

DESCRIPTION

Title of Invention: LIGHT DETECTION DEVICE AND SIGNAL PROCESSING METHOD

Technical Field

[0001]

The present invention relates to a light detection device and a signal processing method, and more particularly, to a light detection device and a signal processing method that reduce crosstalk occurring when a plurality of types of light emitters emit light at a plurality of light emission points.

Background Art

[0002]

There is a light measurement method in which a plurality of types of light emitters are present at a plurality of spatial positions, and a light detection device that separates and detects a light wavelength for each spatial position detects at which position and in what concentration ratio each light emitter is present. An example thereof is a light detection device of a capillary electrophoresis apparatus that includes a plurality of analysis capillaries to analyze a sample labeled with a plurality of types of fluorescent dyes.

[0003]

In analysis using capillary electrophoresis, an analysis target sample is injected into a capillary filled with a separation medium, and a voltage is applied to both ends to perform separation based on a difference in mobility of the analysis target. For example, fluorescence detection is used for detecting the analysis target. The analysis target is labeled with a fluorescent dye, and fluorescence generated by irradiation with excitation light is detected to detect the separated analysis target.

[0004]

As an example, there is a method in which DNA labeled with a fluorescent dye is electrophoresed in a capillary filled with a polymer and separated according to a strand length.

A detection site provided on the capillary is irradiated with excitation light, and generated fluorescence is detected. DNA molecules in a sample move in the capillary and pass through the detection site at different times depending on the strand length. As a result, a strand length distribution of the DNA molecules in the sample is obtained as a fluorescence intensity waveform. When a plurality of types of DNA molecules are present in the sample, and the DNA molecules are distinguished and analyzed, a plurality of types of fluorescent dyes may be used for labeling DNA. The light detection device separates and detects a fluorescence wavelength with a grating or the like, and determines a fluorescent dye type based on a shape of an obtained spectrum.

[0005]

In a capillary electrophoresis apparatus, a plurality of capillaries may be mounted in order to improve measurement throughput. In such a case, for example, capillaries for separation are arranged in a row, and all the capillaries are collectively irradiated with excitation light. Generated fluorescence is acquired by an image sensor. A signal amount is calculated based on a fluorescence image of each capillary to obtain a sample electrophoresis waveform.

[0006]

When a plurality of types of fluorescent dyes and a plurality of capillaries are used as described above, a signal different from a signal to be originally detected may be detected, and a measured concentration ratio of a target may deviate from a true value. For example, there may be a case where light emission of a fluorescent dye B is erroneously recognized even though a fluorescent dye A emits light in a certain capillary, or a case where light emission of a j-th capillary different from an i-th capillary is erroneously recognized even though the i-th capillary emits light. Here, a signal output due to the erroneous recognition is referred to as crosstalk, a signal output due to the erroneous recognition of the dye in the former case is referred to as spectral crosstalk, and a signal output due to the erroneous recognition of the light-emitting capillary in the latter case is referred to as spatial crosstalk.

[0007]

Various causes of occurrence of crosstalk are conceivable. As an example, the spectral crosstalk may occur due to a width of a fluorescent dye emission spectrum. In general,

a fluorescent dye has an emission spectrum with a wavelength width of about several tens of nanometers. When a plurality of dyes are used, an emission spectrum of the dye A and an emission spectrum of the dye B may overlap. When the emission spectrum of the dye A and the dye B overlap each other, even when the dye A emits light, a portion of the emission spectrum of the dye A corresponding to a detection wavelength band of the dye B may be detected as an emission signal of the dye B.

[0008]

As an example, the spatial crosstalk may be caused by a surface reflection of an adjacent capillary. It is assumed that a plurality of capillaries are arranged in a row in the device, and a detector faces such a capillary array. When a fluorescent dye emits light in the *i*-th capillary (hereinafter, referred to as a capillary *i* as appropriate), fluorescence is emitted in all directions, and a part thereof is introduced into the detector. However, a part of the light hits the *j*-th capillary in the vicinity (hereinafter, referred to as a capillary *j* as appropriate) and a part thereof is further reflected toward the detector. In such a case, the capillary *j* reflecting the fluorescence appears to emit the fluorescence on the detector, and a light emission signal of the capillary *j* is detected.

[0009]

Crosstalk has various adverse effects on analysis by capillary electrophoresis. For example, when fluorescence of the capillary *i* is recognized as the fluorescence of the capillary *j* due to the spatial crosstalk, it may be erroneously recognized that a component in a sample originally analyzed in the capillary *i* is contained in a sample analyzed in the capillary *j*. In addition, due to the spectral crosstalk, although only a component *a* labeled with the fluorescent dye A is originally present in a sample, a component *b* labeled with the dye B may be erroneously recognized as being present in the sample.

[0010]

Crosstalk can be reduced by devising measurement conditions and a device configuration. For example, the spectral crosstalk can be reduced by widening an interval between emission wavelengths of fluorescent dyes to be used, and the spatial crosstalk does not occur if completely independent measurement optical systems are prepared according to the

number of capillaries. However, since a detection wavelength detectable by the detector has an upper limit and a lower limit, if an emission spectrum interval between dyes is widened, the number of fluorescent dyes that can be used decreases. In addition, if the optical systems for measuring each capillary are independent, no spatial crosstalk occurs, but light sources and detectors corresponding to the number of capillaries are required, which is disadvantageous in terms of cost and size of the device.

[0011]

Meanwhile, a method for reducing crosstalk by data processing is also known (PTL 1). In this method, crosstalk when a certain fluorescent dye emits light in a certain capillary is comprehensively acquired in advance, and an inverse matrix of a matrix in which crosstalk components are arranged is generated. The crosstalk is reduced by applying the inverse matrix to an actually acquired signal. With this method, it is possible to reduce crosstalk without changing the device configuration and the measurement conditions.

Citation List

Patent Literature

[0012]

PTL 1: JP7282880B

PTL 2: WO2018/151843

PTL 3: WO2023/276078

Non Patent Literature

[0013]

NPL 1: SeqStudioTMGenetic Analyzer Instrument and Software USER GUIDE

<https://assets.thermofisher.com/TFS->

[Assets/LSG/manuals/MAN0018646_SeqStudioInstSW_UG.pdf](https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0018646_SeqStudioInstSW_UG.pdf)

Summary of Invention

Technical Problem

[0014]

The method disclosed in PTL 1 is based on a premise that ratios of spectrum and spatial crosstalk between dyes and between capillaries do not change between a time when the matrix used for crosstalk reduction processing is generated and a time when a sample is analyzed. This condition is basically satisfied when the same capillary array is used for generating the matrix used for the crosstalk reduction processing and for analyzing the sample. However, in capillary electrophoresis, the capillary array is a consumable, and the capillary array is required to be replaced after being used a certain number of times.

[0015]

When the capillary array is replaced, the ratios of spectral crosstalk and spatial crosstalk change. In the capillary array, capillaries are arranged in a row, and since an arrangement error of about several μm to several tens of μm occurs in each capillary, a light reflection pattern on a capillary surface may change for each capillary array. A position and a tilt of a center of the capillary array relative to the detector may also change before and after replacement. These change the ratio of spatial crosstalk.

[0016]

Since a dye emission spectrum is not changed by the capillary replacement, it can be expected that a change in the spectral crosstalk accompanying the capillary array replacement is smaller than that of the spatial crosstalk. However, spectral crosstalk may also change due to the capillary array replacement. For example, when crosstalk occurs in a path where multiple reflections occur in a detector optical system and a signal of the dye A enters a detection channel of the dye B, a positional change of the capillary array changes a state of the multiple reflections, and as a result, the spectral crosstalk may be changed.

[0017]

For this reason, when the method in PTL 1 is adopted, it is required to generate the matrix used for the crosstalk reduction processing for a new array after the capillary array is replaced. When generating the matrix used for the crosstalk reduction processing, it is required to obtain crosstalk ratios for all dyes and capillaries. A crosstalk ratio when the dye A in the certain capillary i emits light can be obtained by injecting only the dye A into only the capillary

i and performing electrophoresis. Therefore, for example, when four types of dyes are used and a capillary array having eight capillaries is used, electrophoresis is performed $4 \times 8 = 32$ times to generate the matrix used for the crosstalk reduction processing. In view of the fact that it takes about several tens of minutes or longer for one single electrophoresis run including preparation operations such as polymer replacement and pre-electrophoresis for removing unnecessary ions, it takes a long time to generate the matrix used for the crosstalk reduction processing. This time increases as the number of dyes and the number of capillaries increase.

[0018]

PTL 1 also discloses a method for reducing effort and time for generating the matrix used for the crosstalk reduction processing. Specifically, there is disclosed a method in which a timing when a sample is injected into each capillary is staggered to stagger a dye light emission timing such that another capillary does not emit light when a certain capillary emits light. There is also disclosed a method in which fluorescently labeled DNA having different strand lengths is introduced into each capillary to similarly prevent another capillary from emitting light when a certain capillary emits light.

[0019]

However, in the former case, the device is required to be equipped with an operation sequence different from that of normal analysis, and the latter case involves hurdles such as the need to prepare a reagent containing DNA whose strand length is controlled for generating the matrix used for the crosstalk reduction processing. When a dye to be used is changed, it is required to regenerate the matrix used for the crosstalk reduction processing even when the capillary array is not replaced.

[0020]

PTL 2 discloses a method of optimizing, in spectral crosstalk reduction (synonymous with processing for obtaining a ratio of a plurality of dyes having an overlapping spectrum based on a fluorescence spectrum), a matrix for reducing spectral crosstalk based on information obtained by analysis during sample analysis without performing electrophoresis for calibration for the purpose of obtaining information on a matrix used for spectral crosstalk reduction. In this method, based on an initial matrix, the matrix used for the processing for reducing spectral

crosstalk is optimized by repeating a matrix change, a dye concentration correlation evaluation, and determination of whether the matrix is updated, such that a correlation decreases based on correlation information between any two estimated dye concentrations.

