

DESCRIPTION

Title of Invention: CAPILLARY ARRAY ELECTROPHORESIS DEVICE

Technical Field

[0001]

The present disclosure relates to a capillary array electrophoresis device.

Background Art

[0002]

A capillary array electrophoresis device is widely used in which a plurality of capillaries made of quartz glass is filled with an electrophoretic separation medium such as an electrolyte solution, an electrolyte solution containing a polymer gel, or a polymer and in which electrophoresis analysis is performed in parallel. Compared with the conventional capillary electrophoresis devices using one capillary, the capillary array electrophoresis device can not only improve the analysis throughput but also reduce the analysis cost per sample. The most widely used capillary array electrophoresis devices are the 3500 series genetic analyzer and the 3730 series genetic analyzer sold by Thermo Fisher Scientific. The 3500 series genetic analyzer can perform parallel electrophoresis analysis of 8 or 24 capillaries. The 3730 series genetic analyzer can perform

parallel electrophoresis analysis of 48 or 96 capillaries. In any case, the laser irradiation portions of the plurality of capillaries (portions irradiated with the laser in the capillary array) are arranged on the same plane in a state where a polyimide coating is removed. This same plane is referred to as an array plane. An array of a plurality of capillaries is referred to as a capillary array. When the capillary array is composed of N capillaries, capillary numbers of 1 to N are attached to capillaries respectively in order of arrangement from the end. By introducing the laser beam from the side of the array plane during electrophoresis, a plurality of capillaries are simultaneously irradiated. Emitted fluorescence is spectrally dispersed and simultaneously detected from each capillary induced by the irradiation. A method in which a laser beam is incident from the side of the array plane to simultaneously irradiate a plurality of capillaries is called a multi-focusing method, and is described in detail in PTL 1. In the multi-focusing method, each capillary acts as a convex lens, and the laser beam is repeatedly condensed along the array plane to advance in the capillary array, thereby enabling simultaneous irradiation of a plurality of capillaries. As a result, DNA sequences or DNA fragment analysis of the same number of samples as the number of capillaries can be performed in parallel. As described in

PTL 1, in a laser irradiation portion of a plurality of capillaries, when an outer radius of the capillary is R (an outer diameter is $2R$), an inner radius is r (an inner diameter is $2r$), a refractive index of a material of the capillary is n_2 , a refractive index of an external medium of the capillary is n_1 , a refractive index of an internal medium (separation medium) of the capillary is n_3 , a distance between an incident position of a laser beam and an array plane is x ($\leq r$), and $x = r/2$, a refractive angle when the laser beam transmits through one capillary is represented by Expression (1).

[0003]

[Math. 1]

$$\Delta\theta = 2 \cdot \left\{ -\sin^{-1}\left(\frac{r}{2 \cdot R}\right) + \sin^{-1}\left(\frac{r \cdot n_1}{2 \cdot R \cdot n_2}\right) - \sin^{-1}\left(\frac{n_1}{2 \cdot n_2}\right) + \sin^{-1}\left(\frac{n_1}{2 \cdot n_3}\right) \right\} \quad \cdot \cdot \cdot (1)$$

[0004]

Each capillary acts as a concave lens when $\Delta\theta > 0$ is satisfied and acts a convex lens when $\Delta\theta < 0$ is satisfied. By setting the condition of $\Delta\theta < 0$, the multi-focusing functions, and thus it becomes possible to perform the simultaneous irradiation of a plurality of capillaries with laser beams. On the other hand, under the condition of $\Delta\theta > 0$, the multi-focusing does not function, and the laser beam diverges from the array plane, so that it becomes impossible to perform the simultaneous irradiation of the

plurality of capillaries with the laser beam. In general, the material of the capillary is quartz glass, and is fixed at $n_2 = 1.46$. From Expression (1), it can be seen that in order to enhance the convex lens action (weaken the concave lens action) of each capillary, the smaller n_1 is, the better n_3 is. Conversely, the larger n_1 and the smaller n_3 are, the stronger the concave lens action of each capillary is.

[0005]

Even when the multi-focusing functions, due to reflection loss of the laser beam at the interface between the medium outside the capillary and the capillary and due to reflection loss of the laser beam at the interface between the medium inside the capillary and the capillary, the intensity thereof attenuates as the laser beam travels in the capillary array, and the obtained fluorescence intensity also attenuates accordingly. If the fluorescence intensity is greatly different between the capillaries, a plurality of samples cannot be analyzed under equivalent conditions, which is disadvantageous (in the embodiment described later, the fluorescence intensity is used as a representative of the signal intensity, but signal intensity other than the fluorescence intensity, for example, scattering intensity or absorbance may be used). Therefore, in the 3500 series genetic analyzer and the 3730 series genetic analyzer, a laser beam oscillated from one laser light source is divided

into two beams. The two laser beams are made incident from both sides of the array plane, so that the multi-focusing functions for each beam. In this way, the sum of the intensity of the laser beam incident from one of the array planes and the intensity of the laser beam incident from the other of the array planes is made uniform. A configuration in which the laser beam is incident only from one side of the array plane is referred to as one-side irradiation. A configuration in which the laser beam is incident from both sides of the array plane is referred to as both-side irradiation. It is common in both the one-side irradiation and the both-side irradiation whether the multi-focusing functions or does not function. When the capillary array includes N capillaries, in the case of one-side irradiation, the capillary number of the capillary at the end on which the laser beam is incident is 1, and the capillary number of the capillary at the end on which the laser beam is emitted is N. In the case of both-side irradiation, the capillary number of the capillary at one end is 1, and the capillary number of the capillary at the opposite end is N.

[0006]

In DNA sequence or DNA fragment analysis performed by the 3500 series genetic analyzer and the 3730 series genetic analyzer, a polymer solution containing urea as a denaturant at a high concentration is used as a separation medium in

order to electrophoretically separate DNA fragments contained in a sample in a single-stranded state. In fact, each of POP-4, POP-6, and POP-7, which are separation media sold for the 3500 series genetic analyzer and the 3730 series genetic analyzer, contains 8 M urea. While the refractive index of water is 1.33, the refractive index of the above polymer solution containing 8 M urea has increased to $n_3 = 1.41$. This enhances the convex lens action of each capillary, which is an advantageous condition for multi-focusing.

[0007]

With the configuration based on PTL 1, in the 3500 series genetic analyzer, laser irradiation portions of a plurality of capillaries having an outer diameter $2R = 323 \mu\text{m}$ and an inner diameter $2r = 50 \mu\text{m}$ are arranged in the air. That is, $n_1 = 1.00$. At this time, from the above Expression (1), $\Delta\theta = -1.3^\circ$, and it can be seen that each capillary has a convex lens action. Therefore, the multi-focusing functions to enable simultaneous irradiation of 8 or 24 capillaries with laser beams. However, in this configuration, since the reflection loss of the laser beam at the interface between the air layer outside the capillary and the capillary (quartz glass) is large, the number of capillaries that can be simultaneously irradiated is about 24.

[0008]

Therefore, according to the configuration disclosed in PTL 2, the number of capillaries that can be simultaneously irradiated is increased in the following 3730 series genetic analyzer. In the 3730 series genetic analyzer, laser irradiation portions of a plurality of capillaries having an outer diameter $2R = 126 \mu\text{m}$ and an inner diameter $2r = 50 \mu\text{m}$ are arranged in a fluorine solution having a refractive index $n_1 = 1.29$. At this time, from the above Expression (1), $\Delta\theta = -0.69^\circ$ is obtained, and it can be seen that each capillary has a convex lens action and the multi-focusing functions. Further, since the reflection loss of the laser beam at the interface between the fluorine solution layer outside the capillary and the capillary (quartz glass) is reduced, the number of capillaries that can be simultaneously irradiated increases. Therefore, it is possible to perform simultaneous irradiation of 48 or 96 capillaries with laser beams.

[0009]

The configuration described in NPL 1 further increases the number of capillaries that can be simultaneously irradiated. In this configuration, the laser irradiation portions of the plurality of capillaries having the outer diameter $2R = 126 \mu\text{m}$ and the inner diameter $2r = 50 \mu\text{m}$ are arranged in a matching solution having the refractive index $n_1 = 1.46$. Among the plurality of capillaries arranged, an

odd-numbered capillary from one end is used for analysis (hereinafter, the analysis capillary), and an even-numbered capillary is used as a rod lens (hereinafter, the lens capillary). That is, the analysis capillaries and the lens capillaries are alternately arranged. The refractive index of the medium (separation medium) inside the analysis capillary is set to $n_3 = 1.41$. The refractive index of the medium inside the lens capillary is set to $n_4 = 1.53$. The capillary is made of quartz glass and has $n_2 = 1.46$. In addition, since the reflection loss of the laser beam becomes zero at the interface between the matching solution layer outside the capillary and the capillary (quartz glass), the number of capillaries that can be simultaneously irradiated further increases. Further, NPL 1 describes the definition of the maximum number of capillaries that can be simultaneously irradiated with a laser beam from P.2874 to P.2875. When the incident intensity is 100% by one-side irradiation, the maximum number of capillaries that can be simultaneously irradiated is the number obtained by doubling the number of capillaries until the intensity of the laser beam attenuates to 50%. This is because the irradiation intensity of each capillary is expected to be uniform when irradiating the capillary array having the number of capillaries on both sides. According to this definition, the maximum number of capillaries in the configuration of

PTL 2 is 150, and the maximum number of capillaries in the configuration of NPL 1 is 550.

Citation List

Patent Literature

[0010]

PTL 1: JP 3654290 B2

PTL 2: JP 5039156 B2

PTL 3: JP 6113549 B2

Non-patent Literature

[0011]

NPL 1: Electrophoresis 2006, 27, 2869-2879

Summary of Invention

Technical Problem

[0012]

In all of the above known techniques, the separation medium contains a high concentration of urea, and the refractive index is $n_3 = 1.41$. On the other hand, in a capillary electrophoresis device using one capillary, the separation medium does not necessarily contain a high concentration of urea, and various types of separation media are used. For example, a separation medium for electrophoretically separating DNA fragments in a double-stranded state does not contain urea and has a refractive index of $n_3 = 1.33$, which is the same as that of water. That

is, in general, the refractive index of the separation medium used in capillary electrophoresis can have various values of $1.33 \leq n_3 \leq 1.41$. In recent years, in order to increase throughput or reduce cost of electrophoresis analysis using such various types of separation media, it is required to use such various types of separation media in a capillary array electrophoresis device.

[0013]

However, in any of the configurations of the above-described known techniques, when setting to $n_3 = 1.33$, the convex lens action of each capillary is lost, the concave lens action becomes strong, and the multi-focusing does not function. That is, it becomes to perform parallel electrophoresis analysis using a plurality of capillaries. Specifically, it is as follows.

[0014]

In the 3500 series genetic analyzer based on PTL 1, when setting to $n_3 = 1.33$, $\Delta\theta = +1.3^\circ$ is obtained from Expression (1), and it can be seen that each capillary has a concave lens action. Therefore, the multi-focusing does not function, and thus it is not possible to perform simultaneous irradiation of 8 or 24 capillaries with a laser beam.

[0015]

In the 3730 series genetic analyzer based on PTL 2,

when setting to $n_3 = 1.33$, $\Delta\theta = +2.9^\circ$ is obtained from Expression (1), and it can be seen that each capillary has a concave lens action. Therefore, the multi-focusing does not function, and thus it is not possible to perform simultaneous irradiation of 48 or 96 capillaries with a laser beam.

[0016]

In the configuration based on NPL 1, when setting to $n_3 = 1.33$, the refractive angle of one analysis capillary is $\Delta\theta_A = +6.6^\circ$, while the refractive angle of one lens capillary is $\Delta\theta_B = -3.0^\circ$ according to Expression (1). At this time, since $\Delta\theta_A + \Delta\theta_B = +3.6^\circ$ is obtained, one set of one analysis capillary and one lens capillary exhibits a concave lens action, and the multi-focusing does not function. On P.2875 of NPL 1, there is a description that the configuration of NPL 1 is advantageous even in the case of $n_3 = 1.33$. However, according to the definition of the maximum number of capillaries in NPL 1 described above, the maximum number of capillaries in the case of $n_3 = 1.33$ is only about 8 from FIG. 11 of NPL 1. Therefore, in the case of $n_3 = 1.33$, the configuration of NPL 1 does not function.

[0017]

On the other hand, PTL 3 discloses a correction method for equalizing apparent fluorescence intensity by obtaining fluorescence intensity (thereafter, measured fluorescence

intensity) of each capillary obtained by multiplying measured fluorescence intensity (hereinafter, corrected fluorescence intensity) of each capillary by a correction coefficient of each capillary recorded in advance on a computer in a capillary array electrophoresis device. Defining that the capillary numbers n of the N capillary arrays are $n = 1, 2, \dots$, and N in order from the end, the measured fluorescence intensity of the capillary number n is $I(n)$, $I(n)$ at the time of calibration is $I_0(n)$, and the capillary number at which $I(n)$ is maximized is m , the correction coefficient $k(n)$ of the capillary number n is obtained by Expression (2).

[0018]

[Math. 2]

$$k(n) = I_0(m) / I_0(n) \quad \cdots (2)$$

The corrected fluorescence intensity $J(n)$ of the capillary number n is obtained by Expression (3).

[0019]

[Math. 3]

$$J(n) = k(n) \times I(n) \quad \cdots (3)$$

[0020]

Here, the calibration means a step of analyzing a standard sample having a constant concentration in all capillaries before analyzing the actual sample. By the above

correction method, the measured fluorescence intensity that has varied in each capillary in the capillary array is converted into uniform corrected fluorescence intensity. $k(n)$ in Expression (2) is set such that the fluorescence intensity of each capillary is equal to the maximum fluorescence intensity. However, this correction method is based on the premise that $k(n)$ is stable. For example, it is necessary that the acquired $k(n)$ does not greatly change when repeating the calibration a plurality of times.

[0021]

In view of such a situation, the present disclosure proposes a technique that enables electrophoresis analysis even using various separation media (of course, it is also possible to cope with a separation medium having a refractive index outside the range of $1.33 \leq n_3 \leq 1.41$) having any refractive index in the range of $1.33 \leq n_3 \leq 1.41$ in a capillary array electrophoresis device.

Solution to Problem

[0022]

In order to solve the above problems, the present disclosure proposes, for example, a capillary array electrophoresis device including: a laser light source configured to emit a laser beam; a capillary array configured such that laser irradiation portions of N capillaries

collectively irradiated with the laser beam are substantially arranged on a same array plane, where N is an integer of 2 or more; an optical system that collectively measures light emission from the N capillaries; and a computer that performs predetermined processing on a light intensity measured by the optical system and outputs the light intensity. An analysis mode of a capillary electrophoresis using a separation medium having at least a refractive index $n_3 < 1.36$ is provided, $|A| > |B|$ is satisfied. Here, an outer radius of the N capillaries in the laser irradiation portion is R , an inner radius of the N capillaries is r , a refractive index of an external medium is n_1 , a refractive index of a material is n_2 , and a refractive index of an internal medium is n_3 , capillary numbers $n = 1, 2, \dots$, and N are assigned to the respective N capillaries in the laser irradiation portion in an arrangement order from one end of the arrangement, an average value in $1 \leq n \leq N$ of a second derivative indicating a degree of protrusion of $L(n)$ representing a laser irradiation light intensity of the capillary number n as a function of n is A , and an average value in a range of $1 \leq n \leq N$ of a second derivative indicating a degree of protrusion of $H(n)$ representing an output light intensity of the capillary number n by the computer as a function of n when a light emitting substance of equal concentration exists inside the

N capillaries in the laser irradiation portion is B.

[0023]

Other features of the present disclosure will be clear from the description and the accompanying drawings of this specification. In addition, embodiments of the present disclosure are achieved and realized by elements, combinations of various elements, the following detailed description, and the attached claims.

It is necessary to understand that the description of this specification is given only as a typical example, and does not limit the scope of claims or applications of the present disclosure.

Advantageous Effects of Invention

[0024]

According to the technology of the present disclosure, in a capillary array electrophoresis device, it is possible to perform electrophoresis analysis using various separation media having an arbitrary refractive index in a range of $1.33 \leq n_3 \leq 1.41$. In particular, it becomes possible to perform capillary electrophoresis analysis using a separation medium having a low refractive index that is the same as or close to the refractive index 1.33 of water. This makes it possible to greatly expand the application range of the capillary array electrophoresis device capable of

improving the analysis throughput and reducing the analysis cost per sample.

Brief Description of Drawings

[0025]

[FIG. 1] FIG. 1 is a diagram illustrating a configuration example of a capillary array electrophoresis device.

[FIG. 2] FIG. 2 is a diagram illustrating a configuration example of an optical system of the capillary array electrophoresis device.

[FIG. 3] FIG. 3 is a diagram illustrating a configuration example illustrating cooperation between a sensor and a computer.

[FIG. 4] FIG. 4 is a diagram illustrating a configuration of a capillary array and a laser beam tracking result based on PTL 1.

[FIG. 5] FIG. 5 is a diagram illustrating an emission fluorescence intensity distribution and a measured fluorescence intensity distribution of the capillary array based on PTL 1.

[FIG. 6] FIG. 6 is a diagram illustrating a configuration of the capillary array of the present disclosure and a laser beam tracking result.

[FIG. 7] FIG. 7 is a diagram illustrating an emission

fluorescence intensity distribution and a measured fluorescence intensity distribution of the capillary array of the present disclosure.

[FIG. 8] FIG. 8 is a diagram for explaining the definition of an arrangement error ΔZ of the capillary array.

[FIG. 9] FIG. 9 is a diagram illustrating a relationship ($n_3 = 1.41$) between the arrangement error ΔZ of the capillary array and the emission fluorescence intensity distribution.

[FIG. 10] FIG. 10 is a diagram illustrating a relationship ($n_3 = 1.33$) between the arrangement error ΔZ of the capillary array and the emission fluorescence intensity distribution.

[FIG. 11] FIG. 11 is a diagram illustrating a relationship between various fluorescence intensity distributions in the capillary array electrophoresis device.

[FIG. 12] FIG. 12 is a diagram illustrating a relationship ($n_3 = 1.41$, correction reference: $n_3 = 1.41$, $\Delta Z = 0 \text{ } \mu\text{m}$) between the arrangement error ΔZ of the capillary array and an output fluorescence intensity distribution.

[FIG. 13] FIG. 13 is a diagram illustrating a relationship ($n_3 = 1.33$, correction reference: $n_3 = 1.33$, $\Delta Z = 0 \text{ } \mu\text{m}$) between the arrangement error ΔZ of the capillary array and the output fluorescence intensity distribution.

[FIG. 14] FIG. 14 is a diagram illustrating a

relationship ($n_3 = 1.41$, correction reference: $n_3 = 1.41$, $\Delta Z = 6 \mu\text{m}$) between the arrangement error ΔZ of the capillary array and the output fluorescence intensity distribution.

[FIG. 15] FIG. 15 is a diagram illustrating a relationship ($n_3 = 1.33$, correction reference: $n_3 = 1.33$, $\Delta Z = 6 \mu\text{m}$) between the arrangement error ΔZ of the capillary array and the output fluorescence intensity distribution.

[FIG. 16] FIG. 16 is a diagram illustrating a relationship ($n_3 = 1.33$, correction reference: $n_3 = 1.41$, $\Delta Z = 0 \mu\text{m}$) between the arrangement error ΔZ of the capillary array and the output fluorescence intensity distribution.

[FIG. 17] FIG. 17 is a diagram illustrating a relationship among the emission fluorescence intensity distribution, the output fluorescence intensity distribution, and the second-order coefficient of the output fluorescence intensity distribution of the capillary array for various capillary internal refractive indexes n_3 .

[FIG. 18] FIG. 18 is a diagram illustrating the output fluorescence intensity distribution of the capillary array for various correction references and the capillary internal refractive indexes n_3 .

[FIG. 19] FIG. 19 is a diagram illustrating a relationship ($n_3 = 1.41$) among the arrangement error ΔZ of the capillary array, the relative fluorescence intensity, and the coefficient of variation.

