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ONO et al.(10) **Pub. No.: US 2017/0010260 A1**(43) **Pub. Date: Jan. 12, 2017**(54) **ANALYSIS DEVICE AND ANALYSIS METHOD****Publication Classification**(71) Applicant: **JVC KENWOOD Corporation**,
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15/1456 (2013.01); **G01N 15/1434** (2013.01);
G01N 2015/0038 (2013.01)(21) Appl. No.: **15/271,328**(22) Filed: **Sep. 21, 2016****Related U.S. Application Data**(63) Continuation of application No. PCT/JP2015/
057303, filed on Mar. 12, 2015.(30) **Foreign Application Priority Data**

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(57)

ABSTRACT

An analysis device optically scans a surface of a substrate to which particles are fixed, detects a pulse wave included in a detection signal obtained from an optical scanning unit when the optical scanning unit scans the substrate, and counts the particles based on pulse interval between two pulse waves each having pulse width less than first reference value determined depending on first pulse width when the optical scanning unit scans a plurality of particles adjacent to each other when the two pulse waves are detected consecutively.

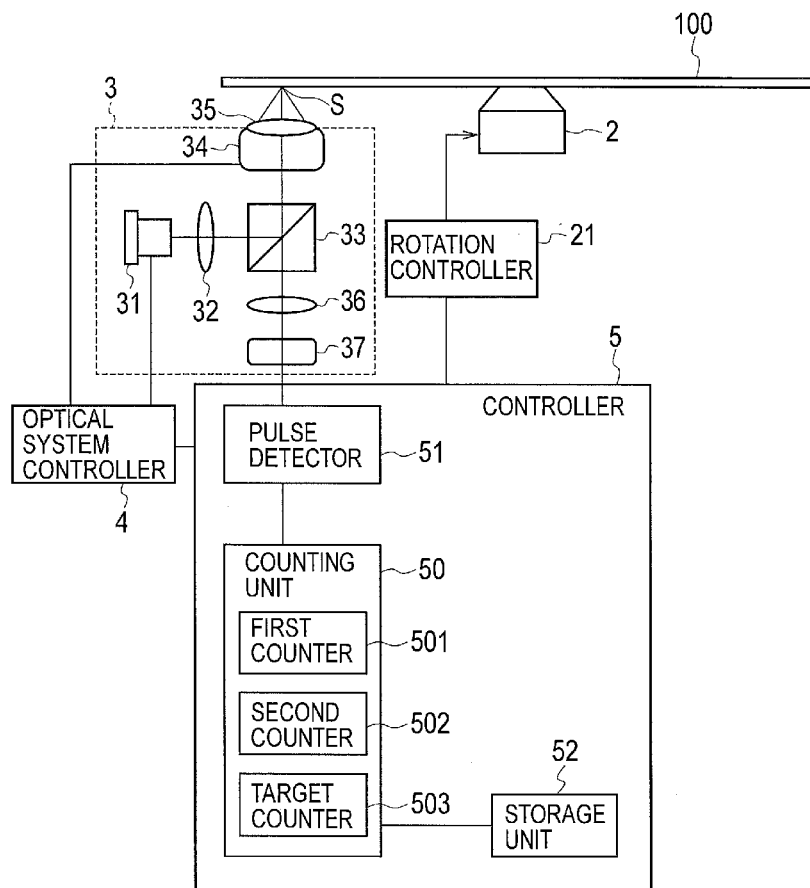
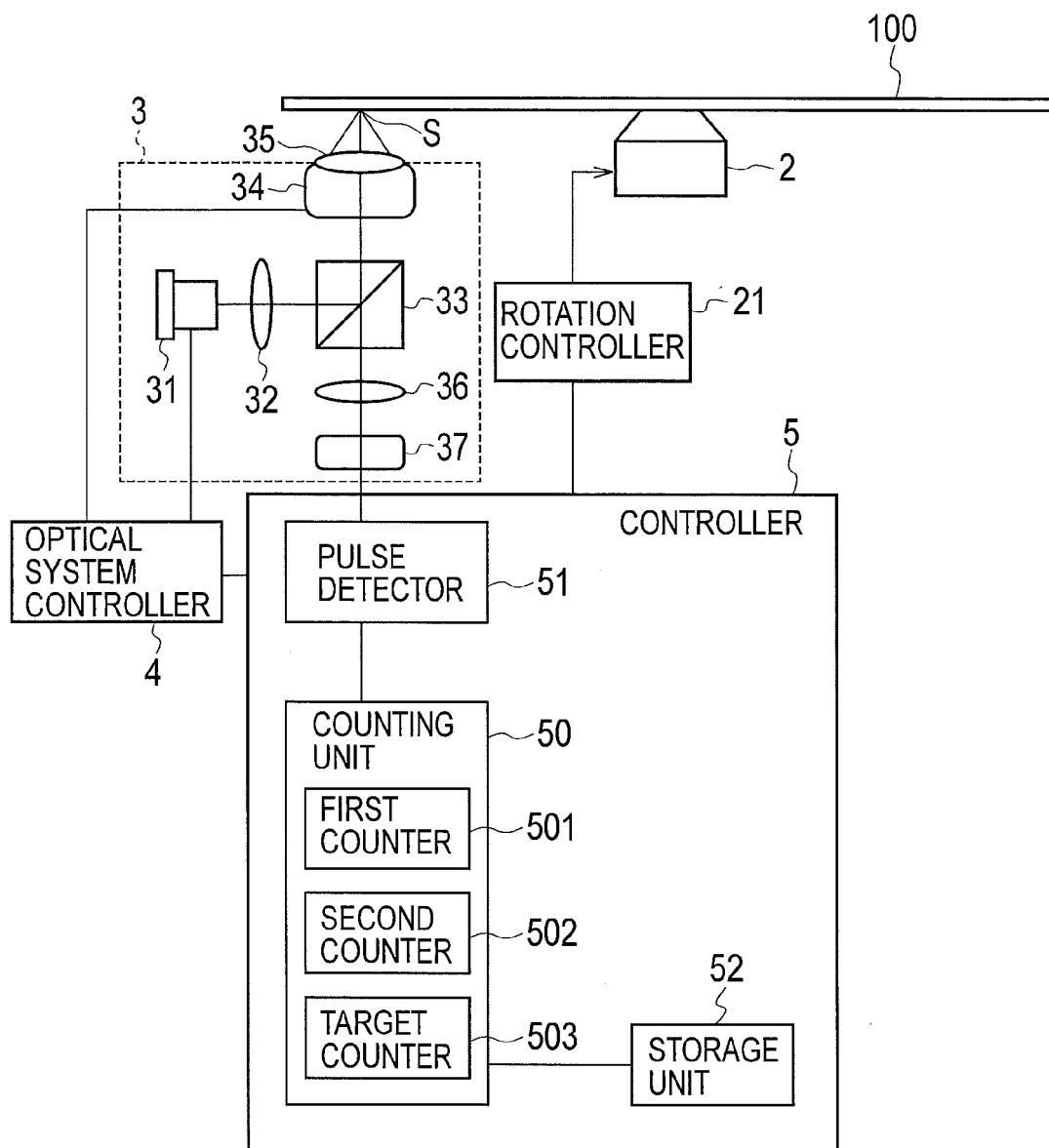


