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(54) Title: METHOD AND APPARATUS FOR DIAGNOSING BLOOD PARASITES

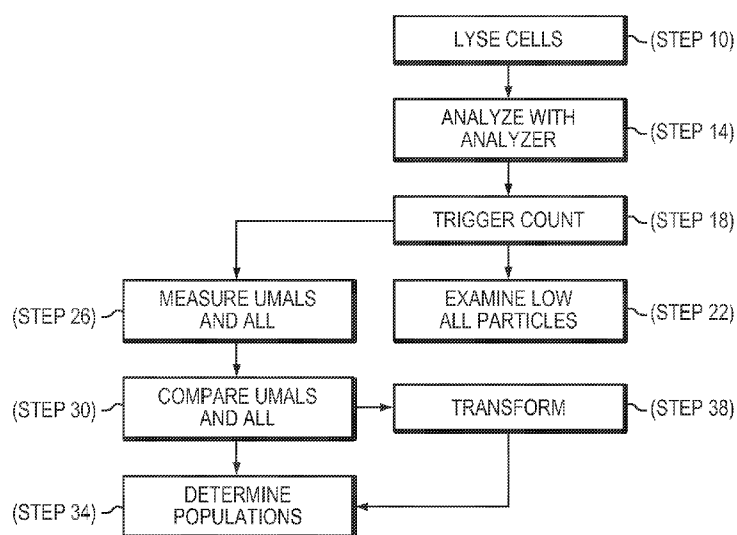


FIG. 2

(57) Abstract: A method and apparatus for detecting parasites in blood samples. The method includes mixing a blood sample with a lytic reagent to lyse red blood cells; measuring particles of the lysed blood sample in a particle analyzer to obtain an axial light loss measurement; and differentiating the parasite particle population in the lysed blood sample in response to the axial light loss measurement. In one embodiment, the method includes measuring light scattering and differentiating a parasite particle population by comparing the light scattering measurement and the axial light loss measurement. The system for detecting a parasite population in a blood sample includes a particle analyzer to obtain an axial light loss measurement; and a computer for using an axial light loss measurement to determine of a parasite particle population.



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METHOD AND APPARATUS FOR DIAGNOSING BLOOD PARASITES

FIELD OF INVENTION

[001] This invention relates to the field of medical diagnostics and more specifically to a method and device for diagnosing infection by a blood parasite.

RELATED APPLICATIONS

[002] This application claims priority to US Provisional Application 61/486,059, which was filed on May 13, 2011 and which is incorporated by reference in its entirety.

BACKGROUND

[003] Parasitic diseases cause death and suffering for many people around the world. One of the major parasitic diseases is malaria. According to the World Health Organization (WHO), malaria is a life-threatening parasitic disease that infects 225 million and kills slightly more than three quarters of a million people each year. A majority of the deaths are children. Most of the victims of this disease live in sub-Saharan Africa, but a significant number of cases occur in Asia, the Middle East, the Americas and Europe.

[004] There are four types of malarial parasites that generally infect humans: *Plasmodium vivax*; *Plasmodium malariae*; *Plasmodium ovale*; and *Plasmodium falciparum*. A fifth type of Plasmodium parasite, *Plasmodium knowlesi*, that causes malaria in monkeys, has been found to infect humans but its prevalence is yet unknown.

[005] With early diagnosis, the disease is treatable using anti-malarial drugs. The World Health Organization (WHO) recommends that prior to treatment, the infection of a patient should be confirmed by a rapid diagnostic test (RDT) or by microscopic blood cell examination. Microscopic examination is slow and labor intensive, requiring highly skilled technicians. RDTs are typically immuno-chromatographic antibody tests, and as a result have issues related to temperature stability, technician skill required and cost.

[006] Attempts to rapidly diagnose malarial infection using cell counters by measuring the white blood cell response to the malarial infection have been somewhat successful. However, it is difficult to establish a reliable relationship between the white cell measurement and the severity of malaria infection. This problem arises because the white cell parameter measured

is the change in white cell volume as lymphocytes and monocytes react to the parasites. Unfortunately, white cell volume does not change immediately when the malaria parasite first infects the patient nor does it immediately return to normal once the parasite is destroyed by treatment. The cell volume change typically lags behind the introduction or removal of the malaria parasites in an unquantifiable fashion. Further, the biological response of lymphocytes and monocytes to the malaria parasites is so complicated that it is hard to reliably quantify the relationship between the change in white cell volume (of lymphocytes and monocytes) with the severity of parasite infection.

[007] What is needed is a rapid diagnostic test for blood parasites that is easily used and interpreted. The present invention addresses this need.

SUMMARY

[008] The disclosed subject matter relates generally to a method and apparatus for detecting parasites in blood samples. In one aspect, the present specification discloses a method for detecting a parasite particle population in a blood sample. In one embodiment the method includes the steps of mixing the blood sample with a lytic reagent to lyse the red blood cells; measuring particles of the mixed blood sample in a particle analyzer to obtain an axial light loss measurement; and differentiating the parasite particle population in the mixed blood sample in response to the axial light loss measurement. In one embodiment, the step of differentiating includes identifying the parasite particle population in the mixed blood sample in response to the axial light loss measurement.

[009] In one embodiment, the differentiated parasite particle population comprises malaria parasites. In another embodiment the method includes performing at least one light scatter measurement and differentiating the parasite particle population in the mixed blood sample in response to the axial light loss measurement and the light scatter measurement. In another embodiment, the method further includes counting the particles in the parasite particle population to determine a coefficient of parasite concentration; and reporting the coefficient of parasite concentration as a count corresponding to a parasite infection. In yet another embodiment, the measuring is part of a nucleated red blood cell (NRBC) analysis. In still yet another embodiment, the at least one light scatter measurement is an upper median angle light scatter measurement.