[FIG. 20] FIG. 20 is a diagram illustrating a relationship ($n_3 = 1.41$) among the arrangement error ΔZ of the capillary array, the digitally corrected relative fluorescence intensity, and the coefficient of variation.

[FIG. 21] FIG. 21 is a diagram illustrating a relationship ($n_3 = 1.33$) among the arrangement error ΔZ of the capillary array, the relative fluorescence intensity, and the coefficient of variation.

[FIG. 22] FIG. 22 is a diagram illustrating a relationship ($n_3 = 1.33$) among the arrangement error ΔZ of the capillary array, the digitally corrected relative fluorescence intensity, and the coefficient of variation.

[FIG. 23] FIG. 23 is a diagram illustrating a configuration of a capillary array (part 2) of the present disclosure and a laser beam tracking result.

[FIG. 24] FIG. 24 is a diagram illustrating an emission fluorescence intensity distribution, a measured fluorescence intensity distribution, and an output fluorescence intensity distribution ($n_3 = 1.41$) of the capillary array (part 2).

[FIG. 25] FIG. 25 is a diagram illustrating an optical system correction coefficient distribution and a digital correction coefficient distribution ($n_3 = 1.41$) of the capillary array (part 2).

[FIG. 26] FIG. 26 is a diagram illustrating an emission fluorescence intensity distribution, a measured fluorescence

intensity distribution, and an output fluorescence intensity distribution ($n_3 = 1.33$) of the capillary array (part 2).

[FIG. 27] FIG. 27 is a diagram illustrating an optical system correction coefficient distribution and a digital correction coefficient distribution ($n_3 = 1.33$) of the capillary array (part 2).

[FIG. 28] FIG. 28 is a diagram illustrating a configuration example of a mode of filling a polymer A with a dual polymer block capillary array electrophoresis device.

[FIG. 29] FIG. 29 is a diagram illustrating a configuration example of a mode of filling a polymer B with the dual polymer block capillary array electrophoresis device.

[FIG. 30] FIG. 30 is a diagram illustrating a modification of the dual polymer block capillary array electrophoresis device.

Description of Embodiments

[0026]

The technology of the present disclosure relates to a capillary array electrophoresis device that simultaneously analyzes a plurality of samples by simultaneously irradiating a plurality of capillaries with a laser beam and simultaneously detecting the emitted fluorescence from each of the capillaries during electrophoresis using the

plurality of capillaries.

[0027]

(A) Summary of the technology of the present disclosure

The present disclosure mainly proposes a technique that makes it possible to use a separation medium having a low refractive index equivalent to the refractive index 1.33 of water or having a refractive index of less than 1.36. When such a separation medium having a low refractive index is used, multi-focusing does not function even if the technology disclosed in any known example (PTLs 1 to 3 and NPL) is used, and thus it is difficult to perform simultaneous irradiation of a plurality of capillaries with laser beams.

[0028]

The present disclosure also proposes a technique for enabling capillary electrophoresis analysis using not only a separation medium having a low refractive index as described above but also a separation medium having a high refractive index, typically a separation medium having a refractive index of 1.36 or more and 1.42 or less. The maximum number of capillaries that can be simultaneously irradiated is preferably larger, and may be 8 or more, or 24 or more depending on the situation. Among the irradiation intensity and the fluorescence intensity of each of the plurality of capillaries in the same capillary array, the lowest irradiation intensity and fluorescence intensity are

preferably larger. In a case where the fluorescence intensity expected when the entire intensity of the laser beam oscillated from the laser light source irradiates the inside of one capillary is set to 1, it is empirically known that practical sensitivity can be obtained when the minimum value MIN (Minimum) of the fluorescence intensity satisfies $\text{MIN} \geq 0.2$. In addition, the smaller the variation in the irradiation intensity and the fluorescence intensity between the plurality of capillaries in the same capillary array is, the better. It has been empirically found that different samples can be analyzed under equivalent conditions if the coefficient of variation CV (Coefficient of Variation) of the irradiation intensity and the fluorescence intensity is $\text{CV} \leq 15\%$, desirably $\text{CV} \leq 10\%$.

[0029]

For each capillary in the capillary array, when setting the capillary outer diameter to $2R = 126 \mu\text{m}$, setting the capillary inner diameter to $2r = 50 \mu\text{m}$, exposing the capillary outside to air and setting to $n_1 = 1.00$, setting the capillary material to quartz glass and $n_2 = 1.46$, and filling the capillary inside with a separation medium and setting to $n_3 = 1.33$, $\Delta\theta = -3.2^\circ$ is obtained from Expression (1), and thus it has been found that each capillary exhibits a convex lens action and multi-focusing functions. In the 3500 series genetic analyzer based on PTL 1, the difference

from the case of $n_3 = 1.33$ is that the outer diameter $2R$ of the capillary is reduced from $323\text{ }\mu\text{m}$ to $126\text{ }\mu\text{m}$. As a result, the concave lens action of each capillary is converted into the convex lens action. As a result of further studies, it has been found that $\Delta\theta < 0$ is obtained and a convex lens acts when setting the outer diameter $2R$ of the capillary to $220\text{ }\mu\text{m}$ or less. Generalizing without limiting to the case where the capillary inner diameter is $2r = 50\text{ }\mu\text{m}$, $\Delta\theta < 0$ is obtained when setting to $R/r \leq 4.4$ and it has been found that a convex lens acts. A low refractive index separation medium of $n_3 = 1.33$ is not examined in PTL 1. That is, this is a condition found for the first time by the technology of the present disclosure.

[0030]

In a case where the capillary outer diameter is $2R = 126\text{ }\mu\text{m}$ and the capillary inner diameter is $2r = 50\text{ }\mu\text{m}$, when the separation medium of the capillary inside has a low refractive index of $n_3 = 1.34$ and 1.35 , $\Delta\theta = -3.5^\circ$ and -3.8° are obtained from Expression (1), and it has been found that each capillary exhibits a convex lens action and multi-focusing functions. As a result of further studies, it has been found that $\Delta\theta < 0$ is obtained and thus a convex lens acts when the outer diameter $2R$ of the capillary is $240\text{ }\mu\text{m}$ or less and $264\text{ }\mu\text{m}$ or less. In general, when setting to $R/r \leq 4.8$ and 5.3 , $\Delta\theta < 0$ is obtained and thus it has been found

that a convex lens acts. Such a low refractive index separation medium is not examined in PTL 1. That is, this is a condition found for the first time by the technology of the present disclosure.

[0031]

As a result of performing the experiment under the condition having the convex lens action described above, multi-focusing functions and it is possible to realize simultaneous irradiation of a plurality of capillaries with a laser beam, and thus $\text{MIN} \geq 0.2$ and $\text{CV} \leq 15\%$ are satisfied, which are practical performances. However, it has become clear that $\text{CV} \leq 10\%$ is not satisfied. The coefficient of variation of the fluorescence intensity is suppressed to be lower in the case of both-side irradiation than in the case of one-side irradiation. But, $\text{CV} \leq 10\%$ is not satisfied, and $\text{CV} \leq 15\%$ is not satisfied in some cases. Therefore, in order to reduce the CV, a known correction method represented by PTL 3 has been applied. However, it has been found that, due to the influence of random variation in the fluorescence intensity of each capillary in the capillary array, there is a case where uniformization of the fluorescence intensity of each capillary does not function, or conversely, the degree of variation in the fluorescence intensity of each capillary increases in some cases.

[0032]

In the case of both-side irradiation, the fluorescence intensity distribution graphically representing the change in the fluorescence intensity with respect to the capillary number of each capillary, forming an uneven shape with the increase in the capillary number, had a downward convex shape as a whole around the vicinity of the center of the capillary array (the capillary array includes N capillaries. When N is an even number, the capillary number is near $N/2$ or $N/2 + 1$. When N is an odd number, the capillary number is near $(N + 1)/2$) (see FIGS. 9 and 10). In general, the coefficient of variation CV increases as the degree of unevenness of the former increases and as the degree of downward protrusion of the latter increases. However, in the fluorescence intensity distribution, since the unevenness of the distribution and the overall downward convex shape are observed to be mixed with each other, they cannot be distinguished from each other, and thus it is difficult to derive the degree thereof. On the other hand, according to the examination using a ray tracing simulation, it has been found that the degree of unevenness of the fluorescence intensity distribution increases as the arrangement error ΔZ of the capillary array increases, whereas the degree of the downwardly convex shape of the fluorescence intensity distribution as a whole increases as n_3 decreases. Here, in the present specification, the arrangement error ΔZ is

defined as follows. First, it is assumed that a Z axis is set in a direction perpendicular to the array plane and that the array plane is located at $Z = 0 \text{ }\mu\text{m}$. In addition, it is assumed that the median value of each Z coordinate of the central axes of the plurality of capillaries in the laser irradiation portion is set to zero. The absolute value of the Z coordinate of the central axis of the capillary farthest from the array plane is defined as ΔZ . At this time, the Z coordinates of the central axes of the capillaries are dispersed within a range of $\pm\Delta Z$. It has been found that the case where $CV \leq 15\%$ is not satisfied occurs when ΔZ is large. Of course, the former factor of increasing the degree of unevenness includes not only the arrangement error ΔZ of the capillary array but also various other experimental errors.

[0033]

In view of the above complicated situation, the present disclosure proposes a technique for most efficiently reducing the CV by applying correction for reducing or eliminating the degree of the downward convex shape as the entire distribution to the fluorescence intensity of each capillary while allowing unevenness in the fluorescence intensity distribution. The reason why the unevenness of the fluorescence intensity is allowed is because it is difficult to obtain ΔZ for each capillary array and in

addition, because the specific shape of the unevenness randomly changes due to various factors even if ΔZ is known. This is the same reason as the reason why the above-described known correction method (PTL 3) does not function. In addition, since it has been clarified by the present disclosure that the degree of the downward convex shape as the entire fluorescence intensity distribution changes depending on n_3 , the present disclosure also proposes a technique for dynamically changing the correction coefficient according to n_3 . Such a correction method is not disclosed in the known correction methods including PTL 3. Hereinafter, each embodiment of the present disclosure will be described in detail. Note that, although each embodiment will be separately described below, the techniques described in the embodiments are not exclusive and can be combined with each other as appropriate.

[0034]

(B) First embodiment

<Configuration example of capillary array electrophoresis device>

FIG. 1 is a diagram illustrating a configuration example of a capillary array electrophoresis device. This capillary array electrophoresis device performs double-stranded DNA fragment analysis in addition to DNA sequence and single-stranded DNA fragment analysis performed in the

conventional capillary array electrophoresis devices. In this embodiment, using 24 capillaries (however, only four capillaries are illustrated in FIG. 1), DNA sequencing of different samples are first performed in each capillary, and then double-stranded DNA fragment analysis of different samples is performed in each capillary. Samples of DNA sequences include single-stranded DNA fragments of various lengths labeled with four fluorophores corresponding to four bases. In addition, the electrophoretic separation medium filled in each capillary when DNA sequencing is performed is a polymer solution containing 8 M urea as a denaturant. Its refractive index is $n_3 = 1.41$. On the other hand, samples for the double-stranded DNA fragment analysis include double-stranded DNA fragments of various lengths labeled with two types of fluorophores. The double-stranded DNA fragment labeled with one fluorophore is a PCR product. The double-stranded DNA fragment labeled with the other fluorophore is a size marker. The electrophoretic separation medium filled in each capillary when the double-stranded DNA fragment analysis is performed is a polymer solution containing no urea as a denaturant. Its refractive index is $n_3 = 1.33$. One analysis session is performed by the following steps (i) to (vi).

[0035]

(i) First, the sample injection ends 2 of the 24

capillaries 1 are immersed in the cathode-side buffer solution 6. The sample elution end 3 is connected to the anode-side buffer solution 7 via the polymer block 9. Here, the 24 sample elution ends 3 are bundled into one to facilitate connection with the polymer block 9.

[0036]

(ii) Next, the valve 10 of the polymer block 9 is closed to pressurize the internal polymer solution by pushing down the piston of the syringe 11 connected to the polymer block 9. Then, the polymer solution is filled into each capillary 1 from the sample elution end 3 toward the sample injection end 2.

[0037]

(iii) Subsequently, the valve 10 is opened to inject a different sample in electrical field into each capillary 1 from the sample injection end 2. Then, a high voltage is applied between the cathode 4 and the anode 5 by the power supply 8 to start capillary electrophoresis. The DNA fragment labeled with the plurality of types of fluorophore is electrophoresed from the sample injection end 2 to the sample elution end 3.

[0038]

(iv) In parallel, a position of each capillary 1 electrophoresed at a certain distance from the sample injection end 2 is defined as a laser irradiation portion 14

The laser irradiation portion 14 is collectively irradiated with the laser beam 13 oscillated from the laser light source 12 by a multi-focusing method. Here, the covering of each of the capillaries 1 in the vicinity of the laser irradiation portion 14 is removed in advance. Each of the capillaries 1 in the vicinity of the laser irradiation portion 14 is arranged on the array plane. The laser beam 13 is condensed and then incident along the array plane from the side of the array plane. In FIG. 1, for the sake of simplicity, it is depicted that one-side irradiation of the laser beam 13 is performed, but actually, both-side irradiation is performed by dividing the laser beam 13 into two.

[0039]

(v) Then, the DNA fragment labeled with a plurality of types of fluorophores is electrophoresed inside each capillary 1. When passing through the laser irradiation portion 14, the labeled fluorophore is excited by irradiation with the laser beam 13 and emits fluorescence. That is, a plurality of types of fluorophores emitted fluorescence from 24 light emission points (laser irradiation portions). Each fluorescence intensity changes from moment to moment with electrophoresis.

[0040]

(vi) Finally, samples injected into each capillary are analyzed by detecting multiple colors of fluorescence

emitted from each light emission point and by analyzing the obtained time-series data.

[0041]

The above steps (i) to (vi) are common in the case of performing DNA sequencing and the case of performing double-stranded DNA fragment analysis, but the polymer solution and the buffer solution are appropriately changed. Further, an analysis session including steps (i) to (vi) can be repeated a plurality of times. For example, a number of different samples can be analyzed by analyzing samples 1 to 24 in a first analysis session and analyzing samples 25 to 48 in a second analysis session to so on. At this time, the DNA sequencing may be repeated using the same polymer solution and buffer solution, or the analysis may be switched to the double-stranded DNA fragment analysis in the middle. Any application can be selected in any analysis session.

[0042]

<Configuration example of optical system for fluorescence detection>

FIG. 2 is a diagram illustrating a cross section of a configuration example of an optical system that performs fluorescence detection of the capillary array electrophoresis device. The present optical system is located on the back side of the laser irradiation portion 14 in FIG. 1. Similarly to FIG. 1, FIG. 2 depicts one-side

irradiation of the four capillary arrays, but this is actually both-side irradiation of the 24 capillary arrays.

[0043]

The capillaries 1 arranged on the array plane are simultaneously irradiated by irradiation of the laser beam 13 with multi-focus. The laser irradiation portions 14 of the respective capillaries 1 serve as fluorescence light emission points 20. The fluorescence 21 emitted from each of the light emission points 20 is collimated collectively by the condenser lens 15. The laser beam is blocked by the laser cut filter 16. The wavelength is dispersed in the direction of the central axis of each capillary by transmitting the laser beam through the transmission type diffraction grating 17. The image forming point 22 is formed on the sensor 19 by the image forming lens 18. The sensor 19 may be an area sensor such as a CCD or a CMOS, a photodiode array, or the like as long as a plurality of image forming points 22 can be simultaneously measured. Each image forming point 22 is actually wavelength-dispersed in the depth direction in FIG. 2, but a single wavelength portion of each image forming point 22 is schematically illustrated in FIG. 2.

[0044]

In such an optical system, as the light emission point 20 moves away from the optical axis 23 of the optical system,

the light collection efficiency of light emission from the light emission point 20 decreases. This is because, as illustrated in FIG. 2, the condensing angle of the fluorescence 21 emitted from the light emission point 20 away from the optical axis 23 is reduced by the vignetting effect of the optical system. Therefore, even if fluorescence of equal intensity is emitted from each light emission point 20, the fluorescence intensity of the corresponding image forming point 22 decreases as the light emission point 20 moves away from the optical axis 23. How much vignetting effect exists, that is, the optical system correction coefficient based on the vignetting effect is determined by the optical system, and can be examined by calculation or experiment. With the optical system correction coefficient based on the vignetting effect, the fluorescence intensity at each image forming point 22 can be calculated from the fluorescence intensity at each light emission point 20.

[0045]

<System configuration example for data analysis and device control>

FIG. 3 is a configuration example illustrating cooperation between a sensor and a computer. The optical system is a part of the capillary array electrophoresis device. The sensor is a part of the optical system. The

computer is connected to a capillary array electrophoresis device. The computer not only performs data analysis but also controls the capillary array electrophoresis device. Data analysis condition setting and capillary array electrophoresis device control condition setting are performed through a touch panel, a keyboard, a mouse, or the like as an input unit. The time-series raw data of the signal output from the sensor is sequentially stored in the memory. The analysis parameter information stored in the database in the HDD is stored in the memory. The CPU analyzes the time-series raw data stored in the memory using the analysis parameter information stored in the memory to derive the time-series analysis data and to sequentially store the time-series analysis data in the memory. The CPU simultaneously displays the time-series analysis data on the monitor which is the display unit. The analysis result can be collated with information on the network through a network interface NIF.

[0046]

<Configuration example of conventional capillary array>

The upper part of FIG. 4 is a cross-sectional view illustrating the configuration of a capillary array of the 3500 series genetic analyzer based on PTL 1. The laser irradiation portions of 24 capillaries having an outer diameter $2R = 323 \mu\text{m}$ and an inner diameter $2r = 50 \mu\text{m}$ are

arranged on the same plane at an interval of 370 μm . The arrangement error is zero ($\Delta Z = 0 \mu\text{m}$). The capillary outside is air and $n_1 = 1.00$. The capillary material is quartz glass and $n_2 = 1.46$. The middle part of FIG. 4 illustrates a laser beam tracking result when a laser beam having a diameter of 50 μm is applied to one side from the left side in a case where the capillary inside is a high refractive index separation medium and $n_3 = 1.41$ under the above conditions. Since multi-focusing is obviously functioning, the insides of all 24 capillaries can be efficiently irradiated. This corresponds to that $\Delta\theta = -1.3^\circ$ according to Expression (1). Each capillary exhibits a convex lens action. On the other hand, the lower part of FIG. 4 illustrates a similar laser beam tracking result when the capillary inside is a low refractive index separation medium and $n_3 = 1.33$ under the above conditions. Since the multi-focusing obviously does not function, the laser beam is diverged from the capillary array, and the entire capillary array cannot be efficiently irradiated. This corresponds to $\Delta\theta = +1.3^\circ$ according to Expression (1), and each capillary exhibits a concave lens action.

[0047]

<Relative fluorescence intensity distribution according to conventional (FIG. 4) capillary array configuration>

The upper part of FIG. 5 is a diagram illustrating the

relative fluorescence intensity of each capillary when the result of one-side irradiation illustrated in the middle part and the lower part of FIG. 4 is remade in the case of both-side irradiation. The capillary number indicates a number sequentially assigned to the leftmost capillary in FIG. 4 as 1 toward the right side. The relative fluorescence intensity means a fluorescence intensity calculated from the irradiation intensity of each capillary in consideration of the laser beam reflection loss assuming that a fluorophore having a constant concentration exists in the laser irradiation portion of each capillary. The fluorescence intensity expected when the entire intensity of the laser beam oscillated from the laser light source irradiates the inside of one capillary is set to 1. In the calculation of the both-side irradiation, it is assumed that half of the entire intensity of the laser beam is irradiated from both sides of the capillary array. When setting to $n_3 = 1.41$, the minimum value of the relative fluorescence intensity is $\text{MIN} = 0.42$ and the coefficient of variation (= standard deviation of relative fluorescence intensity/average value of relative fluorescence intensity) is $\text{CV} = 11\%$ for 24 capillaries. It is found from this result that $\text{MIN} \geq 0.2$ and $\text{CV} \leq 15\%$, which are practical performances, are satisfied. However, $\text{CV} \leq 10\%$, which is a more desirable condition, is not satisfied. The reason why the relative fluorescence

intensity with respect to the capillary number has a downward convex distribution is because the intensity of the laser beam attenuates due to reflection loss as the laser beam travels in the capillary array although multi-focusing is functioning. On the other hand, when setting to $n_3 = 1.33$, $MIN = 0.068$ and $CV = 74\%$ are obtained. It can be seen from this result that neither practical performance is satisfied. [0048]

Here, the relative fluorescence intensity distribution of the capillary array is derived by a simpler method when the multi-focusing is functioning and the arrangement error is zero. This method is realized for the first time in the present disclosure. In order to approximately evaluate the transmittance of the laser beam in consideration of the reflection loss, the incident angle of the laser beam incident on the interface between two types of media having different refractive indexes is assumed to be 0° . Reflectance when light is incident on an interface between a medium having a refractive index n_1 and a medium having a refractive index n_2 at an incident angle of 0° is $ref = \{(n_1 - n_2) / (n_1 + n_2)\}^2$, and transmittance is $tra = 1 - ref$. Thus, the transmittance T when the laser beam passes through one capillary is approximately obtained by the following Expression (4).

