DESCRIPTION

Title of Invention: CAPILLARY ARRAY ELECTROPHORESIS DEVICE

Technical Field

[0001]

The present disclosure relates to a capillary array electrophoresis device.

Background Art

[0002]

Capillary array electrophoresis devices have been widely used in which a plurality of capillaries made of quartz glass is filled with an electrophoretic separation medium such as an electrolyte solution or an electrolyte solution containing a polymer gel or a polymer, to perform electrophoresis analysis in parallel. As compared with the conventional capillary electrophoresis devices using one capillary, the capillary array electrophoresis devices can reduce an analysis cost per sample as well as can improve an analysis throughput. The most widely used capillary array electrophoresis devices are the 3500 Series Genetic Analyzer and the 3730 Series Genetic Analyzer being sold by Thermo Fisher Scientific Inc. 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, laser irradiation portions of a plurality of capillaries (parts of a capillary array irradiated by laser) are arrayed 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 the plurality of capillaries is referred to as the capillary array. By introducing a laser beam from a side of the array plane during electrophoresis, the plurality of capillaries are simultaneously irradiated. Due to this, emitted fluorescence is spectrally dispersed and simultaneously detected from each of the capillaries induced by the irradiation. A method in which the laser beam is incident from the side of the array plane to simultaneously irradiate the plurality of capillaries is called a multi-focus method. Such a multi-focus method is described in detail in PTL 1. In the multi-focus method, each of the capillaries functions as a convex lens. The laser beam is repeatedly condensed along an array plane to advance in a capillary array, thereby enabling simultaneous irradiation of the plurality of capillaries. As a result, it is possible to perform in parallel DNA sequencing or DNA fragment analysis of samples as many as the number of the capillaries. As described in PTL 1, 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 a medium outside the capillary is n_1, a

refractive index of a medium (separation medium) inside the capillary is n_3, a distance between an incident position of the laser beam and the array plane is x (≤ r), and x = r/2 is defined, in laser irradiation portions of the plurality of capillaries, a refraction angle when the laser beam transmits through one capillary is expressed by the following Formula (1).

[0003]

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

[0004]

Each of the capillaries functions as a concave lens when $\Delta\theta > 0$ is satisfied and as a convex lens when $\Delta\theta < 0$ is satisfied. Under a condition satisfying $\Delta\theta < 0$, the multi-focus functions, and thus it becomes possible to perform the simultaneous irradiation of the plurality of capillaries with the laser beam. On the other hand, under a condition satisfying $\Delta\theta > 0$, because the multi-focus does not function and the laser beam diverges from the array plane, 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 $n_2 = 1.46$. It is understood from Formula (1) that it is more preferable as n_1 is smaller and n_3 is larger in order to enhance a convex lens function (weaken a concave lens function) of each of the capillaries. By contrast, the concave lens function of each of the capillaries

is intensified as n_1 is larger and n_3 is smaller n_3.

[0005]

Even when the multi-focus functions, an intensity of the laser beam attenuates as advancing in the capillary array due to reflection losses of the laser beam at an interface between the medium outside the capillary and the capillary and at an interface between the medium inside the capillary and the capillary. Thus, the obtained fluorescence intensity also attenuates accordingly. If the fluorescence intensity is greatly different between the capillaries, it becomes unable to analyze a plurality of samples under equivalent conditions, which is disadvantageous. (Note that the fluorescence intensity is used as a representative of signal intensities in embodiments to be described later, but a signal intensity other than the fluorescence intensity, for example, a scattering intensity or an absorbance may be used.) In this regard, in the 3500 series genetic analyzer and the 3730 series genetic analyzer, the 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-focus functions for each of the laser beams. In this way, the sum of an intensity of the laser beam incident from one side of the array plane and an intensity of the laser beam incident from the other side of the array plane is made uniform. A configuration in which the laser beam is made incident only from

one side of the array plane is referred to as one-side irradiation. A configuration in which the laser beams are made incident from both the sides of the array plane is referred to as two-side irradiation. Whether the multi-focus functions or does not function is common in both the one-side irradiation and the two-side irradiation.

[0006]

In DNA sequencing or DNA fragment analysis performed by the 3500 Series Genetic Analyzer and the 3730 Series Genetic Analyzer, a polymer solution containing a high concentration of urea as a denaturant is used as a separation medium in order to electrophoretically separate DNA fragments contained in a sample into a single-stranded state. In practice, each of POP-4, POP-6, and POP-7, which are separation media being sold for the 3500 Series Genetic Analyzer and the 3730 Series Genetic Analyzer, contains 8 M urea. A refractive index of water is 1.33, whereas a refractive index of the above-described polymer solution containing 8 M urea increases to $n_3 = 1.41$. This enhances a convex lens function of each capillary, which is an advantageous condition for the multi-focus.

[0007]

With the configuration based on PTL 1, laser irradiation portions of a plurality of capillaries each having an outer diameter $2R = 323$ μm and an inner diameter $2r = 50$ μm are disposed in the air in the 3500 Series Genetic Analyzer. That is, n_1

= 1.00 is satisfied. At this time, since $\Delta\theta = -1.3°$ is obtained from the above Formula (1), it is found that each of the capillaries has the convex lens function. Therefore, the multi-focus functions to enable simultaneous irradiation of 8 or 24 capillaries with a laser beam. In this configuration, however, since a reflection loss of the laser beam at an interface between an air layer outside the capillary and the capillary (quartz glass) is great, the number of capillaries that can be simultaneously irradiated is about 24.

[0008]

In this regard, the number of capillaries that can be simultaneously irradiated is increased in the 3730 Series Genetic Analyzer according to a configuration disclosed in PTL 2. In the 3730 Series Genetic Analyzer, laser irradiation portions of a plurality of capillaries each having an outer diameter $2R = 126\ \mu m$ and an inner diameter $2r = 50\ \mu m$ are disposed in a fluorine solution having a refractive index $n_1 = 1.29$. Since $\Delta\theta = -0.69°$ is obtained from the above Formula (1) at this time, it is found that each of the capillaries has a convex lens function and the multi-focus functions. Furthermore, since a reflection loss of a laser beam at an interface between a fluorine solution layer outside the capillary and the capillary (quartz glass) is reduced, the number of capillaries that can be simultaneously irradiated is increased. Therefore, simultaneous irradiation of 48 or 96 capillaries with a laser

beam is possible.

[0009]

A configuration described in NPL 1 further increases the number of capillaries that can be simultaneously irradiated. In this configuration, laser irradiation portions of a plurality of capillaries each having an outer diameter 2R = 126 μm and an inner diameter 2r = 50 μm are disposed in a matching solution having a refractive index n_1 = 1.46. Further, among the plurality of arrayed capillaries, odd-numbered capillaries from one end are used for analysis (hereinafter, analysis capillaries), and even-numbered capillaries are used as rod lenses (hereinafter, lens capillaries). That is, the analysis capillaries and the lens capillaries are alternately arrayed. A refractive index of a medium (separation medium) inside the analysis capillary is set to n_3 = 1.41. A refractive index of a medium inside the lens capillary is set to n_4 = 1.53. All the capillaries are made of quartz glass and have a refractive index n_2 = 1.46. Further, since a reflection loss of a laser beam at an interface between a matching solution layer outside the capillary and the capillary (quartz glass) becomes zero, the number of capillaries that can be simultaneously irradiated is further increased. Furthermore, NPL 1 describes a definition of a maximum number of capillaries that can be simultaneously irradiated with a laser beam from P. 2874 to P. 2875. When an incident intensity is 100% with one-side

irradiation, the maximum number of capillaries that can be simultaneously irradiated is a number obtained by doubling the number of capillaries until the intensity of the laser beam attenuates to 50%. This is because irradiation intensities of the respective capillaries are expected to be uniform when a capillary array having such a number of capillaries is irradiated on both sides. According to this definition, a maximum number of capillaries in the configuration of PTL 2 is 150, and a maximum number of capillaries in the configuration of NPL 1 is 550.

Citation List

Patent Literature

[0010]

PTL 1: Japanese Patent No. 3654290

PTL 2: Japanese Patent No. 5039156

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 $n_3 = 1.41$. On the other hand, in the capillary electrophoresis device using

one capillary, a separation medium does not necessarily contain a high concentration of urea so that various types of separation media are used. For example, a separation medium for electrophoretically separating DNA fragments into a double-stranded state does not contain urea. The separation medium has a refractive index of $n_3 = 1.33$, which is the same as that of water. That is, in general, a refractive index of a separation medium used in capillary electrophoresis can have various values of $1.33 \leq n_3 \leq 1.41$. In recent years, it has been required to use such various types of separation media in capillary array electrophoresis devices in order to achieve a high throughput or a low cost of the electrophoresis analysis using such various types of separation media.

