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(54) Title: SYSTEM AND METHOD FOR FILTER CONFIGURATION

(57) Abstract: Methods and systems for performing a flow cytometry experiment including a plurality of labels having a plurality of emissions spectra using a flow cytometer having a plurality of modifiable filter features defining filter window edges can include determining a filter feature for use in the flow cytometry experiment based at least in part on spectral characteristics of two or more labels and running a flow cytometry experiment using the determined filter feature and the plurality of labels.

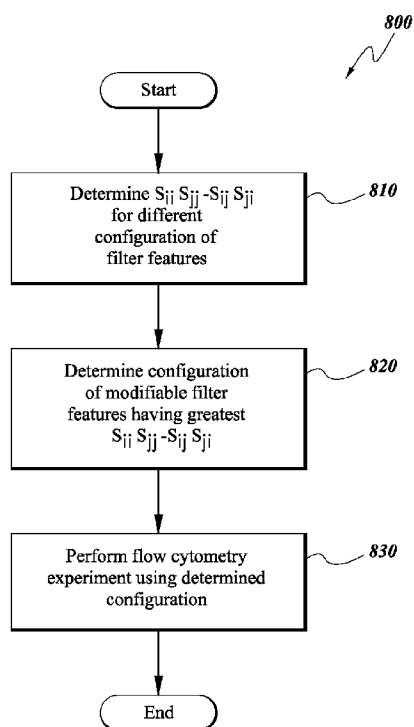


FIG. 8



TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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SYSTEM AND METHOD FOR FILTER CONFIGURATION

Field of the Invention

[0001] This disclosure relates generally to the field of flow cytometry, and more particularly to methods for reducing error in sample analysis.

Description of the Related Art

[0002] Particle analyzers, such as flow and scanning cytometers, are analytical tools that enable the characterization of particles on the basis of optical parameters such as light scatter and fluorescence. In a flow cytometer, for example, particles, such as molecules, analyte-bound beads, or individual cells, in a fluid suspension are passed through a detection region in which the particles are exposed to an excitation light, typically from one or more lasers, and the light scattering and fluorescence properties of the particles are measured. Markers, such as cell surface protein components of cells the presence of which can serve as a distinguishing characteristic, may be recognized by reagents that include fluorescent dyes to facilitate detection, identification, and characterization. Each reagent can include a label, typically a fluorescent molecule or “dye,” conjugated to a detector molecule that will selectively attach to a particular marker, for example, a monoclonal antibody. A multiplicity of different particles or components may be distinguished by using spectrally distinct fluorescent dyes to label the markers. In some implementations, a multiplicity of photodetectors are included in the analyzer. When a particle passes through the laser beam, time correlated pulses on forward scatter (FSC) and side scatter (SSC) detectors, and possibly also fluorescent emission detectors will occur. This is an “event,” and for each event the magnitude of the detector output for each detector, FSC, SSC and fluorescence detectors is stored. The data obtained comprise the signals measured for each of the light scatter parameters and the fluorescence emissions.

[0003] Cytometers may further comprise components for storing the detector outputs and analyzing the data. For example, data storage and analysis may be carried out using a computer connected to the detection electronics. For example, the data can be stored logically in tabular form, where each row corresponds to data for one particle (or one event), and the columns correspond to each of the measured parameters. The use of standard file

formats, such as an “FCS” file format, for storing data from a flow cytometer facilitates analyzing data using separate programs and/or machines. Using current analysis methods, the data typically are displayed in 2-dimensional (2D) plots for ease of visualization, but other methods may be used to visualize multidimensional data.

[0004] The parameters measured using a flow cytometer typically include FSC, which refers to the excitation light that is scattered by the particle along a generally forward direction, SSC, which refers to the excitation light that is scattered by the particle in a generally sideways direction, and the light emitted from fluorescent molecules in one or more channels (frequency bands) of the spectrum, referred to as FL1, FL2, etc., or by the name of the fluorescent dye that emits primarily in that channel. Different cell types can be identified by the scatter parameters and the fluorescence emissions resulting from labeling various cell proteins with dye-labeled antibodies.

[0005] Both flow and scanning cytometers are commercially available from, for example, BD Biosciences (San Jose, Calif.). Flow cytometry is described in, for example, Landy et al. (eds.), *Clinical Flow Cytometry*, Annals of the New York Academy of Sciences Volume 677 (1993); Bauer et al. (eds.), *Clinical Flow Cytometry: Principles and Applications*, Williams & Wilkins (1993); Ormerod (ed.), *Flow Cytometry: A Practical Approach*, Oxford Univ. Press (1997); Jaroszeski et al. (eds.), *Flow Cytometry Protocols*, Methods in Molecular Biology No. 91, Humana Press (1997); and Practical Shapiro, *Flow Cytometry*, 4th ed., Wiley-Liss (2003); all incorporated herein by reference. Fluorescence imaging microscopy is described in, for example, Pawley (ed.), *Handbook of Biological Confocal Microscopy*, 2nd Edition, Plenum Press (1989), incorporated herein by reference.

SUMMARY OF THE INVENTION

[0006] In accordance with one aspect of the present invention, systems and methods are provided for flow cytometer experiments.

[0007] In one embodiment, a system for performing a flow cytometry experiment comprising a plurality of labels having a plurality of emissions spectra is provided. The system includes a flow cytometer having a plurality of modifiable filter features defining filter window edges and processing circuit configured to determine a filter feature for use in the flow cytometry experiment based at least in part on spectral characteristics of two or more

labels. The processing circuit can be further configured to determine, under different configurations of the modifiable filter features, $S_{ii}S_{jj}-S_{ij}S_{ji}$, wherein S_{ii} corresponds to the response of a detector i to a label i , S_{jj} corresponds to the response of a detector j to a label j , S_{ij} corresponds to the response of the detector i to the label j , and S_{ji} corresponds to the response of the detector j to the label i . In some embodiments, the processing circuit is further configured to determine a configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination. In some embodiments, the processing circuit is further configured to, for different configurations of modifiable filter features perform an $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination for different pairs of labels.

[0008] In another embodiment, a method for performing a flow cytometry experiment comprising a plurality of labels having a plurality of emissions spectra using a flow cytometer having a plurality of modifiable filter features defining filter window edges is provided. The method includes determining a filter feature for use in the flow cytometry experiment based at least in part on spectral characteristics of two or more labels and running a flow cytometry experiment using the determined filter feature and the plurality of labels. The method can further include determining, under different configurations of the modifiable filter features, $S_{ii}S_{jj}-S_{ij}S_{ji}$, wherein S_{ii} corresponds to the response of a detector i to a label i , S_{jj} corresponds to the response of a detector j to a label j , S_{ij} corresponds to the response of the detector i to the label j , and S_{ji} corresponds to the response of the detector j to the label i . In some embodiments, the method includes determining a configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination. In some embodiments, the method includes modifying one or more of the plurality of modifiable filter features to achieve the determined configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination. In some embodiments, the method includes, for different configurations of modifiable filter features performing an $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination for different pairs of labels.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1 depicts a flow cytometer in accordance with an illustrative embodiment of the present invention.

[0010] Figure 2 depicts a graph showing examples of the emission spectra of labels and the filter windows of photodetectors in accordance with the present invention.

[0011] Figure 3 depicts a graph showing an example of a selection of filter windows for measuring the emission spectra of a plurality of labels in a flow cytometry experiment.

[0012] Figure 4 depicts a graph showing an example of a selection of filter windows for measuring the emission spectra of a plurality of labels in a flow cytometry experiment.

[0013] Figure 5 depicts a graph showing an example of vectors representing the detector measurements in a flow cytometry experiment.

[0014] Figure 6 depicts a graph showing an example of a selection of filter windows for maximizing fluorescence sensitivity in accordance with an illustrative embodiment with an illustrative embodiment of the present invention.

[0015] Figure 7 depicts a flowchart of one embodiment of a process of performing a flow cytometer experiment in accordance with the present invention.