FIG. 1



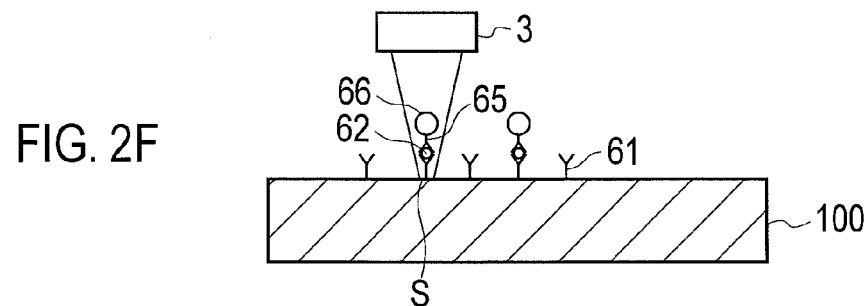
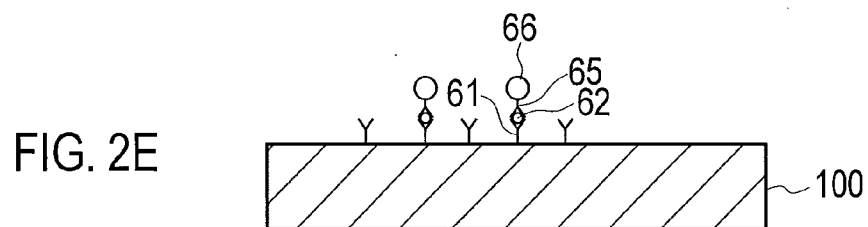
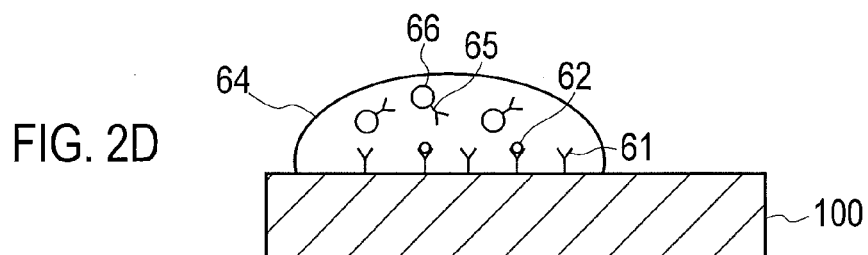
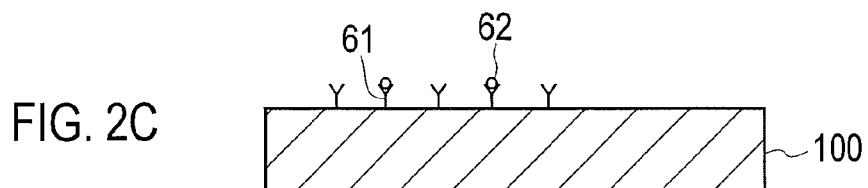
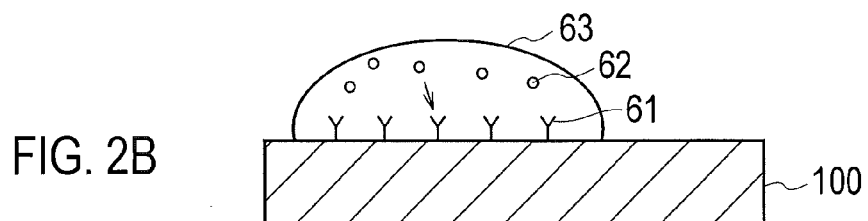
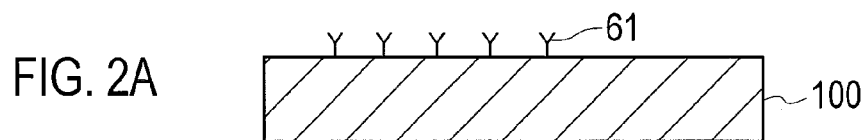