[0049]

[Math. 4]

$$T = \left\{ 1 - \left(\frac{n_1 - n_2}{n_1 + n_2} \right)^2 \right\}^2 \cdot \left\{ 1 - \left(\frac{n_2 - n_3}{n_2 + n_3} \right)^2 \right\}^2 \cdot \cdot \cdot (4)$$

[0050]

Under the condition of the 3500 series genetic analyzer based on PTL 1 illustrated in the middle part of FIG. 4, since $n_1 = 1.00$, $n_2 = 1.46$ and $n_3 = 1.41$ are satisfied, $T = 93\%$ is calculated by Expression (4). In practice, since components of the laser beam whose incident angle is not 0° are included, their transmittances are slightly smaller than the value of Expression (4). Therefore, Expression (4) illustrates ideal transmittance. In the case of one-side irradiation, assuming that the laser irradiation intensity incident on the capillary of the capillary number $n = 1$ is 1, the laser irradiation intensity $L(n)$ of the capillary of the capillary number n is expressed by the following Expression (5).

[0051]

[Math. 5]

$$L(n) = T^{n-1} \cdot \cdot \cdot (5)$$

[0052]

That is, in the 3500 series genetic analyzer, when the number of capillaries is set to $N = 24$, since the laser irradiation intensity attenuates to 93% every time one capillary in the capillary array passes through, the laser

irradiation intensity of the capillary of $n = 24$ decreases to 0.19. On the other hand, in the case of both-side irradiation, assuming that the laser irradiation intensities incident on the capillaries with the capillary numbers $n = 1$ and $n = N$ are set to 0.5 respectively, the laser irradiation intensity $L(n)$ of the capillary with the capillary number n is expressed by the following Expression (6).

[0053]

[Math. 6]

$$L(n) = 0.5 \cdot (T^{n-1} + T^{N-n}) \quad \cdot \cdot \cdot (6)$$

[0054]

Unlike the case of one-side irradiation, the attenuation of the intensity of the laser beams incident from both sides of the array plane is canceled, so that the uniformity of the laser irradiation intensity of each capillary is improved and the lowest laser irradiation intensity is increased. However, the laser irradiation intensity of the capillaries ($n = 1$ and $n = N$) arranged at both ends of the capillary array is the highest, and the laser irradiation intensity of the capillary ($n = (N + 1)/2$ when N is odd, $n = N/2$ and $n = N/2 + 1$ when N is even) arranged at the center of the capillary array is the lowest. That is, as illustrated in the relative fluorescence intensity distribution in the case of $n_3 = 1.41$ in the upper part of FIG. 5, when a graph is formed with the horizontal

axis n and the vertical axis $L(n)$, the distribution becomes a downward convex distribution. Under the condition of the 3500 series genetic analyzer described above, when the number of capillaries is set to $N = 24$, according to Expression (6), the laser irradiation intensity of the capillaries ($n = 1$ and $n = 24$) arranged at both ends of the capillary array becomes 0.60, and the laser irradiation intensity of the capillaries ($n = 12$ and $n = 13$) arranged at the center of the capillary array becomes 0.44 ($\text{MIN} = 0.44$). Thus, $\text{MIN} \geq 0.2$ of the practical performance is satisfied. In addition, since the coefficient of variation of the laser irradiation intensity of the 24 capillaries becomes 11% ($\text{CV} = 11\%$), $\text{CV} \leq 20\%$ and $\text{CV} \leq 15\%$ of the practical performance are satisfied. The above results substantially coincide with $\text{MIN} = 0.42$ and $\text{CV} = 11\%$, which are the results in the case of $n_3 = 1.41$ in the upper part of FIG. 5 described above. That is, when multi-focusing is functioning and the arrangement error is zero, the laser irradiation intensity distribution $L(n)$ can be obtained by the above simple method.

[0055]

The lower part of FIG. 5 illustrates the optical system corrected relative fluorescence intensity of each capillary obtained by adding the vignetting effect of the optical system of the 3500 series genetic analyzer to the result of the upper part of FIG. 4, that is, multiplying the optical

system correction coefficient based on the vignetting effect. When setting to $n_3 = 1.41$, since the downward convex distribution of the relative fluorescence intensity and the distribution of the optical system correction cancel each other with respect to the capillary number, the optical system corrected relative fluorescence intensity has a flat distribution. As a result, the minimum value $MIN = 0.42$ of the fluorescence intensity does not change, but the coefficient of variation significantly decreases to $CV = 0.76\%$. Further, $MIN \geq 0.2$ and $CV \leq 15\%$, which are practical performances, and further $CV \leq 10\%$ are satisfied. On the other hand, when setting to $n_3 = 1.33$, since $MIN = 0.066$ and $CV = 61\%$ do not change much, there is no change in that the practical performance is not satisfied.

[0056]

<Configuration example of capillary array according to present embodiment>

The upper part of FIG. 6 is a cross-sectional view of the configuration of the capillary array according to the present embodiment. The laser irradiation portions of 24 capillaries having an outer diameter $2R = 126 \mu\text{m}$ and an inner diameter $2r = 50 \mu\text{m}$ are arranged on the same plane at an interval of $155 \mu\text{m}$. The arrangement error is zero ($\Delta Z = 0 \mu\text{m}$). The capillary outside is air and $n_1 = 1.00$. The capillary material is quartz glass and $n_2 = 1.46$. The middle

part of FIG. 6 illustrates a laser beam tracking result when a laser beam having a diameter of 50 μm is applied to one side from the left side in a case where the capillary inside comprises a high refractive index separation medium having $n_3 = 1.41$ under the above conditions. Since multi-focusing is obviously functioning, the insides of all 24 capillaries can be efficiently irradiated. This corresponds to that $\Delta\theta = -5.8^\circ$ according to Expression (1), and thus each capillary exhibits a convex lens action.

[0057]

On the other hand, the lower part of FIG. 6 illustrates a similar laser beam tracking result when the capillary inside comprises a low refractive index separation medium having $n_3 = 1.33$ under the above conditions. Also in this case, since multi-focusing clearly functions, the insides of all the 24 capillaries can be efficiently irradiated. This corresponds to that $\Delta\theta = -3.2^\circ$ according to Expression (1), and thus each capillary exhibits a convex lens action. As described above, in both the high refractive index separation medium ($n_3 \geq 1.36$) and the low refractive index separation medium ($n_3 < 1.36$), the fact that each capillary exhibits a convex lens action and the multi-focusing functions cannot be realized in any known example, but is realized only by the technology of the present disclosure. That is, in the capillary array electrophoresis device of the present

embodiment, the multi-focusing functions in both the analysis mode of $n_3 < 1.36$ and the analysis mode of $n_3 \geq 1.36$. Meanwhile, in each analysis mode, it is effective to appropriately change the condition of the electrophoresis analysis according to each purpose. As changeable conditions of the electrophoresis analysis, there are a control temperature of the capillary, an electric field intensity at the time of electrophoresis, an electric field intensity at the time of sample injection and a sample injection time, a laser irradiation intensity, an exposure time of the sensor, and the like. For example, it is effective to change the control temperature of the capillary in each analysis mode, such as adjusting the temperature of the capillary to 30°C in one analysis mode and adjusting the temperature of the capillary to 60°C in another analysis mode.

[0058]

<Relative fluorescence intensity distribution by capillary array configuration of present embodiment (FIG. 6)>

The upper part of FIG. 7 illustrates the relative fluorescence intensity of each capillary when the result of one-side irradiation illustrated in the middle part and the lower part of FIG. 6 is remade in the case of both-side irradiation. When setting to $n_3 = 1.41$, the minimum value of the relative fluorescence intensity is obtained as MIN =

0.42 and the coefficient of variation is obtained as $CV = 11\%$ for 24 capillaries. It is found that $MIN \geq 0.2$ and $CV \leq 15\%$, which are practical performances, are satisfied. However, similarly to the upper part of FIG. 5, $CV \leq 10\%$, which is a more desirable condition, is not satisfied. On the other hand, in the case of $n_3 = 1.33$, unlike the upper part of FIG. 5, since $MIN = 0.40$ and $CV = 12\%$ are obtained, it can be seen that the practical performance is satisfied. However, also in this case, $CV \leq 10\%$ is not satisfied.

[0059]

The lower part of FIG. 7 illustrates the optical system corrected relative fluorescence intensity of each capillary obtained by adding the vignetting effect of the optical system of the 3500 series genetic analyzer to the upper part of FIG. 7, that is, multiplying the result by the optical system correction coefficient based on the vignetting effect. The optical system correction used here is the same as that used in FIG. 5. As a result of the optical system correction, since $MIN = 0.42$ and $CV = 9.0\%$ are obtained when setting to $n_3 = 1.41$, and $MIN = 0.40$ and $CV = 10\%$ are obtained when setting to $n_3 = 1.33$, $MIN \geq 0.2$ and $CV \leq 15\%$, which are practical performances, and further $CV \leq 10\%$ are satisfied.

[0060]

According to FIG. 7, unlike the case of FIG. 5, the relative fluorescence intensity does not change much

depending on the presence or absence of optical system correction. This is because the overall width of the capillary array in FIG. 4 is the interval of $370\text{ }\mu\text{m} \times (24 - 1)$ capillaries = 8.5 mm, whereas the overall width of the capillary array in FIG. 6 is the interval of $155\text{ }\mu\text{m} \times (24 - 1)$ capillaries = 3.6 mm, which is narrow, that is, the distance from the optical axis of each capillary is short, and thus the vignetting effect of the optical system is small. As a result, when setting to $n_3 = 1.41$ in the capillary array of the 3500 series genetic analyzer illustrated in FIG. 4, $\text{CV} = 11\%$ is greatly reduced to $\text{CV} = 0.76\%$ by optical system correction due to the vignetting effect of the optical system, whereas when setting to $n_3 = 1.41$ in the capillary array of the present disclosure illustrated in FIG. 6, $\text{CV} = 11\%$ is reduced only to $\text{CV} = 9\%$ by optical system correction due to the vignetting effect of the optical system. Similarly, also in the case of $n_3 = 1.33$ in the capillary array of the present disclosure, $\text{CV} = 12\%$ is reduced to only $\text{CV} = 10\%$ by optical system correction due to the vignetting effect of the optical system.

[0061]

However, in the configuration of the present embodiment, it has been found that each capillary exhibits a convex lens action and multi-focusing functions when using a separation medium having an arbitrary refractive index of

$n_3 \geq 1.33$ including $n_3 = 1.41$. In addition, as a modification of the present configuration, when fixing an arbitrary capillary having $R/r \leq 4.4$, for example, an arbitrary capillary having an inner diameter of $2r = 50 \mu\text{m}$ and even when using an arbitrary capillary having an outer diameter $2R \leq 220 \mu\text{m}$, each capillary exhibits a convex lens action under the condition of $n_3 \geq 1.33$. Thus, it is possible to cause multi-focusing to function.

[0062]

(C) Second embodiment

In the first embodiment, the result of examination on the case where the capillary array has an arrangement error of zero ($\Delta Z = 0 \mu\text{m}$) is illustrated. However, in reality, ΔZ is never $0 \mu\text{m}$. Therefore, in the present embodiment, the relationship among the arrangement error, the multi-focusing performance, and the relative fluorescence intensity of each capillary will be systematically examined. These studies are made for the first time by the technology of the present disclosure.

[0063]

<Definition of arrangement error of capillary array>

FIG. 8 is a diagram illustrating the definition of the arrangement error ΔZ . The upper part of FIG. 8 illustrates a cross-sectional view of 24 capillary arrays when the arrangement error is 0 ($\Delta Z = 0 \mu\text{m}$). This configuration is

the same as that in the upper part of FIG. 6. With the position of the central axis of the leftmost capillary (the capillary of the capillary number 1) as the origin, the X axis is set along the array plane, and the Z axis is set in the direction perpendicular to the array plane. The Y axis is set along the central axis of the leftmost capillary. The central axis of each capillary is on the X-axis and the Z-coordinate is zero. In contrast, the lower part of FIG. 8 illustrates the same cross-sectional view when there is an arrangement error ($\Delta Z \neq 0 \text{ }\mu\text{m}$). The X-coordinate of the central axis of each capillary is the same as in the case of the upper part of FIG. 8, but the Z-coordinate randomly varies up and down around the X-axis ($Z = 0 \text{ }\mu\text{m}$). Here, ΔZ is defined as the maximum value of the absolute value of each Z coordinate. That is, the distance between the center axis of the capillary farthest from the X axis and the X axis is defined as ΔZ . At this time, the Z coordinates of the central axes of the capillaries are randomly dispersed within a range of $\pm \Delta Z$. ΔZ is an index quantitatively indicating the magnitude of the arrangement error.

[0064]

<Relative fluorescence intensity in case of arrangement error of capillary array>

FIG. 9 is a diagram illustrating the relative fluorescence intensity of each capillary obtained by both-

side irradiation when setting to (a) $\Delta Z = 0 \text{ }\mu\text{m}$, (b) $\Delta Z = 3 \text{ }\mu\text{m}$, (c) $\Delta Z = 6 \text{ }\mu\text{m}$, (d) $\Delta Z = 9 \text{ }\mu\text{m}$, and (e) $\Delta Z = 12 \text{ }\mu\text{m}$, based on the configuration in which $n_3 = 1.41$ is used in the 24 capillary arrays illustrated in the upper part of FIG. 6. For $\Delta Z = 0 \text{ }\mu\text{m}$, a result of obtaining the relative fluorescence intensity for each of 10 sets of capillary arrays of random arrays for ΔZ other than $\Delta Z = 0 \text{ }\mu\text{m}$ is superimposed and displayed. FIG. 9(a) ($\Delta Z = 0 \text{ }\mu\text{m}$) illustrates the same result as $n_3 = 1.41$ in the upper part of FIG. 7. FIG. 9(f) illustrates the relative fluorescence intensity of each of the one set of capillaries for $\Delta Z = 0 \text{ }\mu\text{m}$ and the average of the relative fluorescence intensity of each of the 10 sets of capillaries for ΔZ other than $\Delta Z = 0 \text{ }\mu\text{m}$ in an overlapping manner. It can be seen that as ΔZ increases, the average value and the minimum value of the relative fluorescence intensity decrease and the variation in the relative fluorescence intensity increases. The minimum values of the relative fluorescence intensities for $\Delta Z = 0 \text{ }\mu\text{m}$, $3 \text{ }\mu\text{m}$, $6 \text{ }\mu\text{m}$, $9 \text{ }\mu\text{m}$, and $12 \text{ }\mu\text{m}$ are $\text{MIN} = 0.42, 0.40, 0.33, 0.22, \text{ and } 0.066$, respectively. Coefficients of variation of the relative fluorescence intensities at $\Delta Z = 0 \text{ }\mu\text{m}$, $3 \text{ }\mu\text{m}$, $6 \text{ }\mu\text{m}$, $9 \text{ }\mu\text{m}$, and $12 \text{ }\mu\text{m}$ are $\text{CV} = 11\%, 11\%, 12\%, 17\%, \text{ and } 28\%$, respectively. Here, for $\Delta Z = 0 \text{ }\mu\text{m}$, the minimum value and the coefficient of variation of the relative fluorescence intensity of each capillary of one set of

capillaries are illustrated. For ΔZ other than $\Delta Z = 0 \text{ }\mu\text{m}$, the minimum value and the coefficient of variation of the relative fluorescence intensity of each capillary of the 10 sets are illustrated. When the vignetting effect of the optical system of the 3500 series genetic analyzer is added to the above result of $n_3 = 1.41$, the coefficients of variation in the relative fluorescence intensities at $\Delta Z = 0 \text{ }\mu\text{m}$, $3 \text{ }\mu\text{m}$, $6 \text{ }\mu\text{m}$, $9 \text{ }\mu\text{m}$, and $12 \text{ }\mu\text{m}$ are $CV = 9\%$, 9% , 10% , 14% , and 23% , respectively. That is, it is found that, even if taking into consideration the vignetting effect of the optical system, $CV \leq 10\%$, which is practical performance, is not satisfied under the condition of $\Delta Z \geq 9 \text{ }\mu\text{m}$.

[0065]

FIG. 10 is a diagram illustrating relative fluorescence intensity of each capillary obtained by both-side irradiation when setting to (a) $\Delta Z = 0 \text{ }\mu\text{m}$, (b) $\Delta Z = 3 \text{ }\mu\text{m}$, (c) $\Delta Z = 6 \text{ }\mu\text{m}$, (d) $\Delta Z = 9 \text{ }\mu\text{m}$, and (e) $\Delta Z = 12 \text{ }\mu\text{m}$, based on the configuration in which $n_3 = 1.33$ is set in the 24 capillary arrays illustrated in the upper part of FIG. 6. For $\Delta Z = 0 \text{ }\mu\text{m}$, a result of obtaining the relative fluorescence intensity for each of 10 sets of capillary arrays of random arrays for ΔZ other than $\Delta Z = 0 \text{ }\mu\text{m}$ is superimposed and displayed. FIG. 10(a) ($\Delta Z = 0 \text{ }\mu\text{m}$) illustrates the same result as $n_3 = 1.33$ in the upper part of FIG. 7. FIG. 10(f) illustrates the relative fluorescence

intensity of each of the one set of capillaries for $\Delta Z = 0$ μm and the average of the relative fluorescence intensity of each of the 10 sets of capillaries for ΔZ other than $\Delta Z = 0$ μm in an overlapping manner. It can be seen that, as ΔZ increases, the average value and the minimum value of the relative fluorescence intensity decrease and the variation in the relative fluorescence intensity increases. The minimum values of the relative fluorescence intensity for $\Delta Z = 0$ μm , 3 μm , 6 μm , 9 μm , and 12 μm are MIN = 0.40, 0.39, 0.30, 0.25, and 0.058, respectively. Coefficients of variation of the relative fluorescence intensities at $\Delta Z = 0$ μm , 3 μm , 6 μm , 9 μm , and 12 μm are CV = 12%, 12%, 14%, 16%, and 28%, respectively. Here, for $\Delta Z = 0$ μm , the minimum value and the coefficient of variation of the relative fluorescence intensity of each capillary of one set of capillaries are illustrated. For ΔZ other than $\Delta Z = 0$ μm , the minimum value and the coefficient of variation of the relative fluorescence intensity of each capillary of the 10 sets are illustrated. When the vignetting effect of the optical system of the 3500 series genetic analyzer is added to the above result of $n_3 = 1.33$, the coefficients of variation in the relative fluorescence intensities at $\Delta Z = 0$ μm , 3 μm , 6 μm , 9 μm , and 12 μm are CV = 10%, 10%, 12%, 14%, and 24%, respectively. That is, it is found that, even if taking into consideration the vignetting effect of the

optical system, $CV \leq 10\%$, which is practical performance, is not satisfied under the condition of $\Delta Z \geq 6 \mu\text{m}$.