[0016] Figure 8 depicts a flowchart of one embodiment of a process of performing a flow cytometer experiment in accordance with the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0017] The present invention provides systems and methods for performing flow cytometry experiments. Over the past several years, increases in the number of measurements made for the events of a flow cytometry experiment have been desired, and instrument manufacturers have developed flow cytometer instruments with detection systems and data analysis capabilities of increased complexity and performance. Advances in biochemistry have produced an increasingly large selection of fluorescent labels. Although these advances have made flow cytometry more useful than ever, harnessing that usefulness can still be a challenge. Label selection and instrument configuration are more complex, while at the same time experimental success is more dependent on appropriate experimental design. For example, the choice of fluorescent dyes and the choice of filters used in a cytometry experiment are significant for the accuracy of the conclusions drawn from the data measured because the emission spectra from one fluorescent dye may overlap the detection bands of multiple detectors. Differences in relative brightness between labels and differences

in the relative density of the markers labeled in an experiment can also affect the accuracy of event characterization.

[0018] Figure 1 shows a system 100 for flow cytometry in accordance with an illustrative embodiment of the present invention. The system 100 includes a flow cytometer 110, a controller/processor 190 and a memory 195. The flow cytometer 110 includes one or more excitation lasers 115a-c, a focusing lens 120, a flow chamber 125, a forward scatter detector 130, a side scatter detector 135, a fluorescence collection lens 140, one or more beam splitters 145a-g, one or more bandpass filters 150a-e, one or more longpass (“LP”) filters 155a-b, and one or more fluorescent detectors 160a-f.

[0019] The excitation lasers 115a-c emit light in the form of a laser beam. The wavelengths of the laser beams emitted from excitation lasers 115a-c are 488 nm, 633 nm, and 325 nm, respectively, in the example system of Figure 1. The laser beams are first directed through one or more of beam splitters 145a and 145b. Beam splitter 145a transmits light at 488 nm and reflects light at 633 nm. Beam splitter 145b transmits UV light (light with a wavelength in the range of 10 to 400 nm) and reflects light at 488 nm and 633 nm.

[0020] The laser beams are then directed to a focusing lens 120, which focuses the beams onto the portion of a fluid stream where particles of a sample are located, within the flow chamber 125. The flow chamber is part of a fluidics system which directs particles, typically one at a time, in a stream to the focused laser beam for interrogation. The flow chamber can comprise a flow cell in a benchtop cytometer or a nozzle tip in a stream-in-air cytometer.

[0021] The light from the laser beam(s) interacts with the particles in the sample by diffraction, refraction, reflection, scattering, and absorption with re-emission at various different wavelengths depending on the characteristics of the particle such as its size, internal structure, and the presence of one or more fluorescent molecules attached to or naturally present on or in the particle. The fluorescence emissions as well as the diffracted light, refracted light, reflected light, and scattered light may be routed to one or more of the forward scatter detector 130, the side scatter detector 135, and the one or more fluorescent detectors 160a-f through one or more of the beam splitters 145a-g, the bandpass filters 150a-e, the longpass filters 155a-b, and the fluorescence collection lens 140.

[0022] The fluorescence collection lens 140 collects light emitted from the particle-laser beam interaction and routes that light towards one or more beam splitters and filters. Bandpass filters, such as bandpass filters 150a-e, allow a narrow range of wavelengths to pass through the filter. For example, bandpass filter 150a is a 510/20 filter. The first number represents the center of a spectral band. The second number provides a range of the spectral band. Thus, a 510/20 filter extends 10 nm on each side of the center of the spectral band, or from 500 nm to 520 nm. Shortpass filters transmit wavelengths of light equal to or shorter than a specified wavelength. Longpass filters, such as longpass filters 155a-b, transmit wavelengths of light equal to or longer than a specified wavelength of light. For example, longpass filter 155a, which is a 380 nm longpass filter, transmits light equal to or longer than 380 nm. Filters are often selected to optimize the specificity of a detector for a particular fluorescent dye. The filters can be configured so that the spectral band of light transmitted to the detector is close to the emission peak of a fluorescent dye.

[0023] Beam splitters direct light of different wavelengths in different directions. Beam splitters can be characterized by filter properties such as shortpass and longpass. For example, beam splitter 145g is a 470 LP beam splitter, meaning that the beam splitter 145g transmits wavelengths of light that are 470 nm or longer and reflects wavelengths of light that are shorter than 470 nm in a different direction. In one embodiment, the beam splitters 145a-g can comprise optical mirrors, such as dichroic mirrors.

[0024] The forward scatter detector 130 can be positioned on axis or slightly off axis from the direct beam through the flow cell and is configured to detect diffracted light, the excitation light that travels through or around the particle in mostly a forward direction. The intensity of the light detected by the forward scatter detector is dependent on the overall size of the particle. The forward scatter detector can include a photodiode. The side scatter detector 135 is configured to detect refracted and reflected light from the surfaces and internal structures of the particle, and tends to increase with increasing particle complexity of structure. The fluorescence emissions from fluorescent molecules associated with the particle can be detected by the one or more fluorescent detectors 160a-f. The side scatter detector 135 and fluorescent detectors can include photomultiplier tubes. The signals detected at the forward scatter detector 130, the side scatter detector 135 and the fluorescent

detectors can be converted to electronic signals (voltages) by the detectors. This data can provide information about the sample.

[0025] One of skill in the art will recognize that a flow cytometer in accordance with an embodiment of the present invention is not limited to the flow cytometer depicted in Figure 1, but can include any flow cytometer known in the art. For example, a flow cytometer may have any number of lasers, beam splitters, filters, and detectors at various wavelengths and in various different configurations. In some embodiments, one or more of the components of the flow cytometer may be modifiable. For example, the filter features of the flow cytometer, the beam splitters, bandpass filters, shortpass filters, longpass filters, and in some embodiments, the fluorescent detectors, may be modified to change the range of wavelengths transmitted. In some embodiments, one or more of the filter features may be tunable or adjustable, so that following adjustment, the filter features can transmit and/or reflect different wavelengths of light. An adjustable filter feature may be continuously variable or may have two or more predefined ranges of wavelengths to which it may be set. Alternatively, one or more of the filter features may be replaced with a filter feature transmitting a different range of wavelengths. In other embodiments, the flow cytometer may comprise a spectral flow cytometer, where the filter features may be one or more multianode detectors.

[0026] In operation, cytometer operation is controlled by a controller/processor 190, and the measurement data from the detectors can be stored in the memory 195 and processed by the controller/processor 190. Although not shown explicitly, the controller/processor 190 is coupled to the detectors to receive the output signals therefrom, and may also be coupled to electrical and electromechanical components of the flow cytometer 100 to control the lasers, fluid flow parameters, and the like. Input/output (I/O) capabilities 197 may be provided also in the system. The memory 195, controller/processor 190, and I/O 197 may be entirely provided as an integral part of the flow cytometer 110. In such an embodiment, a display may also form part of the I/O capabilities 197 for presenting experimental data to users of the cytometer 100. Alternatively, some or all of the memory 195 and controller/processor 190 and I/O capabilities may be part of one or more external devices such as a general purpose computer. In some embodiments, some or all of the

memory 195 and controller/processor 190 can be in wireless or wired communication with the cytometer 110. The controller/processor 190 in conjunction with the memory 195 and the I/O 197 can be configured to perform various functions related to the preparation and analysis of a flow cytometer experiment. For example, the controller/processor 190 in conjunction with the memory 195 and the I/O 197 can be configured to perform processes such as those described below with respect to Figures 7 and 8.

[0027] The system of Figure 1 includes six different detectors that detect fluorescent light in six different wavelength bands (which may be referred to herein as a “filter window” for a given detector) as defined by the configuration of filters and/or splitters in the beam path from the flow cell 125 to each detector. Different fluorescent molecules used for a flow cytometer experiment will emit light in their own characteristic wavelength bands. The particular fluorescent labels used for an experiment and their associated fluorescent emission bands may be selected to generally coincide with the filter windows of the detectors. However, as more detectors are provided, and more labels are utilized, perfect correspondence between filter windows and fluorescent emission spectra is not possible. It is generally true that although the peak of the emission spectra of a particular fluorescent molecule may lie within the filter window of one particular detector, some of the emission spectra of that label will also overlap the filter windows of one or more other detectors. This may be referred to as spillover.