[0010] In one embodiment, the step of differentiating includes comparing the measured at least one light scatter measurement and the measured axial light loss measurement and detecting

the parasite particle population by gating respective populations indicated by the comparison. In another embodiment, the method further comprises counting the particles in the parasite particle population to determine a first number; counting said particles in other particle populations resulting from the comparison to determine a second number; and reporting a fraction comprising the first number and the second number as an indication of a parasite infection. In yet another embodiment, the parasite particle population has lower values of the at least one light scatter measurement and lower values of said axial light loss measurement than respective white blood cell populations. In still yet another embodiment, the step of identifying includes comparing the at least one light scatter measurement, the axial light loss measurement, and a third measurement, wherein the third measurement is obtained during said particle measurement; and detecting the parasite particle population by gating respective populations indicated by the comparison. In another embodiment, the third measurement is a direct current (DC) measurement.

[0011] In another embodiment, the method for detecting parasite particles using a blood sample includes the steps of mixing said blood sample with a lytic reagent to lyse red blood cells; measuring particles of the mixed blood sample in a particle analyzer to obtain at least one light scatter measurement, an axial light loss measurement, and a volume measurement; differentiating a first particle population corresponding to the parasite particles in the mixed blood sample based upon the at least one light scatter measurement and the axial light loss measurement; differentiating, based upon the volume measurement, a second particle population corresponding to white blood cells affected by said parasite particles; and reporting a parasite infection in response to the differentiated first and second particle populations. In one embodiment, the steps of differentiating include a first particle population corresponding to the parasite particles in the mixed blood sample based upon the at least one light scatter measurement and the axial light loss measurement and/or identifying a second particle population corresponding to white blood cells affected by the parasite particles. In another embodiment, the step of differentiating the second particle population is further based upon a light scatter measurement.

[0012] In still yet another embodiment, a method of detecting parasites in red blood cells includes the steps of lysing the red blood cells in a blood sample in a manner in which the parasites are preserved; directing a beam of light through the lysed blood sample; measuring the axial light loss from the beam of light as caused by the particle; and determining a population of infected cells from the measured axial light loss measurement. In another embodiment the method includes the steps of measuring the light scattered by a particle in the sample within a predetermined angular range as measured from the direction of the beam of

light; and comparing the light scattered by the particle within the predetermined angular range as measured from the direction of the beam of light against the axial light loss for the particle and determining a population of infected cells from the comparison. In yet another embodiment, the count of the number of particles in the population of infected cells is divided by a count of the number of white blood cells

[0013] In another aspect, the disclosed subject matter relates to a system for detecting a parasite particle population in a blood sample comprising: a particle analyzer to obtain an axial light loss measurement; the particle analyzer triggering the measurement of the light scattered from the axial light lost measurement; and a computer for determining a population indicative of a parasite particle. In one embodiment, the particle analyzer further determines a light scatter measurement and the computer compares the light scattering measurement with the axial light loss measurement, wherein the comparison of the light scatter measurement and an axial light loss measurement quantifies the parasite particle population. In one embodiment, the light scattering measurement is made between 20-45 degrees.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The objects and features of the invention can be better understood with reference to the drawings described below. The drawings are not necessarily drawn to scale; emphasis is instead being placed on illustrating the principles of the disclosed subject matter. The drawings associated with the disclosure are addressed on an individual basis within the disclosure as they are introduced.

[0015] Fig. 1A is a schematic diagram of an embodiment of a cell counter known to the prior art and Fig. 1B is a photomicrograph of a group of red blood cells, some of which are infected by a malarial parasite;

[0016] Fig. 2 is an embodiment of a flow diagram describing the steps of an embodiment of the method of the invention;

[0017] Figs. 3A and 3B show example plots of RUMALS versus ALL for patients infected with *P. vivax* and non-infected patients, respectively;

[0018] Figs. 4A and 4B show example plots of RLALS versus ALL for patients infected with *P. vivax* and non-infected patients, respectively;

[0019] Fig. 5A and 5B show example plots of a measure of parasitic load and a measure of CMC and parasite_particle_population%, respectively, over time for a patient being treated for a *P. vivax* malarial infection;

[0020] Fig. 6A and 6B show example plots of a measure of parasitic load and a measure of CMC and parasite_particle_population%, respectively, over time for a patient being treated for a *P. vivax* malarial infection;

[0021] Figs. 7A and 7B depict plots of RLALS and RUMALS respectively plotted against ALL for a blood sample from a patient infected with *P. vivax*;

[0022] Fig. 7C is a scatter plot of RUMALS and ALL for a blood sample from a patient infected with *P. vivax*, including a 1-dimensional histogram on ALL;

[0023] Figs. 7D and 7E depict plots of cell volume (V) plotted against Rotated Light Scatter (RLSn) and opacity (OP) respectively for a blood sample from the same patient as Figs. 7A and B infected with *P. vivax*;

[0024] Figs. 8A, 8B and 8C depict RLALS versus ALL scatter plots from a patient infected with *P. vivax* being treated with an anti-malarial regimen; and

[0025] Figs. 9A and 9B depict RUMALS and RLALS versus ALL scatter plots for a patient with the *P. falciparum* malarial parasite.

DETAILED DESCRIPTION

[0026] The following description refers to the accompanying drawings that illustrate certain embodiments of the disclosed subject matter. Other embodiments are possible and modifications may be made to the embodiments without departing from the spirit and scope of the disclosed subject matter. Therefore, the following detailed description is not meant to limit the disclosed subject matter. Rather, the scope of the disclosed subject matter is defined by the appended claims. This application incorporates by reference in its entirety US 7,256,048, assigned to the assignee of the instant application.