[0013]

In all of the configurations of the above-described known techniques, however, when $n_3 = 1.33$ is used, the convex lens function of each of the capillaries is lost, the concave lens function becomes stronger, and the multi-focus does not function. That is, it does not become possible to perform the parallel electrophoresis analysis using the plurality of capillaries. Details thereof are as follows.

[0014]

When $n_3 = 1.33$ is satisfied in the 3500 series genetic analyzer based on PTL 1, since $\Delta\theta = +1.3°$ is obtained from Formula (1), it is found that each of the capillaries has the concave

lens function. Therefore, since the multi-focus does not function, it is not possible to perform the simultaneous irradiation of 8 or 24 capillaries with a laser beam.

[0015]

When setting to n_3 = 1.33 in the 3730 series genetic analyzer based on PTL 2, $\Delta\theta$ = +2.9° is obtained from Formula (1) while it is found that each of the capillaries has the concave lens function. Therefore, since the multi-focus does not function, it is not possible to perform the simultaneous irradiation of 48 or 96 capillaries with a laser beam.

[0016]

When setting to n_3 = 1.33 in the configuration based on NPL 1, a refraction angle of one analysis capillary is $\Delta\theta_A$ = +6.6°, whereas a refraction angle of one lens capillary is $\Delta\theta_B$ = -3.0° according to Formula (1). At this time, since $\Delta\theta_A + \Delta\theta_B$ = +3.6° is obtained, one set of one analysis capillary and one lens capillary exhibits the concave lens function and the multi-focus does not function. When setting to n_3 = 1.41 in the configuration of NPL 1, a refraction angle of one analysis capillary is $\Delta\theta_A$ = +2.4°, whereas a refraction angle of one lens capillary is $\Delta\theta_B$ = -3.0° according to Formula (1). At this time, since $\Delta\theta_A + \Delta\theta_B$ = -0.6° is obtained, one set of one analysis capillary and one lens capillary exhibits the convex lens function and the multi-focus functions. In this manner, a method of evaluating the presence or absence of the multi-focus

function by $\Delta\theta_A + \Delta\theta_B$ has been found in the present disclosure. On page 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, a maximum number of capillaries in the case of $n_3 = 1.33$ is only about 8 with reference to FIG. 11 of NPL 1 according to the above-described definition of the maximum number of capillaries in NPL 1. Therefore, the configuration of NPL 1 does not function in the case of $n_3 = 1.33$.

[0017]

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

Solution to Problem

[0018]

In order to solve the above-described problem, 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 as laser irradiation portions of a plurality of capillaries collectively irradiated with the laser beam are substantially

arrayed on the same array plane; and an optical system configured to collectively measure light emission from the plurality of capillaries, wherein $n_1 = 1.00$, $n_2 = 1.46 \pm 0.01$, $n_3 < 1.36$, and $R/r < 5.9$ are satisfied, wherein R denote an outer radius, r denotes an inner radius, n_1 denotes a refractive index of an external medium, and n_2 denotes a refractive index of a material, and wherein n_3 dentoes a refractive index of an internal medium in the plurality of capillaries in the laser irradiation portions.

[0019]

Another characteristic relating to the present disclosure will become apparent from the description of the present specification and the accompanying drawings. Further, aspects of the present disclosure are achieved and realized by elements and combinations of various elements, and the following detailed description and aspects of the appended claims.

It is to be understood that the description in the present specification is merely illustrative and is not intended to limit the scope of the claims or the application of the present disclosure by no means.

Advantageous Effects of Invention

[0020]

According to the technique of the present disclosure, it

is possible to perform the electrophoresis analysis using various separation media having any refractive index in the range of $1.33 \leq n_3 \leq 1.41$ in the capillary array electrophoresis device. In particular, it becomes possible to perform capillary electrophoresis analysis using a separation medium having a low refractive index, which is the same as or close to a refractive index 1.33 of water. This makes it possible to greatly expand an application range of the capillary array electrophoresis device capable of improving an analysis throughput and reducing an analysis cost per sample.

Other objects, configurations, and effects which have not been described above become apparent from embodiments to be described hereinafter.

Brief Description of Drawings

[0021]

[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 view illustrating a configuration

of a capillary array based on PTL 1 and a laser-beam ray tracing result.

[FIG. 5] FIG. 5 is a view illustrating a relative fluorescence intensity distribution of the capillary array based on PTL 1.

[FIG. 6] FIG. 6 is a view illustrating a configuration of a capillary array based on PTL 2 and a laser-beam ray tracing result.

[FIG. 7] FIG. 7 is a view illustrating a relative fluorescence intensity distribution of the capillary array based on PTL 2.

[FIG. 8] FIG. 8 is a view illustrating a configuration of a capillary array of the present disclosure and a laser-beam ray tracing result.

[FIG. 9] FIG. 9 is a view illustrating a relative fluorescence intensity distribution of the capillary array of the present disclosure.

[FIG. 10] FIG. 10 is a view for describing a definition of an array error ΔZ of the capillary array.

[FIG. 11] FIG. 11 is a view for describing a relationship between the array error ΔZ of the capillary array and a production cost.

[FIG. 12] FIG. 12 is a view illustrating a relationship ($n_3 = 1.41$) between the array error ΔZ of the capillary array and the relative fluorescence intensity distribution.

[FIG. 13] FIG. 13 is a view illustrating a relationship (n_3 = 1.41) between the array error ΔZ of the capillary array and the relative fluorescence intensity distribution.

[FIG. 14] FIG. 14 is a view illustrating a relationship (n_3 = 1.33) between the array error ΔZ of the capillary array and the relative fluorescence intensity distribution.

[FIG. 15] FIG. 15 is a view illustrating a relationship (n_3 = 1.33) between the array error ΔZ of the capillary array and the relative fluorescence intensity distribution.

[FIG. 16] FIG. 16 is a view illustrating a relationship (n_3 = 1.41) among the array error ΔZ of the capillary array, a relative fluorescence intensity, and a coefficient of variation.

[FIG. 17] FIG. 17 is a view illustrating a relationship (n_3 = 1.33) among the array error ΔZ of the capillary array, the relative fluorescence intensity, and the coefficient of variation.

[FIG. 18] FIG. 18 is a view illustrating a relationship between an outer diameter of a capillary and the array error ΔZ to be allowed.

Description of Embodiments

[0022]

A technique 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 emitted fluorescence from each of the capillaries during electrophoresis using the plurality of capillaries.

[0023]

(A) Outline of Technique of Present Disclosure

The present disclosure mainly proposes a technique for enabling to use a separation medium having a low refractive index equivalent to a refractive index 1.33 of water or having a refractive index of less than 1.36. In a case where such a separation medium having a low refractive index is used, since multi-focus does not function even if any technique disclosed in known examples (PTLs 1 and 2, and NPL 1) is used, it is difficult to perform simultaneous irradiation of a plurality of capillaries with a laser beam.

[0024]

Further, the present disclosure also proposes a technique for enabling to perform capillary electrophoresis analysis using not only the 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. A maximum number of capillaries that can be simultaneously irradiated is preferably larger, and may be 8 or more, or 24 or more depending on a situation. Among irradiation intensities and

fluorescence intensities of the plurality of capillaries in the same capillary array, the lowest irradiation intensity and fluorescence intensity are preferably higher. When setting to 1 a fluorescence intensity expected when a total intensity of a laser beam oscillated from a laser light source irradiates the inside of one capillary, it is empirically known that practical sensitivity can be obtained when a minimum value MIN (Minimum) of the fluorescence intensity satisfies MIN ≥ 0.2. Further, a variation in the irradiation intensity and fluorescence intensity between the plurality of capillaries in the same capillary array is preferably smaller. It has been empirically known that different samples can be analyzed under equivalent conditions when a coefficient of variation CV in the irradiation intensity and fluorescence intensity is set such that CV ≤ 20% or that CV ≤ 15% depending on a situation. The present disclosure aims to satisfy such practical performances of the capillary array electrophoresis device.