[0028] Figure 2 shows an illustrative example of spillover caused by overlapping emissions spectra for different labels. Figure 2 shows the emission spectra of markers labeled with FITC, represented by the curve extending from a wavelength of approximately 475 nm to 650 nm, and the filter window for a “FITC detector.” One or more filter features, such as bandpass filter 150b as depicted in Figure 1, can be placed in front of the detector, limiting the range of wavelengths that can reach the detector, the range of wavelengths constituting a filter window. The filter window for the FITC detector is 530/30, meaning that the filter window extends from 515 nm to 545 nm. The FITC filter window is represented by the shaded rectangle extending from 515 nm to 545 nm. Figure 2 also shows the emission spectra of markers labeled with PE, represented by the curve extending from approximately 525 nm to approximately 725 nm. One or more filters, such as bandpass filter 150c as

depicted in Figure 1, can be placed in front of the detector. The filter window for the PE detector is 585/42, meaning that the filter window extends from 564 nm to 606 nm. The PE filter window is represented by the shaded rectangle extending from 564 nm to 606 nm. Figure 2 illustrates that a portion of the emissions spectra for FITC overlaps the filter window for the PE detector, labeled as “FITC spillover into PE.” Therefore, some of the fluorescence emission of the FITC label is detected in the PE detector and measured along with the fluorescence emission of the PE label. Spillover can cause inaccurate conclusions to be drawn regarding the abundance of labels present on a particle. This problem can be especially acute for recent uses of flow cytometers as more labels and detectors are utilized, which reduces the separation of fluorescent peaks and filter windows. Given also the increasing number of fluorescent labels available (generally dozens of options are available to an experimenter), with a variety of peak wavelengths, emission intensities and energies, and spectral width characteristics, the variety of marker densities on cells being characterized, as well as in some cases selectable filter windows, it is very challenging to design a suitable set up for a flow cytometer experiment.

[0029] Greater overlap between emission spectra in a filter window can result in a larger degree of fluorescence compensation and increased complexity of measurement. In a flow cytometry experiment having labels with overlapping emission spectra, a trade-off is made between the magnitude of the signal detected by each detector and the degree of overlap between emission spectra. Consequently, preferred filter windows for an experiment may depend on several experimental parameters including label brightness and marker density. For example, in an experiment having relatively bright labels and high marker expression, narrow filter windows may allow for a signal of sufficient magnitude to be detected by each detector. Thus, narrow filter windows may be preferred to provide minimal spillover. However, in an experiment having very dim labels and low marker expression, wider filter windows may be preferable to provide for increased fluorescence signal detection, even though wider filter windows may result in increased spillover. Figure 3 shows an illustrative example of a selection of filter windows for measuring the emission spectra of a plurality of labels in a flow cytometry experiment. Figure 3 shows a first emission spectra 301 representing the emission spectra of markers labeled with FITC and a

second emission spectra 302 representing the emission spectra of markers labeled with PE. For illustrative purposes, emission spectra 301 and 302 have been normalized so that the integrated area, as well as the peak emissions of each, are equal. Figure 3 also shows a first filter window 310 for a first detector D1 (not shown) configured to detect the fluorescence emission of markers labeled with FITC and a second filter window 320 for a second detector D2 (not shown) configured to detect the fluorescence emission of markers labeled with PE. Figure 3 further depicts an area A1 which includes the portion of emission spectrum 301 that falls within the filter window 310. Area A1 represents the fluorescence emission of FITC labels that is detected by detector D1. Figure 3 also depicts an area A4 which includes the portion of emission spectrum 302 that falls within the filter window 320. Area A4 represents the fluorescence emission of PE labels that is detected by the detector D2. The filter windows 310 and 320 are selected to capture as much fluorescent light as possible without detecting fluorescent light from spillover. In the example shown in Figure 3, when using each detector to measure the integrated fluorescence from each label, fluorescence emission from FITC labels is only detected by the FITC detector D1, corresponding to filter window 310, and the fluorescence emission from PE labels is only detected by the PE detector D2, corresponding to the filter window 320. In a flow cytometry experiment performed using the filter windows of Figure 3, some samples having a mixture of the two labels, FITC and PE, can be easily characterized because there is no overlap between the filter windows for detectors D1 and D2. However, a flow cytometry experiment performed using the filter windows of Figure 3 can result in the detection of less fluorescence signal than having filter windows that extend to allow for some overlap of emission spectra. Consequently, a filter window configuration such as that of Figure 3, where filter windows are selected to prevent spillover, may not be preferable in a cytometry experiment including two labels having emission spectra that are relatively close together or in a cytometry experiment having relatively dim labels.

[0030] Figure 4 shows an illustrative example of a selection of filter windows for measuring the emission spectra of a plurality of labels in a flow cytometry experiment. Figure 4 shows the emission spectra 301 and 302 as depicted in Figure 3, with different filter windows. Figure 4 shows a first filter window 410 for detector D1 configured to detect the

fluorescence emission of markers labeled with FITC and a second filter window 420 for detector D2 configured to detect the fluorescence emission of markers labeled with PE. The edges of filter windows 410 and 420 are wider than those of filter windows 310 and 320 respectively. Figure 4 also depicts areas A1 and A4, which are larger in comparison to Figure 3 due to the wider scope of filter windows 410 and 420. Figure 4 further depicts an area A3 which includes the portion of emission spectrum 302 that falls within the filter window 410. Area A3 represents the fluorescence emission of PE labels that is detected by the FITC detector D1. Figure 4 also depicts an area A2 which includes the portion of emission spectrum 301 that falls within the filter window 420. Area A2 represents the fluorescence emission of FITC labels that is detected by the PE detector D2. In a flow cytometry experiment performed using the filter windows of Figure 4, the total fluorescence emission detected by the FITC detector D1 includes the fluorescence emission represented by area A1 in addition to the fluorescence emission represented by area A3, and the total fluorescence emission detected by the PE detector D2 includes the fluorescence emission represented by area A4 in addition to the fluorescence emission represented by area A2. As depicted in Figure 4, the fluorescence emission detected by the FITC detector D1 includes mostly fluorescence emission from FITC labels, but also includes some fluorescence emission from PE labels. The fluorescence emission detected by the PE detector D2 includes mostly fluorescence emission from PE labels, but also includes some fluorescence emission from FITC labels. In comparison to the filter windows shown in Figure 3, more total fluorescence emission is measured when performing an experiment using the filter windows of Figure 4. However, the increased fluorescence emission comes at the expense of some spillover between filter windows.

[0031] Figure 5 shows an illustrative example of a graph showing vectors representing the detector measurements in a flow cytometry experiment. Figure 5 depicts vectors A_1 , A_2 , A_3 , and A_4 , representing the integrated fluorescence of A1, A2, A3, and A4, respectively. Figure 5 also depicts a PE vector representing the sum of the integrated fluorescence of PE detected at each detector, A_3+A_4 , and a FITC vector representing the sum of the integrated fluorescence of FITC detected at each detector, A_1+A_2 . The PE and FITC vectors represent the path a cell would take as the number of labels on the surface of the cell

increase. The length of the PE and FITC vectors increase as more fluorescent emission is measured. Due to the overlap from integrating areas A3 and A2, the detector axes are non-orthogonal. Any measurement of a cell containing a mixture of fluorophores can be expressed as a sum of two vectors parallel to the PE and FITC vectors. This process may be referred to as fluorescence compensation. After comparison to a known standard of brightness, the number of fluorophores can be deduced by comparing the projection of an unknown vector onto either the PE or the FITC axis to the standard.

[0032] In accordance with an illustrative embodiment, filter windows may be determined to maximize the signal-to-noise ratio for each label measured based on the emission spectra of the labels and the expected expression level of the marker of interest. Generally, fluorescence sensitivity may be maximized by collecting as much fluorescent light as possible, and by maximizing the area of the parallelogram bounded by the FITC and PE vectors as described with respect to Figure 5.