[0027] For the purposes of explanation, and in brief overview, referring to Fig. 1A, a cell analyzer 10 as known to the prior art includes a light source 14 that produces a narrow directed beam of light 18 toward a window 22 in a flow cell 26. In various non-limiting embodiments, the

light source is a laser or a laser diode. A carrier fluid 30 carries individual cells from a blood sample through the flow cell 26 thereby allowing each individual cell 34 to interact with the light beam 18.

[0028] A plurality of photosensors are located adjacent the flow cell 26 so as to record the intensity of light scattered at various angles by cells 34 passing through the flow cell 26. One photosensor 38 is positioned directly in the path of the light beam 18. This photosensor 38 measures Axial Light Loss (ALL). Three groups of photosensors 42, 46, 48 are positioned to collect light scattered by the cells in three predetermined angular ranges as measured from the path of the light beam 18. These angles are chosen to best distinguish the various blood components such as red and white cells. Although the nomenclature is somewhat arbitrary, in one embodiment these predetermined angular ranges are respectively less than 10° (termed Low Angle Light Scatter (LALS)) (photodetector 42); from about 10° to about 20° (termed Lower Median Angle Light Scatter (LMALS)) (photodetector 46) from about 20° to about 42° (termed Upper Median Angle Light Scatter (UMALS)) (photodetector 48) and the sum 47 of the detectors for UMALS and the LMALS (10°-42°) (46 and 48 respectively) signals referred to as MALS (Median Angle Light Scatter). Signals from these detectors are transmitted to a processor (not shown), digitized, analyzed and the results displayed.

[0029] The sensor photosensor 38 directly in the beam path 18 measures the amount of light lost from the light beam 18 each time a cell 34 passes through the beam path 18 between the light source 14 and the photosensor 38. This ALL is an indication of the volume and the absorbance of the cell or particle 34 passing through the flow cell 26. The greater the light loss, the larger the cell or particle 34 and the greater its absorbance. The detected LALS, LMALS, and UMALS light provide details about the structure of the particle or cell.

[0030] Another indicator of cell or particle size is the amount of current flowing between electrodes 52 and electrodes 56 in the flow cell 26. As a particle enters the window region 22 of the flow cell 10, current between the electrodes 52 and 56 changes as the cell or particle 34 blocks the current from flowing. The decrease in the amount of current flowing is related to the cell or particle 34 size. The current may be either direct current (DC) or radio frequency (RF) current supplied by a DC or RF source respectively (not shown). The ratio of RF to DC is given by a function of $f(\text{RF}, \text{DC})$, and is termed opacity. In one embodiment, the opacity is given by the expression:

$$\text{Opacity} = f(\text{RF} / \text{DC})$$

where f is a function that deals with shifting and scaling of the data.

[0031] Further, the pulse from the current measurement may, in some embodiments, be used to trigger data collection from the photodetectors.

[0032] The inventors have discovered that in malarial infection there is a population of particles characterized by the ALL. This ALL is indicative of small size and absorbance. The existence of a population of these small size particles as measured by ALL is suggestive of parasitic infection. Thus, by simply measuring the ALL in a sample of lysed blood, one can quickly use this technique to screen a population of patients. Fig. 1B is a photomicrograph of a slide of red blood cells, some of which are infected by malarial parasites. The malarial parasite appears as a dark particle in the red blood cell. After the lysing process, the parasites are preserved and will become evident by measuring the properties of the lysate particles, such as size, density, light scatter and absorbance. Parasites are distinguished from the other cell populations in this manner.

[0033] While quantitative detection of ALL alone may be used in screening for malarial infection, a quantitative measurement of malarial infection can be calculated by measuring the ALL and the UMALS and comparing, for example by plotting, the values against one another. In one embodiment of the method (Fig. 2), the blood sample from a patient is first lysed (Step 10) to remove the red blood cells.

[0034] The lysing step includes forming a 2% solution by diluting, in one embodiment, 14 μ L of an anticoagulated whole blood sample (peripheral blood), with 614 μ L of an isotonic blood diluent, COULTER[®] DxH Diluent[™] (product of Beckman Coulter, Inc., Miami, FL), and mixing the diluted sample 5:1 in one embodiment with 125 μ L of a lytic reagent. About nine seconds after the addition of the lytic reagent the sample mixture is delivered to a flow cell with a sheath fluid, COULTER[®] DxH Diluent[™]. An embodiment of a lytic reagent is an aqueous solution containing active components for lysing red blood cells: in one embodiment, 36 g/L dodecyltrimethylammonium chloride (50% solution), 3.6 g/L tetradecyl-trimethylammonium bromide, and having a pH of about 4.

[0035] The sample is then analyzed by a hematology analyzer (Step 14). In one embodiment, the hematology analyzer is a Beckman Coulter DxH800 hematology analyzer, see, e.g., US 8,094,299, assigned to the assignee of the instant application, and which is hereby incorporated by reference in its entirety. The analyzer may be configured to measure DC and/or RF current, and several light signals, including ALL, LALS, LMALS, and UMALS as each blood cell passes through the flow cell. In one embodiment, the count is triggered by the detection of ALL

particles or other trigger means (Step 18). Then the particles are examined to see the amount of particles corresponding to ALL.

[0036] If quantification is desired, in one embodiment, the analyzer is configured to take angular light scattering measurements when an electric pulse is generated by the ALL photosensor 38 (Step 18) and not when the DC current indicates a particle. In one embodiment, the UMALS and ALL are measured (Step 26) and compared (in one embodiment, plotted) (Step 30). Using this technique, a new population of particles (which is termed a parasite particle population, as explained below) is differentiated (Step 34) and separated from other populations measured by the analyzer. The size of the parasite particle population is then determined to indicate the presence of and severity of a malarial infection.