[0025]

As a result of intensive studies for the above-described problem, it has been found that each of the capillaries exhibits a convex lens function and the multi-focus functions since $\Delta\theta = -3.2°$ is obtained from Formula (1) when a capillary outer diameter $2R = 126\ \mu m$, a capillary inner diameter $2r = 50\ \mu m$, the outside of the capillary is air and has $n_1 = 1.00$, a capillary material is quartz glass and has $n_2 = 1.46$, and the inside of

the capillary is a separation medium and has $n_3 = 1.33$ for each of the capillaries in the capillary array. In the 3500 Series Genetic Analyzer based on PTL 1 described above, a difference from the case of $n_3 = 1.33$ is that the outer diameter 2R of the capillary is reduced from 323 μm to 126 μm. As a result, a concave lens function of each of the capillaries is converted into the convex lens function.

[0026]

Conducting further studies, it has been found that $\Delta\theta < 0$ is obtained if the capillary outer diameter 2R is 220 μm or less so that the convex lens functions. As a generalization without limiting to a case where the capillary inner diameter $2r = 50$ μm, it has been found that $\Delta\theta < 0$ is obtained when $R/r \leq 4.4$ so that the convex lens functions. A separation medium having a low refractive index of $n_3 = 1.33$ is not studied in PTL 1. That is, the above is a condition found for the first time according to the technique of the present disclosure.

[0027]

Further, in a case where the capillary outer diameter is set to $2R = 126$ μm and the capillary inner diameter is set to $2r = 50$ μm, it has been found that $\Delta\theta = -3.5°$, $-3.8°$, and $-4.2°$ is obtained according to Formula (1) when a refractive index of the separation medium inside the capillary is $n_3 = 1.34$, 1.35, and 1.36, respectively, so that each of the capillaries exhibits the convex lens function and the multi-focus functions.

Conducting further studies, it has been found that the convex lens functions since $\Delta\theta < 0$ is satisfied if the outer diameter 2R of the capillary is 240 μm or less, 264 μm or less, and 293 μm or less. As a generalization, it has been found that $\Delta\theta < 0$ is obtained when $R/r \leq 4.8$, $R/r \leq 5.3$, and $R/r \leq 5.9$ are satisfied so that the convex lens functions. That is, it has been found that it is preferable to satisfy $R/r < 5.9$ when a separation medium having a low refractive index of less than 1.36 is used. Such a separation medium having a low refractive index is not studied in PTL 1. That is, the above is a condition found for the first time according to the technique of the present disclosure.

[0028]

Conducting experiments under the above-described conditions having the convex lens function, it has been found that there are cases where it is not possible to always realize simultaneous irradiation of the plurality of capillaries with a laser beam, that is, the multi-focus does not always function. Conducting detailed studies, it has been turned out that an array error of the capillary array adversely affects a performance of the multi-focus. In the present specification, an array error ΔZ is defined as follows. First, it is assumed that a Z axis is set in a direction perpendicular to an array plane and that the array plane is located at $Z = 0$ μm. Further, a median value of Z-coordinates of central axes of the plurality

of capillaries in a laser irradiation portion is set to zero. Then, an 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 $\pm \Delta Z$.

[0029]

PTL 1 assumes that there is no array error, that is, $\Delta Z = 0$ μm. However, it is practically not possible to produce a capillary array having $\Delta Z = 0$ μm. Therefore, there are cases where logic is different from experimental results as described above. It is obvious that the adverse effect on the multi-focus performance decreases as the array error ΔZ of the capillary array is smaller. However, production of a capillary array having a smaller array error ΔZ requires a higher cost. Therefore, it is necessary to quantitatively clarify a tolerance of the array error ΔZ of the capillary array in order to obtain the required multi-focus performance.

[0030]

PTL 2 provides a configuration different from the ones of PTL 1 and the technique of the present disclosure in that the outside of a capillary is not air ($n_1 = 1.00$) but a fluorine solution ($n_1 = 1.29$), but describes that $\Delta Z = 6$ μm in the specification. However, since PTL 2 does not teach a relationship between an array error and a multi-focus performance, a condition of the array error for satisfying a

target performance is unknown. Further, it is also unknown whether or not $\Delta Z = 6\ \mu m$ is a condition for satisfying the target performance (the relationship between ΔZ and the performance is not clearly described).

[0031]

NPL 1 provides a configuration different from the ones of PTL 1 and the technique of the present disclosure in that the outside of a capillary is not air ($n_1 = 1.00$) but a matching solution ($n_1 = 1.46$) and that an analysis capillary and a lens capillary are alternately arrayed, but describes that an allowable array error is $\Delta Z \leq 0.3\ \mu m$ in the specification. Here, it is assumed that a maximum value ΔZ of the array error is three times a standard deviation σ of the array error distribution ($\Delta Z = 3\sigma$). However, it is extremely difficult to produce a capillary array having $\Delta Z \leq 0.3\ \mu m$. Further, there is a possibility that a production cost increases even if such a capillary array can be produced.

[0032]

In this regard, the technique of the present disclosure will clarify conditions for causing the multi-focus to function in the capillary array using the separation medium having a low refractive index, after systematically studying the relationship between the array error ΔZ and the multi-focus performance and a relationship between the array error ΔZ and the production cost of the capillary array. That is, in the

capillary array electrophoresis device using the separation medium having a low refractive index, the present disclosure will derive conditions such as a refractive index of a medium outside the capillary array, an outer diameter, an inner diameter, and an array error for obtaining a target multi-focus performance while keeping the production cost of the capillary array within an allowable range. In general, the production cost increases as a production yield decreases. Using as a reference a production cost when the production yield is 100%, the allowable range is a production cost of about twice to three times the reference.

[0033]

Specifically, in order to set a variation in laser beam irradiation efficiency of the respective capillaries such that CV ≤ 20% is obtained while keeping the production cost of the capillary array within twice the reference in a 24-capillary array electrophoresis device, it is preferable to satisfy n_1 = 1.00, n_2 = 1.46, n_3 = 1.33, 2R = 100 to 200 μm, 2r = 50 μm (R/r = 2 to 4), and ΔZ = 9 μm. Alternatively, in order to set a variation in laser beam irradiation efficiency of the respective capillaries such that CV ≤ 15% is obtained while keeping the production cost of the capillary array within twice the reference in a 24-capillary array electrophoresis device, it is preferable to satisfy n_1 = 1.00, n_2 = 1.46, n_3 = 1.33, 2R = 100 to 175 μm, 2r = 50 μm (R/r = 2 to 4), and ΔZ = 6 μm.

[0034]

Hereinafter, each of the embodiments of the present disclosure will be described in detail. Note that the respective embodiments will be separately described below, but techniques described in the respective embodiments are not exclusive and can be combined with each other as appropriate.

[0035]

(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 sequencing and single-stranded DNA fragment analysis performed in the conventional capillary array electrophoresis devices. The present embodiment uses 24 capillaries (however, only four capillaries are illustrated in FIG. 1) to perform DNA sequencing of different samples in each of the capillaries first and then to perform double-stranded DNA fragment analysis of different samples in each of the capillaries. The samples of DNA sequencing include single-stranded DNA fragments having various lengths and labeled with four fluorophores corresponding to four bases. Further, an electrophoretic separation medium, filled in each of the capillaries when

performing the DNA sequencing, is a polymer solution containing 8 M urea as a denaturant. Its refractive index is n_3 = 1.41. On the other hand, the samples of the double-stranded DNA fragment analysis include double-stranded DNA fragments having various lengths and 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. An electrophoretic separation medium, filled in each of the capillaries when performing the double-stranded DNA fragment analysis, is a polymer solution not containing urea as a denaturant. Its refractive index is n_3 = 1.33. One analysis session was performed by the following steps (i) to (vi).

[0036]

(i) First, sample injection ends 2 of 24 capillaries 1 were immersed in a cathode-side buffer solution 6. Sample elution ends 3 were connected to an anode-side buffer solution 7 via a polymer block 9.

[0037]

(ii) Next, a valve 10 of the polymer block 9 was closed. Then, an internal polymer solution was pressurized by pushing down a piston of a syringe 11 connected to the polymer block 9 to fill the inside of each of the capillaries 1 with the polymer solution from the sample elution ends 3 toward the sample injection ends 2.

[0038]

(iii) Subsequently, the valve 10 was opened. Different samples were injected into the respective capillaries 1 from the sample injection ends 2 by an electric field. Thereafter, a high voltage was applied between a cathode 4 and an anode 5 by a power supply 8 to start capillary electrophoresis. DNA fragments labeled with a plurality of types of fluorophores were electrophoresed from the sample injection ends 2 toward the sample elution ends 3.