[0066]

Each of the distributions of the relative fluorescence intensity with respect to the capillary number illustrated in FIGS. 9 and 10 has a downward convex shape. As ΔZ increases, the degree of downward protrusion increases. On the other hand, as ΔZ increases, the degree to which the relative fluorescence intensity with respect to the capillary number fluctuates unevenly also increases.

[0067]

Similarly to the results of the first embodiment, the results of FIGS. 9 and 10 indicate that the effect of reducing the coefficient of variation of the relative fluorescence intensity by the optical system correction based on the vignetting effect of the optical system is insufficient and that, when the arrangement error ΔZ increases, the situation becomes more remarkable and thus the practical performance cannot be satisfied. Therefore, the present embodiment proposes applying the digital correction by the computer to the relative fluorescence intensity in addition to the optical system correction based on the vignetting effect of the optical system or instead of the optical system correction based on the vignetting effect of the optical system, thereby reducing the coefficient of

variation of the relative fluorescence intensity in consideration of the arrangement error ΔZ to enable to satisfy $CV \leq 10\%$, which is practical performance.

[0068]

<Process of obtaining practical output fluorescence intensity distribution for electrophoresis analysis>

FIG. 11 is a diagram illustrating a process of obtaining a practical output fluorescence intensity distribution for electrophoresis analysis. Specifically, FIG. 11 illustrates steps starting from the concentration of any type of fluorophore present in the laser irradiation portion of each capillary constituting the capillary array to the fluorescence intensity of the fluorophore of the capillary output by the computer. In FIG. 11, expressions of the fluorophore and the fluorescence are used in accordance with the present embodiment, but the present embodiment is not limited to the fluorophore and the fluorescence. Therefore, the fluorophore and the fluorescence in FIG. 11 can be replaced with a light emitter and emission, a scatterer and scattered light, or an absorber and absorbance.

[0069]

Hereinafter, FIG. 11 will be described in detail below. Note that the capillary numbers of the respective capillaries of the capillary array including the N capillaries are set

to $n = 1, 2, \dots$, and N in order from the end. All of the functions $C(n)$, $L(n)$, $I(n)$, $J(n)$, $M(n)$, $K(n)$, and $H(n)$ of n appearing in FIG. 11 hold at any time and for any type of fluorophore. That is, these functions are also functions for time and fluorophore type (however, a function having a small change depending on the time and the type of the fluorophore is also included), but are expressed as a function of only " n " for simplicity in FIG. 11. In addition, the values of these functions have meaning in the relative value for n , that is, the distribution of the function values for n , and have no meaning in the absolute value. Therefore, a coefficient for deriving an absolute value from a relative value is omitted. Further, when the distribution of the function value with respect to n of an arbitrary function is substantially constant or sufficiently flat, the function and the function value can be replaced with 1 regardless of n . Therefore, in the study based on FIG. 11, $CV \leq 15\%$ and $CV \leq 10\%$ are considered without considering $MIN \geq 0.2$ of the practical performance.

[0070]

(i) Emission fluorescence intensity distribution

The emission fluorescence intensity distribution represents an intensity distribution of emission fluorescence obtained from the capillary array. The concentration of the fluorophore in the laser irradiation

portion of the capillary n is referred to as $C(n)$, and $C(n)$ with respect to n is referred to as a fluorophore concentration distribution. $C(n)$ represents the concentration of fluorophore in the capillary number n at a certain time. $C(n)$ is a function that changes with the lapse of time. On the other hand, the irradiation intensity of the laser beam of the capillary n is referred to as $L(n)$. $L(n)$ with respect to n is referred to as a laser irradiation intensity distribution. $L(n)$ is a function indicating the laser irradiation intensity at the capillary number n at a certain time. At this time, it is possible to express the emission fluorescence intensity emitted from the light emission point of the capillary n as $I(n) = L(n) \times C(n)$. $I(n)$ with respect to n is referred to as an emission fluorescence intensity distribution. Again, coefficients that are usually multiplied by the right side of the above expression are omitted. The above $C(n)$, $L(n)$, and $I(n)$ indicate events inside the capillary array and each capillary.

[0071]

(ii) Measured fluorescence intensity distribution

The measured fluorescence intensity distribution is a (measured) fluorescence intensity distribution actually detected by the sensor, and indicates a distribution of the intensity of fluorescence detected by the sensor after the emission fluorescence from the capillary array passes

through a predetermined optical system (that is, the distribution is a fluorescence intensity distribution obtained by actually measuring fluorescence corrected by the optical system.). The optical system correction coefficient of the capillary n based on the vignetting effect of the optical system described in FIG. 2 is defined as $J(n)$. $J(n)$ with respect to n is defined as an optical system correction coefficient distribution. The optical system having any configuration physically changes the light passing through the optical system, but the optical system correction coefficient mathematically (as a numerical value) represents a physical phenomenon occurring in the optical system. In general, the optical system correction coefficient of the capillary away from the optical axis is smaller than that of the capillary close to the optical axis of the optical system. At this time, the fluorescence intensity at the image forming point where the light emission point of the capillary n is formed on the sensor by the optical system, that is, the measured fluorescence intensity by the sensor is represented as $M(n) = J(n) \times I(n)$. $M(n)$ with respect to n is referred to as a measured fluorescence intensity distribution. $J(n)$ and $M(n)$ described above indicate events inside the optical system. The optical system correction coefficient is a function of the capillary number n and is also a function of the coordinate x of the X axis illustrated in FIG. 8. As

illustrated in FIG. 8, when the X coordinate of the central axis of the capillary of $n = 1$ is set to $x = 0$ and the arrangement interval of the capillaries is defined as p , n and x can be converted by $x = (n - 1) \times p$.

[0072]

(iii) Output fluorescence intensity distribution

The output fluorescence intensity distribution is a fluorescence intensity distribution obtained by applying predetermined digital correction processing according to the present embodiment to the measured fluorescence intensity distribution. A digital correction coefficient of the capillary n , which is provided on the computer with respect to the measured fluorescence intensity output from the sensor, is defined as $K(n)$. $K(n)$ with respect to n is referred to as a digital correction coefficient distribution. At this time, the output fluorescence intensity of the capillary n output by the computer based on the digital correction is represented as $H(n) = K(n) \times M(n)$. $H(n)$ with respect to n is referred to as an output fluorescence intensity distribution.

[0073]

FIGS. 5, 7, 9, and 10 will be described again based on FIG. 11. The upper part of FIG. 5, the upper part of FIG. 7, FIG. 9, and FIG. 10 illustrate the emission fluorescence intensity distribution $I(n) = L(n)$ when the fluorophore concentration distribution is constant, that is, $C(n) = 1$.

On the other hand, the lower part of FIG. 5 and the lower part of FIG. 7 illustrate the measured fluorescence intensity distribution $M(n) = J(n) \times L(n)$ obtained by performing optical system correction on the emission fluorescence intensity distribution. $J(n)$ used here is an optical system correction coefficient distribution derived by an experiment for the optical system of the 3500 series genetic analyzer. In addition, since the digital correction is not performed here and $K(n) = 1$ is set, it can also be said that the lower part of FIG. 5 and the lower part of FIG. 7 illustrate the output fluorescence intensity distribution $H(n) = J(n) \times L(n)$.

[0074]

Based on FIG. 11, the digital correction will be studied, which is provided for reducing the coefficient of variation of the emission fluorescence intensity distribution $I(n) = L(n)$ illustrated in FIGS. 9 and 10. As described above, since the optical system correction in this configuration is sufficiently small, $J(n) = 1$ is set in this study. When $J(n) = 1$ cannot be regarded, $K(n)$ derived below may be replaced with $K(n)/J(n)$. In FIGS. 9 and 10, since $C(n) = 1$ is used, $H(n) = K(n) \times L(n)$ is obtained. That is, $K(n)$ such that the output fluorescence intensity distribution $H(n)$ approaches flatness is examined. As long as the laser irradiation intensity distribution $L(n)$ is very

stable, $H(n) = 1$ is obtained by setting $K(n) = 1/L(n)$, so that the coefficient of variation of the relative fluorescence intensity is reduced. For example, $I(n) = L(n)$ may be obtained in advance in a calibration experiment. The reciprocal thereof may be set as $K(n)$ and applied to the relative fluorescence intensity obtained in the subsequent experiment. This is the same digital correction method as in PTL 3. However, as illustrated in FIGS. 9 and 10, in practice, $I(n) = L(n)$ is not stable at all. The variation in the relative fluorescence intensity illustrated in FIGS. 9 and 10 is mainly caused by the refractive index n_3 of the medium of the capillary inside and the arrangement error ΔZ of the capillary array, but is not limited thereto. It is known that the variation also occurs due to a change in environmental temperature or humidity, slight deformation of the components of the device due to vibration, load, or the like, and the variation also randomly changes. One criterion is required to perform digital correction. In PTL 3, the fluorescence intensity distribution acquired at the time of calibration is used as a reference. In FIGS. 9 and 10, a total of 92 curves of the emission fluorescence intensity distribution are illustrated. But, it is not clear which one of these emission fluorescence intensity distributions should be selected as a reference. When an inappropriate emission fluorescence intensity distribution is selected as

a reference, the coefficient of variation of the output fluorescence intensity distribution is increased by the digital correction, and the digital correction may have an adverse effect.

[0075]

<Example of digital correction for fluorescence intensity distribution>

Based on the above, in the present embodiment, as a correction method, digital correction, based on the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ } \mu\text{m}$ in FIGS. 9(a) and 10(a), will be described. However, the digital correction coefficient distribution is changed between the case of $n_3 = 1.41$ in FIG. 9 and the case of $n_3 = 1.33$ in FIG. 10. More generally, the digital correction coefficient distribution is changed by the value of n_3 . This is because it has been found that the degree of downward protrusion of the emission fluorescence intensity distribution increases as n_3 decreases. On the other hand, it is allowable that, as ΔZ increases, the degree of downward protrusion of the emission fluorescence intensity distribution increases and the degree of unevenness change of the relative fluorescence intensity with respect to the capillary number increases.

[0076]

FIGS. 12(a) to 12(f) illustrate output fluorescence intensity distributions $H(n) = K(n) \times L(n)$ obtained by

performing digital correction based on the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ }\mu\text{m}$ illustrated in FIG. 9(a) with respect to each emission fluorescence intensity distribution $I(n) = L(n)$ illustrated in FIGS. 9(a) to 9(f) in the case of $n_3 = 1.41$. The digital correction coefficient distribution $K(n)$ used here is defined as $K(n) = 1/L_0(n)$ when defining as $L_0(n)$ the emission fluorescence intensity distribution with $\Delta Z = 0 \text{ }\mu\text{m}$ in FIG. 9(a). Therefore, as a matter of course, the output fluorescence intensity distribution in FIG. 12(a) is $H(n) = 1$. On the other hand, although the same digital correction coefficient distribution $K(n)$ as described above is used, the output fluorescence intensity distributions in FIGS. 12(b) to 12(e) are also flattened as compared with the emission fluorescence intensity distributions in FIGS. 9(b) to 9(e). As a result, for each of $\Delta Z = 0 \text{ }\mu\text{m}$, $3 \text{ }\mu\text{m}$, $6 \text{ }\mu\text{m}$, $9 \text{ }\mu\text{m}$, and $12 \text{ }\mu\text{m}$, the coefficients of variation of the relative fluorescence intensity in FIG. 9 are $CV = 11\%$, 11% , 12% , 17% , and 28% , whereas the coefficients of variation of the digitally corrected relative fluorescence intensity in FIG. 12 are significantly reduced to $CV = 0\%$, 0.4% , 3% , 8% , and 19% . Therefore, $CV \leq 10\%$ of practical performance is satisfied for any capillary array of $\Delta Z \leq 9 \text{ }\mu\text{m}$ by this digital correction.

[0077]

FIGS. 13(a) to 13(f) illustrate output fluorescence intensity distributions $H(n) = K(n) \times L(n)$ obtained by applying digital correction based on the emission fluorescence intensity distribution of $\Delta Z = 0 \mu\text{m}$ illustrated in FIG. 10(a) to each emission fluorescence intensity distribution $I(n) = L(n)$ illustrated in FIGS. 10(a) to 10(f) in the case of $n_3 = 1.33$. The digital correction coefficient distribution $K(n)$ used here is $K(n) = 1/L_0(n)$ when defining as $L_0(n)$ the emission fluorescence intensity distribution with $\Delta Z = 0 \mu\text{m}$ in FIG. 10(a). It is an important feature of the technology of the present disclosure that $L_0(n)$ is different from the emission fluorescence intensity distribution of $\Delta Z = 0 \mu\text{m}$ in FIG. 9(a) described above. Therefore, as a matter of course, the output fluorescence intensity distribution in FIG. 13(a) is $H(n) = 1$. On the other hand, although the same digital correction coefficient distribution $K(n)$ as described above is used, the output fluorescence intensity distributions in FIGS. 13(b) to 13(e) are also flattened as compared with the emission fluorescence intensity distributions in FIGS. 10(b) to 10(e). As a result, for each of $\Delta Z = 0 \mu\text{m}$, $3 \mu\text{m}$, $6 \mu\text{m}$, $9 \mu\text{m}$, and $12 \mu\text{m}$, the coefficients of variation of the relative fluorescence intensity in FIG. 9 are $CV = 12\%$, 12% , 14% , 16% , and 28% , whereas the coefficients of variation of the digitally corrected relative fluorescence intensity in FIG. 13 are

significantly reduced to $CV = 0\%$, 0.5% , 3% , 6% , and 18% . Therefore, $CV \leq 10\%$ of practical performance is satisfied for any capillary array of $\Delta Z \leq 9 \text{ } \mu\text{m}$ by this digital correction.

It is also possible to simply obtain, using the above-described Expressions (4) and (6), $L_0(n)$ for the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ } \mu\text{m}$ in FIG. 9(a) and $L_0(n)$ for the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ } \mu\text{m}$ in FIG. 10(a). Here, in the case of FIG. 9(a), $n_1 = 1.00$, $n_2 = 1.46$, $n_3 = 1.41$, and $N = 24$ may be set. In the case of FIG. 10(a), $n_1 = 1.00$, $n_2 = 1.46$, $n_3 = 1.33$, and $N = 24$ may be set. In this way, the digital correction coefficient distribution $K(n) = 1/L_0(n)$ is obtained for the first time in the present disclosure.

[0078]

In the present embodiment, it is assumed that the optical system correction coefficient distribution is $J(n) = 1$. The optimum digital correction coefficient distribution $K(n)$ is derived in which the coefficient of variation of the output fluorescence intensity distribution $H(n)$ is reduced. As described above, when $J(n) = 1$ cannot be regarded, $K(n)$ derived above may be replaced with $K(n)/J(n)$. That is, by digital correction and optical system correction, that is, by $K(n) \times J(n)$, the emission fluorescence intensity distribution $I(n)$ of $\Delta Z = 0 \text{ } \mu\text{m}$ at

desired n_3 may just be flattened most. Therefore, it is also effective to change $J(n)$ instead of $K(n)$ according to n_3 .

[0079]

The configuration has been examined, in the above description, in which the 24 capillary arrays illustrated in the upper part of FIG. 6 and FIG. 8 are irradiated on both sides with the laser beam. But, the same effect can be obtained with other configurations.

[0080]

(D) Third embodiment

In the second embodiment, it has been illustrated that, in order to flatten the output fluorescence intensity distribution, it is optimal to apply digital correction based on the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ } \mu\text{m}$ for the same n_3 to the emission fluorescence intensity distribution. However, the technology of the present disclosure is not limited to the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ } \mu\text{m}$, and also exerts an effect in a case where other values are used as a reference. Therefore, in the present embodiment, as an example, digital correction based on an average emission fluorescence intensity distribution of $\Delta Z = 6 \text{ } \mu\text{m}$ will be described. In the present embodiment, similarly to the second embodiment, a configuration will be considered in which the 24 capillary arrays illustrated in the upper part of FIG. 6 and FIG. 8

are irradiated on both sides with a laser beam. But other configurations may be used.

[0081]

<Example of digital correction for fluorescence intensity distribution: when average emission fluorescence intensity distribution of $\Delta Z = 6 \mu\text{m}$ is used as a reference>

FIGS. 14(a) to 14(f) illustrate output fluorescence intensity distributions $H(n) = K(n) \times L(n)$ obtained by applying digital correction based on the average emission fluorescence intensity distribution of $\Delta Z = 6 \mu\text{m}$ illustrated in FIG. 9(f) to each emission fluorescence intensity distribution $I(n) = L(n)$ illustrated in FIGS. 9(a) to 9(f) in the case of $n_3 = 1.41$. The digital correction coefficient distribution $K(n)$ used here is $K(n) = 1/L_0(n)$ when defining as $L_0(n)$ the average emission fluorescence intensity distribution with $\Delta Z = 6 \mu\text{m}$ in FIG. 9(f). Therefore, as a matter of course, the average output fluorescence intensity distribution with $\Delta Z = 6 \mu\text{m}$ in FIG. 14(f) is $H(n) = 1$.

[0082]

On the other hand, although the same digital correction coefficient distribution $K(n)$ as described above is used, the output fluorescence intensity distributions in FIGS. 14(a) to 14(e) are also flattened as compared with the emission fluorescence intensity distributions in FIGS. 9(a) to 9(e). As a result, for each of $\Delta Z = 0 \mu\text{m}$, $3 \mu\text{m}$, $6 \mu\text{m}$, 9

μm , and $12\ \mu\text{m}$, the coefficients of variation of the relative fluorescence intensity in FIG. 9 are $\text{CV} = 11\%$, 11% , 12% , 17% , and 28% , whereas the coefficients of variation of the digitally corrected relative fluorescence intensity in FIG. 14 are significantly reduced to $\text{CV} = 1\%$, 1% , 3% , 7% , and 18% .
[0083]

Therefore, $\text{CV} \leq 10\%$ of practical performance is satisfied for any capillary array of $\Delta Z \leq 9\ \mu\text{m}$ by this digital correction. As compared with the case of using the emission fluorescence intensity distribution of $\Delta Z = 0\ \mu\text{m}$ of the second embodiment as a reference, the CV of $\Delta Z = 0\ \mu\text{m}$ to $3\ \mu\text{m}$ increases remarkably. From the above, from the viewpoint of reducing the coefficient of variation of the relative fluorescence intensity, the case of using the emission fluorescence intensity distribution of $\Delta Z = 0\ \mu\text{m}$ as a reference is more superior comprehensively. But, it is also effective to use the average emission fluorescence intensity distribution of $\Delta Z = 6\ \mu\text{m}$ as a reference.

[0084]

FIGS. 15(a) to 15(f) illustrate output fluorescence intensity distributions $H(n) = K(n) \times L(n)$ obtained by applying digital correction based on the average emission fluorescence intensity distribution of $\Delta Z = 6\ \mu\text{m}$ illustrated in FIG. 10(f) to each emission fluorescence intensity distribution $I(n) = L(n)$ illustrated in FIGS. 10(a) to 10(f)

in the case of $n_3 = 1.33$. The digital correction coefficient distribution $K(n)$ used here is $K(n) = 1/L_0(n)$ when defining as $L_0(n)$ the average emission fluorescence intensity distribution with $\Delta Z = 6 \mu\text{m}$ in FIG. 10(f). Similarly to the second embodiment, this $L_0(n)$ is different from the average emission fluorescence intensity distribution of $\Delta Z = 6 \mu\text{m}$ in FIG. 9(f) described above, which is one of the important features of the technology of the present disclosure. Therefore, as a matter of course, the average output fluorescence intensity distribution with $\Delta Z = 6 \mu\text{m}$ in FIG. 15(f) is $H(n) = 1$.

[0085]

On the other hand, although the same digital correction coefficient distribution $K(n)$ as described above is used, the output fluorescence intensity distributions in FIGS. 15(a) to 15(e) are also flattened as compared with the emission fluorescence intensity distributions in FIGS. 10(a) to 10(e). As a result, for each of $\Delta Z = 0 \mu\text{m}$, $3 \mu\text{m}$, $6 \mu\text{m}$, $9 \mu\text{m}$, and $12 \mu\text{m}$, the coefficients of variation of the relative fluorescence intensity in FIG. 10 are $CV = 12\%$, 12% , 14% , 16% , and 28% , whereas the coefficients of variation of the digitally corrected relative fluorescence intensity in FIG. 15 are significantly reduced to $CV = 2\%$, 2% , 2% , 5% , and 16% .

