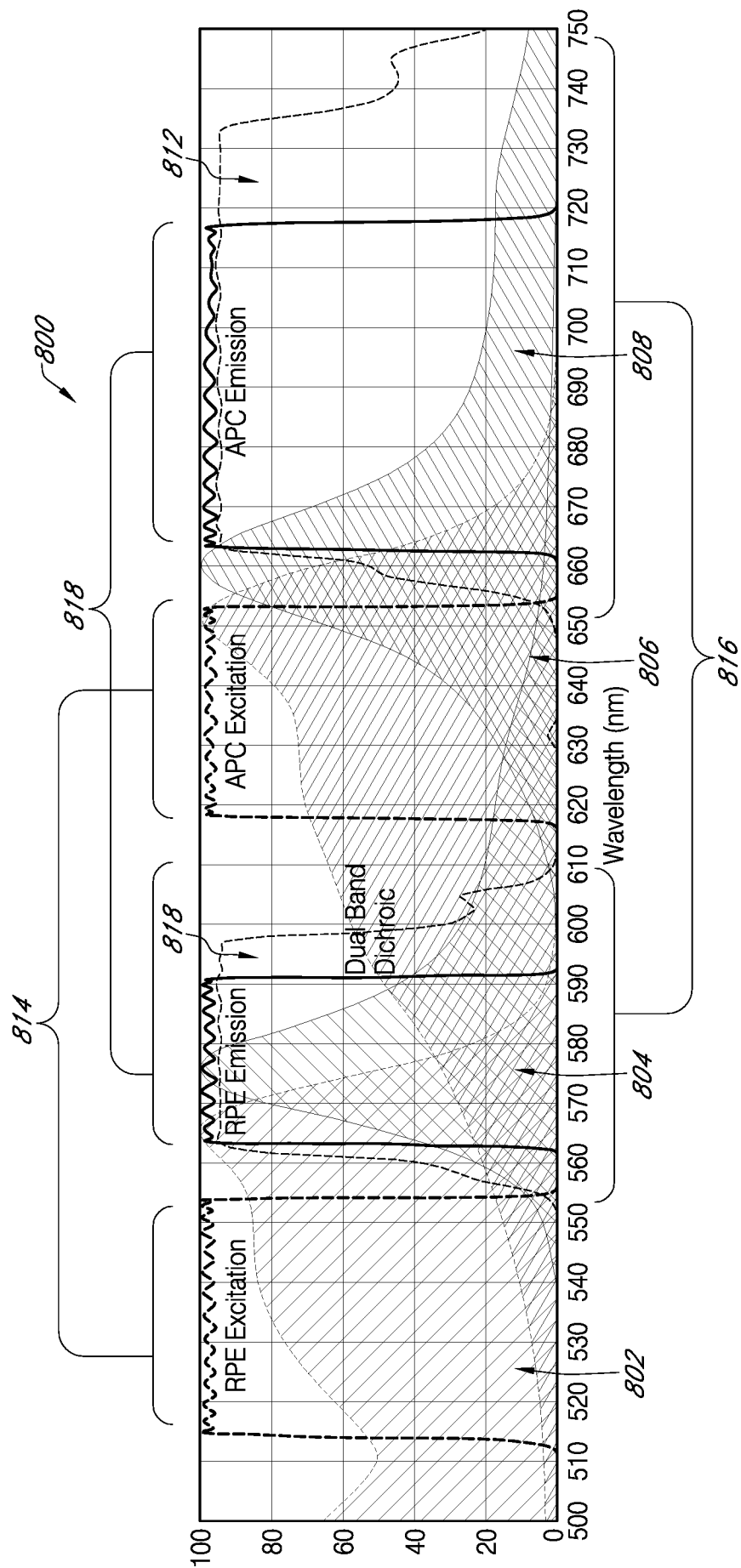


FIG. 8

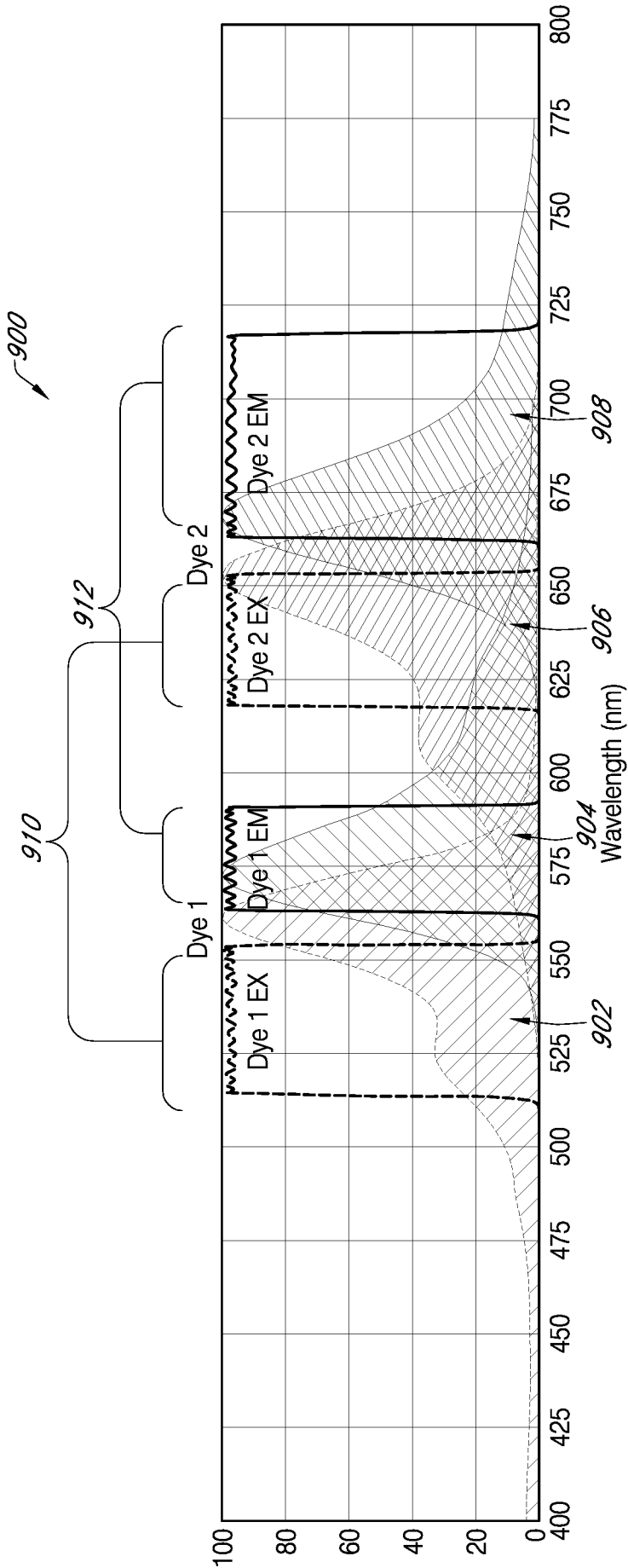


FIG. 9

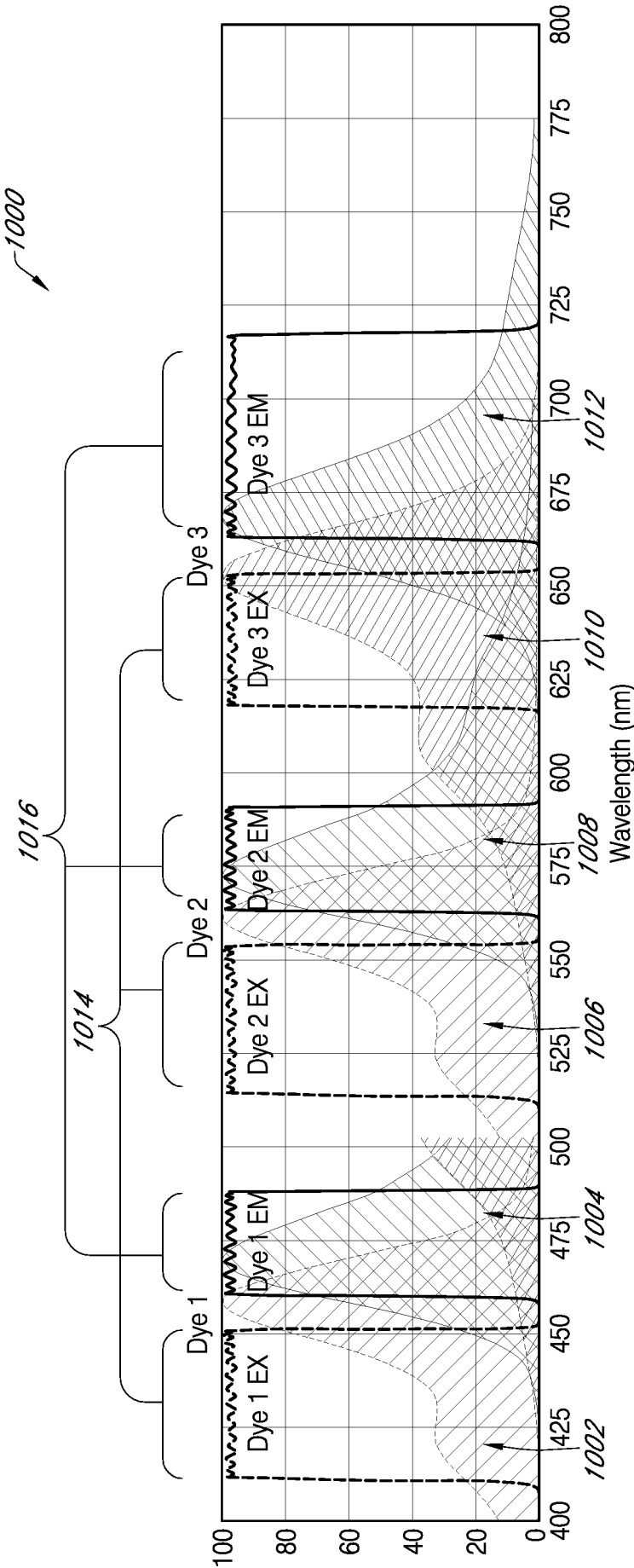


FIG. 10

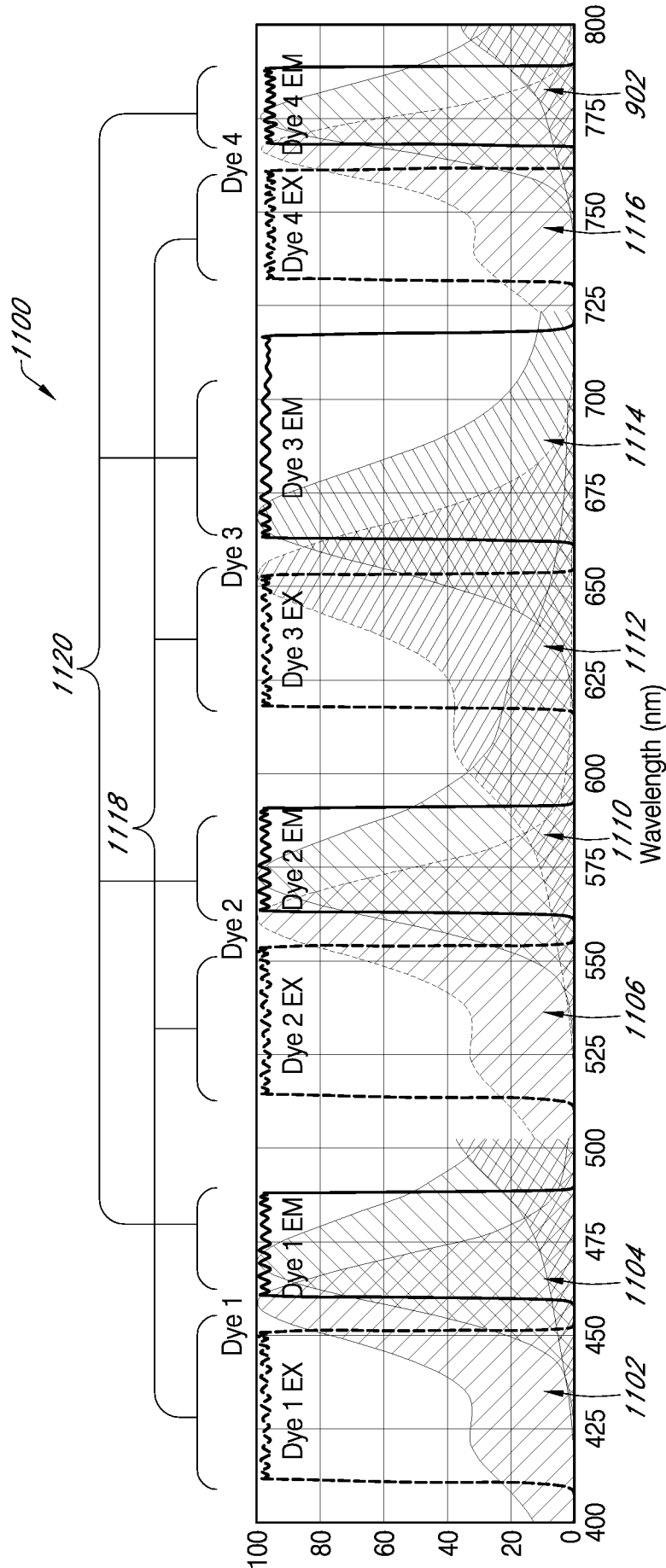


FIG. 11