[0021]

In this method, it is required to remove an outlier in order to correctly obtain a correlation between two dyes caused by spectral crosstalk. That is, it is required to distinguish between spectral crosstalk relative to the dye B when the dye A is present and a case where the dye B is actually present in a sample. It is generally expected that such distinction can be made with respect to the spectral crosstalk. In general, when a plurality of dyes are used, the dye A and the dye B label different analysis targets (molecules) to distinguish the different analysis targets. Therefore, when a sample including both the analysis target labeled with the dye A and the analysis target labeled with the dye B is electrophoresed to obtain fluorescence spectrum time-series data, it can be expected that the dye A and the dye B emit light at different timings in many cases. Meanwhile, the spectral crosstalk relative to the dye B occurring due to the dye A occurs at the same time when the dye A emits light. Since it is rare that the analysis target labeled with the dye A and the analysis target labeled with the dye B accidentally emit light at the same time, when correlation information between estimated dye concentrations of the dye A and the dye B is calculated, it is sufficient to remove a rare value as an outlier whose trend differs from that of the majority of other data.

[0022]

However, even when the method in PTL 2 is extended to spatial crosstalk, prerequisite conditions for removing the outlier as described above are not satisfied. When the method in PTL 2 is extended to spatial crosstalk, it is required to distinguish between spatial crosstalk relative to the dye A in the capillary j when the dye A emits light in the capillary i and a signal when the dye A in the capillary j actually emits light. Similarly to the above case, fluorescence of the dye A caused by the spatial crosstalk in the capillary j occurs at the same timing when the dye A in the capillary i emits light. Here, it is assumed that the dye A labels the same analysis target α in a sample analyzed in the capillary i and a sample analyzed in the capillary j. In this case, a timing when the dye A in the sample actually emits light is the same between the capillary

i and the capillary j. Therefore, there is no guarantee that the spatial crosstalk occurring relative to the dye A in the capillary j when the dye A emits light in the capillary i is always distinguished from the signal actually generated by the light emission of the dye A in the capillary j.

[0023]

This is a serious obstacle in the following cases, for example. As an example, the analysis target α labeled with the dye A and an analysis target β labeled with the dye B are considered. Only the analysis target α is present in a sample 1. It is assumed that the analysis target β is contained in a sample 2, and it is desired to determine whether a minute amount of the analysis target α is present in the sample 2. This is, for example, a case where the sample 1 is a pure substance and a trace component (analysis target α) present in the sample 2 is analyzed based on data of the sample 1.

[0024]

Here, the sample 1 is analyzed in the capillary i, and the sample 2 is analyzed in the capillary j. At this time, a strong signal of the dye A is obtained in the capillary i, and a strong signal of the dye B is obtained in the capillary j. Here, it is desired to determine whether a weak signal of the dye A is generated in the capillary j, but since it is common that the analysis target α is labeled with the dye A, the fluorescence of the dye A is obtained at substantially the same time between the capillary i and the capillary j regardless of whether the fluorescence is due to spatial crosstalk or an actual signal of the analysis target α . Therefore, assuming that a weak signal of the dye A is obtained in the capillary j, it cannot be distinguished, only based on a data trend, whether the signal is generated due to the spatial crosstalk or there is actually a minute amount of the analysis target α labeled with the dye A.

[0025]

Therefore, by combining the method in PTL 1 with the method in PTL 2, it is not possible to optimize a matrix for reducing both spectral crosstalk and spatial crosstalk during analysis and to eliminate the need for reacquisition of a crosstalk reduction matrix at the time of capillary array replacement.

[0026]

In a capillary electrophoresis apparatus disclosed in NPL 1, an option of using a matrix

initially set as a spectral crosstalk reduction matrix is shown (Factory Calibration disclosed in p217). However, this matrix is not optimized for each individual device and can be used only for applications where residual spectral crosstalk is tolerable due to incomplete information.

[0027]

Therefore, the invention has been made in view of such circumstances, and an object thereof is to provide a light detection device and a signal processing method that can perform crosstalk reduction processing using crosstalk information used for the crosstalk reduction processing generated once even when a plurality of light emitter holders (for example, capillary array) are replaced.

Solution to Problem

[0028]

In order to solve the above problems, a light detection device according to the invention includes: a plurality of replaceable light emitter holders where a plurality of types of light emitters emit light therein; an optical sensor having a plurality of detection channels for detecting the light emitted from the plurality of types of light emitters in a plurality of wavelength bands, respectively; a spatial filter configured to fix an incident position of the light emitted from the plurality of types of light emitters on the optical sensor; and a computer configured to process a signal output from the optical sensor, in which the computer includes crosstalk information for reducing crosstalk between the plurality of detection channels therein, performs, using the crosstalk information, a calculation on an output from the optical sensor corresponding to the plurality of wavelength bands for each of a plurality of light emission points where the plurality of types of light emitters emit light to reduce the crosstalk between the detection channels of the optical sensor and derive a concentration ratio or a signal amount ratio of each of the plurality of types of light emitters for each of the plurality of light emitter holders, and performs the calculation using the crosstalk information even when each light emitter holder is replaced with another light emitter holder.

[0029]

A signal processing method according to the invention includes: preparing a light

detection device that includes a plurality of replaceable light emitter holders where a plurality of types of light emitters emit light therein, an optical sensor having a plurality of detection channels for detecting the light emitted from the plurality of types of light emitters in a plurality of wavelength bands, respectively, a spatial filter configured to fix an incident position of the light emitted from the plurality of types of light emitters on the optical sensor, and a computer configured to process a signal output from the optical sensor; measuring light emission of the plurality of types of light emitters by the optical sensor; performing, using crosstalk information stored in the computer in order to reduce crosstalk between the plurality of detection channels, a calculating for an output from the optical sensor corresponding to the plurality of wavelength bands for each of a plurality of light emission points where the plurality of types of light emitters emit light to reduce the crosstalk between the detection channels of the optical sensor and derive a concentration ratio or a signal amount ratio of each of the plurality of types of light emitters for each of the plurality of light emitter holders; and performing the calculation using the crosstalk information even when each light emitter holder is replaced.

Advantageous Effects of Invention

[0030]

According to the light detection device and the signal processing method in the invention, even when a plurality of light emitter holders are replaced, it is possible to perform crosstalk reduction processing using crosstalk information used for the crosstalk reduction processing generated once. Therefore, it is possible to reduce effort and time for regenerating the crosstalk information to be used for the crosstalk reduction processing along with the replacement of the plurality of light emitter holders.

Problems, configurations, and effects other than those described above will be clarified by the following description of embodiments.

Brief Description of Drawings

[0031]

[FIG. 1A] FIG. 1A is a schematic diagram of a light detection device to which a

crosstalk reduction method disclosed in PTL 1 can be applied.

[FIG. 1B] FIG. 1B is a schematic diagram of a spectrum image on a light image sensor of the light detection device in FIG. 1A.

[FIG. 2A] FIG. 2A is a schematic diagram of a crosstalk simulation model.

[FIG. 2B] FIG. 2B is a flowchart of a simulation executed using the simulation model in FIG. 2A.

[FIG. 3A] FIG. 3A is a diagram showing a crosstalk simulation result.

[FIG. 3B] FIG. 3B is a diagram showing a result when crosstalk reduction processing in PTL 1 is performed on the simulation result in FIG. 3A.

[FIG. 4A] FIG. 4A shows a crosstalk simulation result assuming capillary array replacement.

[FIG. 4B] FIG. 4B is a diagram showing a result when the crosstalk reduction processing in PTL 1 is performed on the simulation result in FIG. 4A.

[FIG. 5] FIG. 5 is a schematic diagram of a light detection device according to a first embodiment.

[FIG. 6] FIG. 6 is a schematic diagram showing an occurrence path of crosstalk occurring on a capillary side relative to an optical fiber.

[FIG. 7A] FIG. 7A is a diagram showing a simulation model of the light detection device to which crosstalk reduction processing according to the first embodiment is applied.

[FIG. 7B] FIG. 7B shows a crosstalk simulation result in the simulation model in FIG. 7A.

[FIG. 7C] FIG. 7C is a diagram showing a result when the crosstalk reduction processing is performed on the simulation result in FIG. 7B.

[FIG. 8A] FIG. 8A is a schematic diagram showing a connection method of a calibration light source for acquiring a matrix used for the crosstalk reduction processing according to the first embodiment.

[FIG. 8B] FIG. 8B is a schematic diagram showing a structure example of the calibration light source in FIG. 8A.

[FIG. 9] FIG. 9 is a diagram showing an influence of detector saturation on the crosstalk

reduction processing according to the first embodiment.

[FIG. 10] FIG. 10 is a diagram showing elements of a matrix G and a matrix F.

[FIG. 11] FIG. 11 is a schematic diagram showing a structure example of a calibration light source for acquiring a matrix used for crosstalk reduction processing according to a second embodiment.

[FIG. 12] FIG. 12 is a flowchart showing an operation of a light detection device when acquiring the matrix used for the crosstalk reduction processing according to the second embodiment.

[FIG. 13] FIG. 13 is a schematic diagram of a light detection device according to a modification.

Description of Embodiments

[0032]

In the following embodiments, for the sake of convenience, the description may be made by being divided into a plurality of sections or embodiments, but unless otherwise specified, they are not unrelated to one another, and one has a relation with all or a part of modifications, details, supplementary explanations, and the like of the other. In the following embodiments, when referring to the number of elements (including the number, a numerical value, an amount, a range, or the like) or the like, the number of elements is not limited to a specific number, and may be the specific number or more or the specific number or less, unless otherwise specified or except for a case where the number is apparently limited to a specific number in principle.

[0033]

Further, in the following embodiments, it is needless to mention that components (also including element steps and the like) are not necessarily essential unless otherwise specified or unless clearly considered to be essential in principle. Similarly, in the following embodiments, when a shape, a positional relation, or the like of a component or the like is referred to, the shape or the like is substantially approximate or similar to the shape or the like unless otherwise specified or clearly considered to be inappropriate in principle. The same applies to the above-

described numerical value and range.