[0039]

(iv) In parallel, a position of each of the capillaries 1 electrophoresed by a certain distance from the sample injection end 2 was defined as a laser irradiation portion 14. The laser irradiation portions 14 were collectively irradiated with a laser beam 13 oscillated from a laser light source 12 by a multi-focus method. Here, coatings of the respective capillaries 1 near the laser irradiation portion 14 were removed in advance. Respective capillaries 1 near the laser irradiation portion 14 were arrayed on an array plane. The laser beam 13 was condensed and then incident along the array plane from the side of the array plane. Although FIG. 1 depicts that one-side irradiation of the laser beam 13 is performed for the sake of simplicity, two-side irradiation was actually performed by dividing the laser beam 13 into two beams.

[0040]

(v) Then, when the DNA fragments labeled with the plurality of types of fluorophores were electrophoresed inside the respective capillaries 1 and the DNA fragments passed through the laser irradiation portions 14, the labeled fluorophores were excited by the irradiation with the laser beam 13 to emit fluorescence. That is, the plurality of types of fluorophores emitted the fluorescence from 24 light emission points (laser irradiation portions). Ffluorescence intensities thereof changed from moment to moment with the electrophoresis.

[0041]

(vi) Finally, the samples injected into the respective capillaries were studied by detecting multiple colors of fluorescence emitted from each of the light emission points and then analyzing time-series data thus obtained.

[0042]

Although the above steps (i) to (vi) are common between a case of performing DNA sequencing and a case of performing double-stranded DNA fragment analysis, the polymer solution and the buffer solution are appropriately changed. That is, the capillary array electrophoresis device of the present embodiment can switch and execute a plurality of analysis modes having different conditions including, for example, a first analysis mode for the double-stranded DNA fragment analysis and a second analysis mode for the DNA sequencing. In each of the

analysis modes, it is effective to appropriately change conditions of the electrophoresis analysis according to each purpose. The changeable conditions of the electrophoresis analysis include a capillary control temperature, an electric field intensity at the time of electrophoresis, an electric field intensity and a sample injection time at the time of sample injection, a laser irradiation intensity, a sensor exposure time, and the like. It is effective to change the capillary control temperature in each of the analysis modes, for example, by adjusting the temperature of the capillary to 30°C in the first analysis mode and adjusting the temperature of the capillary to 60°C in the second analysis mode, or the like. Note that the terms "first" and "second" are merely added for convenience to distinguish between the analysis modes and the terms do not indicate the order in which the analysis modes are executed. In the above-described example, a refractive index of an electrophoretic separation medium in the DNA fragment analysis is $n_3 = 1.33$. A refractive index of an electrophoretic separation medium in the DNA sequencing is $n_3 = 1.41$. Therefore, the refractive index of the electrophoretic separation medium in the first analysis mode is $n_3 < 1.36$. The refractive index of the electrophoretic separation medium in the second analysis mode is $n_3 \geq 1.36$. Further, an analysis session including the steps (i) to (vi) can be repeated a plurality of times. For example, it is possible to analyze a large number of different

samples by analyzing Samples 1 to 24 in a first analysis session and analyzing Samples 25 to 48 in a second analysis session, and so on. At this time, the DNA sequencing may be repeated using the same polymer solution and buffer solution, or may be switched to the double-stranded DNA fragment analysis in the middle. Any application can be selected in any analysis session.

[0043]

<Configuration Example of Optical System for Fluorescence Detection>

FIG. 2 is a cross-sectional view illustrating 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. Although FIG. 2 depicts one-side irradiation of a four-capillary array, two-side irradiation of a 24-capillary arrays was actually performed, which is similar to FIG. 1. The capillaries 1 arrayed on an array plane were simultaneously irradiated by irradiation of the laser beam 13 with the multi-focus. The laser irradiation portions 14 of the respective capillaries 1 serve as light emission points 20 of fluorescence. Emitted fluorescence 21 from each of the light emission points 20 was collimated collectively by a condenser lens 15 A laser beam was blocked by a laser cut filter 16 and transmitted through

a transmissive diffraction grating 17 to be wavelength-dispersed in a direction of a central axis of each of the capillaries. An imaging point 22 was formed on a sensor 19 by an imaging 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 the imaging points 22 can be simultaneously measured. The respective imaging points 22 are actually wavelength-dispersed in a depth direction in FIG. 2, but a single wavelength part of each of the imaging points 22 is schematically depicted in FIG. 2.

[0044]

In such an optical system, a light collection efficiency decreases as a distance from an optical axis 23 of the optical system increases. This is because a convergence angle of the emitted fluorescence 21 from the light emission points 20 farther from the optical axis 23 becomes smaller due to a vignetting effect of the optical system as illustrated in FIG. 2. Therefore, even if fluorescence of equal intensity is emitted from each of the light emission points 20, a fluorescence intensity of the corresponding imaging point 22 decreases as the light emission point 20 is separated far from the optical axis 23. Any degree of the vignetting effect, that is, an 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 of the imaging points 22 can be calculated from the fluorescence intensity at each of the light emission points 20.

[0045]

<System Configuration Example for Data Analysis and Device Control>

FIG. 3 is a diagram illustrating a configuration example illustrating cooperation between the 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 the capillary array electrophoresis device. The computer not only performs data analysis but also controls the capillary array electrophoresis device. Conditions for the data analysis and conditions for the control of the capillary array electrophoresis device are set through a touch panel, a keyboard, a mouse, or the like which is an input unit. Pieces of time-series raw data of signals output from the sensor are sequentially stored in a memory. Further, analysis parameter information stored in a database in an HDD is stored in the memory. A CPU analyzes the time-series raw data stored in the memory using the analysis parameter information stored in the memory to derive pieces of time-series analysis data. The CPU sequentially stores the pieces of time-series analysis data in the memory and

simultaneously displays the pieces of time-series analysis data on a monitor which is a display unit. Further, an analysis result can be collated with information on a network through a network interface NIF.

[0046]

<Configuration Example of Capillary Array of Related Art (PTL 1)>

FIG. 4(a) is a cross-sectional view illustrating a configuration of a capillary array of the 3500 Series Genetic Analyzer based on PTL 1. Laser irradiation portions of 24 capillaries each having an outer diameter 2R = 323 μm and an inner diameter 2r = 50 μm are arrayed on the same plane at an interval of 370 μm. An array error is zero (ΔZ = 0 μm). The outside of the capillary is air and has n_1 = 1.00. A capillary material is quartz glass and has n_2 = 1.46.

[0047]

FIG. 4(b) is a view illustrating a laser-beam ray tracing result when a laser beam having ϕ 50 μm is applied to one side from the left side in a case where the inside of the capillary is a separation medium having a high refractive index with n_3 = 1.41 under the above-described conditions. Since the multi-focus obviously functions, the insides of all the 24 capillaries have been efficiently irradiated. This corresponds to a result that Δθ = -1.3° according to Formula (1) and each of the capillaries exhibits a convex lens function.

[0048]

On the other hand, FIG. 4(c) is a view illustrating a similar laser-beam ray tracing result in a case where the inside of the capillary is a separation medium having a low refractive index with n_3 = 1.33 under the above-described conditions. Since the multi-focus obviously does not function, the laser beam is diverged from the capillary array and the entire capillary array has not been efficiently irradiated. This corresponds to a result that $\Delta\theta$ = +1.3° according to Formula (1) and each of the capillaries exhibits a concave lens function.