[0033] Figure 6 shows an illustrative example of a selection of filter windows for maximizing fluorescence sensitivity in accordance with an illustrative embodiment. Figure 6 shows the emission spectra 301 and 302 as depicted in Figure 3, with different filter windows. Figure 6 shows a first filter windows 610 for detector D1 configured to detect the fluorescence emission of markers labeled with FITC and a second filter window 620 for detector D2 configured to detect the fluorescence emission of markers labeled with PE. A vector plot of Figure 6 results in FITC and PE vectors similar to those shown in Figure 5. The area A of a parallelogram formed by the FITC and PE vectors is given by the equation:

$$A = (A_1 A_4) - (A_2 A_3) \quad (1)$$

[0034] The edges of filter windows 610 and 620 are selected so that the filter windows meet at a common wavelength, shown as wavelength λ_C . Different selections of λ_C result in different areas A1, A2, A3, and A4. In order to maximize fluorescence sensitivity, λ_C may be chosen to maximize the area A . Differentiation with respect to λ yields:

$$\begin{aligned} \frac{\partial A}{\partial \lambda} &= \frac{\partial}{\partial \lambda}(A_1 A_4) - \frac{\partial}{\partial \lambda}(A_2 A_3) \\ \frac{\partial A}{\partial \lambda} &= A_1 \frac{\partial A_4}{\partial \lambda} + A_4 \frac{\partial A_1}{\partial \lambda} - \left[A_3 \frac{\partial A_2}{\partial \lambda} + A_2 \frac{\partial A_3}{\partial \lambda} \right] \end{aligned} \quad (2)$$

A_1 and A_2 are inversely related, as are A_3 and A_4 , such that an increase of one results in a decrease of the other, as demonstrated by the following equations:

$$\frac{\partial A_1}{\partial \lambda} = -\frac{\partial A_2}{\partial \lambda} \quad (3)$$

And

$$\frac{\partial A_4}{\partial \lambda} = -\frac{\partial A_3}{\partial \lambda} \quad (4)$$

Substituting equations (3) and (4) into equation (2) results in the following equations:

$$\begin{aligned} \frac{\partial A}{\partial \lambda} &= -\left(A_1 \frac{\partial A_3}{\partial \lambda} + A_4 \frac{\partial A_2}{\partial \lambda} + A_3 \frac{\partial A_2}{\partial \lambda} + A_2 \frac{\partial A_3}{\partial \lambda} \right) \\ \frac{\partial A}{\partial \lambda} &= -\left[(A_1 + A_2) \frac{\partial A_3}{\partial \lambda} + (A_4 + A_3) \frac{\partial A_2}{\partial \lambda} \right] \end{aligned} \quad (5)$$

The area A is maximal for λ when the derivative is zero, implying:

$$\begin{aligned} 0 &= -\left[(A_1 + A_2) \frac{\partial A_3}{\partial \lambda} + (A_4 + A_3) \frac{\partial A_2}{\partial \lambda} \right] \\ (A_1 + A_2) \frac{\partial A_3}{\partial \lambda} &= -(A_4 + A_3) \frac{\partial A_2}{\partial \lambda} \end{aligned} \quad (6)$$

at the wavelength of λ_C that maximizes fluorescence sensitivity. The summed areas, $A_1 + A_2$ and $A_3 + A_4$, which represent the total areas of each of the respective emission spectra for FITC and PE, are invariant. A configuration of modifiable filter features can be determined that results in the filter windows 610 and 620 corresponding to the wavelength of λ_C that maximizes fluorescence sensitivity.

[0035] Figure 7 depicts a flowchart of one embodiment of a process 700 of performing a flow cytometry experiment having a plurality of labels having a plurality of emission spectra using a flow cytometer having a plurality of modifiable filter features defining filter window edges. The process 700 begins at a step 710, wherein, for at least one pair of labels having overlapping emission spectra, a wavelength λ_C is determined, as described with respect to Figure 6, that results in a pair of filter windows that maximize fluorescent sensitivity.

[0036] After the wavelength λ_C is determined, the process 700 moves to a step 720, where a configuration of modifiable filter features having filter windows corresponding to the determined wavelength λ_C is determined.

[0037] After the configuration of modifiable filter features is determined, the process 700 moves to a step 730, wherein a flow cytometry experiment is performed using the determined configuration of modifiable filter features. The process then concludes. In some embodiments, one or more of the modifiable filter features in the flow cytometer are replaced to assemble the determined configuration of modifiable filter features. In some embodiments, one or more of the modifiable filter features are tuned or adjusted to assemble the determined configuration of modifiable filter features.

[0038] Although the above embodiments describe a determination of a configuration of modifiable filter features based on a single pair of labels having overlapping emission spectra, in some embodiments, a determination of a configuration of modifiable filter features can be based on multiple pairs of labels having overlapping emission spectra. In some embodiments, a wavelength λ_C that maximizes fluorescent sensitivity may be determined for each pair of labels having overlapping emission spectra. In some embodiments, a set of wavelengths, such as wavelength λ_C , can be determined for each pair of labels having overlapping emission spectra, such that a summation of the area A for each pair of labels having overlapping emission spectra is maximized.

[0039] In some embodiments, the possible filter windows resulting from different configurations of modifiable filter features may be limited by the modifiable filter features available. Consequently, filter features may not always be available to assemble filter windows corresponding to a determined wavelength λ_C for maximizing fluorescence sensitivity. In some embodiments, a determination may be made, based on different configurations of available modifiable filter features, which combination of modifiable filter features results in the greatest area A . This determination can also be described as the configuration of modifiable filter features that will result in the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$, where S_{ii} corresponds to the response of a detector i to a label i , S_{jj} corresponds to the response of a detector j to a label j , S_{ij} corresponds to the response of the detector i to the label j , and S_{ji} corresponds to the response of the detector j to the label i . This response may be due to a particular filter in front of a detector, or may be due to an inherent property of the given detector.

[0040] Figure 8 depicts a flowchart of one embodiment of a process 800 of performing a flow cytometry experiment having a plurality of modifiable filter features defining filter window edges.

[0041] The process 800 begins at a step 810, wherein, for at least one pair of labels having overlapping emission spectra, $S_{ii}S_{jj}-S_{ij}S_{ji}$ is determined for different combinations of modifiable filter features.

[0042] After $S_{ii}S_{jj}-S_{ij}S_{ji}$ is determined for different combinations of modifiable filter features, the process 800 moves to a step 820, wherein the configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination is determined.

[0043] After the configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination is determined, the process 800 moves to a step 830, wherein a flow cytometry experiment is performed using the determined configuration of modifiable filter features. The process then concludes. In some embodiments, one or more of the modifiable filter features in the flow cytometer are replaced to assemble the determined configuration of modifiable filter features. In some embodiments, one or more of the modifiable filter features are tuned or adjusted to assemble the determined configuration of modifiable filter features.

[0044] In some embodiments, an $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination may be determined under different configurations of modifiable filter features for each pair of labels having overlapping emission spectra. For each configuration of modifiable filter features, the sum of the $S_{ii}S_{jj}-S_{ij}S_{ji}$ determinations for each pair of labels having overlapping emission spectra may be determined. In some embodiments, the configuration of modifiable filter features having the greatest sum of the $S_{ii}S_{jj}-S_{ij}S_{ji}$ determinations for each pair of labels having overlapping emission spectra may be used in performing a cytometry experiment.

[0045] As used herein, the terms “determine” or “determining” encompass a wide variety of actions. For example, “determining” may include calculating, computing, processing, deriving, investigating, looking up (e.g., looking up in a table, a database or another data structure), ascertaining and the like. Also, “determining” may include receiving (e.g., receiving information), accessing (e.g., accessing data in a memory) and the like. Also, “determining” may include resolving, selecting, choosing, establishing, and the like.

[0046] As used herein, the terms “provide” or “providing” encompass a wide variety of actions. For example, “providing” may include storing a value in a location for subsequent retrieval, transmitting a value directly to the recipient, transmitting or storing a reference to a value, and the like. “Providing” may also include encoding, decoding, encrypting, decrypting, validating, verifying, and the like.

[0047] As used herein, a phrase referring to “at least one of” a list of items refers to any combination of those items, including single members. As an example, “at least one of: a, b, or c” is intended to cover: a, b, c, a-b, a-c, b-c, and a-b-c.

[0048] Those of skill in the art would understand that information and signals may be represented using any of a variety of different technologies and techniques. For example, data, instructions, commands, information, signals, bits, symbols, and chips that may be referenced throughout the above description may be represented by voltages, currents, electromagnetic waves, magnetic fields or particles, optical fields or particles, or any combination thereof.

[0049] Those of skill in the art would further appreciate that the various illustrative logical blocks, modules, circuits, and algorithm steps described in connection with the embodiments disclosed herein may be implemented as electronic hardware, computer software, or combinations of both. To clearly illustrate this interchangeability of hardware and software, various illustrative components, blocks, modules, circuits, and steps have been described above generally in terms of their functionality. Whether such functionality is implemented as hardware or software depends upon the particular application and design constraints imposed on the overall system. Skilled artisans may implement the described functionality in varying ways for each particular application, but such implementation decisions should not be interpreted as causing a departure from the scope of the present invention.