FIG. 3

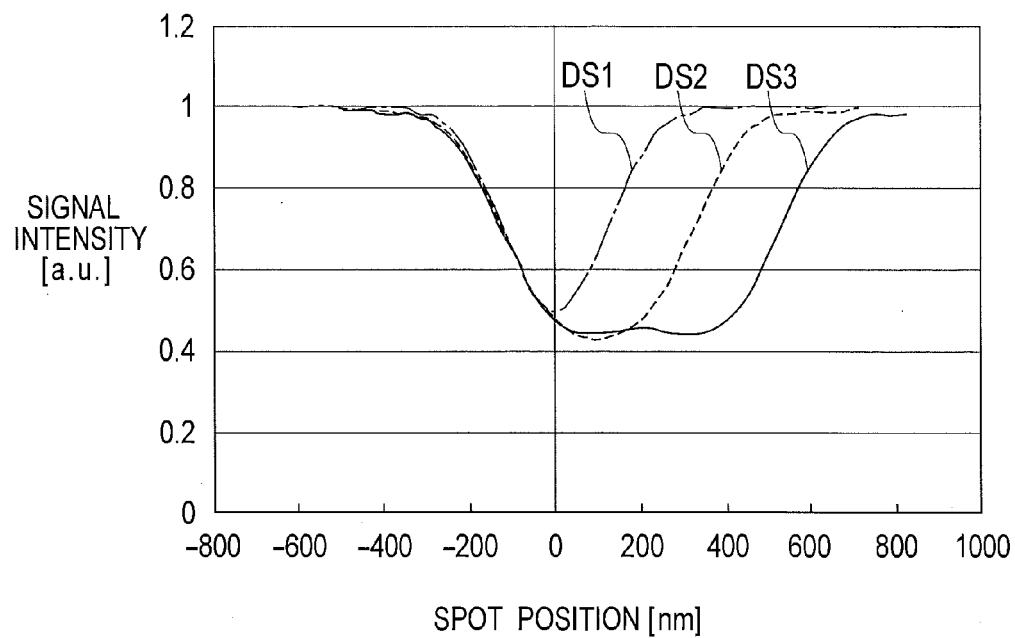


FIG. 4

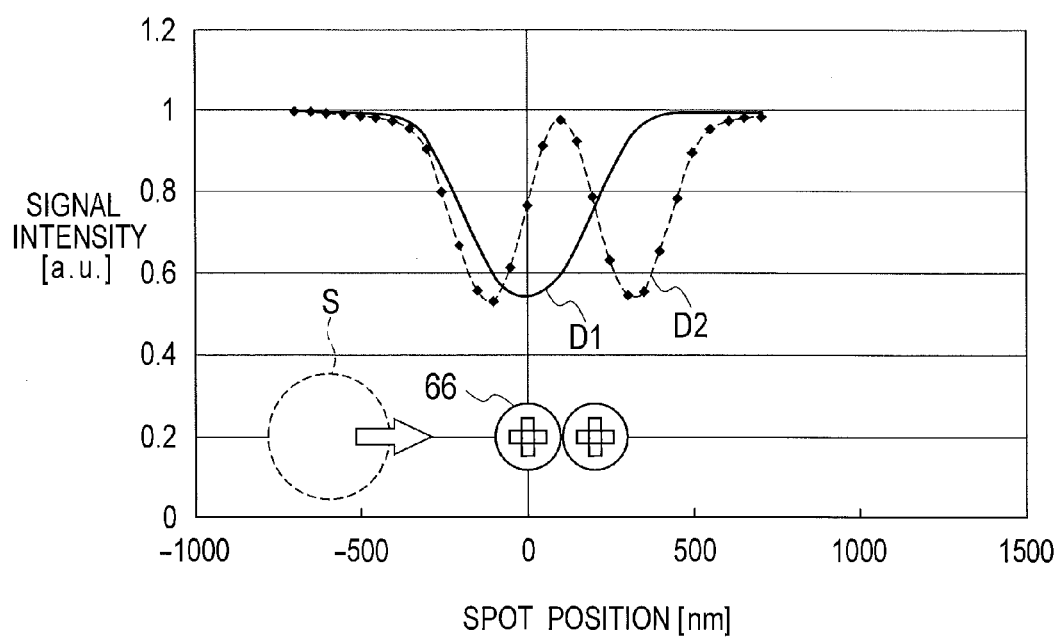


FIG. 5

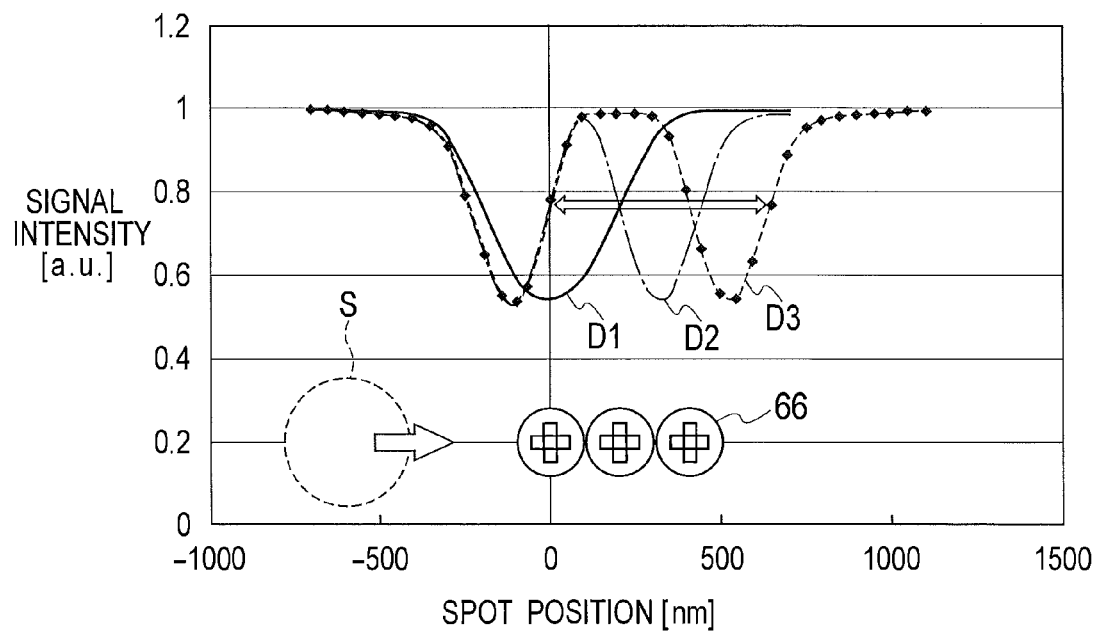


FIG. 6

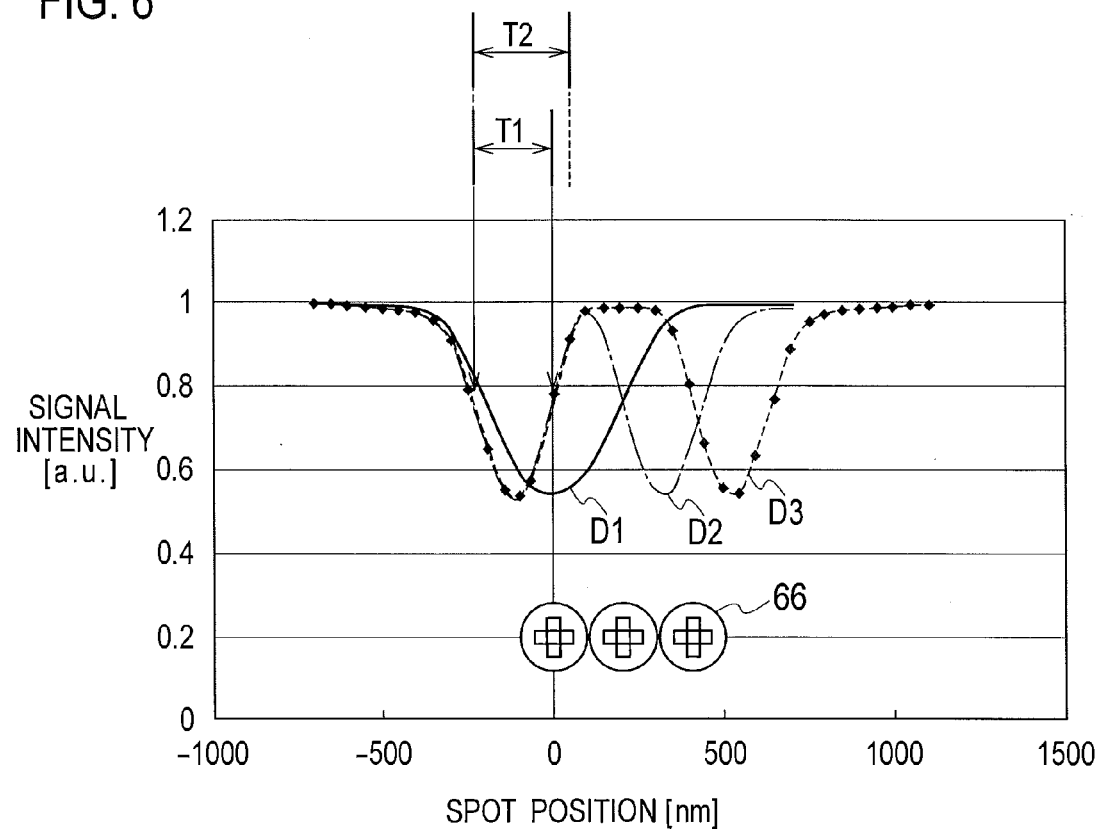


FIG. 7

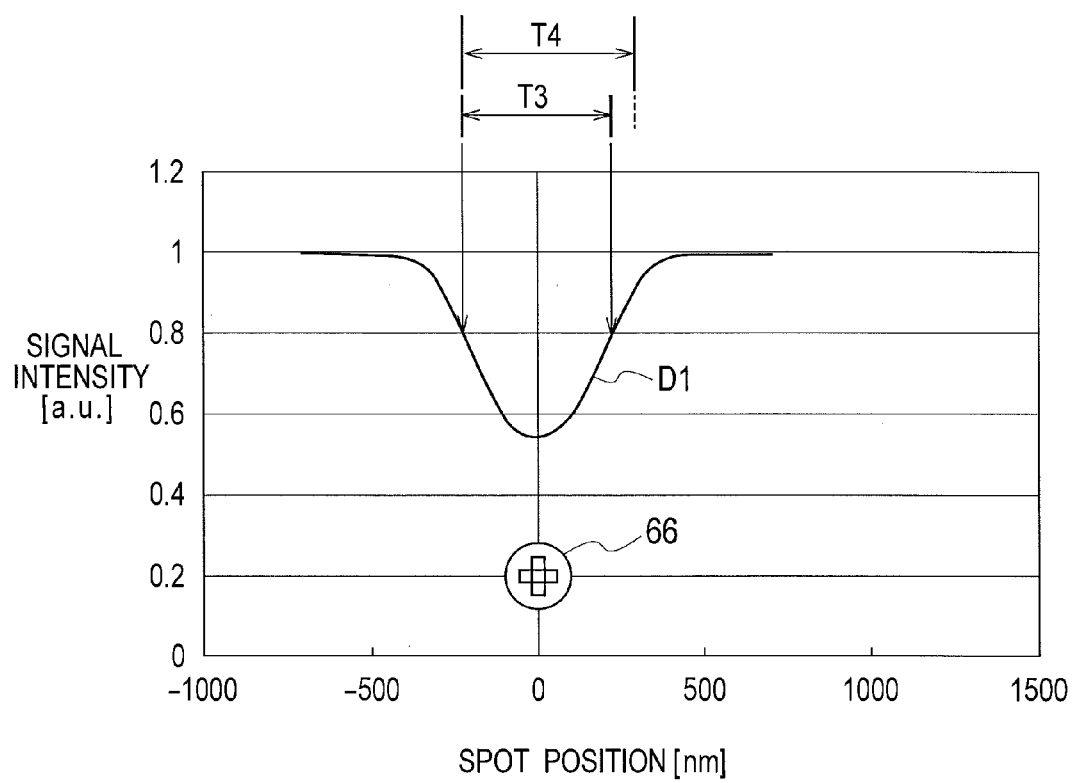


FIG. 8

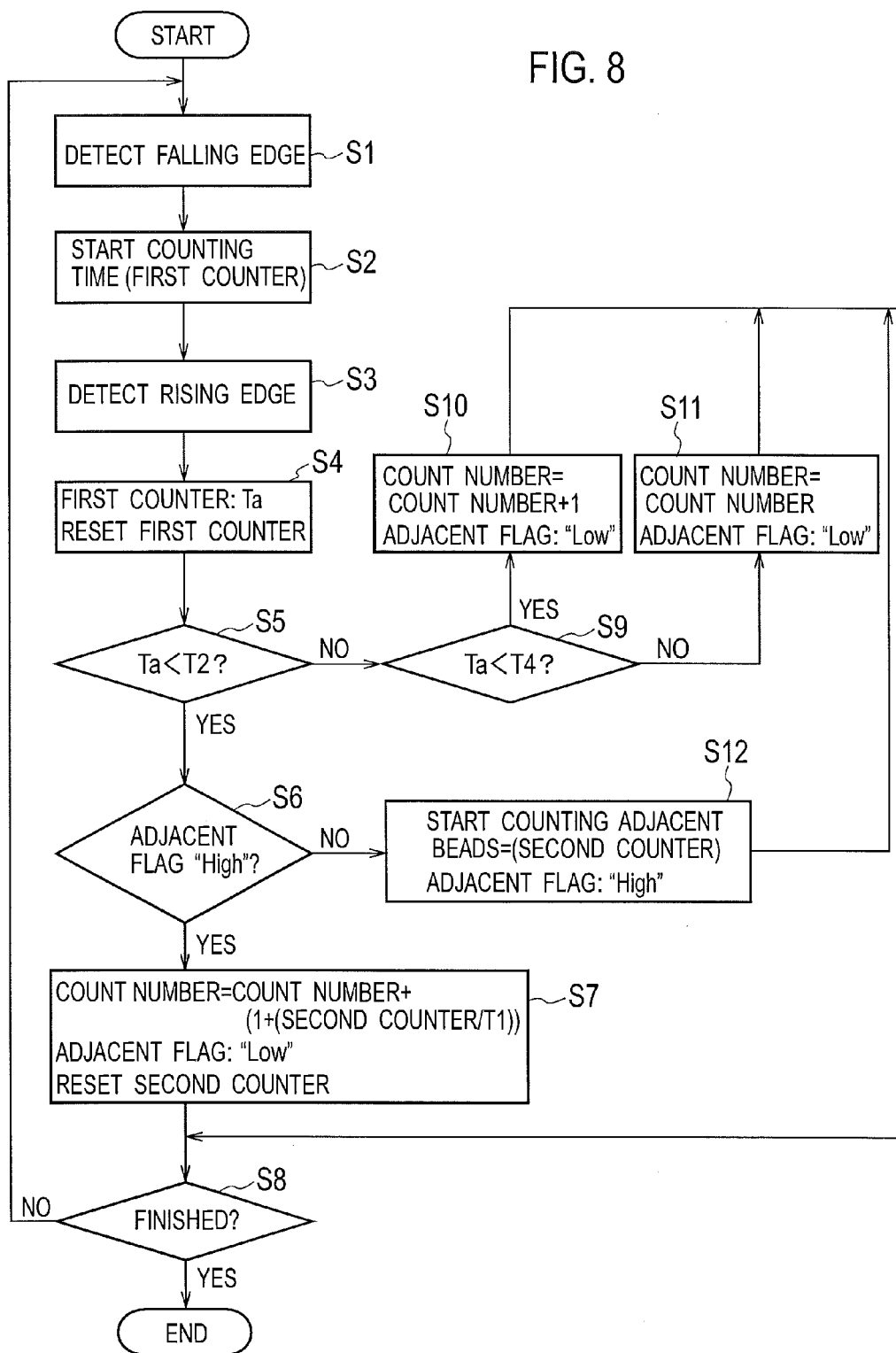


FIG. 9

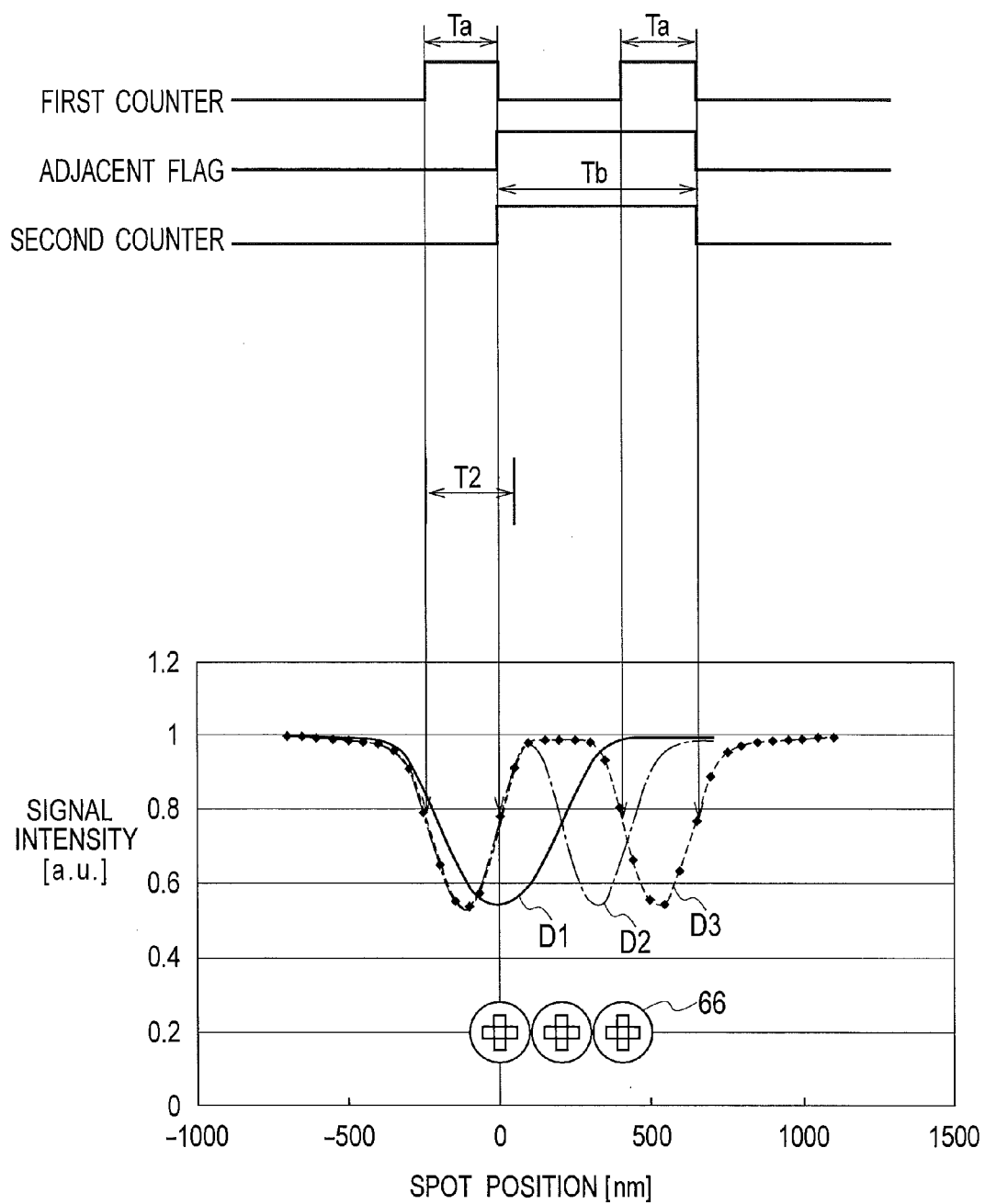


FIG. 10

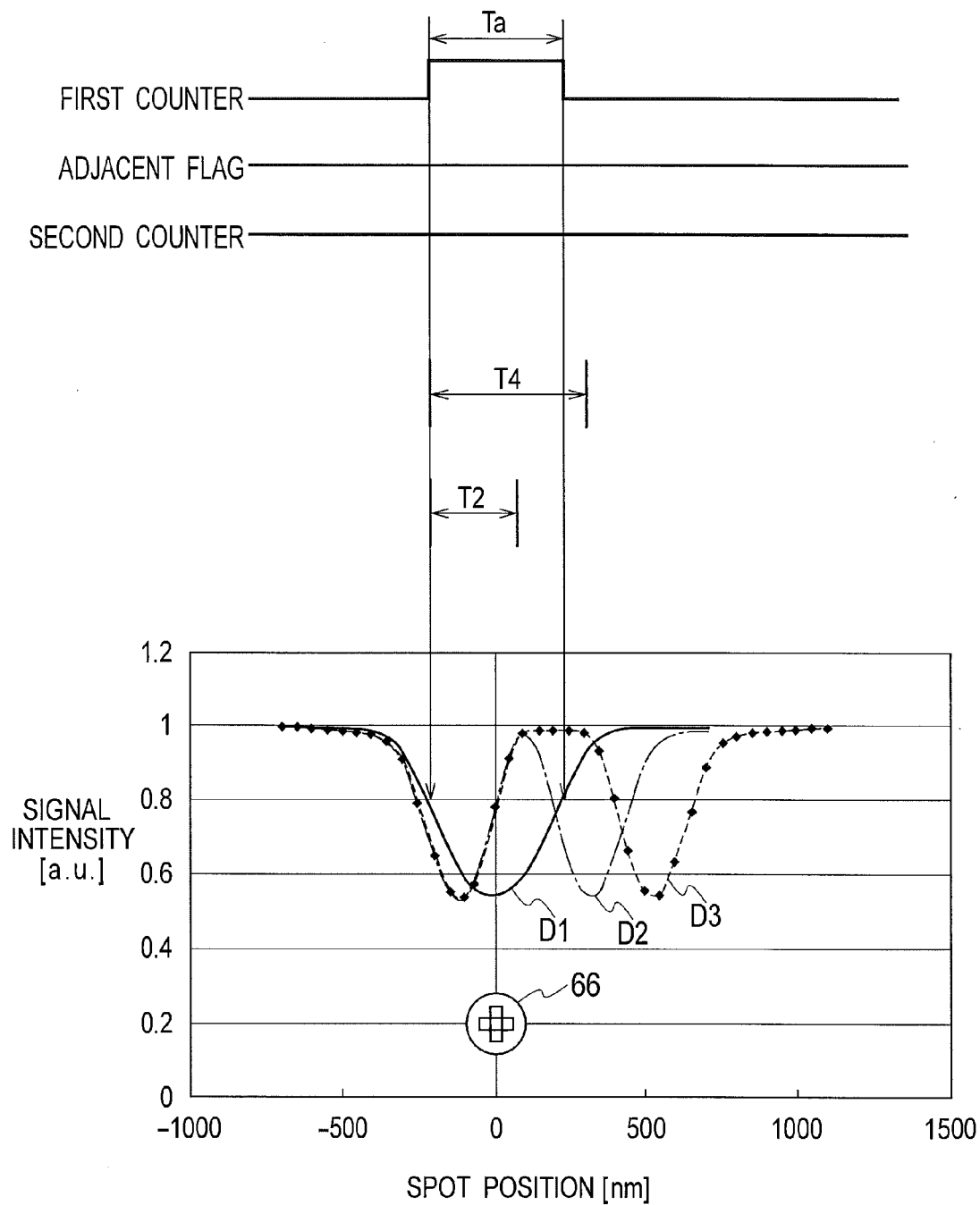
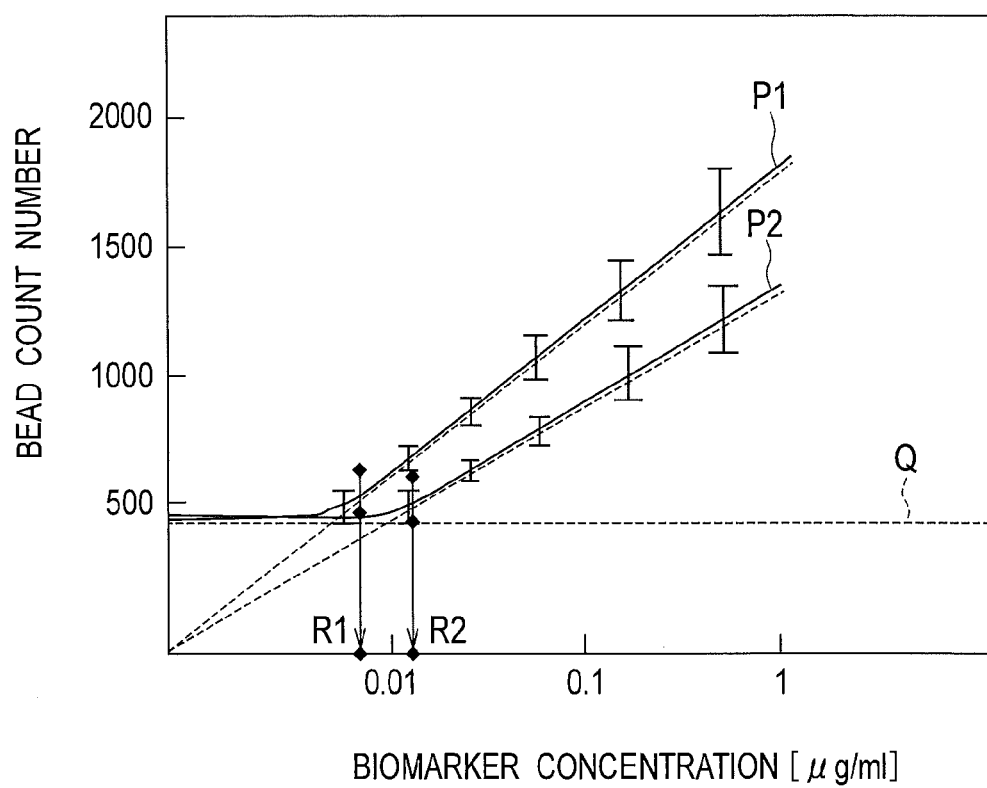


FIG. 11



ANALYSIS DEVICE AND ANALYSIS METHOD

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application is a Continuation of PCT Application No. PCT/JP2015/057303, filed on Mar. 12, 2015, and claims the priority of Japanese Patent Application No. 2014-072536, filed on Mar. 31, 2014, the entire contents of both of which are incorporated herein by reference.

BACKGROUND

[0002] The present disclosure relates to an analysis device and an analysis method for analyzing biomaterials such as antibodies and antigens.

[0003] Immunoassays are known that quantitatively analyze disease detection and therapeutic effects by detecting particular antigens or antibodies as biomarkers associated with diseases. One of the immunoassays is an enzyme-linked immunosorbent assay (ELISA) for detecting antigens or antibodies labeled by enzymes, which is widely used because of having the advantage of low costs. The ELISA requires a long period of time, such as from several hours to a day, to complete a series of multiple steps including pretreatment, antigen-antibody reaction, bound/free (B/F) separation, and enzyme reaction.