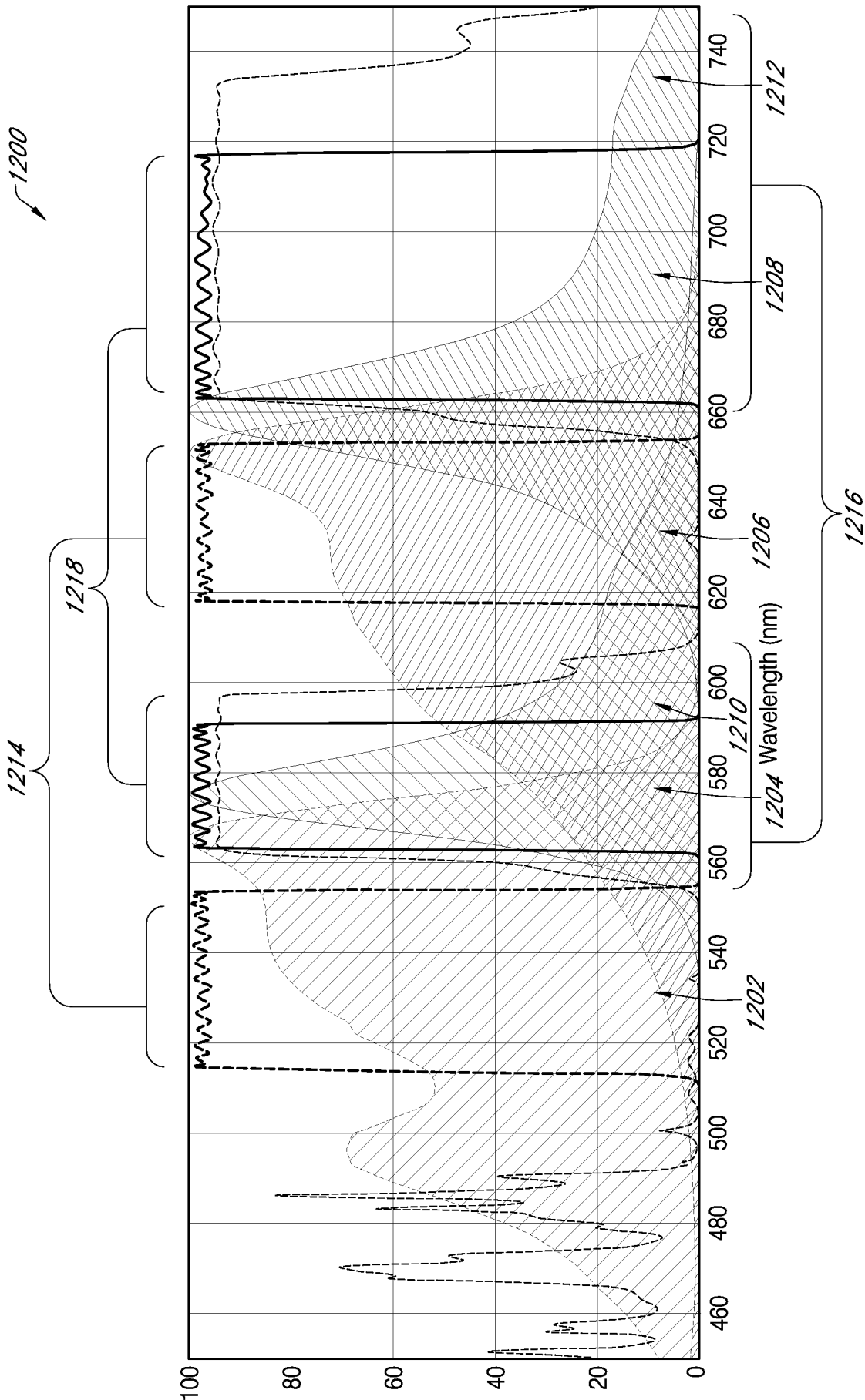


FIG. 12

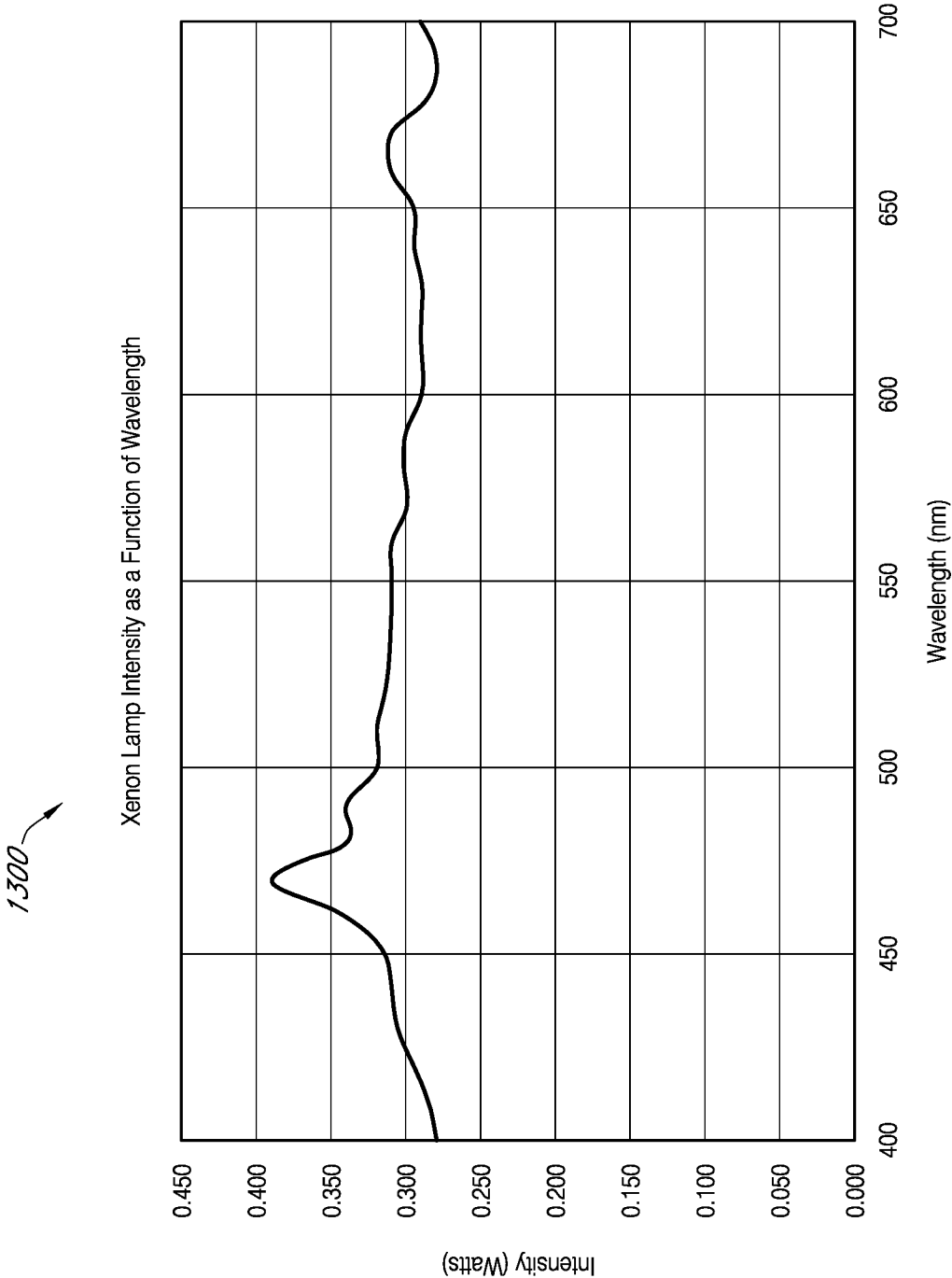


FIG. 13

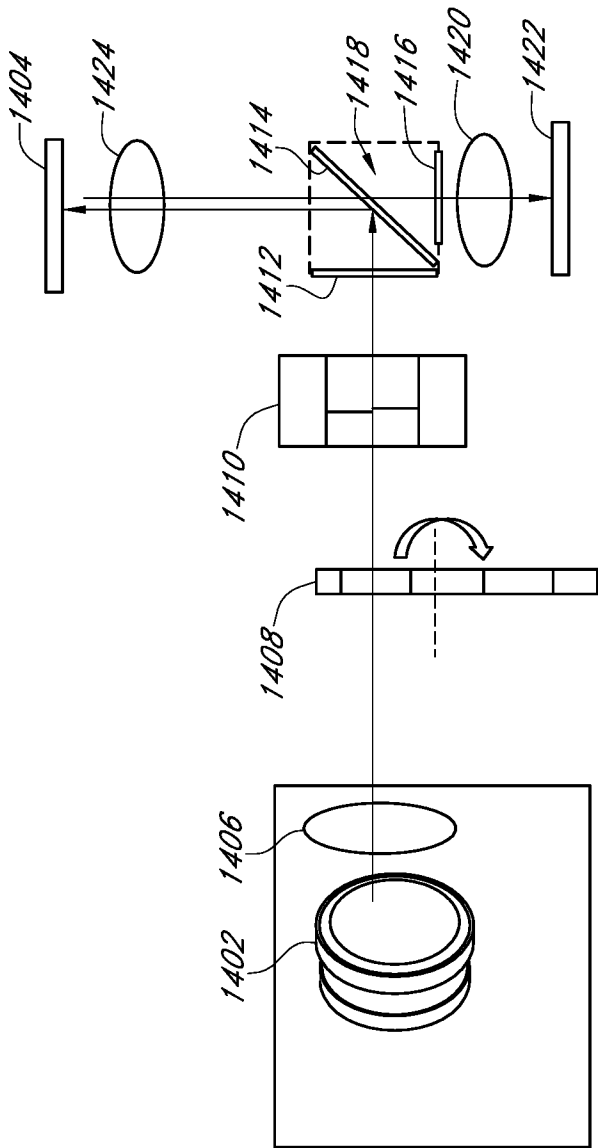


FIG. 14

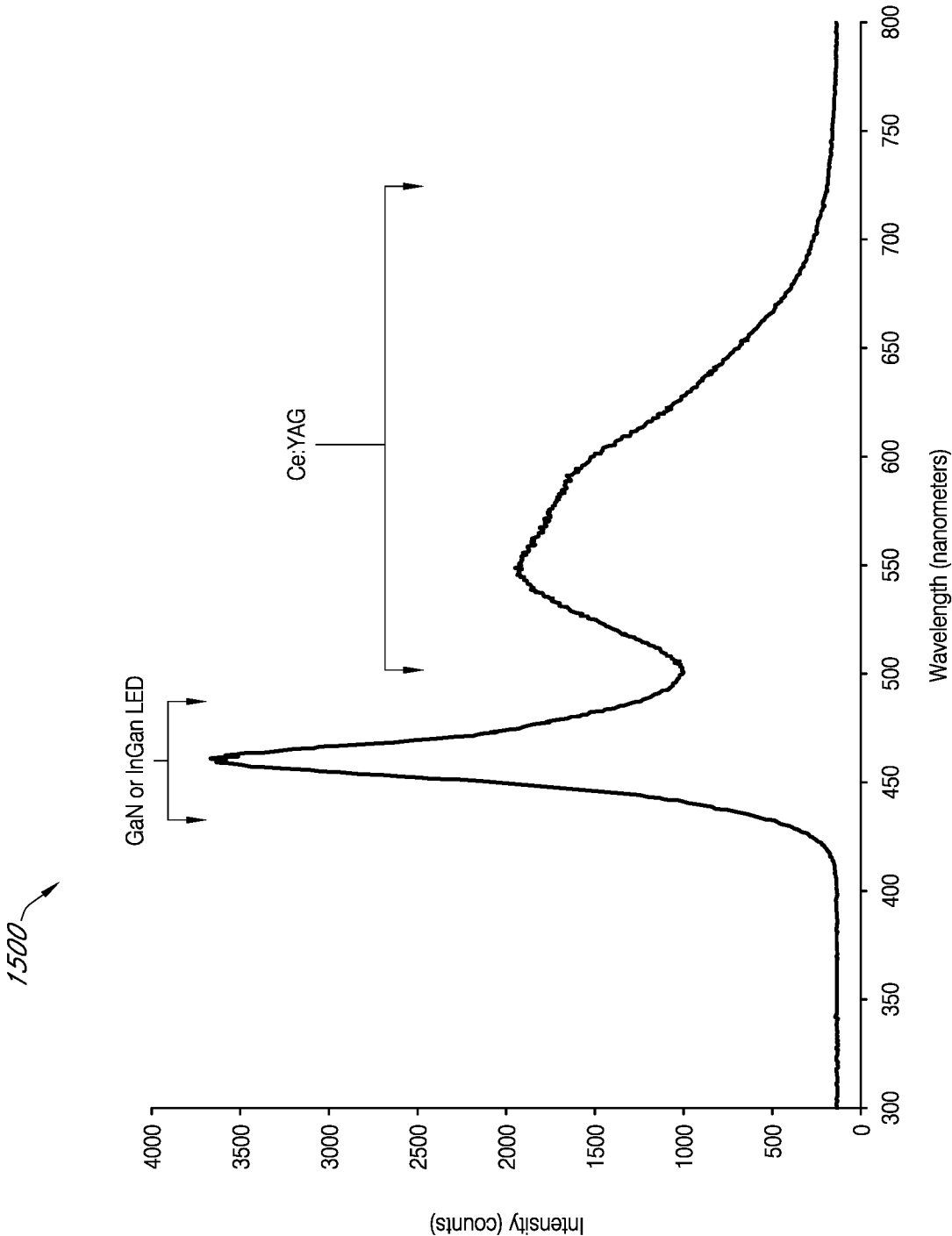