[0050] The techniques described herein may be implemented in hardware, software, firmware, or any combination thereof. Such techniques may be implemented in any of a variety of devices such as general purposes computers, wireless communication devices, or integrated circuit devices having multiple uses including application in wireless communication device handsets and other devices. Any features described as modules or

components may be implemented together in an integrated logic device or separately as discrete but interoperable logic devices. If implemented in software, the techniques may be realized at least in part by a computer-readable data storage medium comprising program code including instructions that, when executed, performs one or more of the methods described above. The computer-readable data storage medium may form part of a computer program product, which may include packaging materials. The computer-readable medium may comprise memory or data storage media, such as random access memory (RAM) such as synchronous dynamic random access memory (SDRAM), read-only memory (ROM), non-volatile random access memory (NVRAM), electrically erasable programmable read-only memory (EEPROM), FLASH memory, magnetic or optical data storage media, and the like. The computer-readable medium may be a non-transitory storage medium. The techniques additionally, or alternatively, may be realized at least in part by a computer-readable communication medium that carries or communicates program code in the form of instructions or data structures and that can be accessed, read, and/or executed by a computer, such as propagated signals or waves.

[0051] The program code may be executed by a processor, which may include one or more processors, such as one or more digital signal processors (DSPs), general purpose microprocessors, an application specific integrated circuits (ASICs), field programmable logic arrays (FPGAs), or other equivalent integrated or discrete logic circuitry. Such a processor may be configured to perform any of the techniques described in this disclosure. A general purpose processor may be a microprocessor, but in the alternative, the processor may be any conventional processor, controller, microcontroller, or state machine. A processor may also be implemented as a combination of computing devices, e.g., a combination of a DSP and a microprocessor, a plurality of microprocessors, one or more microprocessors in conjunction with a DSP core, or any other such configuration. Accordingly, the term "processor," as used herein may refer to any of the foregoing structure, any combination of the foregoing structure, or any other structure or apparatus suitable for implementation of the techniques described herein. In addition, in some aspects, the functionality described herein may be provided within dedicated software modules or hardware modules configured for encoding and decoding, or incorporated in a combined video encoder-decoder (CODEC).

[0052] The methods disclosed herein comprise one or more steps or actions for achieving the described method. The method steps and/or actions may be interchanged with one another without departing from the scope of the claims. In other words, unless a specific order of steps or actions is specified, the order and/or use of specific steps and/or actions may be modified without departing from the scope of the claims.

[0053] Various embodiments of the invention have been described. These and other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A system for performing a flow cytometry experiment comprising a plurality of labels having a plurality of emissions spectra, comprising:
 - a flow cytometer having a plurality of modifiable filter features defining filter window edges; and
 - a processing circuit configured to determine a filter feature for use in the flow cytometry experiment based at least in part on spectral characteristics of two or more labels.
2. The system of claim 1 wherein the processing circuit is configured to determine, under different configurations of the modifiable filter features, $S_{ii}S_{jj}-S_{ij}S_{ji}$, wherein S_{ii} corresponds to the response of a detector i to a label i, S_{jj} corresponds to the response of a detector j to a label j, S_{ij} corresponds to the response of the detector i to the label j, and S_{ji} corresponds to the response of the detector j to the label i.
3. The system of claim 2, wherein the processing circuit is further configured to determine a configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination.
4. The system of claim 2, wherein the processing circuit is further configured to, for different configurations of modifiable filter features:
 - perform an $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination for different pairs of labels.
5. The system of claim 1, wherein the plurality of modifiable filter features comprises one or more filters.
6. The system of claim 1, wherein the plurality of modifiable filter features comprises one or more beam splitters.
7. The system of claim 1, wherein the plurality of modifiable filter features comprises one or more detectors.
8. A method for performing a flow cytometry experiment comprising a plurality of labels having a plurality of emissions spectra using a flow cytometer having a plurality of modifiable filter features defining filter window edges, comprising:
 - determining a filter feature for use in the flow cytometry experiment based at least in part on spectral characteristics of two or more labels; and

running a flow cytometry experiment using the determined filter feature and the plurality of labels.

9. The method of claim 8, comprising determining, under different configurations of the modifiable filter features, $S_{ii}S_{jj}-S_{ij}S_{ji}$, wherein S_{ii} corresponds to the response of a detector i to a label i , S_{jj} corresponds to the response of a detector j to a label j , S_{ij} corresponds to the response of the detector i to the label j , and S_{ji} corresponds to the response of the detector j to the label i .

10. The method of claim 9, comprising determining a configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination.

11. The method of claim 10, comprising modifying one or more of the plurality of modifiable filter features to achieve the determined configuration of modifiable filter features having the greatest $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination.

12. The method of claim 9, comprising, for different configurations of modifiable filter features:

performing an $S_{ii}S_{jj}-S_{ij}S_{ji}$ determination for different pairs of labels.

13. The method of claim 8, wherein the plurality of modifiable filter features comprises one or more filters.

14. The method of claim 8, wherein the plurality of modifiable filter features comprises one or more beam splitters.

15. The method of claim 8, wherein the plurality of modifiable filter features comprises one or more detectors.

16. A method for performing a flow cytometry experiment comprising a plurality of labels having a plurality of emissions spectra using a flow cytometer having a plurality of modifiable filter features defining filter window edges, comprising:

determining for at least one pair of labels having overlapping emission spectra, a common wavelength λ_C that would maximize fluorescent sensitivity if a filter window for each label of the pair of labels has a filter window edge at λ_C ;

determining at least one filter feature for use in the flow cytometry experiment based at least in part on the determined λ_C ; and

running a flow cytometry experiment using the determined filter feature and the plurality of labels.

17. The method of Claim 16, further comprising determining a configuration of modifiable filter features corresponding to the determined λ_C ; and

running a flow cytometry experiment using the determined configuration of modifiable filter features.

18. The method of Claim 16, comprising determining λ_C for each pair of labels of a plurality of pairs of labels having overlapping emission spectra.