[0034]

In all drawings for describing the embodiments, the same members are denoted by the same reference numerals in principle, and repeated descriptions thereof will be omitted.

[0035]

In the following embodiments, use in a capillary electrophoresis apparatus will be described as a typical application example of a light detection device of the invention. This is for more specifically describing the configuration and effect of the invention, and the light detection device of the invention is not limited to the capillary electrophoresis apparatus.

[0036]

(Overview of Capillary Electrophoresis Apparatus of Embodiment)

In the configuration of the capillary electrophoresis apparatus of the embodiment, as an example, a spatial filter is provided at a light collection portion of a spectrometer that acquires a spectrum by dispersing fluorescence from a fluorescent dye, and the light collection position to the spectrometer is fixed. As an example, the spectrometer includes a first lens that collimates incident light, a grating that separates light by wavelength, a second lens that forms an image of light passing through the grating on an optical sensor, and a light image sensor that detects light. The spatial filter is, as an example, a multimode optical fiber, and one end of the optical fiber is fixed to a light incident position of the spectrometer. The other end of the optical fiber is disposed in the vicinity of a capillary to collect fluorescence emitted from the inside of the capillary. The light image sensor is, as an example, a CCD or CMOS image sensor.

[0037]

Although crosstalk may occur due to a plurality of factors, with the above-described configuration, a ratio of crosstalk occurring on the spectrometer side relative to the optical fiber can be fixed without being affected by replacement of a light emitter holder (capillary array). Fluorescence generated in the capillary is collected into the optical fiber and guided to the spectrometer. Examples of causes of occurrence of crosstalk inside the spectrometer include spatial profile spread of a fluorescence spectrum, multiple reflections between surfaces of elements such as lenses, and a grating anomaly. If there is no optical fiber and the capillary is

directly provided at a light introduction position of the spectrometer, a positional shift of each capillary due to the capillary array replacement may affect a spatial profile of fluorescence, an occurrence state of multiple reflections and anomalies, and the like, and a crosstalk ratio may change.

[0038]

By fixing the incident position to the spectrometer by the optical fiber, the spatial profile of the fluorescence, the occurrence state of multiple reflections, anomalies, and the like are fixed. A change in a position of the capillary with respect to the other end of the optical fiber on an opposite side of the spectrometer affects an amount of light incident on the optical fiber and does not affect the ratio of crosstalk occurring inside the spectrometer. Since the change in the position of the capillary does not change the crosstalk ratio, even when the capillary array is replaced, the same information as that before the replacement can be used for crosstalk reduction processing.

[0039]

Meanwhile, crosstalk occurring on the capillary side relative to the optical fiber may still change due to the capillary array replacement. For example, since crosstalk occurring due to a fluorescence reflection on a capillary surface is affected by an arrangement error of capillaries constituting the capillary array, the crosstalk is changed by capillary array replacement. Such crosstalk cannot be completely removed only by providing the above-described optical fiber. It is also possible to sufficiently reduce the crosstalk that occurs on the capillary side and varies due to the capillary array replacement. This is achieved, as an example, by making each capillary independent by partitioning with a light-shielding wall. Alternatively, an interval between capillaries may be increased to such an extent that the crosstalk can be ignored. There is also known a method of limiting an incident angle of light to be detected by placing a pinhole at an outlet of an optical fiber (PTL 3).

[0040]

Although the crosstalk that occurs on the capillary side and varies due to the capillary array replacement cannot be removed only by the optical fiber as described above, an effect of the invention is not reduced. Even when the crosstalk occurring on the capillary side of the

optical fiber is not completely removed, a residual component may be low enough to be allowable for the purpose of analysis.

[0041]

(Known Technique in PTL 1)

Prior to the detailed description of the embodiments, a known technique in PTL 1, which is the background of the invention, and problems thereof will be organized. FIG. 1A is a configuration diagram of a light detection device 100 to which the technique disclosed in PTL 1 can be applied. The light detection device 100 includes a capillary array 101, two lenses 102, a grating 103, and a light image sensor 104. The light detection device 100 disperses and detects fluorescence emitted from the capillary array 101. An arrow in the drawings indicates an irradiation direction of excitation laser light L that excites the fluorescence. The laser light L is emitted to penetrate all capillaries of the capillary array from a lateral direction (side surface direction) of the capillary array 101 to excite dyes inside all the capillaries.

[0042]

In the light detection device 100, the grating 103 disperses a wavelength in a direction perpendicular to the sheet. As shown in FIG. 1B, spectrum images 105 corresponding to the respective capillaries are arranged on the light image sensor 104. The light image sensor 104 outputs a light intensity measurement result for each pixel. Light intensity measurement results are summed up for each wavelength interval of each capillary. The wavelength interval means each interval of 500 nm to 510 nm, 510 nm to 520 nm, ..., 690 nm to 700 nm when, for example, a measurement wavelength range is 500 nm to 700 nm and light intensity values for each wavelength are summed in 10 nm intervals to obtain 20 data points. That is, a set of values obtained by summing outputs from pixels in each section (channel 106) delineated by dotted lines in FIG. 1B is obtained as spectrum information of each capillary.

[0043]

A crosstalk reduction method in the related art disclosed in PTL 1 will be organized in consideration of the light detection device 100. As previously defined, the crosstalk includes two types, that is, spectral crosstalk where a signal of a dye A is erroneously recognized as a signal of a dye B, and spatial crosstalk where a signal emitted by a capillary i is erroneously

recognized as a signal emitted by a capillary j. Crosstalk reduction methods of PTL 1 and the invention are based on a premise that the crosstalk is linear with respect to a signal vector (a vector in which signals corresponding to each capillary and each wavelength interval obtained by the light image sensor 104 are arranged). This is normally satisfied since the light detection device 100 does not include an element that induces a nonlinear effect on light to be detected and is not used under a condition where the nonlinear effect appears (generally, a fairly high light intensity is required).

[0044]

In the above contents, when a signal when the dye A emits light in the capillary i is denoted by S_{Ai} and crosstalk detected in a region where the dye B in the capillary j is to be detected when the dye A emits light in the capillary i is denoted by $S_{Bj \leftarrow Ai}^{CT}$, a relationship therebetween can be expressed by the following formula (1) using a constant c.

$$S_{Bj \leftarrow Ai}^{CT} = cS_{Ai} \quad (1)$$

[0045]

In the crosstalk reduction method in the related art disclosed in PTL 1, a process of obtaining the constant c is first performed. It is assumed that the dye A is injected into the capillary i and nothing is injected into the other capillaries. In this situation, the signal S_{Ai} when the dye A emits light in the capillary i is acquired, and the crosstalk $S_{Bj \leftarrow Ai}^{CT}$ detected in the region where the dye B in the capillary j is to be detected when the dye A emits light in the capillary i is acquired. Based on a ratio therebetween, the constant c is obtained by the following formula (2).

$$c = S_{Bj \leftarrow Ai}^{CT} / S_{Ai} \quad (2)$$

[0046]

Next, a sample to be analyzed is measured. A sample labeled with the dye A is injected into the capillary i. A sample labeled with the dye B is injected into the capillary j. A signal acquired in the capillary i is S_{Ai} . In the capillary j, it is desirable to obtain a signal S_{Bj} when the dye B emits light, but since the crosstalk $S_{Bj \leftarrow Ai}^{CT}$ is superimposed on the signal, a signal S_{Bj}^{act} actually measured is calculated by the following formula (3).

$$S_{Bj}^{act} = S_{Bj} + S_{Bj \leftarrow Ai}^{CT} \quad (3)$$

[0047]

Here, the crosstalk component in formula (3) is expressed by formula (1). In formula (1), the constant c is obtained by formula (2). In addition, S_{Ai} is obtained as the signal acquired in the capillary i . Therefore, the true signal S_{Bj} to be obtained when there is no crosstalk can be obtained by the following formula (4) using formula (1) and formula (3).

$$S_{Bj} = S^{\text{act}}_{Bj} - S^{\text{CT}}_{Bj \leftarrow Ai} = S^{\text{act}}_{Bj} - cS_{Ai} \quad (4)$$

[0048]

The above description is a simplified version of the method disclosed in PTL 1. In practice, the crosstalk may include a component generated from a capillary other than the capillary i and the capillary j and a dye other than the dye A and the dye B. In the above description, fluorescence generated from the dye A and the dye B is referred to as a signal. Actually, the light image sensor 104 outputs a light intensity in each wavelength band as a signal for each capillary.

[0049]

Based on the above, the method disclosed in PTL 1 will be organized. For example, it is assumed that fluorescent dyes of L colors are used for measurement, there are N capillaries for measurement, and the light image sensor 104 measures M wavelength bands. As for the wavelength band, for example, when $M = 20$ and a detection wavelength range is 500 nm to 700 nm, the light image sensor 104 outputs 20 outputs for each capillary, that is, a signal of fluorescence having a wavelength of 500 nm to 510 nm, a signal of fluorescence having a wavelength of 510 nm to 520 nm, ..., and a signal of fluorescence having a wavelength of 690 nm to 700 nm. Hereinafter, a signal (or crosstalk) generated for a wavelength interval k of the capillary i when an l -th dye is introduced into the capillary j is denoted by $S_{ik \leftarrow jl}$.