[0037] In one embodiment, to help distinguish this parasite particle population from other populations measured by the analyzer, the UMALS measurement is transformed using a non-linear function (Step 38) prior to the determination of populations. The transformed value is termed rotated UMALS (RUMALS) and is given by a function of $f(\text{DC}, \text{UMALS})$. In one embodiment, RUMALS is given by the expression:

$$\text{RUMALS} = (C) \text{ARCTAN}(\text{DC} / \text{UMALS})$$

where (C) is a scaling factor, and (DC) is the DC current value. Other types of transformations well known to those skilled in the art may be used to accomplish the same effect.

[0038] This arctangent function attempts to decorrelate the volume measurement as indicated by the DC current value and UMALS value by causing a logarithmic compression of the value of the DC/UMALS ratio that is proportional to a transformation function of these physical quantities. The result of this transformation is shown in the graph of RUMALS against ALL for an infected individual as shown in Fig. 3A. The parasite particle population is shown in the lower-left corner of the plot. This is in contrast to the lower-left region in the RUMALS plotted against ALL plot (Fig. 3B) of a sample from a non-infected individual. The lower-left corner in this plot is almost empty.

[0039] In another embodiment, instead of UMALS, the LALS measurement is used. In one embodiment, to help distinguish this parasite particle population from other populations measured by the analyzer, the LALS measurement is transformed using a non-linear function prior to the determination of populations. In another embodiment, the LALS measurement may be transformed by using the ARCTAN function and the LALS measurement as shown with respect to the UMALS. This transformation value is termed the Rotated LALS (RLALS) and is given by a function of $f(\text{DC}, \text{LALS})$. In one embodiment, RLALS is given by the expression:

$$RLALS = (C) \text{ ARCTAN } (DC / LALS)$$

where (C) is a proportionality constant, and (DC) is the DC current. The result of this measurement and transformation in an infected and non-infected individual is shown in Fig. 4A and Fig. 4B respectively. Other types of transformations well known to those skilled in the art may be used to accomplish the same effect.

[0040] In another embodiment, the MALS measurement which is the sum of LAMLS and UMALS is used. In one embodiment, to help distinguish the parasite particle population from other populations measured by the analyzer, the MALS measurement is transformed using a non-linear function prior to the determination of populations. This transformation value is termed the Rotated MALS (RMALS) and is given by a function of $f(DC, \text{MALS})$. In one embodiment, RMALS is given by the expression:

$$\text{Rotated MALS} = f(\log(\text{MALS}) / DC)$$

where f is a function that deals with shifting and scaling of the data. Other types of transformations well known to those skilled in the art may be used to accomplish the same effect.

[0041] The new population is termed the parasite particle population because after the lysing process of the RBC blood cells, the parasites and the parasitized red blood cells will remain and are differentiated from the other cells types such as the leukocytes, nucleated red blood cells, debris, platelets, and other cellular components in a unique region due to the detected properties. This parasite particle population which appears in a malarial infection is characterized by the ALL measurement and can be further differentiated or enumerated using at least one additional measurement parameter of the UMALS, the LALS, or the DC current.

[0042] The quantification of the parasite particle population allows two additional calculations to be made. The first is termed the “parasite_particle population%” and is a relative measure of the amount of parasite particles normalized to the white blood cell count. The parameter is given by the equation:

$$\begin{aligned} \text{parasite_particle_population\%} = \\ (\text{parasite_particle population event count} / \text{WBC event count}) 100\% \end{aligned}$$

[0043] A second parameter, the Coefficient of Malaria Concentration (CMC) (or Coefficient of Parasite Concentration) is calculated as the absolute count of the parasite particle population in per μL .

[0044] Two studies have evaluated the ability of these two new parameters, the CMC and parasite particle population%, to evaluate the parameters' ability to distinguish non-infected samples from infected samples. Using the Area Under the Curve (AUC) statistical technique, in which the closer the AUC value is to one, the better the discriminator, one study of 139 normal and 89 infected samples found AUC values for the parasite_particle_population% of 0.98 and an AUC for CMC of 0.98. A second study of the same 89 infected samples and 1680 normal or non-infected samples found AUC values of 0.94 for the parasite_particle_population% and 0.96 for the CMC. Thus, both studies showed the two parameters to be good discriminators of malarial parasite infection.

[0045] As a result, both parameters can be used to track the efficacy of treatment. Fig. 5 and Fig. 6 track the reduction of CMC (squares) (Fig. 5A and Fig. 6A) and RBC_Debris% (squares) (Fig. 5B and Fig. 6B) over time, compared with a manual count of parasite burden (diamonds) in patients being treated for malaria. As can be seen, the values of CMC and RBC_Debris% track the manual measure of parasite burden very well. It can also be seen that the treatment results in almost a 100-fold decrease in parasite infection over a four day treatment period.

EXAMPLES

[0046] In one embodiment, 1761 complete blood cell count (CBC) samples were analyzed using the Beckman Coulter DxH800TM hematology analyzer (Beckman Coulter, Brea, CA). (See Sensitive detection and accurate monitoring of *Plasmodium vivax* parasites on routine complete blood count using automatic blood analyzer (DxH800TM), H.K. Lee et al, *Int. Jnl. Lab. Hem.* 24, 201-207.) This collection included 123 blood samples from 52 *P. vivax* malarial patients, 1504 non-malarial samples and 134 samples from normal, healthy subjects. The non-malarial samples included 509 blood samples from patients with leucopenia. The malarial blood samples were taken once, at the time of diagnosis, in 27 patients and 2-6 times from 25 patients. Diagnoses of malarial infection were made by microscopic examination.

[0047] The scatter plots of nucleated red blood cells (nRBC) were screened to detect malarial signals. If *P. vivax* signals were detected, five different scatter plots were reviewed to differentiate the malarial component of the sample from cell debris. Figs. 7A and B depict nRBC screening plots (RLALS and RUMALS respectively plotted against ALL) for a blood sample from a patient with malaria which depict the malarial population (shown by the arrow) and the nRBCs and white cells. Fig. 7C is a scatter plot of RUMALS and ALL for a blood sample from a patient infected with *P. vivax*, including a 1-dimensional histogram on ALL. One can distinguish separate populations by comparing the peak values against the minima.