19. The method of Claim 16, wherein the plurality of modifiable filter features comprises one or more filters.

20. The method of Claim 16, wherein the plurality of modifiable filter features comprises one or more detectors.

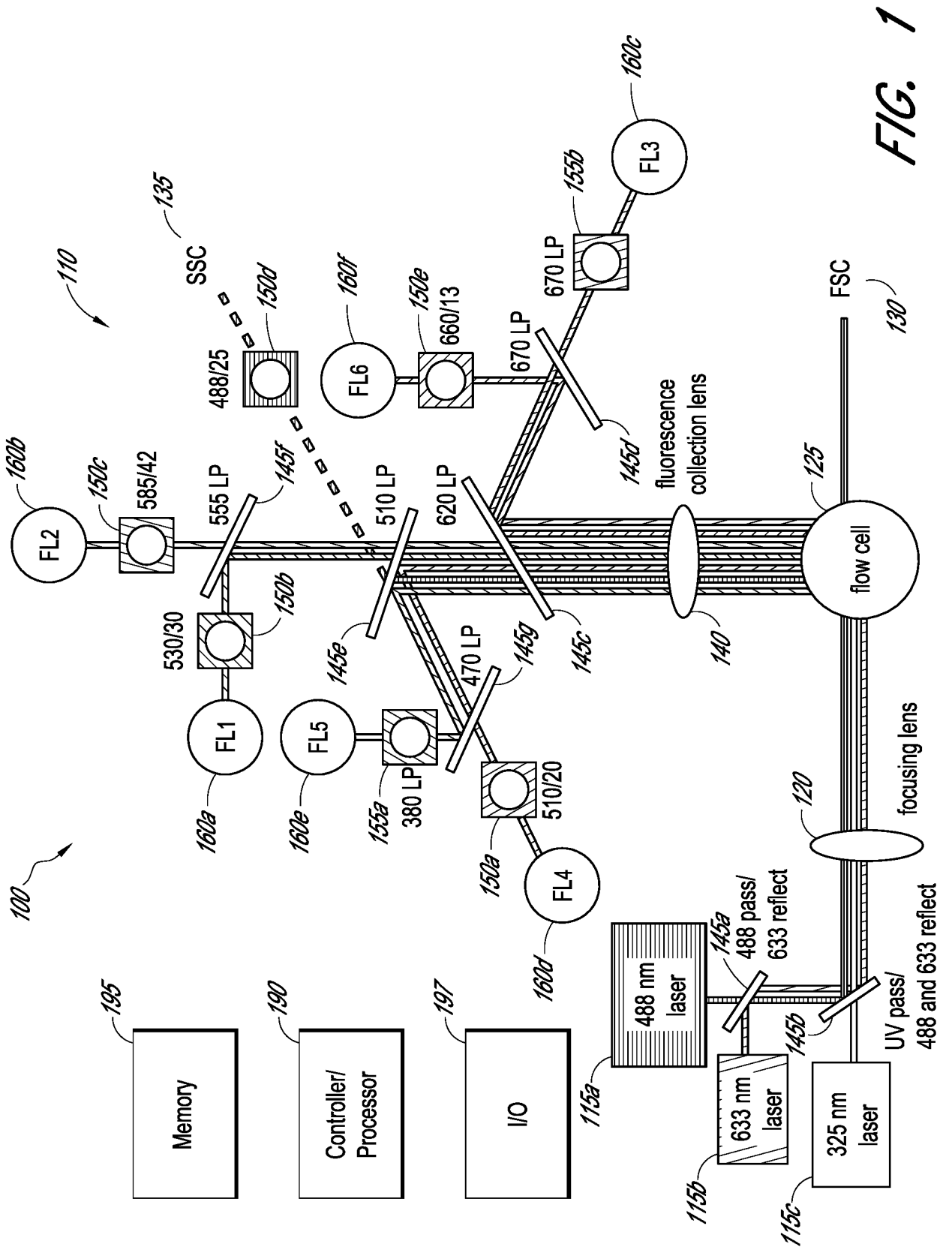


FIG. 1

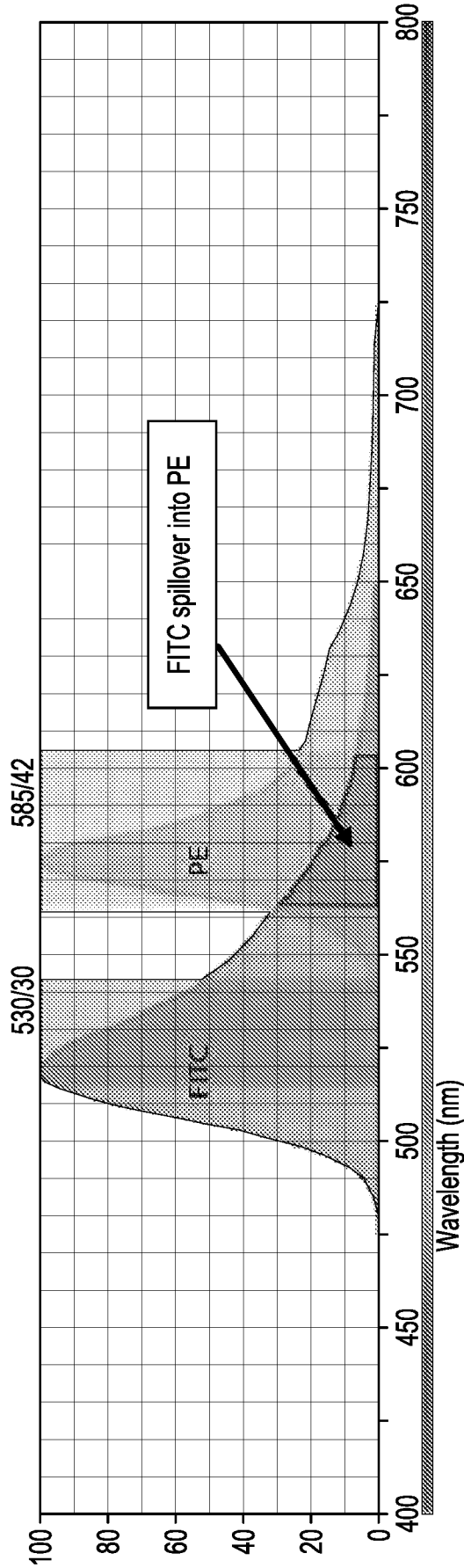


FIG. 2

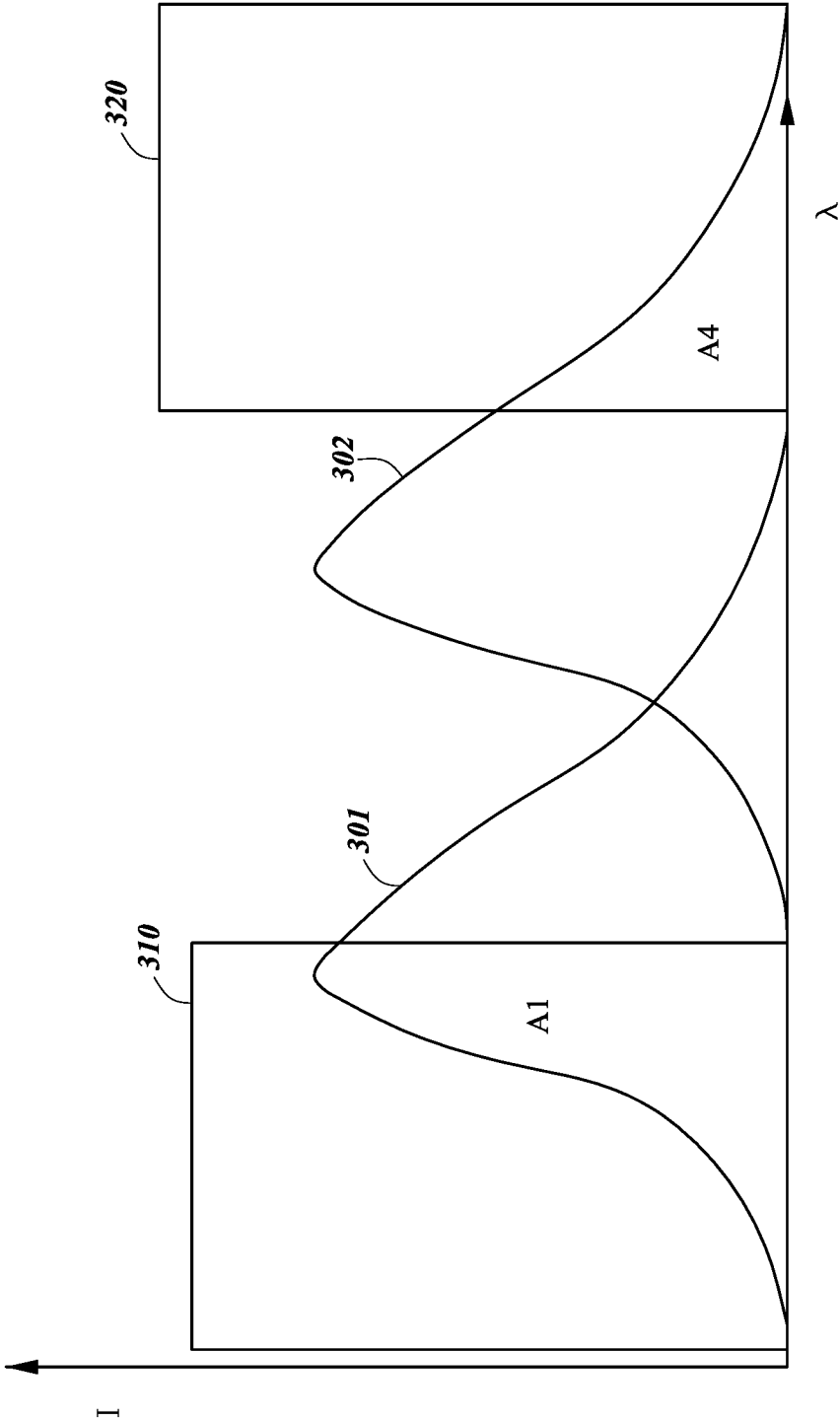


FIG.3

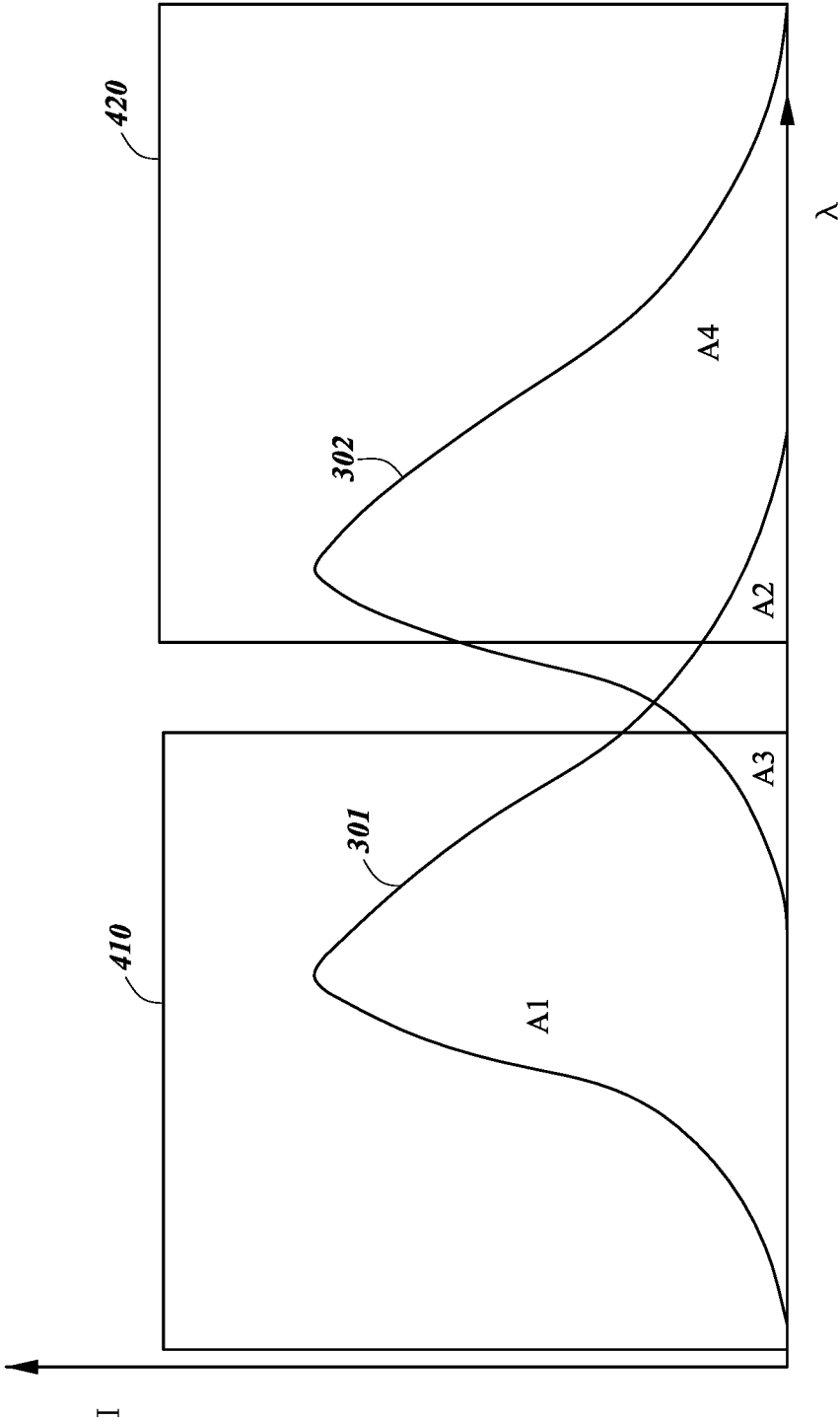


FIG. 4