[0050]

Here, a matrix having $S_{ik \leftarrow jl}$ as an element is described as C . $S_{ik \leftarrow jl}$ has four subscripts and is two-dimensionally arranged such that a ζ, η element $C_{\zeta\eta}$ of the matrix C satisfies $\zeta = Mi + k$ and $\eta = Mj + l$. A specific value of $S_{ik \leftarrow jl}$ may be obtained as an output from the light image sensor 104 by sequentially supplying dyes to be used only to the capillary j one type at a time. The value of $S_{ik \leftarrow jl}$ may be appropriately normalized by a signal or the like when a dye used has

a reference concentration. The matrix C is a matrix in which spectra of the dyes to be used are arranged at positions corresponding to the respective capillaries in the matrix. Here, prior to measurement of a sample to be actually measured, a generalized inverse matrix C^- of the matrix C is obtained from the following formula (6).

$$C^- = [C^t C]^{-1} C^t \quad (6)$$

[0051]

Next, a situation where the sample to be analyzed is analyzed is assumed. At this time, the signal of the wavelength interval k of the capillary i is denoted by S_{ik} . Here, a signal vector s is created by arranging S_{ik} . Specifically, S_{ik} is arranged such that a ζ component s_ζ of the signal vector s satisfies $\zeta = M_i + k$. At this time, a vector d having a ratio of each dye as an element can be obtained by the following formula (7).

$$d = C^- s \quad (7)$$

[0052]

The formula (7) is obtained by extending the description related to single crosstalk component removal described above to a case where there is spatial crosstalk between a plurality of capillaries and spectral crosstalk between a plurality of dyes. The above is the crosstalk reduction method in the related art disclosed in PTL 1. In analysis by capillary electrophoresis, since the vector s is output from the light image sensor at each time, a signal waveform of each dye in each capillary can be obtained by performing a calculation of formula (7) at each time.

[0053]

When the above-described crosstalk reduction method is applied, it is required that the matrix C does not change between a timing when the matrix C is obtained and a timing when the signal is actually measured. PTL 1 discloses that the above is satisfied when the same capillary array is used for acquiring the crosstalk matrix C and measuring the actual sample. However, in capillary electrophoresis, the capillary array is a consumable and is required to be replaced with a new array after a certain number of times of use. PTL 1 does not describe whether the above prerequisite condition is still satisfied at this time or whether the crosstalk reduction method functions.

[0054]

(Simulation)

If various parameters such as a diameter of each capillary and an installation position and an angle of each capillary are completely the same before and after the capillary array replacement, the above-described condition is satisfied. However, it is practically impossible to satisfy this condition, and the above-described condition is invalid when the capillary array is replaced. Hereinafter, a specific description will be given using a simulation. In the following simulation, an influence on crosstalk when an error in a relative position of the capillaries in each capillary array changes due to the capillary array replacement is evaluated. The error in the relative positions of the capillaries means a deviation from ideal positions of the capillaries supposed to be arranged at equal intervals on the same plane. The capillary array is produced by arranging and fixing the capillaries on a substrate or the like, and an interval between the capillaries and a distance to each capillary from the substrate are not completely the same for each capillary array due to individual differences between substrates or a variation in a fixing process.

[0055]

FIG. 2A shows a simulation model. In this simulation, for simplification of the description, wavelength separation is not performed and only spatial crosstalk is considered. This model includes a capillary array 201, two lenses 202, and a light image sensor 203. The capillary array 201 is set to have an inner diameter of 50 μm and an outer diameter of 343 μm , and capillaries are arrayed at a pitch of 370 μm . A light-emitting region having a length of 44 μm is set inside each capillary. An image of the capillary array 201 is formed on the light image sensor 203 by the two lenses 202. The number of capillaries in the capillary array 201 is eight.

[0056]

FIG. 2B shows a simulation flow. When the crosstalk reduction processing is performed (S200: Yes), first, the above-described matrix C^- is calculated. A capillary array for obtaining the matrix C^- is assumed, and a relative position error is set in the capillary array (S201). After a position of each capillary is set, a fluorescence image on the light image sensor 203 is calculated by a ray tracing method (S202). In this step, the ray tracing is performed by

generating fluorescence from only one capillary of capillaries 1 to 8. This step is repeated eight times by changing a capillary that emits light, and an output from the light image sensor 203 when each of the capillaries 1 to 8 emits light is calculated. Next, a point spread function is convolved with a calculation result of the ray tracing (S203). The point spread function is actually measured by a spectrometer including a lens, a grating, and a light image sensor, and represents "blurring" of an optical system relative to a capillary arraying direction (X direction in FIG. 2A). In the ray tracing using the model in FIG. 2A, the lens 202 is treated as an ideal thin lens, and a deviation from an ideal situation in an actual optical system is expressed by the point spread function. Next, with respect to the output from the light image sensor 203 obtained as a calculation result, signals in regions corresponding to the respective capillaries are integrated to obtain crosstalk values. The generalized inverse matrix C^{-} is calculated for the matrix C generated by arranging the crosstalk values (S204). Since this model does not perform wavelength separation, the matrix C is a square matrix in which spatial crosstalk between the capillaries is two-dimensionally arranged.

[0057]

Next, a capillary array actually used for sample analysis is assumed, and crosstalk is calculated. When the crosstalk reduction processing is not performed (S200: No), the calculation is started from this step. A relative position error setting for the capillary array (S205), a calculation by ray tracing (S206), and point spread function convolution (S207) are the same as those in S201, S202, and S203 described above. Thereafter, the signal vector s is calculated based on an obtained output from the light image sensor 104 (S208). When the crosstalk reduction processing is performed (S209: Yes), the crosstalk reduction processing is performed by formula (7) (S210). When the crosstalk reduction processing is not performed (S209: No), this step (S210) is skipped. After the calculation, a result is output (S211). The vector d is output when the crosstalk reduction processing is performed, and the vector s is output when the processing is not performed. When there are a plurality of types of dyes and a dye concentration ratio is calculated based on a spectrum, the vector d and the vector s are different types of information (a dye ratio for each capillary and a light intensity of each wavelength interval), but when light separation in a wavelength direction is not considered, the

two become the same type of information (a light intensity of each capillary directly represents a dye concentration ratio), and thus an effect of presence or absence of the crosstalk processing can be evaluated by comparing the two.

[0058]

(Result of Crosstalk Reduction Processing Without Relative Position Error)

FIG. 3A is a diagram showing crosstalk obtained by the simulation method described above in a case where the crosstalk reduction processing is not performed, and FIG. 3B is a diagram showing crosstalk in a case where the crosstalk reduction processing is performed. In FIGS. 3A and 3B, it is assumed that there is no relative position error in the capillary array, and the generation of the matrix C^{-} and the sample analysis are performed in the same capillary array. In FIG. 3A, a horizontal axis represents a capillary that emits light. Each bar with different hatching indicates a value of crosstalk detected in each of regions (referred to as channels 1 to 8) for detecting the capillaries 1 to 8.

[0059]

According to FIG. 3A, it can be seen that, in the current model, crosstalk is maximized in a channel for detecting an adjacent capillary, and crosstalk of about more than 1% occurs. The crosstalk is mainly caused by a light reflection on a capillary surface and fluorescence leakage to the adjacent channel due to blurring (represented by a point spread function) of an imaging optical system. In addition to the above, it is conceivable that an actual cause of occurrence of the spatial crosstalk may be multiple reflections between elements constituting a spectroscopic system, a grating anomaly, scattering due to dust, or the like, but this model reflects only the above two causes.

[0060]

According to FIG. 3B, when the capillary array is the same, it can be seen that the crosstalk is reduced by the crosstalk reduction processing in the related art. The crosstalk of about more than 1% is reduced to 0.01% or less by the crosstalk reduction processing. In general, a dynamic range of the light image sensor is about 3 to 4 digits, and the crosstalk of 0.01% or less means that the crosstalk is equal to or less than a detection lower limit. This time, models used for generating the matrix C^{-} and calculating the crosstalk are completely the

same, and a reason why the crosstalk value does not reach zero in FIG. 3B is due to a calculation error in the ray tracing method.

[0061]

(Result of Crosstalk Reduction Processing with Relative Position Error)

FIGS. 4A and 4B are diagrams showing crosstalk when the crosstalk reduction processing is not performed and crosstalk when the crosstalk reduction processing is performed, similarly to FIGS. 3A and 3B. However, in this calculation, an error in an XY direction in FIG. 2A is given to each capillary based on a Gaussian distribution with a standard deviation of 10 μm . Assuming that different capillary arrays are used for generating the matrix C^- and analyzing the sample, values of relative position errors of the capillaries set in the capillary arrays are different when generating the matrix C^- and when calculating the crosstalk. Considering that the outer diameter of the capillary is 343 μm , the above relative position error can be said to be relatively small. In this model, the outer diameter of the capillary is completely the same, but the outer diameter of the capillary generally has a tolerance of about $\pm 10 \mu\text{m}$. Assuming that a change in the diameter of the capillary causes a shift in a center position depending on a fixing method, it can be seen that the above relative position error has a value that may actually occur.

[0062]

According to FIG. 4A, it can be seen that even when an arraying error is introduced, there is crosstalk of about more than 1% as in the case where the arraying error is zero. Meanwhile, according to FIG. 4B, it can be seen that when there is an arraying error, crosstalk of about 0.2% at maximum remains even when the crosstalk reduction processing in the related art is performed. In addition, it can be seen that the crosstalk has a negative value in a part of channels, and the crosstalk is excessively corrected. The calculation is repeated nine times with different arraying errors, and an average of maximum values of the residual crosstalk has a value of 0.28%. This indicates that, since the crosstalk value varies due to the arraying error, an effect of the crosstalk reduction processing in the related art cannot be correctly obtained when arrays having different arraying errors are used for generating the matrix C^- and analyzing the sample.

[0063]

This time, an influence of the arraying error on the crosstalk reduction processing is shown using a model in which a cause of occurrence of crosstalk is limited. In practice, as described above, there are crosstalk occurrence causes that are not incorporated into the model. In addition, in capillary replacement, there are factors that increase a crosstalk change in addition to arraying errors such as a change in a position of the entire capillary array, a change in a tilt of the capillary array, a variation in inner and outer diameters of capillaries, and dust on capillaries. As a variation in crosstalk for each capillary array increases, it becomes more difficult to obtain the effect of the crosstalk reduction processing in the related art.