[0004] Another technology is disclosed in which antibodies fixed to an optical disc are allowed to bind to antigens in a specimen, and the antigens are further bound to particles having antibodies and then scanned with an optical head, so as to count the particles captured on the disc in a short period of time (Japanese Unexamined Patent Application Publication No. H05-005741). Still another technology is disclosed in which biosamples or particles are adsorbed to a surface of an optical disc on which a tracking structure is formed, so as to detect changes in signal by an optical pickup (Japanese Translation of PCT International Application Publication No. 2002-530786).

SUMMARY

[0005] The technology disclosed in Japanese Unexamined Patent Application Publication No. H05-005741 or Japanese Translation of PCT International Application Publication No. 2002-530786, however, may fail to obtain detection signals corresponding to particles depending on the type and arrangement of the particles used. Such failure leads to inaccurate counting results, which may decrease the performance of quantitative analysis of analytes.

[0006] A first aspect of the present embodiment provides an analysis device including: an optical scanning unit configured to optically scan a surface of a substrate to which particles are fixed; a pulse detector configured to detect a pulse wave and a pulse width of the pulse wave included in a detection signal obtained from the optical scanning unit when the optical scanning unit scans the substrate; and a counting unit configured to count the particles based on a pulse interval between two pulse waves each having a pulse width less than a first reference value when the pulse detector consecutively detects the two pulse waves.

[0007] A second aspect of the present embodiment provides an analysis method including: optically scanning a surface of a substrate to which particles are fixed; detecting a pulse wave and a pulse width of the pulse wave included