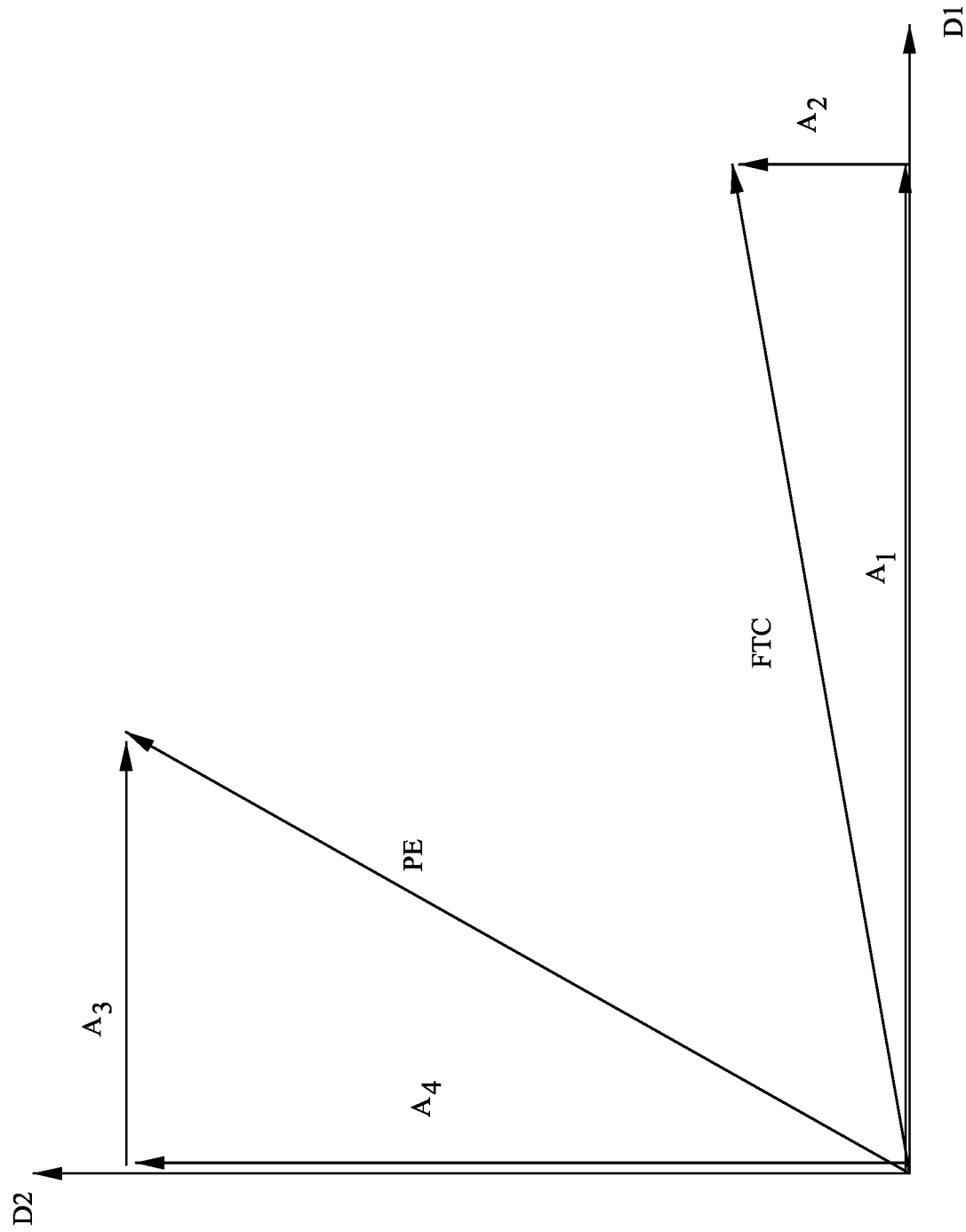


FIG.5

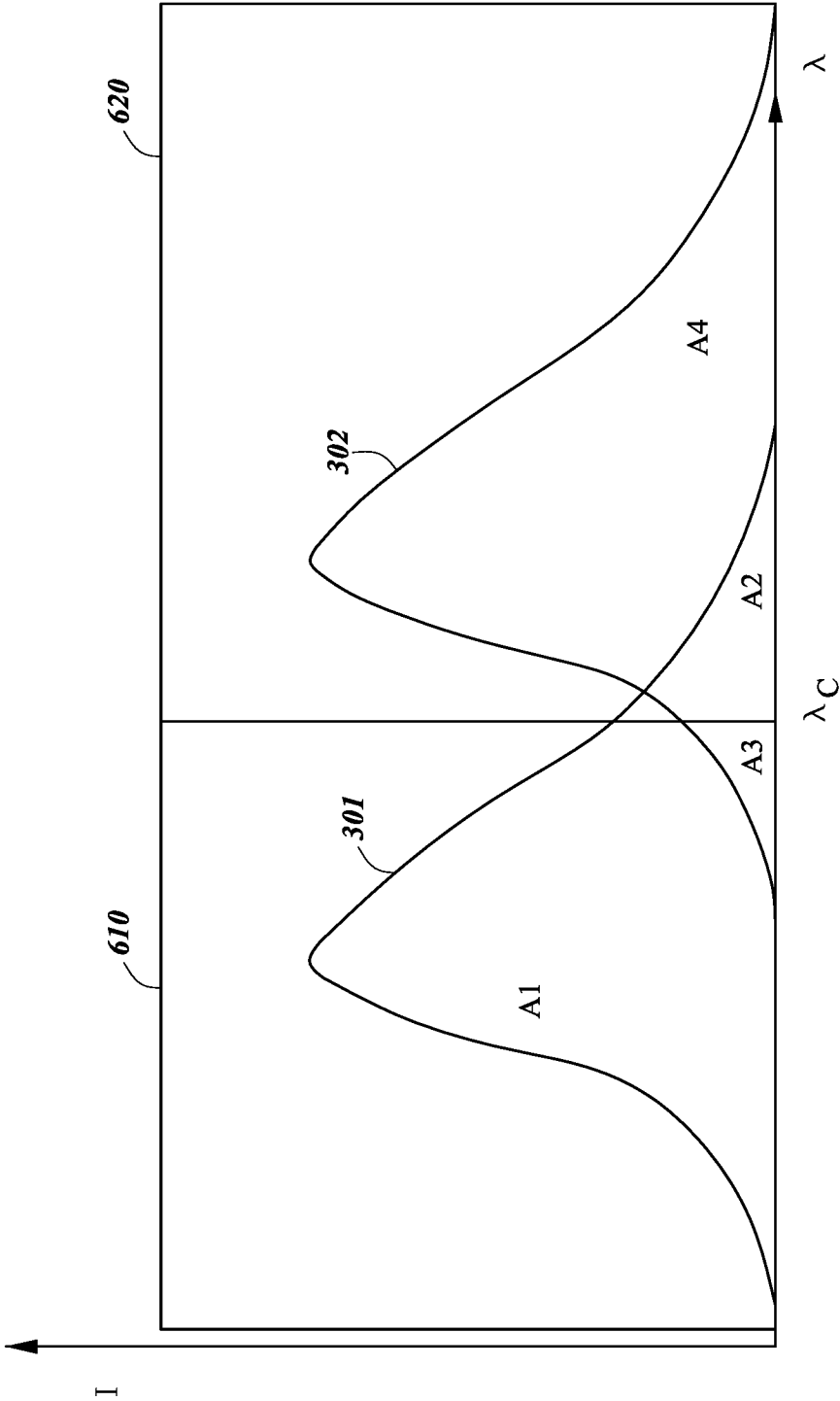
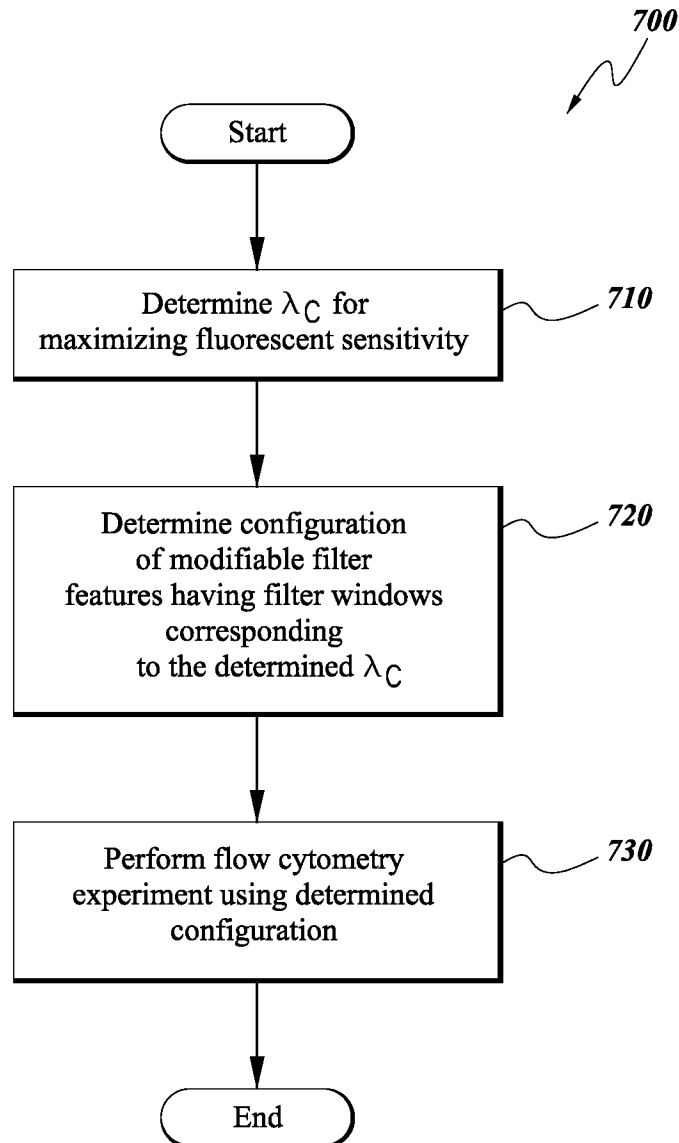
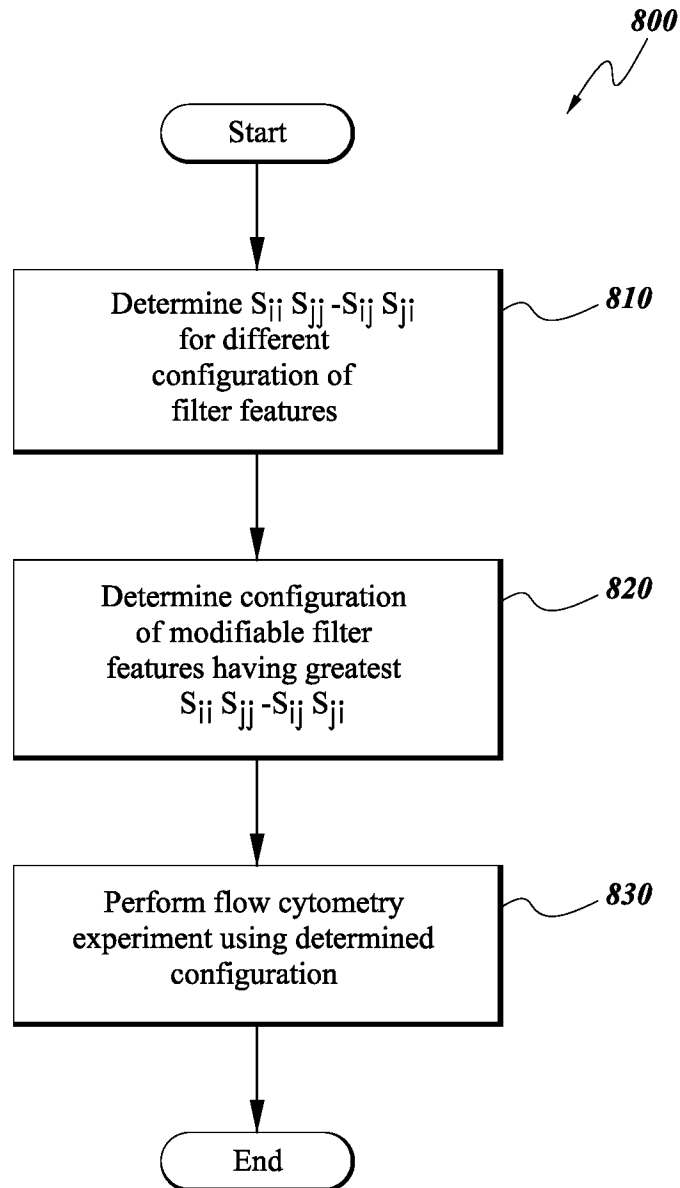


FIG. 6

**FIG. 7**

**FIG. 8**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/051738

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N15/14
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, COMPENDEX, INSPEC, IBM-TDB, WPI Data

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Further documents are listed in the continuation of Box C.



See patent family annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

30 November 2016

Date of mailing of the international search report

08/12/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Liefrink, Feike

INTERNATIONAL SEARCH REPORT

International application No

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