[0064]

As described above, the inventors have newly found that the crosstalk reduction processing method in the related art is required to regenerate the matrix C^- when the capillary array is replaced. Crosstalk information used for generating the matrix C^- is measured by injecting a sample labeled with each dye one type at a time into each capillary and performing electrophoresis, as disclosed in PTL 1, for example. Therefore, when fluorescent dyes of L colors are used and there are N capillaries to be measured, it is required to perform electrophoresis $L \times N$ times. Since one single electrophoresis run takes about several tens of minutes, a long time is required to generate the matrix C^- . PTL 1 also discloses a method for reducing effort and time for generating the matrix C^- . However, in order to use the disclosed method, use of a special sample or an operation of a special device that is not performed in normal analysis is required.

[0065]

The invention eliminates a need for regenerating the matrix C^- accompanying capillary array replacement, thereby solving the above problems.

[0066]

<First Embodiment>

FIG. 5 is a configuration diagram of a light detection device 500 according to a first embodiment. The light detection device 500 includes a capillary array 501, an optical fiber array 510 (spatial filter), a spectrometer 505, and a computer 550. The capillary array 501

includes a plurality of capillaries 501a, and a plurality of light emitters emit light inside each capillary 501a. The capillary array 501 is replaceable. A light image sensor 504 constituting the spectrometer 505 includes a plurality of detection channels (see FIG. 1B) for detecting light emitted from the plurality of light emitters in a plurality of wavelength bands, respectively. The optical fiber array 510 is a spatial filter that fixes an incident position of light emitted from the plurality of light emitters on the spectrometer 505. The computer 550 processes a signal output from the light image sensor 504.

[0067]

The light detection device 500 is different from the light detection device 100 in that the optical fiber array 510 is provided. The optical fiber array 510 is an optical fiber array 510 including a plurality of optical fibers 510a each provided corresponding to a respective one of light emission points of the plurality of capillaries 501a. The optical fiber array 510 collects fluorescence emitted from each capillary 501a in the capillary array 501 and guides the fluorescence to the spectrometer 505 including a lens 502, a grating 503, and the light image sensor 504. The light detection device 500 is assumed to be a detection device of a capillary electrophoresis apparatus, actually operates integrally with a temperature control device, a high voltage application device, an automatic sample stage, a computer for processing a signal, and the like, and performs analysis by capillary electrophoresis of a sample. However, descriptions of portions other than the light detection device 500 will be omitted.

[0068]

Crosstalk reduction processing of the invention can be applied even when the spectrometer 505 has a different form. Specifically, a light wavelength separation method may be performed by a prism instead of a grating, or may be performed by a plurality of dichroic mirrors.

[0069]

A crosstalk reduction processing method according to the first embodiment is the same as the above formula (7) and the description related thereto. However, as processing conditions, it is required that a position of fluorescence introduction to the spectrometer 505 by the optical fiber array 510 is fixed and crosstalk occurring on a capillary side relative to the optical fiber

array 510 is sufficiently reduced by another method. The fixing of the position of fluorescence introduction to the spectrometer 505 may be implemented by a pinhole array, a slit array, or the like other than the optical fiber array 510.

[0070]

The light detection device 500 includes the computer 550. The computer 550 includes a processor 551, a main storage unit 552, an auxiliary storage unit 553, and an input and output interface 554 (hereinafter, an interface is abbreviated as I/F). The processor 551 is a central processing calculation device that controls an operation of each unit of the computer 550. The processor 551 is, for example, a central processing unit (CPU), a graphics processing unit (GPU), a digital signal processor (DSP), or an application specific integrated circuit (ASIC). The processor 551 loads a program stored in the auxiliary storage unit 553 to a work area of the main storage unit 552 in an executable manner. The main storage unit 552 stores a program to be executed by the processor 551, data to be processed by the processor, and the like. The main storage unit 552 is a flash memory, a random access memory (RAM), a read only memory (ROM), or the like. The auxiliary storage unit 553 stores various programs such as an operating system (OS) and various types of data. The auxiliary storage unit 553 is, for example, a solid state drive (SSD), a hard disk drive (HDD), or a combination thereof. The auxiliary storage unit 553 includes crosstalk information (for example, the matrix C^{-} described above) for reducing crosstalk between a plurality of detection channels. The input and output I/F 554 is communicably connected to the light image sensor 504 and a display unit 560.

[0071]

The computer 550 reduces the crosstalk between the detection channels of the light image sensor 504 and derives a concentration ratio or a signal amount ratio of each of a plurality of types of light emitters for each of the plurality of capillaries by performing, using the crosstalk information (for example, the above-described matrix C^{-}), a calculation on an output from the light image sensor 504 corresponding to a plurality of wavelength bands of each of the plurality of light emission points where the plurality of light emitters emit light. Even when the capillary array 501 is replaced with another capillary array, the computer 550 performs the above-described calculation using the same crosstalk information.

[0072]

The crosstalk information is the generalized inverse matrix C^- or information equivalent to the generalized inverse matrix C^- of the matrix C including information on the crosstalk between the plurality of detection channels. The information equivalent to the generalized inverse matrix C^- is, for example, information that is not in a matrix format and is information for reducing the crosstalk between the plurality of detection channels. The calculation for deriving the concentration ratio or the signal amount ratio executed by the computer 550 is a calculation for deriving C^- s by applying, from the left, the matrix C^- to the vector s of the output from the light image sensor 504 (see the above formula (7)) or a calculation equivalent thereto.

[0073]

The computer 550 sequentially supplies the plurality of light emitters to each of the plurality of capillaries 501a, records the output from the light image sensor 504 in a state where a single light emitter among the plurality of light emitters emits light in a single capillary 501a among the plurality of capillaries 501a, arranges outputs from the light image sensor 504 to generate the matrix C , and calculates and stores the generalized inverse matrix C^- of the matrix C by $[C^t C]^{-1} C^t$ (see the above formula (6)) or a calculation equivalent thereto.

[0074]

The crosstalk occurring on the capillary side relative to the optical fiber array 510 occurs, for example, in a path where fluorescence generated from a certain capillary directly enters an optical fiber for detecting another capillary, or a path where fluorescence is reflected on a surface of another capillary and enters the optical fiber for detecting the capillary, as shown in FIG. 6. Reduction of the crosstalk occurring in such a path is implemented by, for example, setting a sufficiently large capillary pitch in the capillary array 501. Alternatively, a method is known in which a pinhole slightly smaller than a core diameter of the optical fiber 510a is provided at an end of the optical fiber array 510 on the capillary array 501 side to limit an angle at which fluorescence is collected to the optical fiber 510a and reduce crosstalk. There is also known a method of providing a light-shielding wall between the capillaries in the capillary array 501.

[0075]

A simulation result of effects of the crosstalk reduction method in the first embodiment will be described with reference to FIGS. 7A to 7C. FIG. 7A is a diagram showing a model used for the simulation. The model in FIG. 7A includes a capillary array 701, two lenses 702, and a light image sensor 703, similarly to the model in FIG. 2A. In FIG. 7A, an optical fiber array 710 is added to the model in FIG. 2A. As the optical fiber array 710, optical fibers having a core diameter of 200 μm and an NA of 0.5 are set, and eight optical fibers are arranged at the same intervals as the capillaries in the capillary array 701. In addition, a light-shielding wall 720 is set between the capillaries as a unit for removing crosstalk occurring before the optical fiber array 710. A flow of the simulation is the same as that shown in FIG. 2B.

[0076]

FIGS. 7B and 7C are graphs showing a result of the crosstalk simulation using the model in FIG. 7A. FIG. 7B is a graph showing crosstalk when the crosstalk reduction processing is not performed, and FIG. 7C is a graph showing crosstalk when the crosstalk reduction processing is performed. In this simulation, as in the case of FIG. 4, assuming that different capillary arrays are used for generating the matrix C^{-} and analyzing the sample, values of relative position errors of the capillaries set in the capillary arrays 701 are different when generating the matrix C^{-} and when calculating the crosstalk.

[0077]

As can be seen from FIG. 7B, the model in FIG. 7A has crosstalk of about 0.5%. On the other hand, when the crosstalk reduction processing of formula (7) is performed, it can be seen that the crosstalk is reduced to 0.01% or less as shown in FIG. 7C. The calculation is repeated nine times with different relative position errors, and an average of maximum values of the residual crosstalk has a value of 0.006%.

[0078]

From a comparison between the result in FIG. 4B and the result in FIG. 7C, it can be seen that the crosstalk reduction processing acts correctly even when using the capillary arrays having different capillary positional deviation values using the method in the first embodiment. Such an effect is not due to the installation of the light-shielding wall 720. In order to show

this, the light-shielding wall 720 is added to FIG. 2A and the same simulation is performed. The calculation is repeated nine times with different relative position errors, and an average of maximum values of the residual crosstalk has a value of 0.11%, which is 10 times or more the result in FIG. 7C.

[0079]

As described above, according to the crosstalk reduction method in the first embodiment, the effect of the crosstalk reduction method can be obtained without regenerating the matrix C^- when the capillary array 501 is replaced. This is an effect obtained by fixing the incident position of fluorescence to the spectrometer 505 by the optical fiber array 510. Even when a position of each capillary in the capillary array 501 changes, the incident position of the fluorescence to the spectrometer 505 is fixed by the optical fiber array 510. Crosstalk occurring in the spectrometer 505 (for example, occurring due to multiple reflections or spectrum image blurring) is not affected by the position of the capillary since the fluorescence incident point is fixed. Therefore, the crosstalk value is not changed by the capillary array replacement, and re-creation of the matrix C^- is not required.