in a detection signal obtained by scanning the substrate; and counting the particles based on a pulse interval between two pulse waves each having a pulse width less than a first reference value when the two pulse waves are consecutively detected in the detection signal.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. 1 is a schematic block diagram for describing a fundamental configuration of an analysis device according to an embodiment.

[0009] FIG. 2A to FIG. 2F are enlarged cross-sectional views each schematically showing a substrate of the analysis device according to the embodiment, for describing an example of a method of fixing antibodies, antigens, and beads to the substrate.

[0010] FIG. 3 is a view showing simulation results of detection of adjacent beads while varying the number of beads, for describing characteristics between a spot position and signal intensity when scanning the substrate.

[0011] FIG. 4 is a view for describing characteristics between a spot position and signal intensity when scanning the substrate of the analysis device according to the embodiment, in which adjacent beads are detected while varying the number of beads.

[0012] FIG. 5 is a view for describing characteristics between a spot position and signal intensity when scanning the substrate of the analysis device according to the embodiment, in which adjacent beads are detected while varying the number of beads.

[0013] FIG. 6 is a view for describing a method of determining a reference value stored in a storage unit included in the analysis device according to the embodiment.

[0014] FIG. 7 is a view for describing a method of determining a reference value stored in the storage unit included in the analysis device according to the embodiment.

[0015] FIG. 8 is a flowchart for describing the operation of the analysis device according to the embodiment.

[0016] FIG. 9 is a view for describing the operation of the analysis device according to the embodiment.