FIG. 15

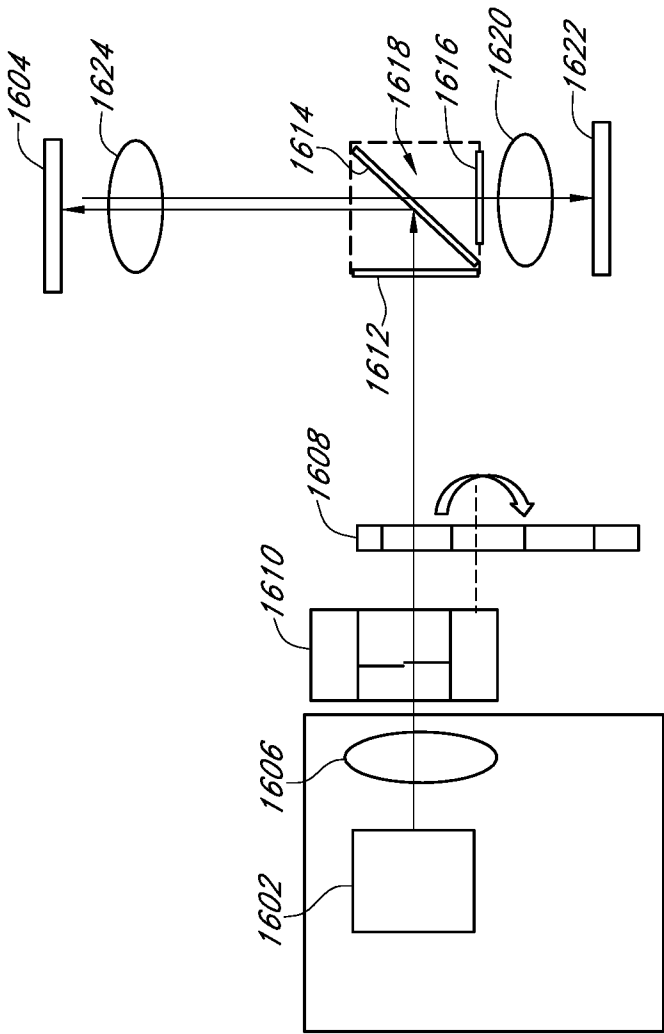


FIG. 16

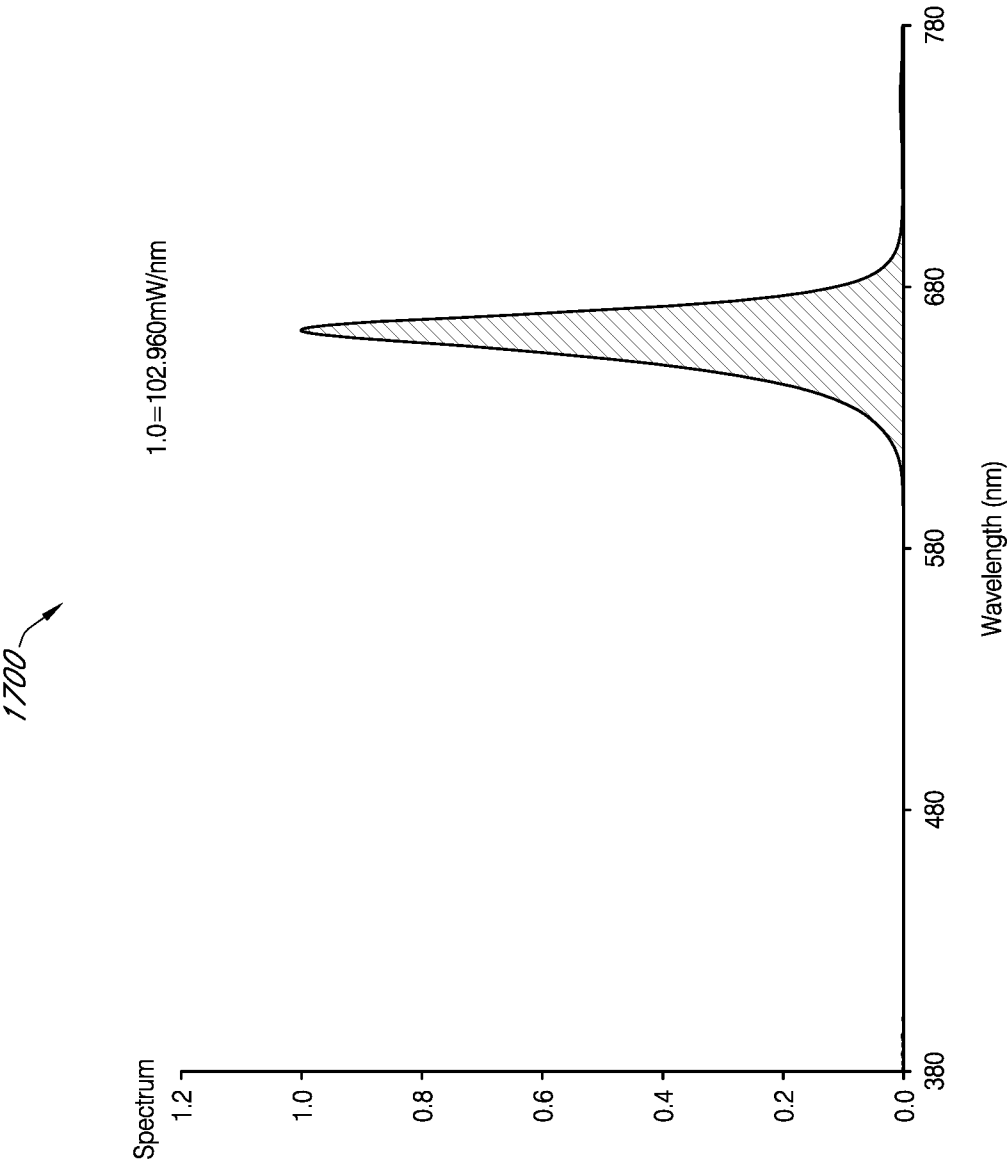


FIG. 17

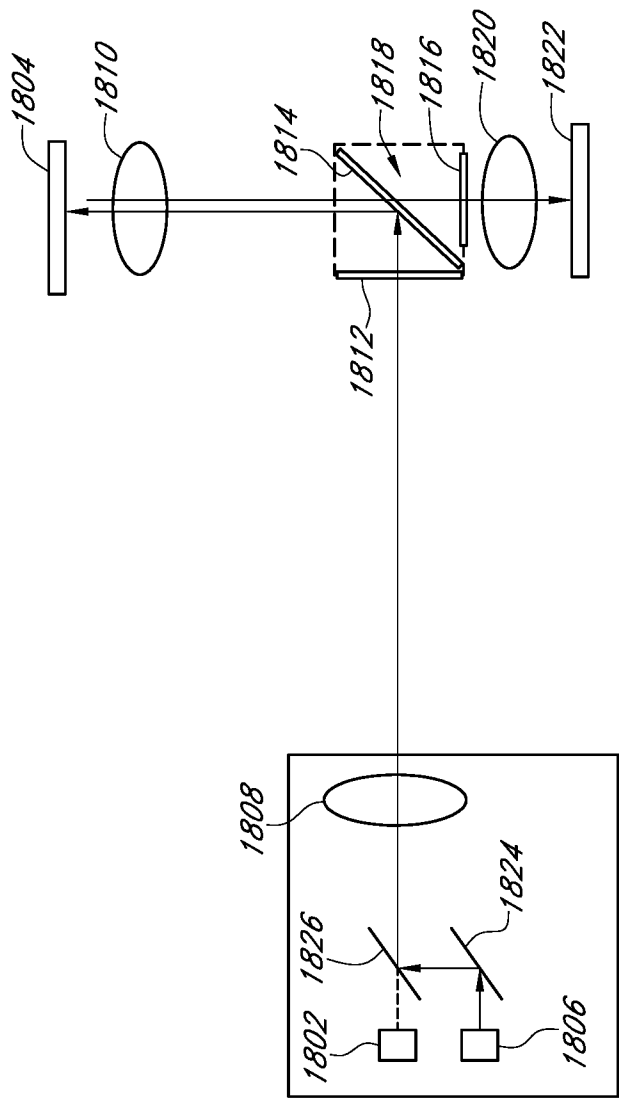


FIG. 18

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2024/034357

A. CLASSIFICATION OF SUBJECT MATTER INV. G01N21/64 C12Q1/686 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2018/372640 A1 (KANEKO YASUHISA [JP]) 27 December 2018 (2018-12-27) abstract; figures 1-12 paragraph [0003] - paragraph [0020] paragraph [0046] - paragraph [0092] -----	1 - 26
A	US 2012/168644 A1 (FENG WENYI [US] ET AL) 5 July 2012 (2012-07-05) abstract; figure 11 paragraph [0089] - paragraph [0093] -----	1 - 26
A	WO 2022/247946 A1 (SHANGHAI RUIYU BIOTECH CO LTD [CN]) 1 December 2022 (2022-12-01) abstract; figures 1-4 -----	1 - 26
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
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Date of the actual completion of the international search 27 September 2024		Date of mailing of the international search report 10/10/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Pisani, Francesca

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2024/034357

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