[0086]

Therefore, $CV \leq 10\%$ of practical performance is satisfied for any capillary array of $\Delta Z \leq 9 \mu\text{m}$ by this digital correction. As compared with the case of using the emission fluorescence intensity distribution of $\Delta Z = 0 \mu\text{m}$ of the second embodiment as a reference, the CV of $\Delta Z = 0 \mu\text{m}$ to $3 \mu\text{m}$ increases remarkably. From the above, from the viewpoint of reducing the coefficient of variation of the relative fluorescence intensity, the case of using the emission fluorescence intensity distribution of $\Delta Z = 0 \mu\text{m}$ as a reference is more superior comprehensively. But, it is also effective to use the average emission fluorescence intensity distribution of $\Delta Z = 6 \mu\text{m}$ as a reference.

[0087]

<Example of digital correction with respect to fluorescence intensity distribution: Case of using emission fluorescence intensity distribution with $\Delta Z = 0 \mu\text{m}$ as reference>

Subsequently, digital correction based on the emission fluorescence intensity distribution of $\Delta Z = 0 \mu\text{m}$ for different n_3 is considered. FIGS. 16(a) to 16(f) illustrate output fluorescence intensity distributions $H(n) = K(n) \times L(n)$ obtained by performing digital correction based on the emission fluorescence intensity distribution of $\Delta Z = 0 \mu\text{m}$ in the case of $n_3 = 1.41$ illustrated in FIG. 9(a) with respect to each emission fluorescence intensity distribution $I(n) = L(n)$ illustrated in FIGS. 10(a) to 10(f) in the case of $n_3 =$

1.33. The digital correction coefficient distribution $K(n)$ used here is $K(n) = 1/L_0(n)$ when defining as $L_0(n)$ the emission fluorescence intensity distribution with $\Delta Z = 0 \text{ }\mu\text{m}$ in FIG. 9(a). As already mentioned, this $L_0(n)$ is different from the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ }\mu\text{m}$ in FIG. 10(a), which is one of the important features in the technology of the present disclosure. Therefore, unlike the result of FIG. 13(a), the output fluorescence intensity distribution of FIG. 16(a) does not satisfy $H(n) = 1$ and has a slightly downward convex shape.

[0088]

Similarly, all the output fluorescence intensity distributions in FIGS. 16(a) to 16(f) also have a downward convex shape. But, all are flattened as compared with the emission fluorescence intensity distributions in FIGS. 10(a) to 10(f). As a result, for each of $\Delta Z = 0 \text{ }\mu\text{m}$, $3 \text{ }\mu\text{m}$, $6 \text{ }\mu\text{m}$, $9 \text{ }\mu\text{m}$, and $12 \text{ }\mu\text{m}$, the coefficients of variation of the relative fluorescence intensity in FIG. 10 are $CV = 12\%$, 12% , 14% , 16% , and 28% , whereas the coefficients of variation of the digitally corrected relative fluorescence intensity in FIG. 16 are significantly reduced to $CV = 1\%$, 1% , 4% , 7% , and 19% .

[0089]

Therefore, $CV \leq 10\%$ of practical performance is satisfied for any capillary array of $\Delta Z \leq 9 \text{ }\mu\text{m}$ by this

digital correction. However, as compared with the digital correction based on the emission fluorescence intensity distribution of $\Delta Z = 0 \text{ }\mu\text{m}$ in the case of $n_3 = 1.33$ in FIG. 13, the coefficient of variation increases as a whole. From the above, from the viewpoint of reducing the coefficient of variation of the relative fluorescence intensity, the case of using the same n_3 emission fluorescence intensity distribution as a reference is superior comprehensively. But, it is also effective to use different n_3 average emission fluorescence intensity distributions as a reference.

[0090]

(E) Fourth embodiment

In the present embodiment, the examination of the third embodiment will be further deepened, and the relationship between the standard of digital correction and the result of digital correction for various n_3 will be examined in detail. Similarly to the second embodiment, a configuration will be examined in which the 24 capillary arrays illustrated in the upper part of FIG. 6 and FIG. 8 are irradiated on both sides with a laser beam. But, other configurations may be used.

[0091]

<Example of digital correction coefficient of relative fluorescence intensity corresponding to each refractive index when $\Delta Z = 0 \text{ }\mu\text{m}$ >

FIG. 17(a) is a diagram illustrating changes in

relative fluorescence intensity with respect to capillary numbers of 13 types when changing, from 1.30 to 1.42 in increments of 0.01, the refractive index n_3 of the medium of the capillary inside in a case where the arrangement error of the capillary array is regarded as $\Delta Z = 0 \text{ } \mu\text{m}$. That is, FIG. 17(a) illustrates the emission fluorescence intensity distribution $I(n) = L(n)$ when setting to $C(n) = 1$ in FIG. 11. In FIG. 17(a), the emission fluorescence intensity distribution $I(n) = L(n)$ is indicated by a solid line when the numerical value of the second decimal place of n_3 is an even number, and is indicated by a dotted line when the numerical value of the second decimal place of n_3 is an odd number. The emission fluorescence intensity distributions of $n_3 = 1.41$ and $n_3 = 1.33$ therein are the same as the emission fluorescence intensity distributions illustrated in FIGS. 9(a) and 10(a), respectively. However, in FIG. 17(a), the scale on the vertical axis is enlarged. It can be seen that, as n_3 decreases, the degree of downward protrusion of the emission fluorescence intensity distribution $L(n)$ increase, that is, the curvature of the downward convex curve monotonously increases. FIG. 17(b) is a diagram illustrating digital correction coefficients for five types of capillary numbers based on $L(n)$ of $n_3 = 1.30, 1.33, 1.36, 1.39$, and 1.42 in FIG. 17(a). That is, FIG. 17(b) illustrates the digital correction coefficient distribution

$K(n)$ of FIG. 11. Also in the present embodiment, it is assumed that the optical correction coefficient distribution in FIG. 11 is $J(n) = 1$. Each $K(n)$ is the inverse of the corresponding $L(n)$, where $K(n) = 1/L(n)$.

Each emission fluorescence intensity distribution $I(n) = L(n)$ in FIG. 17(a) can also be simply obtained using the above-described Expressions (4) and (6). Here, $n_1 = 1.00$, $n_2 = 1.46$, $n_3 = 1.30$ to 1.42 , and $N = 24$ may be set. In this way, the digital correction coefficient distribution $K(n) = 1/L(n)$ is obtained for the first time in the present disclosure.

[0092]

<Example of output fluorescence intensity distribution $H(n)$ after digital correction>

FIG. 18(a) is a diagram illustrating an output fluorescence intensity distribution $H(n) = K(n) \times L(n)$ obtained by performing digital correction with the digital correction coefficient distribution $K(n)$ of $n_3 = 1.30$ illustrated in FIG. 17(b) on the 13 types of emission fluorescence intensity distributions $I(n) = L(n)$ illustrated in FIG. 17(a). Similarly, FIG. 18(b), FIG. 18(c), FIG. 18(d), and FIG. 18(e) are diagrams illustrating the output fluorescence intensity distributions $H(n) = K(n) \times L(n)$ obtained by applying digital correction using the digital correction coefficient distributions $K(n)$ of $n_3 = 1.33, 1.36,$

1.39, and 1.42 illustrated in FIG. 17(b) to the 13 types of emission fluorescence intensity distributions $I(n) = L(n)$ illustrated in FIG. 17(a). All the output fluorescence intensity distributions $H(n)$ illustrated in FIG. 18 are flattened compared to the emission fluorescence intensity distribution $L(n)$ illustrated in FIG. 17(a). However, when the reference n_3 of the digital correction matches the target n_3 of the digital correction, the output fluorescence intensity distribution $H(n)$ is most flattened. Assuming that n_3 in the digital correction reference is denoted by n_3 (correction reference) and n_3 in the digital correction target is denoted by n_3 (correction target), the output fluorescence intensity distribution is $H(n) = 1$ in the case of n_3 (correction reference) = n_3 (correction target), the output fluorescence intensity distribution $H(n)$ is an upward convex curve in the case of n_3 (correction reference) < n_3 (correction target), and the output fluorescence intensity distribution $H(n)$ becomes a downward convex curve in the case of n_3 (correction reference) > n_3 (correction target). In addition, the larger the difference between n_3 (correction reference) and n_3 (correction target) is, the larger the degree of upward protrusion or downward protrusion is. For example, as illustrated in FIG. 18(a), when executing the digital correction for setting $H(n) = 1$ for $n_3 = 1.30$, the relative fluorescence intensity when the same correction is

executed for $H(n)$ of each refractive index is illustrated. $H(n)$ when $n_3 = 1.30$ is flat. But, in the case of other refractive indexes, the correction is not appropriately performed as the difference from 1.30 increases. As can be seen from FIG. 18(a), in the case of $n_3 = 1.42$, the correction is excessive.

[0093]

<Change in second-order coefficient when each output fluorescence intensity distribution $H(n)$ before and after digital correction is approximated by quadratic function>

The degree of upward protrusion and the degree of downward protrusion on the output fluorescence intensity distribution $H(n)$ can be expressed by a second-order coefficient when each output fluorescence intensity distribution $H(n)$ is approximated by a quadratic function. In the present embodiment, the output fluorescence intensity distribution is approximated by a quadratic function, but the degree of upward protrusion and the degree of downward protrusion on $H(n)$ may be expressed by other functions or other methods.

[0094]

FIG. 17(c) is a diagram illustrating a change in a secondary coefficient when approximating, by a quadratic function, each of the emission fluorescence intensity distribution $I(n)$ before digital correction in FIG. 17(a)

and the output fluorescence intensity distribution $H(n)$ after digital correction according to each correction reference in FIG. 18 with respect to the refractive index n_3 of the medium of the capillary inside. When the second-order coefficient indicated on the vertical axis is zero, the output fluorescence intensity distribution becomes $H(n) = 1$ and is completely flat. On the other hand, as the second-order coefficient is larger than zero, the degree of downward protrusion of the output fluorescence intensity distribution $H(n)$ increases. On the other hand, the degree of upward protrusion of the output fluorescence intensity distribution $H(n)$ increases as the second-order coefficient becomes smaller than zero. The second-order coefficient before correction gradually decreases together with n_3 . This corresponds to the fact that the degree of downward protrusion of the emission fluorescence intensity distribution $L(n)$ decreases as n_3 increases in FIG. 17(a). Each of the corrected second-order coefficients indicates a smaller value than the second-order coefficient before correction. In addition, each of the absolute values of the second-order coefficients after the correction indicates a smaller value than the absolute value of the second-order coefficient before correction. These results indicate that both digital corrections exhibit the effect of flattening the emission fluorescence intensity distribution.

[0095]

In FIG. 17(c), five types of curves indicated by $n_3 = 1.30$ (correction reference), $n_3 = 1.33$ (correction reference), $n_3 = 1.36$ (correction reference), $n_3 = 1.39$ (correction reference), and $n_3 = 1.42$ (correction reference) after correction correspond to FIGS. 18(a), 18(b), 18(c), 18(d), and 18(e), respectively. In any of the changes in the second-order coefficient of the output fluorescence intensity distribution according to the five types of correction references, the second-order coefficient is zero when n_3 (correction reference) = n_3 (correction target) is satisfied. The second-order coefficient is negative when n_3 (correction reference) < n_3 (correction target) is satisfied. The second-order coefficient becomes positive when n_3 (correction reference) > n_3 (correction target) is satisfied. Here, the horizontal axis in FIG. 17(c) indicates n_3 (correction target). The absolute value of the second-order coefficient increases as the difference between n_3 (correction reference) and n_3 (correction target) increases. The above corresponds to the change in the degree of upward protrusion and downward protrusion illustrated in FIG. 18.

[0096]

As described above, when digital correction is performed, it is most effective to set to n_3 (correction reference) = n_3 (correction target). Alternatively, it is

most effective to minimize the difference between n_3 (correction reference) and n_3 (correction target). When n_3 (correction reference) < n_3 (correction target) is satisfied, the digital correction is excessive so that the output fluorescence intensity distribution becomes an upward convex curve. When n_3 (correction reference) > n_3 (correction target) is satisfied, the digital correction is insufficient so that the output fluorescence intensity distribution becomes a downward convex curve. However, these cases are also effective because they are flattened as compared with the downward convex curve of the emission fluorescence intensity distribution before correction. When using separation media having a plurality of different types of n_3 at different timings, it is effective to perform digital correction according to n_3 to be used at each timing. That is, the correction reference and the digital correction coefficient distribution are changed according to n_3 to be used. The features of the digital correction according to the present embodiment have been described above.

[0097]

Although having studied above the case where the arrangement error of the capillary array is $\Delta Z = 0 \text{ }\mu\text{m}$, it is possible to obtain the same effect by the same means also in the case of $\Delta Z \neq 0 \text{ }\mu\text{m}$. For example, when $\Delta Z = 6 \text{ }\mu\text{m}$ is set as a common condition, a result similar to that in FIG. 17(c)

is obtained. That is, each correction reference may be acquired under the condition of $\Delta Z = 6 \mu\text{m}$, and the emission fluorescence intensity distribution to be corrected may be acquired under the condition of $\Delta Z = 6 \mu\text{m}$. On the other hand, when ΔZ of each correction reference is different from ΔZ of the emission fluorescence intensity distribution to be corrected, some deformation occurs. In FIG. 17(c), when $n_3 = 1.33$ (correction reference) at $\Delta Z = 0 \mu\text{m}$ is replaced with $n_3 = 1.33$ (correction reference) at $\Delta Z = 6 \mu\text{m}$, the corresponding curve shifts in the lower left direction. That is, when n_3 (correction target) on the horizontal axis is 1.33, the second-order coefficient on the vertical axis becomes 0 when performing digital correction with $n_3 = 1.33$ (correction reference) at $\Delta Z = 0 \mu\text{m}$, but the second-order coefficient on the vertical axis becomes negative when performing digital correction with $n_3 = 1.33$ (correction reference) at $\Delta Z = 6 \mu\text{m}$. Although such a curve shift occurs, the overall trend is similar. Even when digital correction is performed according to any reference, when n_3 (correction target) on the horizontal axis is set to a large value, for example, when setting to $n_3 = 1.42$, the second-order coefficient on the vertical axis becomes negative. On the other hand, when n_3 (correction target) on the horizontal axis is set to a small value, for example, when setting to $n_3 = 1.30$, the second-order coefficient on the vertical axis

becomes positive.

[0098]

(F) Fifth embodiment

In a fifth embodiment, the effect of the digital correction is evaluated for various ΔZ capillary arrays, and ΔZ satisfying $\text{MIN} \geq 0.2$ and $\text{CV} \leq 15\%$ or $\text{CV} \leq 10\%$, which are practical performances, is also clarified. Also, the present embodiment will consider a configuration in which the 24 capillary arrays illustrated in the upper part of FIG. 6 and FIG. 8 are irradiated on both sides with a laser beam. But, a configuration other than this may be used.

[0099]

<Results of optical correction and digital correction>

(i) When refractive index $n_3 = 1.41$

FIG. 19 is a diagram illustrating the relative fluorescence intensity of each capillary obtained by both-side irradiation at the time of changing ΔZ to 0.0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0, 1.41, and 15.0 μm in the case of $n_3 = 1.35$ on the upper side, and the coefficient of variation thereof on the lower side. The right side of each of the upper and lower parts in FIG. 19 is an enlarged view of the left side. For $\Delta Z = 0.0 \mu\text{m}$, one capillary array is used. For each ΔZ of $\Delta Z \neq 0.0 \mu\text{m}$, a capillary array composed of 100 random arrays is used. When creating the graph in the upper part of FIG. 19, 24 pieces of relative fluorescence

intensity data are used for $\Delta Z = 0.0 \text{ }\mu\text{m}$, and 24 pieces $\times$ 100 sets = 2400 pieces of relative fluorescence intensity data are used for each ΔZ of $\Delta Z \neq 0.0 \text{ }\mu\text{m}$. Then, in the graph, a black circle plot indicates an average value, an error bar indicates $\pm$ standard deviation, a black triangle plot indicates a maximum value, and a black square plot indicates a minimum value. As can be seen from FIG. 19(a), as ΔZ increases, all of the average value, the maximum value, and the minimum value decrease, and the standard deviation increases.

[0100]

When creating the graph in the lower part of FIG. 19, one set of coefficients of variation of the relative fluorescence intensity is used for $\Delta Z = 0.0 \text{ }\mu\text{m}$, and 100 sets of coefficients of variation of the relative fluorescence intensity are used for each ΔZ of $\Delta Z \neq 0.0 \text{ }\mu\text{m}$. Then, in the graph, a black circle plot indicates an average value, an error bar indicates $\pm$ standard deviation, a black triangle plot indicates a maximum value, and a black square plot indicates a minimum value. As can be seen from FIG. 19(c), as ΔZ increases, all of the average value, the maximum value, and the minimum value increase, and the standard deviation increases.

[0101]

When defining as MIN the minimum value of the relative

fluorescence intensity in the upper part of FIG. 19, it can be seen that $\Delta Z \leq 7.2 \mu\text{m}$ is sufficient to satisfy $\text{MIN} \geq 0.2$ which is practical performance. On the other hand, when defining as CV the average value + standard deviation of the relative fluorescence intensity in the lower part of FIG. 19, it can be seen that $\Delta Z \leq 6.4 \mu\text{m}$ is sufficient to satisfy $\text{CV} \leq 15\%$ which is practical performance. On the other hand, $\text{CV} \leq 10\%$ cannot be satisfied.

[0102]

FIG. 20 is a diagram illustrating a result of performing optical system correction and digital correction on all of the emission fluorescence intensity distributions $I(n) = L(n)$ of the 1001 kinds of capillary arrays illustrated in FIG. 19. The optical system correction coefficient distribution $J(n)$ is obtained by actually measuring the optical system correction coefficient distribution of the 3500 series genetic analyzer. $J(n)$ has a maximum value of 1 at the center of the capillary array, that is, in the middle of $n = 12$ and $n = 13$, and decreases as it goes away from the center. Therefore, it is possible to mutually compare the absolute values of the emission fluorescence intensity distribution $I(n)$ and the measured fluorescence intensity distribution $M(n)$.

[0103]

On the other hand, the reference of the digital

correction is set to the measured fluorescence intensity distribution $M(n) = J(n) \times L(n)$ of $\Delta Z = 0 \text{ }\mu\text{m}$ and $n_3 = 1.41$ in the lower part of FIG. 7 obtained by performing the optical system correction on the emission fluorescence intensity distribution $I(n) = L(n)$ of $\Delta Z = 0 \text{ }\mu\text{m}$ and $n_3 = 1.41$ in the upper part of FIG. 7. At this time, as in the second and third embodiments, the digital correction coefficient distribution may be $K(n) = 1/M(n)$, but in the present embodiment, may be $K(n) = \alpha \times \{[n - (N + 1)/2] \times p\}^2 + 1$. Here, α denotes a coefficient, setting to $\alpha = -0.074$, N denotes the total number of capillaries, setting to $N = 24$, and p denotes the arrangement interval of the capillaries, setting to $p = 0.155 \text{ mm}$. $K(n)$ has a maximum value of 1 at the center of the capillary array, that is, in the middle of $n = 12$ and $n = 13$, and decreases as it goes away from the center. Therefore, it is possible to mutually compare the absolute values of the measured fluorescence intensity distribution $M(n)$ and the output fluorescence intensity distribution $H(n)$. At this time, when this digital correction coefficient distribution $K(n)$ is applied to the measured fluorescence intensity distribution $M(n)$ of $n_3 = 1.41$ in the lower part of FIG. 7, the output fluorescence intensity distribution $H(n) = K(n) \times M(n)$ is most flattened. The coefficient of variation of the emission fluorescence intensity distribution in the upper part of FIG. 7 is $CV =$

11%. On the other hand, the coefficient of variation of the measured fluorescence intensity distribution in the lower part of FIG. 7 is $CV = 9\%$. Further, the coefficient of variation of the output fluorescence intensity distribution to which the digital correction is applied is reduced to $CV = 0.6\%$.