[0049]

<Relative Fluorescence Intensity Distribution According to Capillary Array Configuration of Related Art (PTL 1)>

FIG. 5(a) is a view illustrating relative fluorescence intensities of the respective capillaries obtained when the results of the one-side irradiation illustrated in FIGS. 4(b) and 4(c) are remade into the case of two-side irradiation. Capillary numbers indicate numbers sequentially assigned to the right side with a capillary on the leftmost side in FIGS. 4(a) to 4(c) as 1. The relative fluorescence intensity indicates a fluorescence intensity calculated from an irradiation intensity of each of the capillaries in consideration of a laser beam reflection loss assuming that fluorophores at a constant concentration exist in the laser irradiation portion of each

of the capillaries. A fluorescence intensity, expected when a total intensity of the laser beam oscillated from a laser light source irradiates the inside of one capillary, is set to 1. In the calculation of the two-side irradiation, it is assumed that half of the total intensity of the laser beam is irradiated from two sides of the capillary array. When setting to $n_3 = 1.41$, a result is obtained for the 24 capillaries, that a minimum value of the relative fluorescence intensity is MIN = 0.42 and a coefficient of variation (= standard deviation of relative fluorescence intensity/average value of relative fluorescence intensity) is CV = 11% is obtained. It is found from the result that MIN ≥ 0.2 and CV ≤ 20%, which are practical performances, are satisfied. The reason why the relative fluorescence intensity with respect to the capillary number has a distribution protruding downward is because the intensity of the laser beam attenuates due to a reflection loss as the laser beam travels in the capillary array although the multi-focus is functioning. On the other hand, when setting to $n_3 = 1.33$, MIN = 0.068 and CV = 74% are obtained and it is found that the practical performances are not satisfied.

[0050]

FIG. 5(b) is a view illustrating optical system-corrected relative fluorescence intensities of the respective capillaries obtained by adding a vignetting effect of an optical system of the 3500 Series Genetic Analyzer to the results of

FIG. 5(a), that is, by multiplying the results by an optical system correction coefficient based on the vignetting effect. When setting to n_3 = 1.41, since the distribution protruding downward of the relative fluorescence intensity and a distribution of 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 did not change, but the coefficient of variation significantly decreased to CV = 0.76%. Of course, the practical performances are satisfied. On the other hand, when setting to n_3 = 1.33, MIN = 0.066 and CV = 61% are obtained with little change. The practical performances are still not satisfied.

[0051]

<Configuration Example of Capillary Array of Related Art (PTL 2)>

FIG. 6(a) is a cross-sectional view illustrating a configuration of a capillary array of the 3730 Series Genetic Analyzer based on PTL 2. Laser irradiation portions of 96 capillaries, each having an outer diameter 2R = 126 μm and an inner diameter 2r = 50 μm, are arrayed on the same plane at an interval of 155 μm. An array error is zero (ΔZ = 0 μm). The outside of the capillary is a fluorine solution and has n_1 = 1.29. A capillary material is quartz glass and has n_2 = 1.46.

[0052]

FIG. 6(b) is a view illustrating a laser-beam ray tracing result when a laser beam having ϕ 50 μm is applied to one side from the left side in a case where the inside of the capillary is a separation medium having a high refractive index with n_3 = 1.41 under the above-described conditions. Since the multi-focus functions, the insides of all the 96 capillaries have been efficiently irradiated. This corresponds to a result that $\Delta\theta$ = -0.69° is obtained according to Formula (1) and that each of the capillaries exhibits the convex lens function.

[0053]

On the other hand, FIG. 6(c) is a view illustrating a similar laser-beam ray tracing result in a case where the inside of the capillary is a separation medium having a low refractive index with n_3 = 1.33 under the above-described conditions. Since the multi-focus does not function, the laser beam is diverged from the capillary array and the entire capillary array has not been efficiently irradiated. This corresponds to a result that $\Delta\theta$ = +2.9° is obtained according to Formula (1) and each of the capillaries exhibits a concave lens function.

[0054]

<Relative Fluorescence Intensity Distribution According to Capillary Array Configuration of Related Art (PTL 2)>

FIG. 7(a) is a view illustrating relative fluorescence intensities of the respective capillaries obtained when the

results of the one-side irradiation illustrated in FIGS. 6(b) and 6(c) are remade into the case of two-side irradiation. When setting to $n_3 = 1.41$, a result is obtained for the 96 capillaries, that a minimum value of the relative fluorescence intensity is MIN = 0.63 and a coefficient of variation is CV = 3.2%. It is found from the result that MIN ≥ 0.2 and CV ≤ 20%, which are the practical performances, are satisfied. On the other hand, when setting to $n_3 = 1.33$, MIN = 0.00067 and CV = 192% are obtained and it is found that the practical performances are not satisfied.

[0055]

FIG. 7(b) is a view illustrating optical system-corrected relative fluorescence intensities of the respective capillaries obtained by adding a vignetting effect of an optical system of the 3730 Series Genetic Analyzer to the results of FIG. 7(a), that is, by multiplying the results by an optical system correction coefficient based on the vignetting effect. When setting to $n_3 = 1.41$, the distribution of the relative fluorescence intensity slightly protrudes downward with respect to the capillary number, whereas the optical system-corrected relative fluorescence intensity has a distribution protruding upward. As a result, the minimum value of the fluorescence intensity slightly decreased to MIN = 0.54, and the coefficient of variation slightly increased to CV = 4.6%. However, the practical performances are satisfied. On the

other hand, when setting to n_3 = 1.33, MIN = 0.00066 and CV = 183% are obtained with little change. The practical performances are still not satisfied.

[0056]

<Configuration Example of Capillary Array According to First Embodiment>

FIG. 8(a) is a cross-sectional view illustrating a configuration example of a capillary array according to the first embodiment. Laser irradiation portions of 24 capillaries, each having an outer diameter 2R = 126 μm and an inner diameter 2r = 50 μm, are arrayed on the same plane at an interval of 155 μm. An array error is zero (ΔZ = 0 μm). The outside of the capillary is air and has n_1 = 1.00. A capillary material is quartz glass and has n_2 = 1.46.

[0057]

FIG. 8(b) is a view illustrating a laser-beam ray tracing result when a laser beam having ϕ 50 μm is applied to one side from the left side in a case where the inside of the capillary is a separation medium having a high refractive index with n_3 = 1.41 under the above-described conditions. Since the multi-focus obviously functions, the insides of all the 24 capillaries have been efficiently irradiated. This corresponds to a result that Δθ = -5.8° is obtained according to Formula (1) and each of the capillaries exhibits the convex lens function.

[0058]

On the other hand, FIG. 8(c) is a view illustrating a similar laser-beam ray tracing result in a case where the inside of the capillary is a separation medium having a low refractive index with $n_3 = 1.33$ under the above-described conditions. In this case as well, since the multi-focus functions, the insides of all the 24 capillaries have been efficiently irradiated. This corresponds to a result that $\Delta\theta = -3.2°$ is obtained according to Formula (1) and each of the capillaries exhibits the convex lens function. In this manner, it is hardly realized in any known technique that each of the capillaries exhibits the convex lens function and the multi-focus functions in both the separation medium having a high refractive index ($n_3 \geq 1.36$) and the separation medium having a low refractive index ($n_3 < 1.36$), but the multi-focus by each of the capillaries is realized only by the technique of the present disclosure. That is, in the capillary array electrophoresis device of the present embodiment, the multi-focus functions in both the first analysis mode in which $n_3 < 1.36$ is set and the second analysis mode in which $n_3 \geq 1.36$ is set.

[0059]

<Relative Fluorescence Intensity Distribution According to Capillary Array Configuration of First Embodiment (FIG. 8)>

FIG. 9(a) is a view illustrating relative fluorescence intensities of the respective capillaries obtained when the

results of the one-side irradiation illustrated in FIGS. 8(b) and 8(c) are remade into the case of two-side irradiation. When setting to $n_3 = 1.41$, a result is obtained for the 24 capillaries, that a minimum value of the relative fluorescence intensity is MIN = 0.42 and a coefficient of variation is CV = 11%. It is found from the result that MIN ≥ 0.2 and CV ≤ 20%, which are the practical performances, are satisfied. On the other hand, since MIN = 0.40 and CV = 12% are obtained in the case of $n_3 = 1.33$, it is understood that the practical performances are also satisfied.

[0060]

FIG. 9(b) is a view illustrating optical system-corrected relative fluorescence intensities of the respective capillaries obtained by adding the vignetting effect of the optical system of the 3500 Series Genetic Analyzer to the results of FIG. 9(a), that is, by multiplying the results 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(b), but the relative fluorescence intensity does not change much depending on the presence or absence of the optical system correction, unlike the case of FIG. 5(b). When setting to $n_3 = 1.41$, MIN = 0.42 and CV = 9.0% are obtained. When setting to $n_3 = 1.33$, MIN = 0.40 and CV = 10% are obtained. This is because the vignetting effect of the optical system is small since an overall width of the capillary

array in FIG. 8 is narrow as the interval of 155 μm × (24 - 1) = 3.6 mm, that is, since a distance from an optical axis of each of the capillaries is short, while, by contrast, an overall width of the capillary array in FIG. 4 is the interval of 370 μm × (24 - 1) = 8.5 mm.