[0080]

However, as described above, in the method in the invention, the crosstalk occurring on the capillary side relative to the optical fiber array 510 is required to be sufficiently small. Since the method in the first embodiment reduces an invariant component in the crosstalk (basically, the crosstalk of the spectrometer 505), a component that varies due to replacement of the capillary array 501 cannot be removed. For example, when an amount of crosstalk caused by a surface reflection changes due to a change in an arrangement error of the capillary array, crosstalk corresponding to the change remains even after the crosstalk reduction processing. In order to show this, the light-shielding wall 720 is removed from the model in FIG. 7A and the same simulation is performed. The calculation is repeated nine times with different relative position errors, and an average of maximum values of the residual crosstalk has a value of 0.12%. Therefore, an optical system to which the method in the invention is applied is required to be designed such that a variation in the crosstalk occurring on the capillary side relative to the optical fiber array 510 is sufficiently smaller than an allowable value.

[0081]

In the light detection device 500 and the light detection method in the first embodiment, since the matrix C^{-} is basically not changed, for example, the matrix C^{-} may be generated after completion of device production or after installation at a use place. The matrix generation can be performed by a method similar to the method disclosed in PTL 1. For example, a sample labeled with a certain dye is injected into a certain capillary and electrophoresis is performed to record a signal from the light image sensor 504. This is repeated for all dyes in all capillaries. Then, measurement results are arranged to form the matrix C . Specifically, outputs of wavelength intervals of the capillaries when a signal of a target dye is maximized in a target capillary are arranged. Thereafter, the generalized inverse matrix C^{-} of the matrix C is generated.

[0082]

At the time of sample analysis, the processing of formula (7) is performed on the output from the light image sensor 504 at each time to obtain information on a ratio of each dye at each time. An electrophoretic waveform of an analysis target labeled with each dye is obtained by arranging ratios of dyes in a time series.

[0083]

The matrix C^{-} is basically not changed, but the matrix C^{-} may be acquired again for any reason. For example, a position of a component of the spectrometer 505 may be shifted due to device relocation or long-term use. In such a case, since a ratio of crosstalk inside the spectrometer may also change, the matrix C^{-} may be regenerated.

[0084]

The matrix C may include partial information in order to reduce effort at the time of generating the matrix C . That is, the matrix C may be a combination of elements of a plurality of matrices C_n each including only crosstalk information between a part of light emitter holders or dyes. For example, from the simulation result in FIGS. 7A to 7C, it can be seen that spatial crosstalk strongly occurs in a channel for measuring an adjacent capillary in this case. In such a case, it is also possible to generate the matrix C while ignoring crosstalk from capillaries that are two or more capillary positions apart.

[0085]

A configuration in FIG. 7A will be described as an example. It is assumed that each matrix C_n is a matrix generated by arranging signals acquired from a channel for measuring each capillary when a dye is injected into a capillary n . When the matrix C is generated, the dye is simultaneously supplied to capillaries in each set among a set of a capillary 1, a capillary 4, and a capillary 7, a set of a capillary 2, a capillary 5, and a capillary 8, and a set of a capillary 3 and a capillary 6. Then, the matrix C_n is generated using signals of channels corresponding to a capillary $n + 1$, the capillary n , and a capillary $n - 1$, and signals of channels corresponding to the other capillaries are zero. That is, when the dye is simultaneously injected into the capillary 1, the capillary 4, and the capillary 7, C_1 is generated using values of a channel 1 and a channel 2, C_4 is generated using values of a channel 3, a channel 4, and a channel 5, and C_7 is generated using values of a channel 6, a channel 7, and a channel 8. After all matrices C_n (in the above case, C_1 to C_8) are generated, the matrices C_n are sequentially arranged to obtain the matrix C . By generating the matrix C using this method, the number of times of electrophoresis for obtaining information can be reduced.

[0086]

Although spatial crosstalk between a capillary pair having a small spatial crosstalk value is ignored in the above description, the same method can be adopted similarly also in a case where emission spectra of a certain dye pair are significantly different and there is almost no overlap. That is, in the case of the dye pair whose fluorescence spectra do not overlap, the matrix C can be generated using data obtained by simultaneously measuring the dye pair.

[0087]

The matrix C may not necessarily be generated by injecting the dye into the light emitter holder, that is, the capillary. The crosstalk information (matrix C') may be acquired by connecting a calibration light source 801 to the spatial filter. The calibration light source 801 sequentially introduces calibration light to each emitted light collection position of the spatial filter. The calibration light is light having the same spectrum as an emission spectrum of the plurality of light emitters. FIG. 8A shows a case where the matrix C is generated using the calibration light source 801. The calibration light source 801 sequentially introduces light

having the same spectrum as an emission spectrum of each dye into the optical fiber in the optical fiber array 510. The matrix C may be generated from the output from the spectrometer 505 in the same manner as the method described above.

[0088]

FIG. 8B shows a structure example of the calibration light source 801. The calibration light source 801 includes an excitation light source 802 therein. The excitation light source 802 irradiates a flow path 803 with fluorescence excitation light. The dyes are sequentially injected into the flow path 803 by a dye injection mechanism 804. Fluorescence generated in the flow path 803 is collimated by a lens 805, and the excitation light is removed by a wavelength filter 806. The collimated fluorescence is introduced into one fiber in an output fiber array 809 by mirrors 807 and a lens 808. Angles of a part of the mirrors 807 are changed to allow a fiber to which the fluorescence is introduced to be changed.

[0089]

The crosstalk reduction method in the first embodiment is based on a premise that there is a linear relationship between crosstalk and a signal that is a source of the crosstalk. Therefore, when this relationship breaks down due to detector saturation, the method in the first embodiment does not function. FIG. 9 is a schematic graph showing an influence of the detector saturation. In the graph in FIG. 9, a horizontal axis represents a concentration of a dye to be measured, and a vertical axis represents an amount of crosstalk. In a spectroscopic system having crosstalk, the dye concentration and the crosstalk are in a proportional relationship. In an ideal situation where there is no crosstalk, the crosstalk is always zero regardless of the dye concentration.

[0090]

According to the crosstalk reduction method in the first embodiment, the crosstalk is ideally completely zero. Therefore, in the spectroscopic system in which the crosstalk is present and the crosstalk reduction processing of the first embodiment is performed, the crosstalk is zero up to a certain dye concentration regardless of the concentration in an ideal state (even under realistic conditions where slight crosstalk remains even after the reduction processing, the crosstalk after the reduction has a value smaller than an original value).

However, when a detector is saturated in a capillary that measures the dye to be measured, an output signal is constant even when the dye concentration further increases. Meanwhile, since the crosstalk is proportional to a fluorescence intensity, the crosstalk continues to increase in proportion to the concentration. As a result, an actual amount of crosstalk exceeds an amount of crosstalk estimated based on a detector output. In this case, the crosstalk cannot be completely removed by the crosstalk reduction method in the first embodiment, and the crosstalk value is not zero. Therefore, the crosstalk reduction method in the first embodiment is required to be used within a range where the detector is not saturated. When a certain channel of the detector is saturated, it is useful to warn a user that there is a possibility that other channels where the detector is not saturated do not exhibit original performance.

[0091]

Specifically, when the output from the light image sensor 504 is saturated, the computer 550 may display a warning on the display unit 560.

[0092]

<Signal Processing Method>

Here, a signal processing method using the light detection device 500 of the first embodiment will be described.

[0093]

The signal processing method in the present embodiment includes preparing the light detection device 500, measuring light emitted from a plurality of light emitters by the light image sensor 504, performing, using the crosstalk information (matrix C') for reducing crosstalk between a plurality of detection channels stored in the computer 550, a calculation on the output from the light image sensor 504 corresponding to a plurality of wavelength bands of a plurality of light emission points where the plurality of light emitters emit light to reduce the crosstalk between the detection channels of the light image sensor 504 and derive a concentration ratio or a signal amount ratio of each of the plurality of types of light emitters for each of the plurality of light emitter holders (capillaries 501a), and

performing the calculation with the same crosstalk information even when the capillary

array 501 is replaced.

[0094]

The crosstalk information (matrix C^-) is the generalized inverse matrix C^- of the matrix C including information on crosstalk or information equivalent thereto. The calculation for deriving the concentration ratio or the signal amount ratio is a calculation for deriving C^- s by applying, from the left, the matrix C^- to the vector s of the output from the light image sensor 504 (see the above formula (7)) or a calculation equivalent thereto.

[0095]

<First Embodiment: Summary>

The light detection device 500 according to the first embodiment includes the capillary array 501, the optical fiber array 510, the lens 502, the grating 503, and the light image sensor 504. In addition, the computer 550 that processes the output from the light image sensor 504 is provided. The computer 550 retains therein the generalized inverse matrix C^- of the matrix C generated based on the output from the light image sensor 504 when the sample labeled with the dye to be used is sequentially injected into each of all the capillaries in the capillary array 101 and measured in the light detection device 500. When a measurement target is analyzed, the computer 550 obtains each dye ratio by applying the generalized inverse matrix C^- to the output from the light image sensor 504 at each time. The same value is used as the generalized inverse matrix C^- even when the capillary array 501 is replaced.

[0096]

<Second Embodiment>

In the first embodiment, the concentration ratio of each dye is obtained by collectively processing the output from the light image sensor 104 with the generalized inverse matrix C^- . In a second embodiment, spectral crosstalk caused by a dye, spectral crosstalk caused by a spectrometer, and spatial crosstalk are separately processed. In a crosstalk reduction method according to the second embodiment, processing of reducing crosstalk occurring inside a spectrometer is performed using a matrix acquired using a calibration light source, and spectral crosstalk caused by dye fluorescence spectrum overlap is reduced using a matrix acquired by measuring a fluorescence spectrum based on a dye introduced into a capillary. Therefore, the

crosstalk reduction processing has a form different from the formula (7). With this processing format, it is possible to easily change or add a dye type.