[0104]

FIG. 20 illustrates a result of applying the above optical system correction and digital correction not only to the case of $\Delta Z = 0 \mu\text{m}$ but also to the emission fluorescence intensity distributions of all ΔZ . When the upper part of FIG. 20 is compared with the upper part of FIG. 19, it can be seen that the minimum value of the relative fluorescence intensity distribution does not change, but the maximum value and the average value decrease, and the difference between the maximum value and the minimum value and the standard deviation decrease. On the other hand, when comparing the lower part of FIG. 20 with the lower part of FIG. 19, it can be seen that the coefficient of variation is greatly reduced not only for $\Delta Z = 0 \mu\text{m}$ but also for all ΔZ . When defining as MIN the minimum value of the relative fluorescence intensity in the upper part of FIG. 20, the condition satisfying $\text{MIN} \geq 0.2$, which is the practical performance, is $\Delta Z \leq 7.2 \mu\text{m}$, which is the same as the result in the upper part of FIG. 19. On the other hand, when defining as CV the

average value + standard deviation of the relative fluorescence intensity in the lower part of FIG. 20, it can be seen that $\Delta Z \leq 9.0 \text{ } \mu\text{m}$ is sufficient to satisfy $\text{CV} \leq 15\%$ which is practical performance. On the other hand, it can be seen that $\Delta Z \leq 7.8 \text{ } \mu\text{m}$ is sufficient to satisfy $\text{CV} \leq 10\%$.
[0105]

The above-described allowable range of ΔZ is greatly enlarged as compared with FIG. 19. That is, it can be seen that the allowable range of ΔZ satisfying the practical performance is greatly expanded by the digital correction according to the present disclosure.

[0106]

(ii) When refractive index is set to $n_3 = 1.33$

FIG. 21 illustrates in the upper part, for the case of $n_3 = 1.33$, the relative fluorescence intensity of each capillary obtained by both-side irradiation when ΔZ is changed to 0.0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0, 13.5, and 15.0 μm , and illustrates in the lower part the coefficient of variation thereof. The right side of each of the upper and lower parts in FIG. 21 is an enlarged view of the left side. For $\Delta Z = 0.0 \text{ } \mu\text{m}$, one capillary array is used, and for each ΔZ of $\Delta Z \neq 0.0 \text{ } \mu\text{m}$, a capillary array composed of 100 random arrays is used. When creating the graph in the upper part of FIG. 21, 24 pieces of relative fluorescence intensity data are used for $\Delta Z = 0.0 \text{ } \mu\text{m}$, and 24×100 sets

= 2400 pieces of relative fluorescence intensity data are used for each ΔZ of $\Delta Z \neq 0.0 \mu\text{m}$. In the graph, a black circle plot indicates an average value, an error bar indicates $\pm$ standard deviation, a black triangle plot indicates a maximum value, and a black square plot indicates a minimum value. As can be seen from FIG. 21(a), as ΔZ increases, all of the average value, the maximum value, and the minimum value decrease, and the standard deviation increases.

[0107]

When creating the graph in the lower part of FIG. 21, one set of coefficients of variation of the relative fluorescence intensity is used for $\Delta Z = 0.0 \mu\text{m}$, and 100 sets of coefficients of variation of the relative fluorescence intensity are used for each ΔZ of $\Delta Z \neq 0.0 \mu\text{m}$. In the graph, a black circle plot indicates an average value, an error bar indicates $\pm$ standard deviation, a black triangle plot indicates a maximum value, and a black square plot indicates a minimum value. As can be seen from FIG. 21(c), as ΔZ increases, all of the average value, the maximum value, and the minimum value increase, and the standard deviation increases. When defining as MIN the minimum value of the relative fluorescence intensity in the upper part of FIG. 21, it can be seen that $\Delta Z \leq 7.9 \mu\text{m}$ is sufficient to satisfy $\text{MIN} \geq 0.2$ which is practical performance.

[0108]

On the other hand, when defining as CV the average value + standard deviation of the relative fluorescence intensity in the lower part of FIG. 19, it can be seen that $\Delta Z \leq 5.7 \mu\text{m}$ is sufficient to satisfy $CV \leq 15\%$ which is practical performance. However, $CV \leq 10\%$ cannot be satisfied.

[0109]

FIG. 22 is a diagram illustrating a result of performing optical system correction and digital correction on all of the emission fluorescence intensity distributions $I(n) = L(n)$ of the 1001 kinds of capillary arrays illustrated in FIG. 21. The optical system correction coefficient distribution $J(n)$ is obtained by actually measuring the optical system correction coefficient distribution of the 3500 series genetic analyzer, and is the same as that used in FIG. 20. $J(n)$ has a maximum value of 1 at the center of the capillary array, that is, in the middle of $n = 12$ and $n = 13$, and decreases as it goes away from the center. Therefore, it is possible to mutually compare the absolute values of the emission fluorescence intensity distribution $I(n)$ and the measured fluorescence intensity distribution $M(n)$.

[0110]

On the other hand, the reference of the digital correction is set to the measured fluorescence intensity

distribution $M(n) = J(n) \times L(n)$ of $\Delta Z = 0 \text{ }\mu\text{m}$ and $n_3 = 1.33$ in the lower part of FIG. 7 obtained by performing the optical system correction on the emission fluorescence intensity distribution $I(n) = L(n)$ of $\Delta Z = 0 \text{ }\mu\text{m}$ and $n_3 = 1.33$ in the upper part of FIG. 7. The digital correction coefficient distribution may be set to $K(n) = 1/M(n)$ similarly to the second and third embodiments, but in the present embodiment, is set to $K(n) = \alpha \times [\{n - (N + 1)/2\} \times p]^2 + 1$. Here, α is a coefficient, setting to $\alpha = -0.081$, N is the total number of capillaries, setting to $N = 24$, and p is the arrangement interval of the capillaries, setting to $p = 0.155 \text{ mm}$. That is, the only difference from the digital correction applied in FIG. 20 is that α changes from -0.074 to -0.081 . This difference corresponds to n_3 changing from 1.41 to 1.33 . $K(n)$ has a maximum value of 1 at the center of the capillary array, that is, in the middle of $n = 12$ and $n = 13$, and decreases as it goes away from the center. Therefore, it is possible to mutually compare the absolute values of the measured fluorescence intensity distribution $M(n)$ and the output fluorescence intensity distribution $H(n)$. At this time, when this digital correction coefficient distribution $K(n)$ is applied to the measured fluorescence intensity distribution $M(n)$ of $n_3 = 1.33$ in the lower part of FIG. 7, the output fluorescence intensity distribution $H(n) = K(n) \times M(n)$ is most flattened. The coefficient of

variation in the emission fluorescence intensity distribution in the upper part of FIG. 7 is $CV = 12\%$, whereas the coefficient of variation in the measured fluorescence intensity distribution in the lower part of FIG. 7 is $CV = 10\%$. Further, the coefficient of variation of the output fluorescence intensity distribution, to which the digital correction is applied, is reduced to $CV = 0.8\%$.

[0111]

FIG. 22 illustrates a result of applying the above optical system correction and digital correction not only to the case of $\Delta Z = 0 \mu m$ but also to the emission fluorescence intensity distributions of all ΔZ . When comparing the upper part of FIG. 22 with the upper part of FIG. 21, the minimum value of the relative fluorescence intensity distribution does not change, but the maximum value and the average value decrease, and the difference between the maximum value and the minimum value and the standard deviation decrease. On the other hand, when comparing the lower part of FIG. 22 with the lower part of FIG. 21, not only $\Delta Z = 0 \mu m$ but also the coefficient of variation is greatly reduced for all ΔZ . When defining as MIN the minimum value of the relative fluorescence intensity in the upper part of FIG. 22, the condition satisfying $MIN \geq 0.2$, which is the practical performance, is $\Delta Z \leq 7.9 \mu m$, which is the same as the result in the upper part of FIG. 21. On the other hand, when

defining as CV the average value + standard deviation of the relative fluorescence intensity in the lower part of FIG. 22, it can be seen that $\Delta Z \leq 10.1 \mu\text{m}$ is sufficient to satisfy $\text{CV} \leq 15\%$ which is practical performance. On the other hand, it can be seen that $\Delta Z \leq 8.5 \mu\text{m}$ is sufficient to satisfy $\text{CV} \leq 10\%$. The above-described allowable range of ΔZ is greatly enlarged as compared with FIG. 21. That is, it can be seen that the allowable range of ΔZ satisfying the practical performance is greatly expanded by the digital correction according to the present disclosure.

[0112]

(G) Sixth embodiment

In NPL 1 described above, the refractive index of the medium (separation medium) inside the analysis capillary is set to $n_3 = 1.41$, and the refractive index of the medium inside the lens capillary is set to $n_4 = 1.53$. The capillary is made of quartz glass and has $n_2 = 1.46$. At this time, from the above Expression (1), the refractive angle of one analysis capillary is $\Delta\theta_A = +2.4^\circ$, while the refractive angle of one lens capillary is $\Delta\theta_B = -3.0^\circ$. At this time, since $\Delta\theta_A + \Delta\theta_B = -0.61^\circ$ is obtained, one set of one analysis capillary and one lens capillary exhibits a convex lens action, and multi-focusing functions. As described above, a method for evaluating the presence or absence of the multi-focusing function by $\Delta\theta_A + \Delta\theta_B$ has been found for the first

time in the technology of the present disclosure.

[0113]

<Configuration example of capillary array in which analysis capillaries and lens capillaries are alternately arranged>

The upper part of FIG. 23 illustrates a configuration example of the capillary array of the present disclosure that enables simultaneous irradiation of a larger number of capillaries. The laser irradiation portions of 192 capillaries having an outer diameter $2R = 126\ \mu\text{m}$ and an inner diameter $2r = 50\ \mu\text{m}$ are arranged on the same plane at an interval of $155\ \mu\text{m}$. The arrangement error is zero ($\Delta Z = 0\ \mu\text{m}$). The capillary outside is a low refractive index solution with $n_1 = 1.25$, and the capillary material is quartz glass with $n_2 = 1.46$. As the low refractive index solution, for example, Fluorinert of 3M can be used. An odd-numbered 96 capillaries from the left side of the capillary array are defined as analysis capillaries, and the refractive index of the internal medium is set to n_3 . In addition, capillary numbers 1 to 96 each are assigned to analysis capillaries in order from the left side. On the other hand, the even 96 capillaries from the left side of the capillary array are defined as lens capillaries, and the refractive index of the internal medium is set to $n_4 = 1.46$. No capillary number is assigned to each lens capillary.

[0114]

The middle part of FIG. 23 illustrates the laser beam tracking result when the laser beam having a diameter of $\phi 50 \mu\text{m}$ is applied to one side from the left side in the case of $n_3 = 1.41$ under the above conditions. At this time, multi-focusing functions, and the insides of all the 192 capillaries can be efficiently irradiated. From the above Expression (1), the refractive angle of one analysis capillary is $\Delta\theta_A = -1.4^\circ$, and the refractive angle of one lens capillary is $\Delta\theta_B = -3.3^\circ$. At this time, since $\Delta\theta_A + \Delta\theta_B = -4.7^\circ$ is obtained, one set of one analysis capillary and one lens capillary exhibits a convex lens action, and multi-focusing functions.

[0115]

The lower part of FIG. 23 illustrates the laser beam tracking result when the laser beam having a diameter of $\phi 50 \mu\text{m}$ is applied to one side from the left side in the case of $n_3 = 1.33$ under the above conditions. At this time, multi-focusing functions, and the insides of all the 192 capillaries can be efficiently irradiated. From the above Expression (1), the refractive angle of one analysis capillary is $\Delta\theta_A = + 2.0^\circ$, and the refractive angle of one lens capillary is $\Delta\theta_B = -3.3^\circ$. At this time, since $\Delta\theta_A + \Delta\theta_B = -1.3^\circ$ is obtained, one set of one analysis capillary and one lens capillary exhibits a convex lens action, and multi-focusing functions.

[0116]

<Examples of relative fluorescence intensity, optical system corrected relative fluorescence intensity, and digital correction & optical system corrected relative fluorescence intensity, for $n_3 = 1.41$ >

FIG. 24(a) illustrates the emission fluorescence intensity distribution $I(n) = L(n)$, which is the relative fluorescence intensity of the analysis capillaries of the capillary numbers 1 to 96 when setting to $n_3 = 1.41$, in the case where the result of the one-side irradiation illustrated in the middle part of FIG. 23 is remade in the case of the both-side irradiation. The relative fluorescence intensity of the lens capillary is not illustrated. The minimum value of the relative fluorescence intensity of $MIN = 0.30$ and the coefficient of variation of $CV = 20\%$ are obtained for the 96 analysis capillaries. It is found that $MIN \geq 0.2$, which is practical performance, is satisfied, but $CV \leq 15\%$ and $CV \leq 10\%$ are not satisfied.

[0117]

FIG. 25(a) illustrates an optical system correction coefficient distribution $J(n)$ when taking into consideration the vignetting effect of the optical system of the 3730 series genetic analyzer used in the present embodiment. Note that the optical system of the 3730 series genetic analyzer is different from the optical system of the 3500 series

genetic analyzer in that the respective vignetting effects are different. FIG. 25(a) illustrates the optical system correction coefficient distribution when setting to $a = -0.25635$, $b = 1$, and $c = 11.736815$ in $J(x) = a + (b - a) \times \exp\{-1 \times x^2 / (2 \times c^2)\}$. Here, the capillary number n and the spatial coordinate x are associated with each other by $x = \{n - (N + 1)/2\} \times p$. N is the total number of analysis capillaries, setting to $N = 96$, and p is the arrangement interval of the analysis capillaries, setting to $p = 0.155 \text{ mm} \times 2 = 0.310 \text{ mm}$. x is a spatial coordinate along the X axis illustrated in FIG. 8, but unlike FIG. 8, the origin of the X axis is arranged at the center of the capillary array, that is, between $n = 48$ and $n = 49$. As illustrated in FIG. 25(a), $J(n)$ has a maximum value of 1 at the center of the capillary array and decreases as it goes away from the center. Therefore, it is possible to mutually compare the absolute values of the emission fluorescence intensity distribution $L(n)$ and the measured fluorescence intensity distribution $M(n)$.

[0118]

FIG. 24(b) illustrates a measured fluorescence intensity distribution $M(n) = J(n) \times L(n)$ obtained by multiplying $J(n)$ in FIG. 25(a) by $L(n)$ in FIG. 24(a). The measured fluorescence intensity distribution in FIG. 24(b) represents an upward convex curve unlike the measured

fluorescence intensity distribution in the lower part of FIG. 7. This is because the optical system correction is large since the overall width of the capillary array is wide. The minimum value of the relative fluorescence intensity in the measured fluorescence intensity distribution in FIG. 24(b) is set to $\text{MIN} = 0.17$, and the coefficient of variation is set to $\text{CV} = 14\%$. Therefore, while $\text{MIN} \geq 0.2$, which is practical performance, is not satisfied, $\text{CV} \leq 15\%$ is satisfied, but $\text{CV} \leq 10\%$ is not satisfied.

[0119]

FIG. 25(b) illustrates a digital correction coefficient distribution $K(n)$ for reducing the coefficient of variation of the measured fluorescence intensity distribution of FIG. 24(b). This digital correction coefficient distribution represents a downward convex curve unlike the digital correction coefficient distribution in FIG. 17(b). This is to flatten the measured fluorescence intensity distribution convex upward in FIG. 24(b). FIG. 25(b) illustrates the digital correction coefficient distribution when setting to $a = -0.25635$, $b = 1$, and $c = 16.8$ in $K(x) = 1/[a + (b - a) \times \exp\{-1 \times x^2/(2 \times c^2)\}]$. Here, the capillary number n and the spatial coordinate x are associated with each other by $x = \{n - (N + 1)/2\} \times p$. N is the total number of analysis capillaries, setting to $N = 96$, and p is the arrangement interval of the analysis

capillaries, setting to $p = 0.155 \text{ mm} \times 2 = 0.310 \text{ mm}$. x is a spatial coordinate along the X axis illustrated in FIG. 8, but unlike FIG. 8, the origin of the X axis is arranged at the center of the capillary array, that is, between $n = 48$ and $n = 49$. As illustrated in FIG. 25(b), $K(n)$ has a maximum value of 1 at both ends of the capillary array, that is, at the positions of $n = 1$ and $n = 96$, and decreases toward the center. Therefore, it is possible to mutually compare the absolute values of the measured fluorescence intensity distribution $M(n)$ and the output fluorescence intensity distribution $H(n)$.

[0120]

FIG. 24(c) illustrates an output fluorescence intensity distribution $H(n) = K(n) \times M(n)$ obtained by multiplying the digital correction coefficient distribution $K(n)$ in FIG. 25(b) by the measured fluorescence intensity distribution $M(n)$ in FIG. 24(b). As can be seen from the comparison with the measured fluorescence intensity distribution $M(n)$ in FIG. 24(b), the output fluorescence intensity distribution $H(n)$ in FIG. 24(c) is flattened. The minimum value of the relative fluorescence intensity in the output fluorescence intensity distribution of FIG. 24(c) is $\text{MIN} = 0.17$, and the coefficient of variation is $\text{CV} = 2\%$. Therefore, while $\text{MIN} \geq 0.2$, which is practical performance, is not satisfied, both $\text{CV} \leq 15\%$ and $\text{CV} \leq 10\%$ are satisfied.

In the present embodiment, by enhancing the laser output intensity by 50%, $\text{MIN} = 0.26$ is effectively satisfied, and $\text{MIN} \geq 0.2$ of practical performance is satisfied. From the above, all the practical performance is satisfied.

[0121]

FIG. 26(a) illustrates the emission fluorescence intensity distribution $I(n) = L(n)$, which is the relative fluorescence intensity of the analysis capillaries of the capillary numbers 1 to 96 when $n_3 = 1.33$ in the case where the result of the one-side irradiation illustrated in the lower part of FIG. 23 is reprinted in the case of the both-side irradiation. The relative fluorescence intensity of the lens capillary is not illustrated. The minimum value of the relative fluorescence intensity of $\text{MIN} = 0.23$ and the coefficient of variation of $\text{CV} = 27\%$ are obtained for the 96 analysis capillaries, and it is found that $\text{MIN} \geq 0.2$, which is practical performance, is satisfied, but $\text{CV} \leq 15\%$ and $\text{CV} \leq 10\%$ are not satisfied.

[0122]

<Examples of relative fluorescence intensity, optical system corrected relative fluorescence intensity, and digital correction & optical system corrected relative fluorescence intensity, for $n_3 = 1.33$ >

FIG. 27(a) illustrates an optical system correction coefficient distribution $J(n)$ when taking into consideration

the vignetting effect of the optical system of the 3730 series genetic analyzer used in the present embodiment, which is the same as $J(n)$ illustrated in FIG. 25(a). FIG. 26(b) illustrates a measured fluorescence intensity distribution $M(n) = J(n) \times L(n)$ obtained by multiplying $J(n)$ in FIG. 27(a) by $L(n)$ in FIG. 26(a). The measured fluorescence intensity distribution in FIG. 26(b) has an upward convex curve as in FIG. 24(b), but is flattened as compared with FIG. 24(b). The minimum value of the relative fluorescence intensity in the measured fluorescence intensity distribution in FIG. 26(b) is $\text{MIN} = 0.17$, and the coefficient of variation is $\text{CV} = 9\%$. Therefore, while $\text{MIN} \geq 0.2$, which is practical performance, is not satisfied, both $\text{CV} \leq 15\%$ and $\text{CV} \leq 10\%$ are satisfied.

[0123]

FIG. 27(b) illustrates a digital correction coefficient distribution $K(n)$ for further reducing the coefficient of variation of the measured fluorescence intensity distribution of FIG. 26(b). Similarly to FIG. 25(b), this digital correction coefficient distribution represents a downward convex curve when setting to $a = -0.25635$, $b = 1$, and $c = 21.8$ in $K(x) = 1/[a + (b - a) \times \exp\{-1 \times x^2/(2 \times c^2)\}]$. Here, the capillary number n and the spatial coordinate x are associated with each other by $x = \{n - (N + 1)/2\} \times p$. The difference from $K(n)$ in FIG. 25(b)

is only the value of c .