[0061]

<Summary of First Embodiment>

From the above, it has become clear that each of the capillaries exhibits the convex lens function and that the multi-focus functions when a separation medium having an arbitrary refractive index of $n_3 \geq 1.33$ including $n_3 = 1.41$ is used in the configuration of the first embodiment. Further, as a modification of the present configuration, each of the capillaries exhibits a convex lens function under the condition of $n_3 \geq 1.33$ when using any capillary having $R/r \leq 4.4$ is used, for example, any capillary having an outer diameter of $2R \leq 220$ μm in a case where an inner diameter $2r = 50$ μm is fixed. Thus, it is possible to cause the multi-focus to function.

[0062]

(C) Second Embodiment

The first embodiment illustrates the result of studies in the case where the capillary array has the array error of zero ($\Delta Z = 0$ μm). However, ΔZ is not 0 μm practically. In this regard, a second embodiment systematically studies a relationship between (i) an array error and (ii) a multi-focus

performance and a relative fluorescence intensity of each of capillaries. Furthermore, the second embodiment also studies a relationship between the array error and a production cost of a capillary array. These studies are conducted for the first time by the technique of the present disclosure.

[0063]

<Definition of Array Error of Capillary Array>

FIG. 10 is a view for describing a definition of an array error ΔZ. FIG. 10(a) is a cross-sectional view illustrating a 24-capillary array when the array error is 0 ($\Delta Z = 0$ μm). This configuration is the same as that of FIG. 8(a). An X axis is set along an array plane. A Z axis is set in a direction perpendicular to the array plane. Further, a Y axis is set in a direction parallel to a central axis of each of the capillaries. The central axis of each of the capillaries is on the X-axis and has a Z-coordinate being zero.

[0064]

On the other hand, FIG. 10(b) is a cross-sectional view illustrating a capillary array when there is an array error ($\Delta Z \neq 0$ μm). X-coordinates of central axes of the respective capillaries are the same as those in the case of FIG. 10(a), but Z-coordinates randomly vary up and down around the X axis ($Z = 0$ μm). Here, ΔZ is a maximum value of absolute values of the respective Z-coordinates. That is, a distance between the X axis and the central axis of the capillary farthest from 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 ± ΔZ. An index quantitatively indicating a magnitude of the array error is denoted by ΔZ.

[0065]

<Relationship Between Array Error of Capillary Array and Production Cost>

FIG. 11 is a view illustrating a result of studying the relationship between the array error ΔZ of the capillary array and the production cost. A structure of a capillary array is the same as that in FIG. 8(a). Laser irradiation portions of 24 capillaries, each having an outer diameter 2R = 126 μm and an inner diameter 2r = 50 μm, are arrayed on the same plane at an interval of 155 μm. The outside of the capillary is air and has n_1 = 1.00. A capillary material is quartz glass and has n_2 = 1.46. Under the above conditions, a large number of capillary arrays were produced such that the array error approaches zero as much as possible. The array error ΔZ of each of the produced capillary arrays was measured using a laser microscope.

[0066]

FIG. 11(a) is a view illustrating a result of fitting the relationship between the array error ΔZ and a production frequency with Gaussian distribution and setting a mode value to 1. The mode value was obtained when ΔZ = 6.1 μm is used.

A standard deviation was 1.6 μm. The number of capillary arrays with ΔZ = 0 μm was substantially zero.

[0067]

FIG. 11(b) is a view illustrating a result of deriving production yields of the capillary arrays with respect to a maximum allowable array error ΔZ from the result of FIG. 11(a). The production yield was 0% when setting to ΔZ ≤ 1.9 μm, 10% when setting to ΔZ = 4.1 μm, 50% when setting to ΔZ = 6.1 μm, 90% when setting to ΔZ = 8.1 μm, and 100% when setting to ΔZ ≥ 10.3 μm,. The production yield increased as ΔZ increased.

[0068]

FIG. 11(c) is a view illustrating a result of deriving production cost reference ratios with respect to the maximum allowable array error ΔZ from the result of FIG. 11(b). The production cost reference ratio indicates which multiple of the production cost to be required when defining a production cost with a yield of 100% as 1. The production cost reference ratio was 1.0 when setting to ΔZ ≥ 11.4 μm, 2.0 when setting to ΔZ = 6.1 μm, 4.1 when setting to ΔZ = 5.0 μm, and 10.5 when setting to ΔZ = 4.0 μm. The production cost reference ratio increased as ΔZ decreased.

[0069]

<Relationship Between Array Error of Capillary Array and Relative Fluorescence Intensity>

FIGS. 12 and 13 are views illustrating relative

fluorescence intensities of capillaries obtained by two-side irradiation when ΔZ is changed based on the configuration in which n_3 = 1.41 is used in the 24-capillary array illustrated in FIG. 8(a). FIG. 12(a) illustrates a case where ΔZ = 0 μm is set, FIG. 12(b) illustrates a case where ΔZ = 3 μm is set, FIG. 12(c) illustrates a case where ΔZ = 6 μm is set. FIG. 13(a) illustrates a case where ΔZ = 9 μm is set. FIG. 13(b) illustrates a case where ΔZ = 12 μmis set. Results of the relative fluorescence intensities obtained for one capillary array for ΔZ = 0 μm and for ten randomly arrayed capillary arrays for ΔZ other than ΔZ = 0 μm are illustrated in an overlapping manner. FIG. 12(a) (ΔZ = 0 μm) illustrates the same result as the case of n_3 = 1.41 in FIG. 9(a). FIG. 13(c) illustrates the relative fluorescence intensities of the respective capillaries of one set for ΔZ = 0 μm. Fig. 13(c) also illustrates averages of the relative fluorescence intensities of the respective capillaries of the ten sets in an overlapping manner for ΔZ other than ΔZ = 0 μm. It is found that, as ΔZ increases, an average value and a minimum value of the relative fluorescence intensities decrease and that the relative fluorescence intensity greatly varies. The minimum values of the relative fluorescence intensities were obtained as MIN = 0.42 when setting to ΔZ = 0 μm, MIN = 0.40 when setting to ΔZ = 3 μm, MIN = 0.33 when setting to ΔZ = 6 μm, MIN = 0.22 when setting to ΔZ = 9 μm, and MIN = 0.066 when setting to ΔZ = 12

μm. Further, coefficients of variation of the relative fluorescence intensities were obtained as CV = 11% when setting to ΔZ = 0 μm, CV = 11% when setting to ΔZ = 3 μm, CV = 12% when setting to ΔZ = 6 μm, CV = 17% when setting to ΔZ = 9 μm, and CV = 28% when setting to ΔZ = 12 μm. Here, the minimum value and the coefficient of variation of the relative fluorescence intensities of the respective capillaries of the one set were illustrated for ΔZ = 0 μm. The minimum values and the coefficients of variation of the relative fluorescence intensities of the respective capillaries of the ten sets were illustrated for ΔZ other than ΔZ = 0 μm.

[0070]

From the above, it has been found that ΔZ ≤ 9 μm is preferable to satisfy MIN ≥ 0.2, which is the practical performance. Further, it has been found that ΔZ ≤ 9 μm or ΔZ ≤ 6 μm is preferable to satisfy CV ≤ 20% or CV ≤ 15% which is the practical performance.