[0017] FIG. 10 is a view for describing the operation of the analysis device according to the embodiment.

[0018] FIG. 11 is a view for comparing characteristics of biomarker concentration and counting results of beads in the analysis device according to the embodiment with those in a conventional device.

DETAILED DESCRIPTION

[0019] Hereinafter, an embodiment will be described with reference to the drawings. The same or similar elements shown in the drawings are designated by the same or similar reference numerals below, and overlapping descriptions thereof are not repeated herein.

Analysis Device

[0020] As shown in FIG. 1, an analysis device according to the embodiment includes a substrate 100, a motor 2 that rotates the substrate 100, an optical scanning unit 3 that optically scans the substrate 100, and a controller 5 that controls the motor 2 and the optical scanning unit 3.

[0021] The substrate 100 is formed into a circular shape having substantially the same dimensions as optical discs

such as compact discs (CDs), digital versatile discs (DVDs), and Blu-ray discs (BD). The substrate **100** has a track structure on the surface thereof that the optical scanning unit **3** can scan. The track structure includes, for example, grooves, lands, and pits, and is formed into a spiral extending from the inner side to the outer side. The substrate **100** is formed of a hydrophobic resin material, such as polycarbonate resin and cycloolefin polymer, used for common optical discs. The substrate **100** may be, as necessary, provided with a thin film on the surface thereof, or subjected to surface treatment with a silane coupling agent.

[0022] As shown in FIG. 2A, antibodies **61** specifically binding to antigens **62**, which are biomaterials serving as analytes, are fixed to the surface of the substrate **100**. The antigens **62** are labeled with beads (particles) **66** to which antibodies **65** specifically binding to the antigens **62** are adsorbed, so that the antigens **62** and the beads **66** are correlatively fixed to the surface of the substrate **100**. The antigens **62** are specifically bound to the antibodies **61** and **65**, so as to be used as biomarkers serving as indicators of diseases.

[0023] As shown in FIG. 2A, the antibodies **61** are preliminarily fixed to the surface of the substrate **100**. The antibodies **61** are bound to the surface of the substrate **100** due to hydrophobic binding or covalent binding. The antibodies **61** may be fixed to the surface of the substrate **100** via a substance such as avidin. Then, as shown in FIG. 2B, a sample solution **63** including the antigens **62** is applied dropwise to the surface of the substrate **100**. The antigens **62** move through the sample solution **63** by Brownian motion and come into contact with the antibodies **61**, so as to be specifically bound to the antibodies **61** by an antigen-antibody reaction. As shown in FIG. 2C, the surface of the substrate **100** to which the sample solution **63** is applied dropwise is subjected to spin washing with pure water or the like, so as to remove the sample solution **63** including excessive antigens **62** not bound to the antibodies **61** from the surface of the substrate **100**.

[0024] As shown in FIG. 2D, a buffer solution **64** including the beads **66** is applied dropwise to the surface of the substrate **100**. The buffer solution **64** may be applied while the sample solution **63** remains on the surface of the substrate **100**. The antibodies **65** adsorbed to the beads **66** specifically bind to the antigens **62** by the antigen-antibody reaction. The beads **66** are then bound to the antigens **62**, so as to label the antigens **62**.

[0025] The beads **66** are formed of synthetic resin such as polystyrene including a magnetic material such as ferrite, and formed into a substantially spherical shape. A diameter of the beads **66** is in the range of from several tens of nanometers to several hundreds of nanometers, and a particular example of the diameter is 200 nm. When the buffer solution **64** is applied dropwise, the beads **66** are quickly collected to the surface of the substrate **100** such that a magnet is placed on the opposite side of the surface of the substrate **100**, so as to promote the reaction with the antigens **62**. In addition, the time required to label the antigens **62** fixed to the substrate **100** can be reduced to approximately several minutes such that the antigens **62** and the beads **66** are simultaneously applied to the substrate **100**.

[0026] The antibodies **61** and **65** may be any biomaterials having specificity that specifically bind to the antigens **62**. A combination of the antibodies **61** and **65** is selected such that the antibodies **61** and **65** separately bind to different sites.

For example, when membrane vesicles such as exosomes on which several types of antigens **62** are expressed are used as analytes, the types of the antibodies **61** and **65** are chosen differently from each other, so as to detect a biosample including two types of antigens **62**. The antibodies **61** and **65** are, however, not limited thereto, and may be the same type because exosomes, which are different from typical antigens, include multiple antigens of the same kind of protein on the surface thereof.

[0027] As shown in FIG. 2E, the substrate **100** to which the buffer solution **64** is applied dropwise is washed with, for example, pure water, so as to remove the buffer solution **64** including excessive beads **66** not bound to the antigens **62** from the substrate **100**. As shown in FIG. 2F, the substrate **100** is optically scanned by the optical scanning unit **3**, so as to detect the beads **66** to analyze the antigens **62** labeled with the beads **66**.

[0028] As shown in FIG. 1, the optical scanning unit **3** includes a laser oscillator **31**, a collimator lens **32**, a beam splitter **33**, an actuator **34**, an objective lens **35**, a condensing lens **36**, and a light detector **37**. The optical scanning unit **3** is an optical pickup that optically scans the substrate **100**.

[0029] The laser oscillator **31** emits laser light to the collimator lens **32** according to the control by the controller **5**. The laser oscillator **31** is a semiconductor laser oscillator that emits laser light having, for example, a wavelength of 405 nm which is the same as that for reproduction of BD, and output of about 1 mW. The collimator lens **32** collimates the laser light emitted from the laser oscillator **31**. The beam splitter **33** reflects the laser light collimated by the collimator lens **32** toward the objective lens **35**.

[0030] The objective lens **35** concentrates the laser light transmitted via the beam splitter **33** on the surface of the substrate **100**, to which the antibodies **61** are fixed, due to the operation of the actuator **34** according to the control by the controller **5**, so as to image spot S. The objective lens **35** has a numerical aperture of, for example, 0.85. The laser light concentrated by the objective lens **35** is reflected from the substrate **100** and then reaches the beam splitter **33**. The incident laser light passes through the beam splitter **33** and further reaches the light detector **37** via the condensing lens **36**. The condensing lens **36** concentrates the laser light reflected from the substrate **100** into the light detector **37**. The light detector **37** is, for example, a photodiode to output, to the controller **5**, a detection signal corresponding to the volume of the laser light reflected from the substrate **100**.

[0031] The controller **5** controls the operation of the motor **2** via a rotation controller **21**. The motor **2** is controlled by the controller **5** to rotate the substrate **100** at a constant linear velocity (CLV). The linear velocity is, for example, 4.92 m/s.

[0032] The controller **5** controls the operation of the laser oscillator **31** and the actuator **34** via an optical system controller **4**. The actuator **34** is controlled by the controller **5** to move the optical scanning unit **3** in a radial direction of the substrate **100** so as to spirally scan the surface of the rotating substrate **100**. The controller **5** also detects errors such as focus errors (FE) or tracking errors (TE) from the detection signal output from the light detector **37**. The controller **5** controls the actuator **34** and other components to appropriately scan the surface of the substrate **100** depending on the errors detected.

[0033] The controller **5** includes a pulse detector **51**, a storage unit **52**, and a counting unit **50**. The pulse detector

51 inputs the detection signal output from the light detector **37**. The pulse detector **51** detects a pulse wave and a pulse width of the pulse wave included in the detection signal obtained from the optical scanning unit **3**. The pulse detector **51** is a signal processing device such as a digital signal processor (DSP). The storage unit **52** is a memory such as a semiconductor memory. The storage unit **52** stores reference values corresponding to the pulse wave and the pulse width detected by the pulse detector **51**.

[0034] The counting unit **50** counts the number of beads **66** fixed to the surface of the substrate **100** according to the pulse wave detected by the pulse detector **51** and the reference values stored in the storage unit **52**. The counting unit **50** is, for example, a central processing unit (CPU). The counting unit **50** includes, as a logical structure, a first counter **501**, a second counter **502**, and a target counter **503**.

[0035] The first counter **501** measures pulse width T_a of the pulse wave detected by the pulse detector **51**. The second counter **502** measures, depending on the pulse width T_a of the pulse wave detected by the pulse detector **51**, pulse interval T_b between the pulse wave and a pulse wave subsequently detected. The target counter **503** counts the number of beads **66** according to the measurement results by the first counter **501** and the second counter **502** and the reference values stored in the storage unit **52**.