[0048] Once the malarial cells were detected in a sample, the sample underwent five part differential plots (5PD plots) (Figs. 7D and E) to further distinguish the malarial population (again pointed to by the arrow) from the other cells. The 5PD plots can distinguish the five main populations lymphocytes (LY), monocytes (MO), neutrophils (NE), eosinophils (EO) and basophils (BA), plus the non-white cell populations. 5PD plots are designated 5PD1 and 5PD2. In 5PD1, a display of Rotated Light Scatter (RLSn) (graphed along the x-axis) is plotted against volume (V) as shown in Fig. 7D. The Rotated Light Scatter used in this example is Rotated Median Angle Light Scatter (RMALS). In 5PD2 the display is opacity (OP) graphed along the x-axis against volume V graphed along the y-axis) as shown in Fig. 7E. The five part differential scatter plots help distinguish malarial particles from cell debris.

[0049] Figs. 8A, B and C depict RLALS versus ALL scatter plots from a malarial patient being treated with an anti-malarial regimen. The initial blood sample contained 664 parasites / μl and Fig. 8A shows the malarial component (arrow). After two days of treatment, the concentration of malarial parasites reduced to 86 parasites/ μl as shown by the malarial population Fig. 8B. After 12 days of treatment the concentration of parasites was 0 and the population is missing from Fig. 8C. Conventional microscopic examination also failed to detect malaria parasites after 12 days.

[0050] Thus, as discussed in the previous paragraph, one measurement resulting from the technique is that the size of the *P. vivax* signals correlates with the parasite burden. Thus, it is possible to count the number of particles and from that derive the count of the parasite burden in terms of number of parasites per unit volume. As a result, one can actively track the result of treatment and can more accurately predict the result of treatment using anti-malarials.

[0051] This study showed that this cytometric technique can be used to inexpensively and quickly test for blood parasites and monitor treatment of the disease. All of the 52 malarial samples (100%) showed specific characteristics on nRBC scatter plots and were easily identified. One of the 1509 (.07%) samples showed an anomalous signal, which was easily shown as not being the result of malaria infection by performing 5PD (five part differential) plots.

[0052] Thus, the sensitivity of the method was 100% and the specificity was also 100%, making this a very powerful technique. It should also be noted that although the majority of experimental data in this example relates to *P. vivax* malarial infections, the technique will also detect the presence of other malarial parasites such as *P. falciparum* (Figs. 9A and B).

[0053] It is to be understood that the figures and descriptions of the invention have been simplified to illustrate elements that are relevant for a clear understanding of the invention. Those of ordinary skill in the art will recognize, however, that these and other elements may be desirable. However, because such elements are well known in the art, and because they do not facilitate a better understanding of the invention, a discussion of such elements is not provided herein. It should be appreciated that the figures are presented for illustrative purposes and not as construction drawings. Omitted details and modifications or alternative embodiments are within the purview of persons of ordinary skill in the art.

[0054] It can be appreciated that, in certain aspects of the invention, a single component may be replaced by multiple components, and multiple components may be replaced by a single component, to provide an element or structure or to perform a given function or functions. Except where such substitution would not be operative to practice certain embodiments of the invention, such substitution is considered within the scope of the invention.

[0055] The examples presented herein are intended to illustrate potential and specific implementations of the invention. It can be appreciated that the examples are intended primarily for purposes of illustration of the invention for those skilled in the art. There may be variations to these diagrams or the operations described herein without departing from the spirit of the invention. For instance, in certain cases, method steps or operations may be performed or executed in differing order, or operations may be added, deleted or modified.

[0056] Furthermore, whereas particular embodiments of the invention have been described herein for the purpose of illustrating the invention and not for the purpose of limiting the same, it will be appreciated by those of ordinary skill in the art that numerous variations of the details, materials and arrangement of elements, steps, structures, and/or parts may be made within the principle and scope of the invention without departing from the invention as described in the claims.

[0057] Variations, modification, and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the invention as claimed. Accordingly, the invention is to be defined not by the preceding illustrative description, but instead by the spirit and scope of the following claims.

CLAIMS

1. A method for detecting a parasite particle population in a blood sample comprising the steps of:
 - a) mixing said blood sample with a lytic reagent to lyse red blood cells;
 - b) measuring the light loss from a light source caused by particles of the lysed blood sample passing through a particle analyzer to obtain an axial light loss measurement; and
 - c) differentiating using a computer the parasite particle population from other populations in the lysed blood sample in response to the axial light loss measurement.
2. The method of claim 1 further comprising measuring light scattered from a source caused by particles of the lysed blood sample passing through the particle analyzer to obtain a light scatter measurement and differentiating using the computer the parasite particle population in the lysed blood sample in response to the light scattering measurement and the axial light loss measurement.
3. The method of claim 1, wherein the differentiated parasite particle population comprises cells infected with malaria parasites.
4. The method of claim 2, further comprising:
 - a) counting said particles in the parasite particle population to determine a coefficient of parasite concentration; and
 - b) reporting the coefficient of parasite concentration as a count corresponding to a parasite infection.
5. The method of claim 4 further comprising the step of determining the effectiveness of treatment by periodically measuring the parasite concentration in samples of patient blood obtained during treatment with an anti-malarial drug.
6. The method of claim 2, wherein the at least one light scatter measurement is an upper median angle light scatter measurement.
7. The method of claim 2, wherein the differentiating comprises:
 - a) comparing, using the computer, the measured at least one light scatter measurement and the measured axial light loss measurement; and