[0097]

First, crosstalk occurring inside the spectrometer 505 will be organized. The spectrometer 505 is provided with N optical fibers for introducing light, and the light image sensor 504 measures M wavelength bands for each optical fiber. The wavelength band means that, for example, when M = 20 and a detection wavelength range is 500 nm to 700 nm, the light image sensor 504 outputs 20 outputs for each fiber, that is, a signal of fluorescence having a wavelength of 500 nm to 510 nm, a signal of fluorescence having a wavelength of 510 nm to 520 nm, ..., and a signal of fluorescence having a wavelength of 690 nm to 700 nm. Here, an output in a wavelength band m for a fiber n of the light image sensor 504 is denoted by S_{mn} . A one-dimensional array of S_{mn} is defined as the output signal vector s of the light image sensor 104. S_{mn} has two subscripts and is one-dimensionally arrayed such that S_{mn} becomes an $Mn + m$ component s_{Mn+m} of s.

[0098]

Meanwhile, light incident on the fiber n of the spectrometer 505 is considered. Power of light in a wavelength range corresponding to the wavelength band m of the light is denoted by P_{mn} . A vector p of incident power is created by arranging the power P_{mn} . As before, P_{mn} is one-dimensionally arrayed to be an $Mn + m$ component p_{Mn+m} of p.

[0099]

The spectrometer 505 outputs a signal that is linear with respect to the power of incident light. Therefore, a relationship between p and s can be expressed by the following formula (8).

$$s = Gp \quad (8)$$

[0100]

A matrix G is a matrix representing crosstalk occurring in the spectrometer 505. The matrix G is acquired by sequentially introducing monochromatic light corresponding to a wavelength detection interval of the optical sensor (for example, the light image sensor 504) for each emitted light collection position of the spatial filter (for example, the optical fiber array 510). In an ideal spectrometer without crosstalk, the matrix G is an identity matrix (s and p are

normalized such that an output signal is 1 when the light power is 1). When there is crosstalk, a triangular portion shown in the matrix G in FIG. 10 is a region indicating a value of the crosstalk. When sensitivity of the spectrometer is different for each emitted light collection position, a diagonal element in the matrix G may have a value other than 1. For example, when the spectrometer 505 has a configuration as shown in FIG. 5, optical loss in the spectrometer may increase at an emitted light collection position farther from a central axis of the lens 502. In this case, the value of the diagonal element corresponding to the collection position is smaller than 1.

[0101]

Next, a relationship between a dye in a capillary and the power of light incident on each fiber is organized. Now, it is assumed that L types of dyes are used for analysis. A ratio of an l -th dye in an n -th capillary among N capillaries is defined as D_{ln} . A dye ratio vector d is obtained by one-dimensionally arraying D_{ln} . D_{ln} has two subscripts and D_{ln} is arrayed one-dimensionally to be an $Ln + 1$ element d_{Ln+1} of d .

[0102]

Here, it is assumed that a signal generated by the n -th capillary is measured by an n -th optical fiber. The light power P_{mn} measured in the n -th optical fiber is a sum of emission spectra of L types of fluorescent dyes in the n -th capillary, weighted by a ratio D_{ln} . Therefore, a relationship between the incident power vector p and the dye ratio vector d is expressed by the following formula (9).

$$p = Fd \quad (9)$$

Here, a matrix F is a matrix in which fluorescence spectra of dyes are arranged.

[0103]

The following formula (10) is obtained from formula (8) and formula (9).

$$s = GFd \quad (10)$$

Therefore, assuming that an inverse matrix of the matrix G is G^{-1} and a generalized inverse matrix of the matrix F is F^{-} , the following formula (11) is obtained. The generalized inverse matrix F^{-} is obtained by $[F^t F]^{-1} F^t$ or a calculation equivalent thereto.

$$d = F^{-} G^{-1} s \quad (11)$$

This means that if G^{-1} and F^{-} are obtained, the dye ratio d is obtained from the output s from the light image sensor. The calculation of G^{-1} means processing for reducing crosstalk occurring inside the spectrometer, and the calculation of F^{-} means processing for calculating the dye ratio (reducing spectral crosstalk) in consideration of dye fluorescence spectrum overlap.

[0104]

FIG. 10 shows shapes of the matrix G and the matrix F . The matrix G is a square matrix having a size of $M \times N$, and 1 is arranged along diagonal elements. Regions indicated by triangles above and below the diagonal elements represent crosstalk. In an ideal spectrometer without crosstalk, all elements in this portion are 0. The matrix F is a matrix having $M \times N$ rows and $L \times N$ columns. The matrix F is a matrix in which N matrices F_1 each having M rows and L columns are arranged in a diagonal portion and the other regions are 0. In the matrix F_1 , spectra of L dyes are arranged from a first column to an L -th column (rectangles in FIG. 10 represent the spectra).

[0105]

In the crosstalk reduction method in the second embodiment, the calculation is divided into two stages, and the matrix G and the matrix F are separately acquired. The matrix G is acquired using a calibration light source 1101 (see FIG. 11). The calibration light source 1101 is connected to the optical fiber array 510 of the spectrometer 505 in the same manner as the calibration light source 801. The calibration light source 1101 sequentially introduces light having wavelengths corresponding to M wavelength bands into each of N fibers. That is, the calibration light source 1101 first introduces light having a wavelength corresponding to a wavelength band 1 into a fiber 1. Next, light having a wavelength corresponding to a wavelength band 2, light having a wavelength corresponding to a wavelength band 3, ..., and light having a wavelength corresponding to a wavelength band M are introduced. Next, the same operation is performed on a fiber 2. Next, the same operation is performed on a fiber 3, a fiber 4, ..., and a fiber N .

[0106]

The matrix G is generated as follows. When the calibration light source 1101 introduces light of the wavelength interval m into the fiber n , an output value for a fiber k in the

optical fiber array and for a wavelength interval l is denoted by $A_{kl \leftarrow mn}$. At this time, assuming that $\zeta = Mk + l$ and $\eta = Mn + m$, an η, ζ element in the matrix G is $G_{\zeta\eta} = A_{kl \leftarrow mn} / A_{mn \leftarrow mn}$.
[0107]

FIG. 11 shows a structure example of the calibration light source 1101 that performs the above-described operation. The calibration light source 1101 includes a white light source 1102 therein. The white light source 1102 is, for example, a white LED or a halogen lamp. Light from the white light source 1102 is separated for each wavelength by a lens 1103 and a grating 1104. A slit 1105 is placed at an imaging point of the separated light. The slit 1105 extracts only a component of a specific wavelength in the light from the white light source 1102. The grating 1104 can change an angle thereof, and a wavelength of light passing through the slit 1105 is controlled by adjusting the angle. The light passing through the slit 1105 is introduced into a specific fiber in an output fiber array 1108 by a lens 1106 and mirrors 1107. An angle of one of the mirrors 1107 can be changed, and the optical fiber in the output fiber array 1108 to which light is to be introduced is controlled by adjusting the angle.
[0108]

The matrix F may be measured by simultaneously injecting a sample labeled with a dye into all capillaries. In the matrix acquisition method in the first embodiment, it is required to perform signal measurement for N capillaries by injecting a dye into only one capillary, whereas in the matrix acquisition method in the second embodiment, measurement can be simultaneously performed in all capillaries, and thus a time required for measurement is reduced to $1/N$.
[0109]

Since the matrix F is a matrix obtained by repeatedly arraying, for the number of capillaries, the matrix F_1 where spectra of the respective dyes are arranged, the matrix F_1 may be measured by measuring a dye spectrum with a specific capillary and repeatedly arranged for the number of capillaries to obtain the matrix F . In the crosstalk reduction method in the related art and the crosstalk in the first embodiment, information on spectral crosstalk caused by a dye, information on crosstalk caused by the light detection device, and information on detection efficiency of each wavelength are mixed in a single processing matrix. Therefore, the

information is required to be acquired by supplying each dye for each capillary. However, in the crosstalk reduction method in the second embodiment, since information on an effect caused by the spectrometer is consolidated in the matrix G, it is not required to measure the same sample for each capillary at the time of measuring the matrix F.

[0110]

As described above, in the crosstalk reduction method in the second embodiment, it is easy to acquire the processing matrix, particularly, the matrix F including the information on the dye spectrum, as compared to the crosstalk reduction method in the related art disclosed in PTL 1 and the crosstalk reduction method in the first embodiment. This is particularly useful when adding a dye used for analysis. When it is desired to use a new dye having no information in a computer that performs the crosstalk reduction processing, in the crosstalk reduction method in the related art disclosed in PTL 1 or the crosstalk reduction method in the first embodiment, it is required to sequentially introduce the new dye into all capillaries to acquire fluorescence spectrum information on the new dye. On the other hand, in the crosstalk reduction method in the second embodiment, the fluorescence spectrum may be measured by introducing the new dye into all capillaries simultaneously or into a specific capillary.

[0111]

Alternatively, the matrix F may be provided in a form of being stored in a computer in advance. Information on crosstalk depending on each individual spectrometer is contained in the matrix G. Therefore, it is not required to measure the matrix F for each individual light detection device, spectrum information on each dye measured in a specific individual device may be stored in a computer of another individual device, and the matrix F may be generated based on the information. That is, spectrum information on the dye measured by a certain individual light detection device may be disclosed on a web, the user may download the spectrum information on the dye from the web and store the spectrum information in a computer, and the matrix F may be generated based on the stored spectrum information.

[0112]

FIG. 12 shows an example of an operation of the device when the matrix F is acquired. First, the user sets a dye or a set of dyes for which a spectrum is to be acquired (S1201). Next,

the user selects a method for acquiring a fluorescence spectrum (S1202). When a method of measuring a dye spectrum with all capillaries is selected, the device first prepares for electrophoresis (S1203). After the preparation is completed, the user sets a sample labeled with a dye to be measured (S1204). After the setting of the sample is completed, the device injects the sample into a capillary and performs electrophoresis (S1205). A spectrum when the sample electrophoretically migrates to a fluorescence measurement point is acquired (S1206). This cycle is repeated for all dyes for which the spectrum is to be acquired (S1207). If the sample is prepared such that all dye spectra can be collectively acquired, this cycle may be performed once. This is, for example, a sample in which dyes A, B, C, etc., to be measured are labeled with DNA having a strand length of 100 bases, 150 bases, 200 bases, etc., respectively, and mixed. When electrophoresis is performed, the dyes A, B, C, etc., sequentially emit light at different times. Such a calibration sample is commercially available and is relatively easy to obtain. Since the DNA reaches the fluorescence measurement point in order of the strand length due to the electrophoresis, the dyes A, B, and C emit light in order in a time series. After all fluorescence spectra are measured, the fluorescence spectra are arranged to generate the matrix F (S1208).