Reference Values

[0036] FIG. 3 shows simulation results of three detection signals DS1 to DS3 obtained in such a manner as to scan each of one projection pit assuming that one bead is present on the substrate **100**, adjacent two projection pits assuming that two beads are present on the substrate **100**, and adjacent three projection pits assuming that three beads are present on the substrate **100**. The transverse axis represents a position of the spot S corresponding to each front bead **66** in a particular interval, and the vertical axis represents signal intensity obtained such that each signal is normalized by a detection signal detected when there is no bead **66**. The pulse width is assumed to gradually increase as the number of beads **66** increases, as indicated by the detection signal DS1 with one isolated bead **66**, the detection signal DS2 with two beads **66**, and the detection signal DS3 with three beads **66** in this order.

[0037] As shown in FIG. 4, however, a detection signal D2 obtained such that adjacent two beads **66** are actually scanned includes two pulse waves with substantially the same pulse width which is smaller than a pulse width of a detection signal D1 obtained such that one isolated bead **66** is scanned. As shown in FIG. 5, a detection signal D3 obtained such that adjacent three beads are actually scanned includes two pulse waves having substantially the same pulse width as those of the detection signal D2 and having a larger pulse interval than the detection signal D2. A detection signal with adjacent four beads **66** scanned also includes two pulse waves having substantially the same width as those of the detection signal D2 and having a larger pulse interval than the detection signal D3.

[0038] When the diameter of beads **66** is approximately one half of the wavelength of the laser light scanned, and there are a plurality of beads **66** adjacent to each other, the number of beads cannot be counted accurately, which may decrease the performance of quantitative analysis of the analytes. The inventors resolved the effects of light on a structure (particles) with a smaller size than a wavelength of

the light having different pits from common optical discs as described above, by solving Maxwell's equations with regard to times and space variables by a finite-difference time-domain (FDTD) method. The counting unit **50** can count the number of beads **66** with high accuracy when a plurality of beads **66** adjacent to each other are present on the substrate **100**, on the basis of the predetermined reference values stored in the storage unit **52**.

[0039] As shown in FIG. 6, the storage unit **52** preliminarily stores first pulse width T_1 of the respective detection signals D2 and D3 and first reference value T_2 determined depending on the first pulse width T_1 when the optical scanning unit **3** scans a plurality of beads **66** adjacent to each other. The first reference value T_2 is, for example, the sum of the first pulse width T_1 and a predetermined value. The predetermined value added to the first pulse width T_1 may be a jitter value included in the detection signal. The predetermined value added to the first pulse width T_1 may also be approximately 100% to 130% of the jitter value. The first reference value T_2 may be a predetermined percentage of the first pulse width T_1 . For example, the first reference value T_2 is approximately 100% to 130% of the first pulse width T_1 .

[0040] As shown in FIG. 7, the storage unit **52** preliminarily stores second pulse width T_3 of the detection signal D1 and second reference value T_4 determined depending on the second pulse width T_3 when the optical scanning unit **3** scans a bead **66** isolated from other beads **66**. The second reference value T_4 is, for example, the sum of the second pulse width T_3 and a predetermined value. The predetermined value added to the second pulse width T_3 may be a jitter value included in the detection signal. The predetermined value added to the second pulse width T_3 may also be approximately 100% to 130% of the jitter value. The second reference value T_4 may be a predetermined percentage of the second pulse width T_3 . For example, the second reference value T_4 is approximately 100% to 130% of the second pulse width T_3 .

Analysis Method

[0041] An analysis method by the analysis device according to the embodiment is described below with reference to the flowchart shown in FIG. 8, in which the optical scanning unit **3** optically scans the substrate **100**, and the counting unit **50** counts the number of beads **66** fixed to the substrate **100**, so as to analyze the analytes labeled by the beads **66**.

[0042] First, the operator allows the rotation controller **21** and the optical system controller **4** to respectively start operations of the motor **2** and the optical scanning unit **3** according to the control by the controller **5**. The substrate **100** to which antigens **62** and beads **66** are fixed on the surface thereof by the antigen-antibody reaction, is rotated at a constant linear velocity by the motor **2**, so as to be optically scanned by the optical scanning unit **3**. The optical scanning unit **3** detects, with the light detector **37**, the laser light emitted from the laser oscillator **31** and reflected from the surface of the substrate **100**. The light detector **37** outputs a detection signal corresponding to the volume of the detected laser light to the pulse detector **51**.

[0043] In step S1, the pulse detector **51** obtains the detection signal output from the light detector **37** to detect a falling edge of the obtained detection signal. The pulse detector **51** preliminarily holds a threshold set to intensity corresponding to approximately one half of a peak value of

the detection signal detected when beads 66 are scanned, and detects a point where the detection signal falls below the threshold as a falling edge of the detection signal.

[0044] In step S2, the first counter 501 starts measuring time Ta from the point where the falling edge is detected in step S1, as shown in FIG. 9.

[0045] In step S3, the pulse detector 51 detects a rising edge of the detection signal obtained from the light detector 37. The pulse detector 51 preliminarily holds the threshold set to the intensity corresponding to approximately one half of the peak value of the detection signal detected when beads 66 are scanned, and detects a point where the detection signal exceeds the threshold as a rising edge of the detection signal.

[0046] In step S4, the first counter 501 fixes the time Ta from the point where the falling edge is detected in step S1 to the point where the rising edge is detected in step S3, and resets it. The target counter 503 obtains and holds the time Ta fixed by the first counter 501 as a pulse width (half width) Ta of the pulse wave detected in steps S1 to S3.

[0047] In step S5, the target counter 503 reads out the first reference value T2 from the storage unit 52, and determines whether the pulse width Ta held in step S4 is less than the first reference value T2. The target counter 503 sets the process proceeding to step S6 when the pulse width Ta is less than the first reference value T2, or sets the process proceeding to step S9 when the pulse width Ta is greater than or equal to the first reference value T2.

[0048] When the pulse width Ta is less than the first reference value T2 in step S5, the target counter 503 determines whether an adjacent flag is "High" (=1) in step S6. The adjacent flag is a flag set in the target counter 503 in association with the second counter 502. The target counter 503 sets the process proceeding to step S7 when the adjacent flag is "High" in step S6, or sets the process proceeding to step S12 when the adjacent flag is "Low" (=0).

[0049] In the example shown in FIG. 9, it is assumed that the detection signal D3 is input into the pulse detector 51, and the pulse detector 51 detects the rising edge of the first pulse wave in step S3. In such a case, since the adjacent flag is "Low" in step S6, the counting unit 50 sets the process proceeding to step S12.

[0050] In step S12, the second counter 502 starts measuring time Tb from the point where the rising edge is detected in step S3. The target counter 503 sets, in association with the second counter 502, the adjacent flag to "High" from the point where the rising edge is detected in step S3, and the process proceeds to step S8.

[0051] In step S8, the controller 5 determines whether the scanning of the substrate 100 in a predetermined tracking range by the optical scanning unit 3 is finished. The controller 5 ends the process when the scanning is finished, or sets the process returning to step S1 when the scanning is not yet finished.

[0052] In the example shown in FIG. 9, it is assumed that the detection signal D3 is input into the pulse detector 51, and the pulse detector 51 detects the rising edge of the second pulse wave in step S3. In such a case, since the adjacent flag is "High" in step S6, the counting unit 50 sets the process proceeding to step S7.

[0053] In step S7, the second counter 502 determines the time Tb from the point where the first rising edge is detected in step S3 to the point where the second rising edge is detected in the next step S3, and resets it. The target counter

503 obtains and holds the time Tb determined by the second counter 502 as a pulse interval Tb of the two pulse waves detected in the two sets of steps S1 to S3, and sets the adjacent flag to "Low".

[0054] In step S7, the target counter 503 determines that the optical scanning unit 3 has scanned a plurality of beads 66 adjacent to each other, so as to read out the first pulse width T1 from the storage unit 52 to count the number of beads 66 according to $1+(Tb/T1)$. The value obtained from $(Tb/T1)$ is, for example, rounded off to the nearest integer. In the example shown in FIG. 9, when the detection signal D3 is input into the pulse detector 51, the number of beads 66 results in $1+2=3$. As described above, when the two pulse waves each having the pulse width Ta less than the first reference value T2 are detected consecutively, the target counter 503 counts the number of beads 66 according to $1+(Tb/T1)$. The target counter 503 sets the process proceeding to step S8 after step S7.