- b) detecting the parasite particle population by gating respective populations indicated by the comparison.
8. The method of claim 7, further comprising:
- a) counting, using the computer, said particles in the parasite particle population to determine a first number;
 - b) counting, using the computer, said particles in other particle populations to determine a second number; and
 - c) reporting a fraction comprising the first number and the second number as an indication of a parasite infection.
9. The method of claim 2, wherein the parasite particle population has lower values of said at least one light scatter measurement and lower values of said axial light loss measurement than respective white blood cell populations.
10. The method of claim 2, wherein the differentiating comprises:
- a) comparing the at least one light scatter measurement, the axial light loss measurement, and a third measurement, wherein the third measurement is obtained during said particle measurement; and
 - b) detecting the parasite particle population by gating respective populations indicated by the comparison.
11. The method of claim 10, wherein the third measurement is a direct current (DC) measurement.
12. The method of claim 1 further comprising distinguishing a parasite infection from other diseases by performing, using the computer, a 5PD plot on the blood sample.
13. A method of detecting parasite particles using a blood sample, comprising the steps of:
- a) mixing said blood sample with a lytic reagent to lyse red blood cells;
 - b) measuring particles of the lysed blood sample in a particle analyzer to obtain a light scatter measurement of light scattered from a light source by the particles, an axial light loss measurement of the decrease in light intensity from a source caused by the particles, and a volume measurement of each of the particles;
 - c) differentiating using a computer a first particle population corresponding to the parasite particles in the lysed blood sample based upon the at least one light scatter measurement and the axial light loss measurement;

- d) differentiating, using a computer, based upon the volume measurement, a second particle population corresponding to white blood cells affected by said parasite particles; and
 - e) reporting a parasite infection in response to the identified first and second particle populations.
14. The method of claim 13, wherein the identifying the second particle population is further based upon a measurement of light scatter caused by the second particle population.
15. A method of detecting parasites in red blood cells comprising the steps of:
- a) lysing the uninfected red blood cells in a blood sample;
 - b) directing a beam of light through the lysed blood sample;
 - c) measuring the axial light loss from the beam of light as caused by a parasite particle population;
 - d) determining a measure of the parasite particle population in response to the measured axial light loss.
16. The method of claim 15 further comprising the steps of:
- a) measuring the light scattered by a particle in the sample within a predetermined angular range as measured from the direction of the beam of light;
 - b) comparing the light scattered by the particle within the predetermined angular range as measured from the direction of the beam of light against the axial light loss for the particle; and
 - c) determining a population of infected cells from the comparison.
17. The method of claim 16 further comprising the step of determining the effectiveness of treatment by periodically measuring a parasite concentration in samples of patient blood obtained during treatment with an anti-malarial drug.
18. The method of claim 15 wherein the count of the number of particles in the parasite particle population is divided by a count of the number of white blood cells.
19. A system for detecting a parasite particle population in a blood sample comprising:
- a) a lysing solution for lysing red blood cells;
 - b) a particle analyzer to obtain an axial light loss measurement of each lysed red blood cell; and

- b) a computer determining a population of parasite particles from the axial light loss measurement,
wherein the presence of a lower axial light loss measurement is indicative of the parasite population particle.
20. The system of claim 19, wherein the particle analyzer obtains a light scatter measurement and the computer determines a population of parasite particles in response to comparing the light scattering measurement with the axial light loss measurement.
21. The system of claim 20, wherein the light scattering measurement is made between 20-45 degrees.
22. The system of claim 19 further comprising the step of determining a parasite concentration in the blood sample from a determination of the count of the parasite population.
23. The system of claim 22 further comprising the step periodically taking an additional sample of blood and determining the parasite concentration so as to measure the effectiveness of anti-parasite drug treatment on the source of the blood sample.
24. The system of claim 19 further comprising the step of distinguishing a parasite infection from another disease by performing a 5PD plot on the blood sample.
25. A method of detecting parasite particles using a blood sample, comprising the steps of:
- a) mixing said blood sample with a lytic reagent to lyse red blood cells;
 - b) measuring particles of the lysed blood sample in a particle analyzer to obtain a light scatter measurement of light scattered from a light source by the particles;
 - c) measuring particles of the lysed blood sample in a particle analyzer to obtain a volume measurement of the particles;
 - d) differentiating using a computer a first particle population corresponding to the parasite particles in the lysed blood sample in response to the light scatter measurement and the volume measurement;
 - e) differentiating using a computer a second particle population corresponding to white blood cell particles in the lysed blood sample in response to the light scatter measurement and the volume measurement; and
 - f) reporting a parasite infection in response to the identified first and second particle populations.

26. A method of detecting parasite particles using a blood sample, comprising the steps of:
- mixing said blood sample with a lytic reagent to lyse red blood cells;
 - b) measuring particles of the lysed blood sample in a particle analyzer to obtain a measurement of particle opacity;
 - c) measuring particles of the lysed blood sample in a particle analyzer to obtain a volume measurement of the particles;
 - d) differentiating using a computer a first particle population corresponding to the parasite particles in the lysed blood sample in response to the opacity measurement and the volume measurement;
 - e) differentiating using a computer a second particle population corresponding to white blood cell particles in the lysed blood sample in response to the opacity measurement and the volume measurement; and
 - f) reporting a parasite infection in response to the identified first and second particle populations.