[0071]

FIGS. 14 and 15 are views illustrating relative fluorescence intensities of capillaries obtained by two-side irradiation when ΔZ is changed based on the configuration in which n_3 = 1.33 is used in the 24-capillary array illustrated in FIG. 8(a). FIG. 14(a) illustrates a case where ΔZ = 0 μm is set. FIG. 14(b) illustrates a case where ΔZ = 3 μmis set. FIG. 14(c) illustrates a case where ΔZ = 6 μmis set. FIG. 15(a)

illustrates a case where $\Delta Z = 9$ μmis set. FIG. 15(b) illustrates a case where $\Delta Z = 12$ μmis set. Results of the relative fluorescence intensities obtained for one capillary array for $\Delta Z = 0$ μm and for ten randomly arrayed capillary arrays for ΔZ other than $\Delta Z = 0$ μm are illustrated in an overlapping manner. FIG. 14(a) ($\Delta Z = 0$ μm) illustrates the same result as the case of $n_3 = 1.33$ in FIG. 9(a). FIG. 15(c) illustrates the relative fluorescence intensities of the respective capillaries of one set for $\Delta Z = 0$ μm Fig 15(c) also illustrates averages of the relative fluorescence intensities of the respective capillaries of the ten sets in an overlapping manner for ΔZ other than $\Delta Z = 0$ μm. It is found that, as ΔZ increases, an average value and a minimum value of the relative fluorescence intensities decrease and that the relative fluorescence intensity greatly varies. The minimum values of the relative fluorescence intensities were obtained as MIN = 0.40 when setting to $\Delta Z = 0$ μm, MIN = 0.39 when setting to $\Delta Z = 3$ μm, MIN = 0.30 when setting to $\Delta Z = 6$ μm, MIN = 0.25 when setting to $\Delta Z = 9$ μm, and MIN = 0.058 when setting to $\Delta Z = 12$ μm. Further, coefficients of variation of the relative fluorescence intensities were obtained as CV = 12% when setting to $\Delta Z = 0$ μm, CV = 12% when setting to $\Delta Z = 3$ μm, CV = 14% when setting to $\Delta Z = 6$ μm, CV = 16% when setting to $\Delta Z = 9$ μm, and CV = 28% when setting to $\Delta Z = 12$ μm. Here, the minimum value and the coefficient of variation of the relative fluorescence

intensities of the respective capillaries of the one set were illustrated for $\Delta Z = 0$ μm. The minimum values and the coefficients of variation of the relative fluorescence intensities of the respective capillaries of the ten sets were illustrated for ΔZ other than $\Delta Z = 0$ μm.

[0072]

<Summary of Second Embodiment>

From the above, it has been found that $\Delta Z \leq 9$ μm is preferable to satisfy MIN ≥ 0.2, which is the practical performance. Further, it has been found that $\Delta Z \leq 9$ μm or $\Delta Z \leq 6$ μm is preferable to satisfy CV ≤ 20% or CV ≤ 15% which is the practical performance. These results are the same as the results in the case of $n_3 = 1.41$ illustrated in FIGS. 12 and 13.

[0073]

According to the result of FIG. 11(c), a production cost reference ratio when setting to $\Delta Z = 9$ μm is only 1.04, that is, an increase of 4% from the reference. Further, a production cost reference ratio when setting to $\Delta Z = 6$ μm is 2.10, that is, about twice the reference. Therefore, it is found that these production costs are within the allowable range and that the above-described conditions can be realized.

[0074]

In general, a capillary filled with a separation medium having a refractive index of $n_3 = 1.33$ among separation media

having any refractive index of $n_3 \geq 1.33$ exhibits the weakest convex lens function according to Formula (1). That is, the practical performances are satisfied under any condition of $n_3 \geq 1.33$ as long as it is configured such that the practical performances are satisfied under the condition of $n_3 = 1.33$. Therefore, the above study results are also established when using the separation media having any refractive index of $n_3 \geq 1.33$. Further, as a modification of the present configuration, each of the capillaries exhibits a convex lens function under the condition of $n_3 \geq 1.33$ when using any capillary having $R/r \leq 4.4$, for example, using any capillary having an outer diameter of $2R \leq 220$ μm in a case where an inner diameter $2r = 50$ μm is fixed. Thus, it is possible to cause the multi-focus to function, and the practical performances are satisfied.

[0075]

(D) Third Embodiment

A third embodiment studies in more detail the relationship between the array error, the multi-focus performance, and the relative fluorescence intensity of each of capillaries studied in the second embodiment.

[0076]

<Relationship Between Array Error of Capillary Array and Relative Fluorescence Intensity>

FIG. 16(a) is a view illustrating relative fluorescence intensities of capillaries obtained by two-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 based on the configuration in which n_3 = 1.41 is used in the 24-capillary array illustrated in FIG. 8(a). FIG. 16(c) is a view illustrating coefficients of variation of the relative fluorescence intensities under the same conditions. FIGS. 16(b) and 16(d) are enlarged views of FIGS. 16(a) and 16(c), respectively. One capillary array is used for ΔZ = 0.0 μm, and 100 capillary arrays having random arrays are used for each ΔZ where ΔZ ≠ 0.0 μm is set. In FIGS. 16(a) and 16(b), 24 pieces of relative fluorescence intensity data were used for ΔZ = 0.0 μm, and 2400 pieces of relative fluorescence intensity data were used for each ΔZ where ΔZ ≠ 0.0 μm is set. A black circle plot indicates an average value. An error bar indicates ± standard deviation. A black triangle plot indicates a maximum value. A black square plot indicates a minimum value. As ΔZ increases, all of the average value, the maximum value, and the minimum value decrease, but the standard deviation increases. In FIGS. 16(c) and 16(d), one set of coefficients of variation of relative fluorescence intensities was used for ΔZ = 0.0 μm. 100 sets of coefficients of variation of relative fluorescence intensities were used for each ΔZ where ΔZ ≠ 0.0 μm is set. A black circle plot indicates an average value. An error bar indicates ± standard deviation. A black triangle plot indicates a maximum value. A black square plot indicates a minimum value. As ΔZ increases, all of the

average value, the maximum value and the minimum value increase, and the standard deviation increases.

[0077]

When defining as MIN a minimum value of the relative fluorescence intensities in FIGS. 16(a) and 16(b), it is understood that $\Delta Z \leq 7.2$ μm is preferable to satisfy MIN ≥ 0.2 which is the practical performance. Further, when defining as MIN "average value - standard deviation" of the relative fluorescence intensities in FIGS. 16(a) and 16(b), it is understood that $\Delta Z \leq 14.2$ μm is preferable to satisfy MIN ≥ 0.2 which is the practical performance. On the other hand, when defining as CV "average value + standard deviation" of the relative fluorescence intensities in FIGS. 16(c) and 16(d), it is understood that $\Delta Z \leq 8.2$ μm or $\Delta Z \leq 6.4$ μm is preferable to satisfy CV ≤ 20% or CV ≤ 15% which is the practical performance.

[0078]

FIG. 17 is a view illustrating results in a case where $n_3 = 1.41$ in FIG. 16 is changed to $n_3 = 1.33$. In FIGS. 17(a) and 17(b), as ΔZ increases, all of an average value, a maximum value, and a minimum value decrease, but the standard deviation increases as in FIGS. 16(a) and 16(b). In FIGS. 17(c) and 17(d), as ΔZ increases, all of an average value, a maximum value, and a minimum value increase and a standard deviation increases, as in FIGS. 16(c) and 16(d). When defining as MIN a minimum value of relative fluorescence intensities in FIGS. 17(a) and

17(b), it is understood that $\Delta Z \leq 7.8$ μm is preferable to satisfy MIN ≥ 0.2 which is the practical performance. Further, when defining as MIN "average value - standard deviation" of the relative fluorescence intensities in FIGS. 16(a) and 16(b), it is understood that $\Delta Z \leq 14.8$ μm is preferable to satisfy MIN ≥ 0.2 which is the practical performance. On the other hand, when defining as CV "average value + standard deviation" of the relative fluorescence intensities in FIGS. 16(c) and 16(d), it is understood that $\Delta Z \leq 8.3$ μm or $\Delta Z \leq 5.7$ μm is preferable to satisfy CV ≤ 20% or CV ≤ 15% which is the practical performance. [0079]

As described above, $\Delta Z \leq 7.2$ μm is preferable to satisfy MIN ≥ 0.2, which is the practical performance, when defining as MIN the minimum value of the relative fluorescence intensities in the case of using the separation media having any refractive index of $n_3 \geq 1.33$. A production cost reference ratio for $\Delta Z = 7.2$ μm is only 1.32, that is, an increase of 32% from the reference. When defining as CV "average value + standard deviation" of the relative fluorescence intensities, $\Delta Z \leq 8.2$ μm or $\Delta Z \leq 5.7$ μm is preferable to satisfy CV ≤ 20% or CV ≤ 15% which is the practical performance. A production cost reference ratio is 1.10 when setting to $\Delta Z = 8.2$ μm and is 2.48 when setting to $\Delta Z = 5.7$ μm. These production costs are within the allowable range, and the above conditions can be implemented.