[0055] When the pulse width Ta is greater than or equal to the first reference value T2 in step S5, the target counter 503 reads out the second reference value T4 from the storage unit 52, and determines in step S9 whether the pulse width Ta held in step S4 is less than the second reference value T4. The target counter 503 sets the process proceeding to step S10 when the pulse width Ta is less than the second reference value T4, or sets the process proceeding to step S11 when the pulse width Ta is greater than or equal to the second reference value T4.

[0056] As shown in the example of FIG. 10, it is assumed that the detection signal D1 is input into the pulse detector 51, and the pulse detector 51 detects the rising edge of the pulse wave in step S3. In such a case, since the pulse width Ta is greater than or equal to the first reference value T2 in step S5, and the pulse width Ta is less than the second reference value T4 in step S9, the counting unit 50 sets the process proceeding to step S10.

[0057] In step S10, the target counter 503 determines that the optical scanning unit 3 has scanned one bead 66 isolated from other beads 66 and therefore the count of beads 66 results in one. Thus, the target counter 503 determines that the number of beads 66 counted is one when the pulse wave having the pulse width Ta greater than or equal to the first reference value T2 and less than the second reference value T4 is detected. The target counter 503 then sets the adjacent flag to "Low", and the process proceeds to step S8.

[0058] When the pulse width Ta is greater than or equal to the second reference value T4 in step S9, the target counter 503 determines in step S11 that the pulse wave having the pulse width greater than or equal to the second reference value T4 is noise derived from foreign substances or aggregations, so as not to consider the pulse wave when implementing counting processing. The target counter 503 then sets the adjacent flag to "Low", and the process proceeds to step S8.

[0059] It is also assumed that the pulse wave having the pulse width Ta less than the first reference value T2 is detected in the first set of steps S1 to S3, and the pulse wave having the pulse width Ta greater than or equal to the first reference value T2 and less than the second reference value T4 is detected in the next set of steps S1 to S3. In such a case, the target counter 503 determines that the pulse wave detected first is noise derived from foreign substances or aggregations, so as not to consider the pulse wave when implementing counting processing.

[0060] As described above, when the pulse wave having the pulse width T_a less than the first reference value T_2 is detected in the detection signal, the target counter **503** adds the number based on the pulse width T_a and the first pulse width T_1 to count up the number of beads **66**. When the pulse wave having the pulse width T_a greater than or equal to the first reference value T_2 and less than the second reference value T_4 is detected in the detection signal, the target counter **503** adds 1 to count up the number of beads **66**.

Comparative Example

[0061] A comparative example in which the counted results of beads **66** obtained by the analysis device according to the embodiment are compared with the counted results obtained by a conventional method, is described below with reference to FIG. **11**. The transverse axis represents biomarker concentration of analytes, and the vertical axis represents the counted results of beads **66**. The counted results obtained by the analysis device according to the embodiment are indicated by the curved line **P1**, and the counted results obtained by the conventional method are indicated by the curved line **P2**.

[0062] The analysis revealed that the count is entirely smaller in the curved line **P2** than the curved line **P1** regardless of the biomarker concentration, in which the maximum difference therebetween is several tens of percent. As indicated by the broken lines along the respective curved lines **P1** and **P2**, when the biomarker content is zero, the count would ideally result in zero. In the detection method by use of the antigen-antibody reaction, however, nonspecific adsorption appears on the substrate **100** other than the binding by the antigen-antibody reaction. Even when the biomarker concentration is zero, the beads **66** fixed to the surface of the substrate **100** due to the nonspecific adsorption are thus inevitably counted.

[0063] In the respective curved lines **P1** and **P2**, the points of contact (points of intersection) between the lower limits of error and the background noise level Q of the respective curved lines **P1** and **P2** are respectively denoted by the limits of detection **R1** and **R2**. The limit of detection **R1** in the analysis device according to the embodiment is improved compared with the limit of detection **R2** in the conventional method, and it is apparent that the sensitivity of the biomarker detection is improved. Accordingly, the analysis device according to the embodiment can improve the sensitivity for detecting diseases.

[0064] The analysis device according to the embodiment varies the number to be added depending on the pulse width of the detection signal to count up the beads **66** when a plurality of beads **66** adjacent to each other are fixed onto the substrate **100**. Therefore, the analysis device according to the embodiment can count the beads **66** with high accuracy to improve the quantitative analysis of analytes even when irregular pulse waves are detected in the detection signal because of arrangement of the beads **66**.

[0065] Further, since the first reference value T_2 and the second reference value T_4 are determined in view of the jitter value of the detection signal, the analysis device according to the embodiment can count the beads **66** with higher accuracy, so as to reduce the influence of jitter when classifying the pulse width T_a .

Other Embodiments

[0066] While the present invention has been described above by reference to the embodiment, the present invention is not intended to be limited to the descriptions and drawings which form part of the disclosure. Various alternative embodiments, examples, and practical applications will be apparent to those skilled in the art from this disclosure.

[0067] For example, in the embodiment described above, the combination of the biomaterials as analytes and specific biomaterials specifically binding to the analytes is not limited to the combination of the antigens **62** and the antibodies and antibodies **65** fixed to the beads **66**. Examples of specifically-binding combinations include a combination of a ligand and an acceptor (such as enzymatic proteins, lectins, and hormones), and a combination of nucleic acids having complementary base sequences to each other.

[0068] Alternatively, a well formed of, for example, silicone rubber may be provided on the surface of the substrate **100**, and the reaction between the target antibodies **61**, antigens **62** and beads **66** and the removal of materials not reacted by washing may be implemented within the well, so as to exclude the steps of, for example, spin washing and drying to simplify the process. Further, a plurality of wells may be provided in the same radius within the allowable area of the substrate **100**, so as to measure a plurality of specimens simultaneously.

[0069] The present invention includes a program for executing, by a computer, the functions of a notifying device according to the embodiment described above. The program may be read out from a storage medium and input into the computer, or may be transmitted via an electrical communication circuit and input into the computer.

[0070] The present invention, of course, includes other embodiments not described in this description, such as embodiments including the above-described configurations mutually applied. Therefore, the scope of the present invention is defined only by the appropriate features according to the claims in view of the explanations made above.

What is claimed is:

1. An analysis device comprising:

an optical scanning unit configured to optically scan a surface of a substrate to which particles are fixed;
a pulse detector configured to detect a pulse wave and a pulse width of the pulse wave included in a detection signal obtained from the optical scanning unit when the optical scanning unit scans the substrate; and
a counting unit configured to count the particles based on a pulse interval between two pulse waves each having a pulse width less than a first reference value when the pulse detector consecutively detects the two pulse waves.

2. The analysis device according to claim 1, wherein, when the two pulse waves each having the pulse width less than the first reference value are consecutively detected by the pulse detector, the counting unit counts the particles based on a number obtained by dividing the pulse interval between the two pulse waves by a first pulse width detected when the optical scanning unit scans the particles adjacent to each other.

3. The analysis device according to claim 2, wherein the first reference value is a sum of the first pulse width and a predetermined value included in the detection signal.

4. The analysis device according to claim 1, wherein the counting unit counts the particles and determines that a

particle count is one when the pulse detector detects a pulse wave having a pulse width greater than or equal to the first reference value and less than a second reference value.

5. The analysis device according to claim 4, wherein the second reference value is a sum of a second pulse width and a predetermined value included in the detection signal.

6. The analysis device according to claim 4, wherein, when the pulse detector detects the pulse wave having the pulse width less than the first reference value and subsequently detects the pulse wave having the pulse width greater than or equal to the first reference value and less than the second reference value, the counting unit does not implement counting processing with regard to the pulse wave having the pulse width less than the first reference value detected first.

7. The analysis device according to claims 4, wherein, when the pulse detector detects a pulse wave having a pulse width greater than or equal to the second reference value, the counting unit does not implement counting processing with regard to the pulse wave having the pulse width greater than or equal to the second reference value.

8. An analysis method comprising:

optically scanning a surface of a substrate to which particles are fixed;

detecting a pulse wave and a pulse width of the pulse wave included in a detection signal obtained by scanning the substrate; and

counting the particles based on a pulse interval between two pulse waves each having a pulse width less than a first reference value when the two pulse waves are consecutively detected in the detection signal.

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