[0124]

FIG. 26(c) illustrates an output fluorescence intensity distribution $H(n) = K(n) \times M(n)$ obtained by multiplying the digital correction coefficient distribution $K(n)$ in FIG. 27(b) by the measured fluorescence intensity distribution $M(n)$ in FIG. 26(b). As can be seen from the comparison with the measured fluorescence intensity distribution $M(n)$ in FIG. 26(b), the output fluorescence intensity distribution $H(n)$ in FIG. 26(c) is further flattened. The minimum value of the relative fluorescence intensity in the output fluorescence intensity distribution of FIG. 26(c) is $MIN = 0.17$, and the coefficient of variation is $CV = 3\%$. Therefore, while $MIN \geq 0.2$, which is practical performance, is not satisfied, both $CV \leq 15\%$ and $CV \leq 10\%$ are satisfied. In the present embodiment, by enhancing the laser output intensity by 50%, $MIN = 0.26$ is effectively satisfied, and $MIN \geq 0.2$ of practical performance is satisfied. From the above, all the practical performance is satisfied.

[0125]

(H) Seventh embodiment

A seventh embodiment discloses a configuration example of an improved capillary array electrophoresis device.

<Configuration example of capillary array electrophoresis

device>

FIGS. 28 and 29 are diagrams illustrating a configuration example of a capillary array electrophoresis device capable of switching between the polymer solution A 25 and the polymer solution B 28, which are two types of separation media, on the device. By adopting the configuration of the capillary array electrophoresis device of the present embodiment, it is possible to perform electrophoresis analysis using a plurality of types of polymer solutions without attaching and detaching the capillary array to and from the device while using the same capillary array.

[0126]

FIG. 28 illustrates mode A in which the capillary 1 is filled with the polymer solution A 25 and capillary electrophoresis is performed using the polymer solution A 25. FIG. 29 illustrates mode B in which the capillary 1 is filled with the polymer solution B 28 and capillary electrophoresis is performed using the polymer solution B 28. When the analysis session including steps (1) to (6) illustrated in the first embodiment is repeated, an arbitrary mode of mode A and mode B can be selected in each analysis session. For example, after the analysis session of mode A is repeated a plurality of times, the analysis session of mode B may be repeated a plurality of times, or the analysis

session of mode A and the analysis session of mode B may be alternately repeated. These settings can be performed by the input unit of the computer illustrated in FIG. 3, and can be confirmed by the display unit. A separation medium having a refractive index of $1.33 \leq n_3 < 1.36$ is defined as a low refractive index separation medium, and a separation medium having a refractive index of $1.36 \leq n_3 \leq 1.42$ is defined as a high refractive index separation medium. The polymer solution A 25 and the polymer solution B 28 may be either a high refractive index separation medium or a low refractive index separation medium, but in the present embodiment, the polymer solution A 25 is a high refractive index separation medium and the polymer solution B 28 is a low refractive index separation medium. Most parts of FIGS. 28 and 29 are common to FIG. 1, and objects denoted by common reference numerals in each drawing have the same functions by the same configuration. Hereinafter, portions different from those in FIG. 1 will be mainly described. The number of capillaries is not limited to 24 in the first embodiment, and is arbitrary. In the laser irradiation portion 14, the analysis capillaries and the lens capillaries may be alternately arranged (arrangement similar to that in FIG. 22). In this case, it is assumed that an object simply expressed as a capillary hereinafter represents an analysis capillary.

In the present disclosure, in a single analysis mode, it is assumed that the insides of all the capillaries constituting a single capillary array are filled with the same separation medium having the same refractive index. However, in the single analysis mode, the insides of different capillaries constituting a single capillary array may be filled with different separation media having different refractive indices. Even in such a case, the configuration of the present disclosure enables efficient simultaneous irradiation of all the capillaries constituting a single capillary array with the laser beam.

[0127]

In the capillary electrophoresis device illustrated in FIGS. 28 and 29, a dual polymer block 30 is used instead of the polymer block 9 in FIG. 1. When the analysis capillary and the lens capillary are alternately arranged in the laser irradiation portion 14, the lens capillary is not connected to the dual polymer block 30, but only the sample elution end 3 of the analysis capillary is bundled and connected to the dual polymer block 30. A syringe A 24 containing the polymer solution A 25 and a syringe B 27 containing the polymer solution B 28 are connected to the dual polymer block 30. The polymer solution A 25 inside the syringe A 24 is connected to the anode-side buffer solution 7 by a flow path inside the dual polymer block 30 when a valve A 26 is opened

and an anode tank valve 31 is opened. Similarly, the polymer solution B 28 inside the syringe B 27 is connected to the anode-side buffer solution 7 by the flow path inside the dual polymer block 30 when a valve B 29 is opened and the anode tank valve 31 is opened. A cleaning flow 33 used when cleaning the flow path is connected to the anode-side buffer solution 7 by the flow path inside the dual polymer block 30 when a cleaning valve 32 is opened and the anode tank valve 31 is opened. The sample elution end 3 is connected to a flow path between the valve A 26 and the anode tank valve 31, a flow path between the valve B 29 and the anode tank valve 31, and a flow path between the cleaning valve 32 and the anode tank valve 31.

[0128]

<Operation of mode A and mode B in analysis session, and switching operation thereof>

[1] Regarding mode A

First, mode A will be described. As illustrated in FIG. 28, it is assumed that the flow path between the valve A 26 and the anode tank valve 31 is filled with the polymer solution A 25, that is, the sample elution end 3 is in contact with the polymer solution A 25. At this time, the valve A 26 is opened, the valve B 29 is closed, the anode tank valve 31 is closed, the cleaning valve 32 is closed, and the piston of the syringe A 24 is pushed down, thereby

pressurizing the polymer solution A 25 inside the dual polymer block 30 and filling the inside of each capillary 1 with the polymer solution A 25 from the sample elution end 3 toward the sample injection end 2. After filling the polymer solution A 25, the anode tank valve 31 is opened, different samples are injected into each capillary 1 from the sample injection end 2, and then a high voltage is applied between the cathode 4 and the anode 5 by the power supply 8, thereby performing capillary electrophoresis. At this time, the valve A 26 may be closed.

[0129]

[2.1] Regarding switching from mode A to mode B

Next, a method for switching from mode A to mode B will be described. In order to perform a desired electrophoresis analysis, it is necessary that the polymer solution, with which the sample elution end 3 is in contact, is not a mixture of the polymer solution A 25 and the polymer solution B 28, that the polymer solution A 25 or the polymer solution B 28 is diluted, or that bubbles are mixed in the polymer solution inside the dual polymer block 30. Therefore, switching from mode A to mode B is performed in the next step. The valve A 26 is closed, the valve B 29 is opened, the anode tank valve 31 is opened, the cleaning valve 32 is closed, and the piston of the syringe B 27 is pushed down, thereby replacing with the polymer solution B 28 the flow

path between the valve B 29 and the anode-side buffer solution 7 while discharging the polymer solution A 25 existing in the flow path between the valve B 29 and the anode-side buffer solution 7 into the anode-side buffer solution 7 as illustrated in FIG. 29. When the replacement is completed, the piston of the syringe B 27 is stopped from being pushed down. In this step, the amount (volume) by which the syringe B 27 pushes out the polymer solution B 28 is made larger than the internal volume of the flow path between the valve B 29 and the anode-side buffer solution 7, so that the polymer solution, with which the sample elution end 3 is in contact, is the pure polymer solution B 28. In addition, since the amount of the polymer solution A 25 discharged into the anode-side buffer solution 7 is smaller than the amount of the anode-side buffer solution 7, the polymer solution A 25 discharged into the anode-side buffer solution 7 does not adversely affect electrophoresis. Of course, the polymer solution A 25 may be discharged not into the anode-side buffer solution 7 but into the waste liquid tank.

[0130]

[2.2] Regarding more efficient switching from mode A to mode B

Next, a method for more efficiently switching from mode A to mode B will be described. (1) The valve A 26 is closed,

the valve B 29 is closed, the anode tank valve 31 is opened, the cleaning valve 32 is opened, and pure water is injected into the dual polymer block 30 by the cleaning flow 33, thereby replacing with pure water the flow path between the cleaning valve 32 and the anode-side buffer solution 7 while discharging the polymer solution A 25 existing in the flow path between the cleaning valve 32 and the anode-side buffer solution 7 to the waste liquid tank. In this step, the anode-side buffer liquid tank is replaced with a waste liquid tank in advance. By making the amount (volume) of pure water injected in the cleaning flow 33 larger than the internal volume of the flow path between the cleaning valve 32 and the anode-side buffer solution 7, the polymer solution A 25 remaining in the same flow path is brought close to 0. (2) Subsequently, air is injected into the dual polymer block 30 by the cleaning flow 33 while keeping the valve A 26 closed, the valve B 29 closed, the anode tank valve 31 open, and the cleaning valve 32 open, thereby replacing with air the flow path between the cleaning valve 32 and the anode-side buffer solution 7 while discharging pure water existing in the flow path between the cleaning valve 32 and the anode-side buffer solution 7 to the waste liquid tank. As a result, it is possible to avoid that the polymer solution B 28 is finally diluted. (3) Thereafter, the valve A 26 is closed, the valve B 29 is opened, the anode tank valve 31 is opened, the

cleaning valve 32 is closed, and the piston of the syringe B 27 is pushed down, thereby replacing with the polymer solution B 28 the flow path between the valve B 29 and the anode-side buffer solution 7 while discharging the air existing in the flow path between the valve B 29 and the anode-side buffer solution 7 to the waste liquid tank. Finally, as illustrated in FIG. 29, when the waste liquid tank is replaced with the anode-side buffer liquid tank, the shift to mode B is completed. According to the above method, it is also possible to obtain an effect of reducing the amount of the polymer solution B 28 consumed at the time of switching from mode A to mode B. It is allowed to omit one of the injection of pure water and the injection of air by the cleaning flow 33.

[0131]

[3] Regarding mode B

Next, mode B will be described. As illustrated in FIG. 29, the flow path between the valve B 29 and the anode tank valve 31 is filled with the polymer solution B 28, that is, the sample elution end 3 is in contact with the polymer solution B 28. At this time, the valve A 26 is closed, the valve B 29 is opened, the anode tank valve 31 is closed, the cleaning valve 32 is closed, and the piston of the syringe B 27 is pushed down, thereby pressurizing the polymer solution B 28 inside the dual polymer block 30 and filling

the inside of each capillary 1 with the polymer solution B 28 from the sample elution end 3 toward the sample injection end 2. After filling the polymer solution B 28, the anode tank valve 31 is opened, different samples are injected into each capillary 1 from the sample injection end 2, and then a high voltage is applied between the cathode 4 and the anode 5 by the power supply 8, thereby performing capillary electrophoresis. At this time, the valve B 29 may be closed.

[0132]

[4] Regarding switching from mode B to mode A

Finally, a method for switching from mode B to mode A will be described. (1) The valve A 26 is closed, the valve B 29 is closed, the anode tank valve 31 is opened, the cleaning valve 32 is opened, and pure water is injected into the dual polymer block 30 by the cleaning flow 33, thereby replacing with pure water the flow path between the cleaning valve 32 and the anode-side buffer solution 7 while discharging the polymer solution B 28 existing in the flow path between the cleaning valve 32 and the anode-side buffer solution 7 to the waste liquid tank. In this step, the anode-side buffer liquid tank is replaced with a waste liquid tank in advance. By making the amount (volume) of pure water injected in the cleaning flow 33 larger than the internal volume of the flow path between the cleaning valve 32 and the anode-side buffer solution 7, the polymer solution B 28

remaining in the same flow path is brought close to 0. (2) Subsequently, air is injected into the dual polymer block 30 by the cleaning flow 33 while keeping the valve A 26 closed, the valve B 29 closed, the anode tank valve 31 open, and the cleaning valve 32 open, thereby replacing with air the flow path between the cleaning valve 32 and the anode-side buffer solution 7 while discharging pure water existing in the flow path between the cleaning valve 32 and the anode-side buffer solution 7 to the waste liquid tank. As a result, it is possible to avoid that the polymer solution A 25 is finally deluted. (3) Thereafter, the valve A 26 is opened, the valve B 29 is closed, the anode tank valve 31 is closed, the cleaning valve 32 is opened, and the piston of the syringe A 24 is pushed down, replacing with the polymer solution A 25 the flow path between the valve A 26 and the cleaning valve 32 while discharging the air existing in the flow path between the valve A 26 and the cleaning valve 32 to the outside. (4) Subsequently, the valve A 26 is opened, the valve B 29 is closed, the anode tank valve 31 is opened, the cleaning valve 32 is closed, and the piston of the syringe A 24 is pushed down, thereby replacing with the polymer solution A 25 the flow path between the valve A 26 and the anode-side buffer solution 7 while discharging the air existing in the flow path between the valve A 26 and the anode-side buffer solution 7 to the waste liquid tank.

Finally, as illustrated in FIG. 28, when the waste liquid tank is replaced with the anode-side buffer liquid tank, the shift to mode A is completed. According to the above method, it is also possible to obtain an effect of reducing the amount of the polymer solution A 25 consumed at the time of switching from mode B to mode A. It is allowed to omit one of the injection of pure water and the injection of air by the cleaning flow 33.

[0133]

<Structure of dual polymer block 30>

In order to efficiently or reliably perform the switching between mode A and mode B described above, it is effective that the dual polymer block 30 has the following characteristic configurations.

[0134]

The characteristic configuration 1 is that the sample elution end 3 of the capillary 1 is connected to the flow path between the valve A 26 and the anode tank valve 31. The valve A 26 has an opening/closing mechanism closest to the syringe A 24, which is a pressurizing mechanism for the polymer solution A 25. The anode tank valve 31 has an opening/closing mechanism closest to the anode-side buffer solution 7.

[0135]

The characteristic configuration 2 is that the sample

elution end 3 of the capillary 1 is connected to the flow path between the valve B 29 and the anode tank valve 31. The valve B 29 has an opening/closing mechanism closest to the syringe B 27, which is a pressurizing mechanism for the polymer solution B 28. The anode tank valve 31 has an opening/closing mechanism closest to the anode-side buffer solution 7.

[0136]

The characteristic configuration 3 is that the sample elution end 3 of the capillary 1 is connected to the flow path between the cleaning valve 32 and the anode tank valve 31. The cleaning valve 32 has the opening/closing mechanism closest to the injection port of the cleaning flow 33. The anode tank valve 31 has the opening/closing mechanism closest to the anode-side buffer solution 7.

[0137]

The characteristic configuration 4 is that the valve A 26 and the valve B 29 are connected to the flow path between the cleaning valve 32 and the anode tank valve 31. The valve A 26 has an opening/closing mechanism closest to the syringe A 24 which is a pressurizing mechanism for the polymer solution A 25. The valve B 29 has an opening/closing mechanism closest to the syringe B 27 which is a pressurizing mechanism for the polymer solution B 28. The cleaning valve 32 has an opening/closing mechanism closest to the injection

port of the cleaning flow 33. The anode tank valve 31 has an opening/closing mechanism closest to the anode-side buffer solution 7.

[0138]

(I) Eighth embodiment

An eighth embodiment discloses another configuration example of the improved capillary array electrophoresis device.

<Configuration example of capillary array electrophoresis device>

FIG. 30 is a diagram illustrating another configuration example (modification) of the capillary array electrophoresis device in which the polymer solution A 25 and the polymer solution B 28, which are two types of separation media, can be switched on the device, illustrated in FIGS. 28 and 29. By adopting this configuration, it is possible to perform electrophoresis analysis using a plurality of types of polymer solutions without attaching and detaching the capillary array to and from the device while using the same capillary array.

[0139]

In the capillary array electrophoresis device illustrated in FIG. 30, a rotary valve 35 is inserted between the syringe 11 and the polymer block 9 to be connected by piping. The rotary valve 35 is also connected to a container

containing the polymer solution A 25, a container containing the polymer solution B 28, and a container containing pure water 34. The rotary valve 35 can connect any two of the inside of the syringe 11, the polymer solution A 25, the polymer solution B 28, the pure water 34, the air, and the inside of the polymer block 9.

[0140]

<Operation of mode A and mode B in analysis session, and switching operation thereof>

[1] Regarding mode A

First, mode A will be described. As illustrated in FIG. 30, it is assumed that the flow path between the rotary valve 35 and the valve 10 is filled with the polymer solution A 25, that is, the sample elution end 3 is in contact with the polymer solution A 25. At this time, the rotary valve 35 connects the polymer solution A 25 inside the syringe 11 and the polymer solution A 25 inside the polymer block 9, closes the valve 10, and pushes down the piston of the syringe 11 to pressurize the polymer solution A 25 inside the polymer block 9, and fills the inside of each capillary 1 with the polymer solution A 25 from the sample elution end 3 toward the sample injection end 2. After filling the polymer solution A 25, the valve 10 is opened, different samples are injected into each capillary 1 from the sample injection end 2, and then a high voltage is applied between

the cathode 4 and the anode 5 by the power supply 8, thereby performing capillary electrophoresis.

[0141]

[2] Regarding switching from mode A to mode B

Next, a method for switching from mode A to mode B will be described. In order to perform a desired electrophoresis analysis, it is necessary that the polymer solution, with which the sample elution end 3 is in contact, is not a mixture of the polymer solution A 25 and the polymer solution B 28, the polymer solution A 25 or the polymer solution B 28 is diluted, or bubbles are mixed in the polymer solution inside the polymer block 9. Therefore, switching from mode A to mode B is performed in the next step. (1) The rotary valve 35 connects the polymer solution A 25 inside the syringe 11 and the polymer solution A 25 inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11 to the bottom, thereby discharging the entire amount of the polymer solution A 25 inside from the syringe 11. The same amount of the polymer solution A 25 as the discharged amount is discharged from the polymer block 9 to the waste liquid tank. At this time, the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the polymer solution A 25. (2) Subsequently, the rotary valve 35 connects the inside of the syringe 11

and the pure water 34, closes the valve 10, and pushes up the piston of the syringe 11 to the top, thereby filling the inside of the syringe 11 with the pure water 34. (3) Then, the rotary valve 35 connects the pure water 34 inside the syringe 11 and the polymer solution A 25 inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11 to the bottom, thereby discharging the entire amount of the pure water 34 inside from the syringe 11. The same amount of the polymer solution A 25 and the pure water 34 as the discharged amount is discharged from the polymer block 9 to the waste liquid tank. At this time, the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the pure water 34. The steps (2) and (3) are repeated as necessary to prevent the polymer solution A 25 from remaining in the flow path. (4) Subsequently, the rotary valve 35 connects the inside of the syringe 11 and the outside air, closes the valve 10, and pushes up the piston of the syringe 11 to the top, thereby filling the inside of the syringe 11 with air. (5) Then, the rotary valve 35 connects the air inside the syringe 11 and the pure water 34 inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11 to the bottom, thereby discharging the entire amount of the air inside the syringe 11. The same amount of the pure water 34 and the

air as the discharged amount is discharged from the polymer block 9 to the waste liquid tank. At this time, the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the air. The steps (4) and (5) are repeated as necessary to prevent liquid from remaining in the flow path. (6) This time, the rotary valve 35 connects the inside of the syringe 11 and the polymer solution B 28, closes the valve 10, and pushes up the piston of the syringe 11 to the top, thereby filling the inside of the syringe 11 with the polymer solution B 28. (7) Finally, the rotary valve 35 connects the polymer solution B 28 inside the syringe 11 and the air inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11, thereby discharging the entire amount of the air inside the polymer block from the polymer block 9 to the waste liquid tank. When the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the polymer solution B 28, lowering of the piston of the syringe 11 is stopped, and the shift to B mode is completed.