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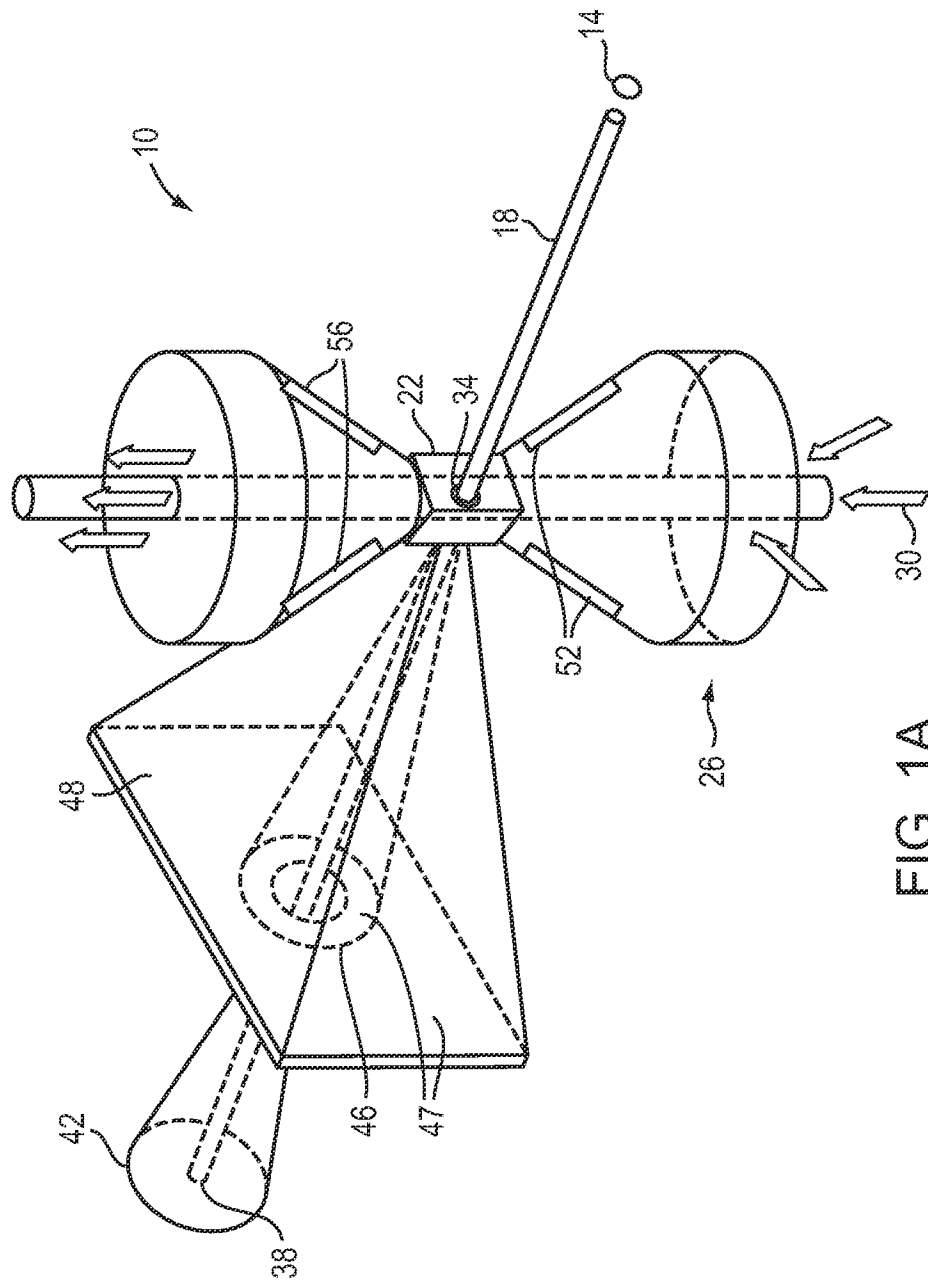
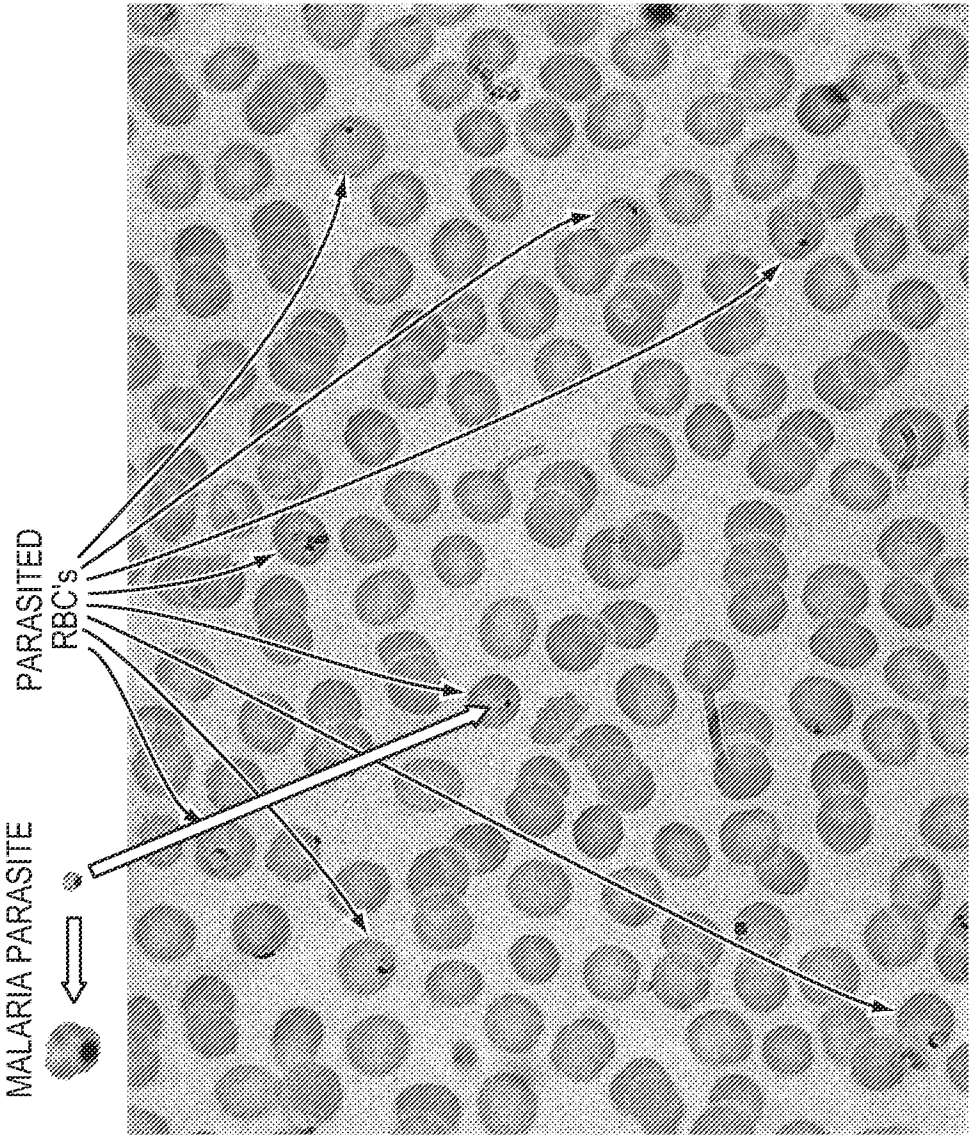