[0080]

<Relationship Between Capillary Outer Diameter and Array Error>

FIG. 18 is a view illustrating results of obtaining a maximum array error ΔZ satisfying CV ≤ 20% or CV ≤ 15%, which is the practical performance as in FIG. 17 when a capillary outer diameter 2R is changed within a range of 75 μm ≤ 2R ≤ 250 μm under the conditions of FIG. 17. An arrangement interval in capillary arrays for each 2R is set to be uniform as 2R + 29 μm. As described above, since each of capillaries exhibits a concave lens function when 2R > 220 μm is obtained according to Formula (1), the multi-focus does not function and thus CV ≤ 20% or CV ≤ 15% is not satisfied. At this time, the array error ΔZ on the vertical axis in FIG. 18 is indicated by zero. From the results of FIG. 18, ΔZ ≤ 9 μm is preferable to satisfy CV ≤ 20%, which is the practical performance, when the capillary outer diameter 2R is appropriately selected within a range of 100 μm ≤ 2R ≤ 200 μm or a ratio R/r of the outer diameter to an inner diameter of the capillary is appropriately selected within a range of 2 ≤ R/r ≤ 4. Further, ΔZ ≤ 6 μm is preferable to satisfy CV ≤ 15%, which is practical performance, when the capillary outer diameter 2R is appropriately selected within a range of 100 μm ≤ 2R ≤ 175 μm, or the ratio R/r of the outer diameter to the inner diameter of the capillary is appropriately selected within a range of 2 ≤ R/r ≤ 3.5. A production cost reference ratio is 1.04 when setting to ΔZ = 9 μm and is 2.10

when setting to $\Delta Z = 6\ \mu m$. These production costs are within the allowable range, and the above conditions can be implemented.

[0081]

As described above, the present disclosure is based on the fact that the insides of all the capillaries constituting the capillary array are filled with the same separation medium having the same refractive index n_3 in a single analysis mode. However, this is not always required. That is, the inside of each of the capillaries constituting the capillary array may be filled with different separation media having different refractive indexes n_3 in a single analysis mode or in single capillary array electrophoresis analysis. This is because each of the capillaries exhibits the convex lens function and the multi-focus functions at least when the refractive indices of the separation media filling the respective capillaries fall within a range of $1.33 \leq n_3 \leq 1.41$. For example, even if using a capillary array, in a single analysis mode, in which capillaries filled with a separation medium having a low refractive index of $n_3 = 1.33$ and capillaries filled with a separation medium having a high refractive index of $n_3 = 1.41$ are alternately arrayed , it is possible to efficiently and simultaneously irradiate with a laser beam all the capillaries constituting the capillary array since each of the capillaries exhibits the convex lens function and the multi-focus functions.

A plurality of the polymer blocks 9 illustrated in FIG. 1 may be used to fill capillaries with different separation media having different refractive indexes n_3. If the sample elution end 3 of each of the capillaries is connected to any one of the plurality of polymer blocks 9, each of the capillaries can be filled with a separation medium having a desired refractive index n_3.

Reference Signs List

[0082]

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 condenser lens

16 laser cut filter

17 transmissive diffraction grating

18 imaging lens

19 sensor

20 light emission point

21 fluorescence

22 imaging point

23 optical axis

CLAIMS

[Claim 1]

A capillary array electrophoresis device comprising:

a laser light source configured to emit a laser beam;

a capillary array configured as laser irradiation portions of a plurality of capillaries collectively irradiated with the laser beam are substantially arrayed on a same array plane; and

an optical system configured to collectively measure light emission from the plurality of capillaries,

wherein $n_1 = 1.00$, $n_2 = 1.46 \pm 0.01$, $n_3 < 1.36$, and $R/r < 5.9$ are satisfied,

wherein R, r, n_1, and n_2 denote an outer radius, an inner radius, a refractive index of an external medium, and a refractive index of a material, respectively and,

wherein n_3 denotes a refractive index of an internal medium in each of the plurality of capillaries in the laser irradiation portions.

[Claim 2]

The capillary array electrophoresis device according to claim 1, wherein $n_3 \leq 1.35$ and $R/r \leq 5.3$ are satisfied.

[Claim 3]

The capillary array electrophoresis device according to claim 1, wherein $n_3 \leq 1.34$ and $R/r \leq 4.8$ are satisfied.

[Claim 4]

The capillary array electrophoresis device according to claim 1, wherein $n_3 = 1.33 \pm 0.01$ and $R/r \leq 4.4$ are satisfied.

[Claim 5]

The capillary array electrophoresis device according to claim 1, wherein $\Delta Z \leq 9$ μm is satisfied,

when defining as $2 \times \Delta Z$, a distance in a direction perpendicular to the array plane between two capillaries, which are farthest from each other in the perpendicular direction among the plurality of capillaries in the laser irradiation portions.

[Claim 6]

The capillary array electrophoresis device according to claim 5, wherein $2 \leq R/r \leq 4$ is satisfied.

[Claim 7]

The capillary array electrophoresis device according to claim 5, wherein $\Delta Z \leq 6$ μm is satisfied.

[Claim 8]

The capillary array electrophoresis device according to claim 5, wherein a condition $2 \leq R/r \leq 3.5$ is satisfied.

[Claim 9]

A capillary array electrophoresis device comprising:

a laser light source configured to emit a laser beam;

a capillary array configured as laser irradiation portions of a plurality of capillaries collectively irradiated with the laser beam are substantially arrayed on a same array

plane; and

an optical system configured to collectively measure light emission from the plurality of capillaries,

wherein $n_1 = 1.00$, $n_2 = 1.46 \pm 0.01$, and $R/r < 5.9$ are satisfied,

wherein an outer radius, an inner radius, a refractive index of an external medium, a refractive index of a material, and a refractive index of an internal medium in each of the plurality of capillaries in the laser irradiation portions are denoted by R, r, n_1, n_2, and n_3, respectively, and

wherein the capillary array electrophoresis device has a plurality of analysis modes including a first analysis mode in which $n_3 < 1.36$ is used and a second analysis mode in which $n_3 \geq 1.36$ is used.

[Claim 10]

The capillary array electrophoresis device according to claim 9, wherein $R/r \leq 5.3$ and $n_3 \leq 1.35$ are satisfied in the first analysis mode.

[Claim 11]

The capillary array electrophoresis device according to claim 9, wherein $R/r \leq 4.8$ and $n_3 \leq 1.34$ are satisfied in the first analysis mode.

[Claim 12]

The capillary array electrophoresis device according to claim 11, wherein $R/r \leq 4.4$ and $n_3 = 1.33 \pm 0.01$ are satisfied

in the first analysis mode.

[Claim 13]

The capillary array electrophoresis device according to claim 9, wherein $\Delta Z \leq 9$ μm is satisfied,

when defining as $2 \times \Delta Z$, a distance in a direction perpendicular to the array plane between two capillaries, which are farthest from each other in the perpendicular direction among the plurality of capillaries in the laser irradiation portions.

[Claim 14]

The capillary array electrophoresis device according to claim 13, wherein $2 \leq R/r \leq 4$ is satisfied.

[Claim 15]

The capillary array electrophoresis device according to claim 13, wherein $\Delta Z \leq 6$ μm is satisfied.

[Claim 16]

The capillary array electrophoresis device according to claim 15, wherein $2 \leq R/r \leq 3.5$ is satisfied.

[Claim 17]

A capillary array electrophoresis device comprising:

a laser light source configured to emit a laser beam;

a capillary array configured as laser irradiation portions of a plurality of capillaries collectively irradiated with the laser beam are substantially arrayed on a same array plane; and

an optical system configured to collectively measure light emission from the plurality of capillaries,

wherein following (i) and (ii) are satisfied:

(i) $n_3 < 1.36$ and

(ii) [Math. 1]

$$-\sin^{-1}\left(\frac{r}{2\cdot R}\right)+\sin^{-1}\left(\frac{r\cdot n_1}{2\cdot R\cdot n_2}\right)-\sin^{-1}\left(\frac{n_1}{2\cdot n_2}\right)+\sin^{-1}\left(\frac{n_1}{2\cdot n_3}\right)<0$$

wherein an outer radius, an inner radius, a refractive index of an external medium, a refractive index of a material, and a refractive index of an internal medium in each of the plurality of capillaries in the laser irradiation portions are denoted by R, r, n_1, n_2, and n_3, respectively, and,

wherein $\Delta Z \leq 9$ μm is satisfied

when defining as $2 \times \Delta Z$, a distance in a direction perpendicular to the array plane between two capillaries, which are farthest from each other in the perpendicular direction among the plurality of capillaries in the laser irradiation portions.

[Claim 18]

The capillary array electrophoresis device according to claim 17, wherein $\Delta Z \leq 6$ μm is satisfied.