[0142]

[3] Regarding mode B

Next, mode B will be described. It is assumed that the flow path between the rotary valve 35 and the valve 10

is filled with the polymer solution B 28, that is, the sample elution end 3 is in contact with the polymer solution B 28. At this time, the rotary valve 35 connects the polymer solution B 28 inside the syringe 11 and the polymer solution B 28 inside the polymer block 9, closes the valve 10, and pushes down the piston of the syringe 11, thereby pressurizing the polymer solution B 28 inside the polymer block 9, and thereby filling the inside of each capillary 1 with the polymer solution B 28 from the sample elution end 3 toward the sample injection end 2. After filling the polymer solution B 28, the valve 10 is opened, different samples are injected into each capillary 1 from the sample injection end 2, and then a high voltage is applied between the cathode 4 and the anode 5 by the power supply 8, thereby performing capillary electrophoresis.

[0143]

[4] Regarding switching from mode B to mode A

Finally, a method for switching from mode B to mode A will be described. (1) The rotary valve 35 connects the polymer solution B 28 inside the syringe 11 and the polymer solution B 28 inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11 to the bottom, thereby discharging the entire amount of the polymer solution B 28 inside from the syringe 11. The same amount of the polymer solution B 28 as the discharged amount is

discharged from the polymer block 9 to the waste liquid tank. At this time, the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the polymer solution B 28. (2) Subsequently, the rotary valve 35 connects the inside of the syringe 11 and the pure water 34, closes the valve 10, and pushes up the piston of the syringe 11 to the top, thereby filling the inside of the syringe 11 with the pure water 34. (3) Then, the rotary valve 35 connects the pure water 34 inside the syringe 11 and the polymer solution B 28 inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11 to the bottom, thereby discharging the entire amount of the pure water 34 inside from the syringe 11. The same amount of the polymer solution B 28 and the pure water 34 as the discharged amount is discharged from the polymer block 9 to the waste liquid tank. At this time, the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the pure water 34. The steps (2) and (3) are repeated as necessary to prevent the polymer solution B 28 from remaining in the flow path. (4) Subsequently, the rotary valve 35 connects the inside of the syringe 11 and the outside air, closes the valve 10, and pushes up the piston of the syringe 11 to the top, thereby filling the inside of the syringe 11

with air. (5) Then, the rotary valve 35 connects the air inside the syringe 11 and the pure water 34 inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11 to the bottom, thereby discharging the entire amount of the air inside the syringe 11. The same amount of the pure water 34 and the air as the discharged amount is discharged from the polymer block 9 to the waste liquid tank. At this time, the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the air. The steps (4) and (5) are repeated as necessary to prevent liquid from remaining in the flow path. (6) This time, the rotary valve 35 connects the inside of the syringe 11 and the polymer solution A 25, closes the valve 10, and pushes up the piston of the syringe 11 to the top, thereby filling the inside of the syringe 11 with the polymer solution A 25. (7) Finally, the rotary valve 35 connects the polymer solution A 25 inside the syringe 11 and the air inside the polymer block 9, opens the valve 10, and pushes down the piston of the syringe 11, thereby discharging the entire amount of the air inside the polymer block from the polymer block 9 to the waste liquid tank. When the flow path from the tip portion of the syringe 11 to the boundary with the anode-side buffer solution 7 of the polymer block 9 is filled with the polymer solution A 25, lowering of the

piston of the syringe 11 is stopped, and the shift to mode A is completed.

[0144]

As described above, in order to efficiently or reliably switch between mode A and mode B, it is effective that the device has the following characteristic configuration. The rotary valve 35 in the eighth embodiment also serves as the valve A 26, the valve B 29, and the cleaning valve 32 in the seventh embodiment. The syringe 11 according to the eighth embodiment also serves as the syringe A 24, the syringe B 27, and the cleaning flow according to the seventh embodiment. Further, the valve 10 in the eighth embodiment has the same role as the anode tank valve 31 in the seventh embodiment. In view of this, it can be said that the characteristic structures 1 to 4 described in the seventh embodiment are also established in the eighth embodiment. Alternatively, an expression specific to the eighth embodiment as follows is also possible. A characteristic configuration is that the sample elution end 3 of the capillary 1 is connected to the flow path between the rotary valve 35, which is an opening/closing mechanism closest to the syringe 11, which is a pressurizing mechanism for any of the polymer solution A 25, the polymer solution B 28, the pure water 34, or air, and the anode tank valve 10 which is an opening/closing mechanism closest to the anode-side buffer solution 7.

[0145]

(J) Conclusion

(i) The optimum correction coefficient for the emission fluorescence intensity distribution varies depending on the refractive index n_3 of the separation medium. On the premise of this fact, when the refractive index n_3 is not defined (in an unknown case), it is unclear what correction coefficient should be used. When an optimum correction coefficient is not used, the correction coefficient itself can be a factor of an error. In the first place, the refractive index n_3 of the separation medium also changes depending on environmental conditions such as temperature. It is not necessarily clear what correction should be made under such conditions. Therefore, in the technology of the present disclosure, the relationship (FIG. 17(c)) between the refractive index n_3 of the separation medium in the capillary array and the second-order coefficient when the relative fluorescence intensity distribution is approximated by a quadratic function is introduced. As illustrated in FIG. 17(a), all the relative fluorescence intensity distributions before correction have a downward convex shape, and as illustrated in FIG. 17(c), the second-order coefficient is positive. In the correction for this, whether the correction is appropriate varies depending on which refractive index n_3 is used as a reference.

For example, when digital correction is performed based on the refractive index $n_3 = 1.36$, in the case of the separation medium having the refractive index $n_3 = 1.36$, the relative fluorescence intensity distribution after correction becomes flat, and the second-order coefficient becomes 0. On the other hand, in the case of a separation medium having a refractive index larger than 1.36, the relative fluorescence intensity distribution after correction has an upward convex shape, and the second-order coefficient is negative. This indicates that the correction is excessive. In addition, in the case of a separation medium having a refractive index smaller than 1.36, the relative fluorescence intensity distribution after correction is convex downward, and the second-order coefficient is positive. This indicates that the correction is insufficient. However, even when the correction is excessive or insufficient, it is considered that the effect of correction can be expected (correction is effective) since the relative fluorescence intensity distribution is flattened as compared with the relative fluorescence intensity distribution before correction. That is, one of the features of the technology of the present disclosure is to change the correction reference and the digital correction coefficient distribution in accordance with the refractive index n_3 of the separation medium to be used.

[0146]

In the present disclosure, the average value in $1 \leq n \leq N$ of the second derivative, indicating the degree of protrusion of the laser irradiation intensity distribution $L(n)$, is defined as A (the average value is used because the second derivative slightly varies depending on the position of the capillary). Here, the laser irradiation light intensity of the capillary number n of the N capillaries in the laser irradiation portion 14 is represented by a function of n (capillary numbers $n = 1, 2, \dots$, and N in sequence order from one end of the capillary array). A is a value uniquely determined under a predetermined condition. In addition, an average value in $1 \leq n \leq N$ of the second derivative, indicating the degree of protrusion of the output fluorescence intensity distribution $H(n)$, is defined as B. Here, the output light intensity of the capillary number n is represented by a function of n when the light emitting substance having the equal concentration exists inside the N capillaries in the laser irradiation portion 14. At this time, the capillary electrophoresis device is configured to have an analysis mode of the capillary electrophoresis using a separation medium having the refractive index $n_3 < 1.36$, and to satisfy $|A| > |B|$. That is, if $|A| > |B|$ is satisfied, in the case of using a separation medium having a refractive index satisfying $n_3 < 1.36$, the relative fluorescence

intensity distribution having a downward convex shape approaches flat, and effective correction can be realized.

[0147]

(ii) More specifically, $|A| > |B|$ can be realized by executing at least one of optical correction by an optical system and digital correction by computer calculation. Although $|A| > |B|$ can be realized by digital correction without particularly devising the configuration of the optical system, the configuration of the optical system may be changed and digital correction by computer calculation may be executed. In addition, $|A| > |B|$ may be realized only by changing the configuration of the optical system.

[0148]

For example, when the number of capillaries is set to 24 and a separation medium having a relatively high refractive index is used, the relative fluorescence intensity distribution is flat only by adopting the correction coefficient $J(n)$ of the actual optical system as illustrated in FIG. 5. In such a case (a case where the refractive index of the separation medium is high), it can be seen that digital correction is not necessary. On the other hand, when a relatively thin capillary (see FIG. 6) is used, such a correction effect cannot be expected even if correction is performed by the optical system regardless of the level of the refractive index. This is because, since

a thin capillary is used, each capillary is close to the central axis of the optical system, and optical correction is not effective. For example, there is a case where the relative fluorescence intensity distribution becomes slightly flat but not perfect due to correction by the optical system. In such a case, flatness is further improved by adding digital correction (see FIG. 11). That is, the relative fluorescence intensity distribution is flattened as a whole by compensating by digital correction an action that is not sufficient by the correction using the optical system and by combining the optical correction and the digital correction. As a simple method, for example, it is conceivable to optimize the digital correction coefficient so that the relative fluorescence intensity distribution becomes flat according to the refractive index. On the other hand, in some cases, a flat relative fluorescence intensity distribution may be realized by artificially changing the optical system (changing the configuration of at least a part of the optical system) to optimize the optical system without performing digital correction.

[0149]

As described above, in the technology of the present disclosure, a flat relative fluorescence intensity distribution may be realized by changing at least a part of the configuration of the optical system, or may be realized

only by digital correction. Further, it may be realized by using both of them.

[0150]

Note that a specific method for controlling the correction coefficient distribution of the optical system is disclosed in, for example, US 7477381 B and PTL 3 (Japanese Patent No. 6113549). In the former, it is disclosed that light emitted from each capillary is received with equal efficiency by inserting into the optical system a cat's-eye type light blocking aperture 112 as illustrated in FIG. 24. In addition, the latter discloses that light emitted from each capillary is uniformly received by inserting into the optical system a variable density filter (a filter in which transmittance of light is changed depending on a position). Both documents show that the light receiving efficiency is made uniform, but this is an example, and it is not always necessary to make the light receiving efficiency uniform. What is desired to show here is that the correction coefficient distribution of the optical system can be controlled by changing the design of each device.

[0151]

(iii) In the present disclosure, a predetermined coefficient of digital correction is stored in a memory corresponding to the value(s) of the refractive indexes n_3 of the separation medium used for analysis. The computer

reads the correction coefficient from the memory (switching tables), and can appropriately correct the measured fluorescence intensity distribution $M(n)$ by applying the correction coefficient. Normally, correction is performed by performing calibration, but since there are cases where correction can be performed and where correction cannot be performed, it is effective to read and use a correction coefficient.

[0152]

(iv) One of the features of the technology of the present disclosure is that the value of B (an average value at $1 \leq n \leq N$ of the second derivative showing the degree of protrusion of $H(n)$ in which the output light intensity is represented by a function of n) changes when introducing into the capillary a separation medium having a refractive index larger or smaller than the assumed refractive index n_3 . For example, when the relative fluorescence intensity distribution is corrected based on the refractive index $n_3 = 1.36$, $B \approx 0$ is obtained for the refractive index $n_3 = 1.36$ from FIG. 17(c) (see the value of the vertical axis). In this case, when intentionally introducing into the capillary a separation medium having a refractive index higher or lower than the refractive index $n_3 = 1.36$, the degree of upward protrusion or downward protrusion in the relative fluorescence intensity distribution changes. Such a

viewpoint has been found for the first time by the technology of the present disclosure.

[0153]

(v) One of the features of the technology of the present disclosure is that the average value B of the second derivative of the output fluorescence intensity distribution $H(n)$, in which the output light intensity of the capillary number n is represented by a function of n , always changes from positive to negative when the refractive index n_3 of the separation medium is sequentially increased. This point is also a feature that cannot be found in the prior art. At this time, when introducing into the capillary a separation medium having a very high refractive index n_3 (for example, the refractive index $n_3 = 1.42$), the value of B becomes negative (the relative fluorescence intensity distribution is convex upward), and when introducing into the capillary a separation medium having a very low refractive index n_3 (for example, the refractive index $n_3 = 1.30$), the value of B becomes positive (the relative fluorescence intensity distribution is convex downward).

[0154]

(vi) Further, one of the features of the technology of the present disclosure is that a plurality of separation media having a predetermined refractive index n_3 is used. The current products can use a plurality of types of

separation media, but the refractive index n_3 is 1.41 for any separation media. On the other hand, it is possible to use, for analysis in the technology of the present disclosure, not only $n_3 = 1.41$ but also separation media (separation media having a plurality of refractive indexes) such as $n_3 = 1.33$ and $n_3 = 1.36$. Such a viewpoint has been found for the first time by the technology of the present disclosure.

Reference Signs List

[0155]

- 1 capillary
- 2 sample injection end
- 3 sample elution end
- 4 cathode
- 5 anode
- 6 cathode side buffer solution
- 7 anode-side buffer solution
- 8 power supply
- 9 polymer block
- 10 valve
- 11 syringe
- 12 laser light source
- 13 laser beam
- 14 laser irradiation portion
- 15 condensing lens
- 16 laser cut filter

- 17 transmission type diffraction grating
- 18 imaging lens
- 19 sensor
- 20 light emission point
- 21 fluorescence
- 22 image forming point
- 23 optical axis
- 24 syringe A
- 25 polymer solution A
- 26 valve A
- 27 syringe B
- 28 polymer solution B
- 29 valve B
- 30 dual polymer block
- 31 anode tank valve
- 32 cleaning valve
- 33 cleaning Flow
- 34 pure water
- 35 rotary valve

CLAIMS

[Claim 1]

A capillary array electrophoresis device comprising:
a laser light source configured to emit a laser beam;
a capillary array configured such that laser irradiation portions of N capillaries collectively irradiated with the laser beam are substantially arranged on a same array plane, where N is an integer of 2 or more;

an optical system configured to collectively measure light emission from the N capillaries; and

a computer configured to perform predetermined processing on a light intensity measured by the optical system and to output the light intensity,

wherein an analysis mode of a capillary electrophoresis using a separation medium having at least a refractive index $n_3 < 1.36$ is provided, $|A| > |B|$ is satisfied,

wherein an outer radius of the N capillaries in the laser irradiation portion is defined as R, an inner radius of the N capillaries is defined as r, a refractive index of an external medium is defined as n_1 , a refractive index of a material is defined as n_2 , and a refractive index of an internal medium is defined as n_3 ,

wherein capillary numbers $n = 1, 2, \dots$, and N are assigned to the respective N capillaries in the laser irradiation portion in an arrangement order from one end of

the arrangement,

wherein an average value in $1 \leq n \leq N$ of a second derivative indicating a degree of protrusion of $L(n)$, representing a laser irradiation light intensity of the capillary number n as a function of n , is defined as A , and

wherein an average value in a range of $1 \leq n \leq N$ of a second derivative indicating a degree of protrusion of $H(n)$, representing an output light intensity of the capillary number n by the computer as a function of n when a light emitting substance of equal concentration exists inside the N capillaries in the laser irradiation portion, is defined as B .

[Claim 2]

The capillary array electrophoresis device according to claim 1, wherein the $|A| > |B|$ is realized by at least one of optical system correction by a vignetting effect of the optical system or digital correction by processing of the computer.

[Claim 3]

The capillary array electrophoresis device according to claim 2, wherein the computer is configured to read a correction coefficient for the digital correction from a memory that stores the correction coefficient in advance, and to execute the digital correction.

[Claim 4]

The capillary array electrophoresis device according to claim 1, wherein an average value B of the second derivative of $H(n)$ decreases as a refractive index n_{3_actual} of a separation medium filled in each of the N capillaries becomes larger than a refractive index n_{3_assume} recognized when the computer executes the predetermined processing, and wherein the average value B increases as the refractive index n_{3_actual} becomes smaller than the refractive index n_{3_assume} .

[Claim 5]

The capillary array electrophoresis device according to claim 2, wherein at least a part of a configuration of the optical system is changed according to the refractive index n_3 of a separation medium filled in each of the N capillaries so as to reduce an absolute value $|B|$ of an average value of the second derivative of $H(n)$, and wherein a correction coefficient of the optical system correction is changed.

[Claim 6]

The capillary array electrophoresis device according to claim 2, wherein a correction coefficient of digital correction by the computer is changed according to the refractive index n_3 of a separation medium filled in each of the N capillaries so as to reduce an absolute value $|B|$ of an average value of the second derivative of $H(n)$.

[Claim 7]

A capillary array electrophoresis device comprising:
a laser light source configured to emit a laser beam;
a capillary array configured such that laser irradiation portions of N capillaries collectively irradiated with the laser beam are substantially arranged on a same array plane, where N is an integer of 2 or more;
an optical system configured to collectively measure light emission from the N capillaries; and
a computer configured to execute predetermined processing on light intensity data measured by the optical system,

wherein a sign of an average value in $1 \leq n \leq N$ of the second derivative of the function $H(n)$ changes from positive to negative with an increase in a refractive index n_3 ,

wherein an outer radius of the N capillaries in the laser irradiation portion is defined as R , an inner radius of the N capillaries is defined as r , a refractive index of an external medium is defined as n_1 , a refractive index of a material is defined as n_2 , and a refractive index of an internal medium is defined as n_3 ,

wherein capillary numbers $n = 1, 2, \dots$, and N are assigned to the respective N capillaries in the laser irradiation portion in an arrangement order from one end of the arrangement,

wherein the output light intensity of the capillary

number n by the computer is $H(n)$, and

wherein $H(n)$ is approximated by a function of n when an equal concentration of a light emitting substance exists inside the N capillaries in the laser irradiation portion.

[Claim 8]

The capillary array electrophoresis device according to claim 7, wherein a change in sign of an average value of the second derivative of $H(n)$ occurs within a range of $1.30 \leq n_3 \leq 1.42$.

[Claim 9]

The capillary array electrophoresis device according to claim 7, wherein when the refractive index $n_3 = 1.42$, a sign of an average value of the second derivative of $H(n)$ is negative.

[Claim 10]

The capillary array electrophoresis device according to claim 7, wherein when the refractive index $n_3 = 1.30$, a sign of an average value of the second derivative of $H(n)$ is positive.

[Claim 11]

A capillary array electrophoresis device comprising:
a laser light source configured to emit a laser beam;
a capillary array configured such that laser irradiation portions of N capillaries collectively irradiated with the laser beam are substantially arranged on

a same array plane, where N is an integer of 2 or more;

an optical system configured to collectively measure light emission from the N capillaries; and

a computer configured to output light intensity measured by the optical system,

wherein a separation medium introduced into the capillary array is selected from a plurality of types of separation media having different refractive indexes of $1.33 \leq n_3 \leq 1.41$,

wherein an outer radius of the N capillaries in the laser irradiation portion is defined as R , an inner radius of the N capillaries is defined as r , a refractive index of an external medium is defined as n_1 , a refractive index of a material is defined as n_2 , and a refractive index of an internal medium is defined as n_3 , and

wherein capillary numbers $n = 1, 2, \dots$, and N are assigned to the respective N capillaries in the laser irradiation portion in an arrangement order from one end of the arrangement.

[Claim 12]

The capillary array electrophoresis device according to claim 11, wherein the plurality of types of separation media include a separation medium having a refractive index of $n_3 < 1.36$.

[Claim 13]

The capillary array electrophoresis device according to claim 11, wherein the computer is configured to display a user interface screen for selecting a separation medium to be used from among the plurality of types of separation media on a display screen.

[Claim 14]

The capillary array electrophoresis device according to claim 13, wherein, when an output light intensity of the capillary number n by the computer is defined as $H(n)$, the $H(n)$ is changed when the type of the separation medium selected by the computer is changed in a state where the refractive index n_3 of the separation medium filled in each of the N capillaries is fixed.

[Claim 15]

The capillary array electrophoresis device according to claim 13, wherein, when an output light intensity of the capillary number n by the computer is defined as $H(n)$, and when a second-order coefficient when $H(n)$ is approximated by a second-order function of n is defined as α ,

wherein the second-order coefficient α is positive when the refractive index n_3 of the separation medium selected by the computer is larger than the refractive index n_3 of the separation medium filled in each of the N capillaries, and

wherein the second-order coefficient α is negative

when the refractive index n_3 of the separation medium selected by the computer is smaller than the refractive index n_3 of the separation medium filled in each of the N capillaries.