[0113]

When a method of measuring the fluorescence spectrum with a single capillary is selected, an operation of the device is the same as the method of measuring the fluorescence spectrum with all capillaries (S1209 to S1214). However, the sample is injected into only a single capillary. When the matrix F is generated, fluorescence spectra acquired by the single capillary are repeatedly arranged.

[0114]

When a method of generating the matrix F based on internal information is selected, a fluorescence spectrum (emission spectrum information) of the set dye is read from a memory (S1215). The fluorescence spectrum may be stored in the computer from the beginning, or spectrum information may be provided through the Internet or the like and downloaded. The matrix F is generated based on the read fluorescence spectrum (S1216). The generated matrix F is stored in the computer (S1217).

[0115]

<Modification>

The invention is not limited to the above-described embodiments and includes various modifications. For example, the above-described embodiments have been described in detail to facilitate understanding of the invention, and the invention is not necessarily limited to those including all the configurations described above. A part of a configuration according to a certain embodiment can be replaced with a configuration according to another embodiment, and a configuration according to another embodiment can be added to a configuration according to a certain embodiment. In addition, another configuration can be added to a part of a configuration of each embodiment, and the part of the configuration of each embodiment can be deleted or replaced with another configuration.

[0116]

For example, in the first embodiment, an example has been described in which the optical fiber array 510 is provided as the spatial filter, and alternatively, the spatial filter may be a filter having a through-hole through which light passes. A shape of the through-hole of the filter may be circular or rectangular. FIG. 13 is a configuration diagram of a light detection device 1300 according to a modification. Unlike the light detection device 500, the light detection device 1300 includes a pinhole array 1310 in which a plurality of pinholes (circular through-holes) are provided in an array to correspond to light emission points of a plurality of light emitter holders (capillaries). The pinhole array 1310 may be a slit array in which slits (rectangular through-holes) are provided in an array.

[0117]

In the above embodiment, the light detection device including the plurality of light emitter holders where the plurality of types of light emitters emit light is used, and alternatively, one light emitter holder may be used when there are a plurality of types of light emitters, and one type of light emitter may be used when there are a plurality of light emitter holders.

[0118]

In the calculation in the above embodiments, various calculations using matrices are performed, and alternatively, an equivalent calculation using information not in a matrix format

may be performed as long as an equivalent result is obtained.

Reference Signs List

[0119]

- 100 light detection device
- 101 capillary array
- 102 lens
- 103 grating
- 104 light image sensor
- 105 spectrum image
- 106 channel
- 201 capillary array
- 202 lens
- 203 light image sensor
- 500 light detection device
- 501 capillary array
- 501a capillary
- 502 lens
- 503 grating
- 504 light image sensor
- 505 spectrometer
- 510 optical fiber array
- 510a optical fiber
- 550 computer
- 551 processor
- 552 main storage unit
- 553 auxiliary storage unit
- 554 input and output interface
- 560 display unit

701 capillary array
702 lens
703 light image sensor
710 optical fiber array
720 light-shielding wall
801 calibration light source
802 excitation light source
803 flow path
804 dye injection mechanism
805 lens
806 wavelength filter
807 mirror
808 lens
809 output fiber array
1101 calibration light source
1102 white light source
1103 lens
1104 grating
1105 slit
1106 lens
1107 mirror
1108 output fiber array
1300 light detection device
1310 pinhole array

CLAIMS

[Claim 1]

A light detection device comprising:

a plurality of replaceable light emitter holders where a plurality of types of light emitters emit light therein;

an optical sensor having a plurality of detection channels for detecting the light emitted from the plurality of types of light emitters in a plurality of wavelength bands, respectively;

a spatial filter configured to fix an incident position of the light emitted from the plurality of types of light emitters on the optical sensor; and

a computer configured to process a signal output from the optical sensor, wherein the computer

includes crosstalk information for reducing crosstalk between the plurality of detection channels therein,

performs, using the crosstalk information, a calculation on an output from the optical sensor corresponding to the plurality of wavelength bands for each of a plurality of light emission points where the plurality of types of light emitters emit light to reduce the crosstalk between the detection channels of the optical sensor and derive a concentration ratio or a signal amount ratio of each of the plurality of types of light emitters for each of the plurality of light emitter holders, and

performs the calculation using the crosstalk information even when each light emitter holder is replaced with another light emitter holder.

[Claim 2]

The light detection device according to claim 1, wherein

the crosstalk information is a generalized inverse matrix C^+ of a matrix C including information on the crosstalk between the plurality of detection channels or information equivalent to the generalized inverse matrix, and

the calculation for deriving the concentration ratio or the signal amount ratio is a

calculation of applying the matrix C^{-} to a vector s of the output from the optical sensor from left to derive $C^{-}s$ or a calculation equivalent thereto.

[Claim 3]

The light detection device according to claim 2, wherein
the matrix C is a combination of elements of a plurality of matrices C_n each including only crosstalk information between a part of the light emitter holders or dyes.

[Claim 4]

The light detection device according to claim 1, wherein
the spatial filter is an optical fiber array including a plurality of optical fibers each provided corresponding to a respective one of the light emission points of the plurality of light emitter holders.

[Claim 5]

The light detection device according to claim 1, wherein
the spatial filter has a plurality of pinholes or slits each provided corresponding to a respective one of the light emission points of the plurality of light emitter holders.

[Claim 6]

The light detection device according to claim 2, wherein
the plurality of types of light emitters are sequentially supplied to each of the plurality of light emitter holders, the output from the optical sensor in a state where a single light emitter among the plurality of types of light emitters emits light in a single holder among the plurality of light emitter holders is recorded, outputs from the optical sensor are arranged to generate the matrix C , and the generalized inverse matrix C^{-} of the matrix C is calculated by $[C^t C]^{-1} C^t$ or a calculation equivalent thereto and stored.

[Claim 7]

The light detection device according to claim 1, wherein
regarding the crosstalk information, when a matrix representing crosstalk occurring due to the light detection device is G and a matrix representing spectral crosstalk occurring due to overlap of emission spectra of dyes is F , a concentration ratio or a signal amount ratio of a dye is obtained by a calculation $F^{-1}G$ or a calculation equivalent thereto using an inverse matrix G^{-1} of the matrix G and a generalized inverse matrix F^{-} of the matrix F obtained by $[F^t F]^{-1} F^t$ or a calculation equivalent thereto.

[Claim 8]

The light detection device according to claim 7, wherein
the matrix G is acquired by sequentially introducing monochromatic light corresponding to a wavelength detection interval of the optical sensor for each emitted light collection position of the spatial filter.

[Claim 9]

The light detection device according to claim 7, wherein
the matrix F is acquired by sequentially supplying the plurality of types of light emitters to all or a part of the plurality of light emitter holders.

[Claim 10]

The light detection device according to claim 7, wherein
the matrix F is generated based on emission spectrum information of the plurality of types of light emitters stored in the computer.

[Claim 11]

The light detection device according to claim 2 or 7, wherein
the crosstalk information is acquired by connecting a calibration light source to the spatial filter, and
the calibration light source sequentially introduces calibration light to each emitted

light collection position of the spatial filter.

[Claim 12]

The light detection device according to claim 11, wherein
the calibration light source introduces light having the same spectrum as an emission spectrum of the plurality of types of light emitters.

[Claim 13]

The light detection device according to claim 11, wherein
the calibration light source introduces monochromatic light corresponding to a wavelength detection interval of the optical sensor.

[Claim 14]

The light detection device according to claim 1, wherein
a warning is displayed when the output from the optical sensor is saturated.

[Claim 15]

A signal processing method comprising:
preparing a light detection device that includes a plurality of replaceable light emitter holders where a plurality of types of light emitters emit light therein, an optical sensor having a plurality of detection channels for detecting the light emitted from the plurality of types of light emitters in a plurality of wavelength bands, respectively, a spatial filter configured to fix an incident position of the light emitted from the plurality of types of light emitters on the optical sensor, and a computer configured to process a signal output from the optical sensor;
measuring the light emitted from the plurality of types of light emitters by the optical sensor;
calculating, using crosstalk information stored in the computer in order to reduce crosstalk between the plurality of detection channels, an output from the optical sensor corresponding to the plurality of wavelength bands for each of a plurality of light emission points

where the plurality of types of light emitters emit light to reduce the crosstalk between the detection channels of the optical sensor and derive a concentration ratio or a signal amount ratio of each of the plurality of types of light emitters for each of the plurality of light emitter holders; and

performing the calculation using the crosstalk information even when each light emitter holder is replaced.

[Claim 16]

The signal processing method according to claim 15, wherein
the crosstalk information is a generalized inverse matrix C^- of a matrix C including information on the crosstalk or information equivalent thereto, and the calculation for deriving the concentration ratio or the signal amount ratio is a calculation of applying the matrix C^- to a vector s of the output from the optical sensor from left to derive C^-s or a calculation equivalent thereto.

[Claim 17]

The signal processing method according to claim 16, wherein
regarding the crosstalk information used for the calculation, when a matrix representing crosstalk occurring in the light detection device is G and a matrix representing spectral crosstalk occurring due to overlap of emission spectra of dyes is F , a concentration ratio or a signal amount ratio of a dye is obtained by a calculation $F^-G^{-1}s$ or a calculation equivalent thereto using an inverse matrix G^{-1} of the matrix G and a generalized inverse matrix F^- of the matrix F obtained by $[F^tF]^{-1}F^t$ or a calculation equivalent thereto.