FIG. 1A



MALARIA PARASITES IN A PATIENT WITH MALARIA
(THE PARASITES ARE INSIDE THE RED CELLS)

FIG. 1B

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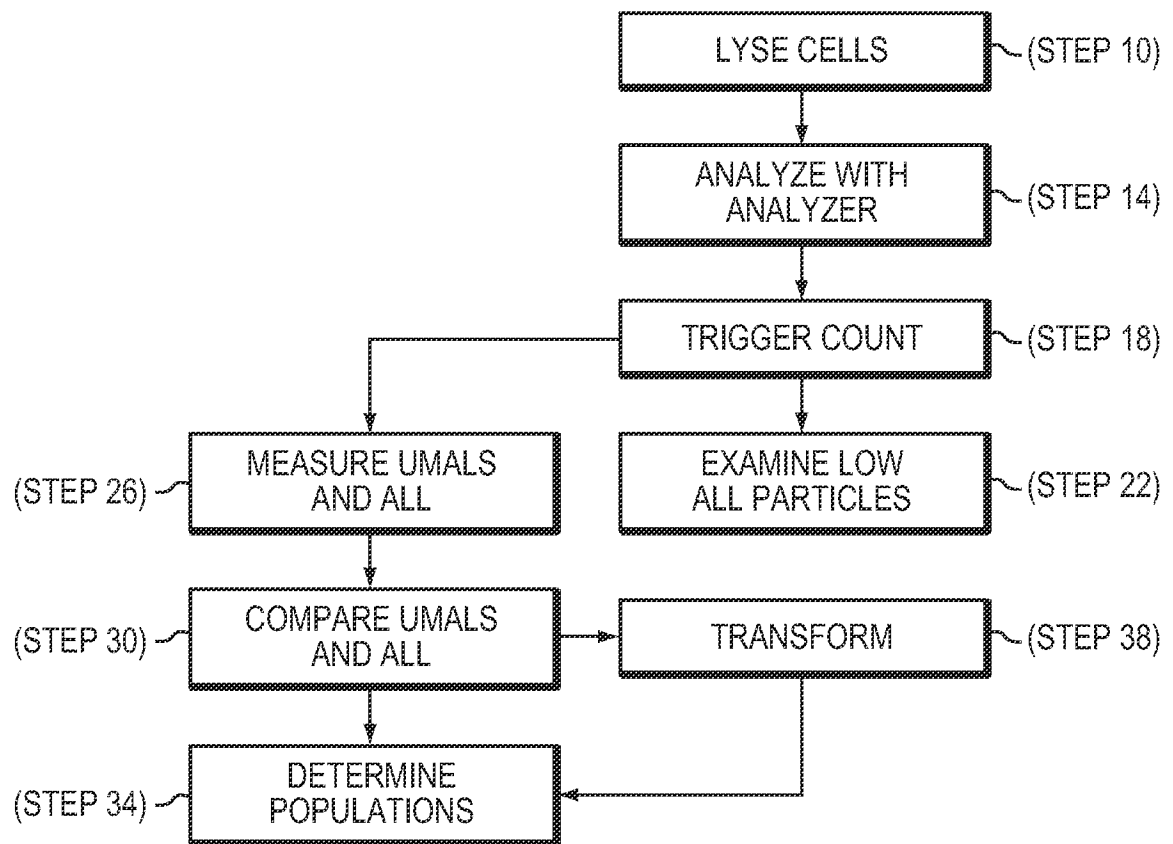


FIG. 2

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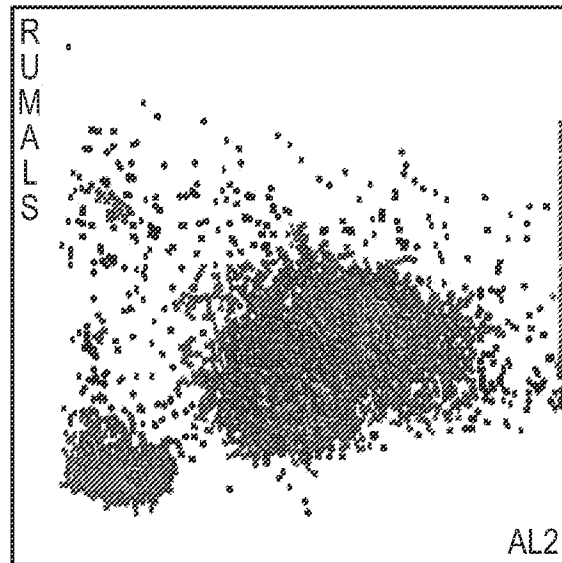


FIG. 3A

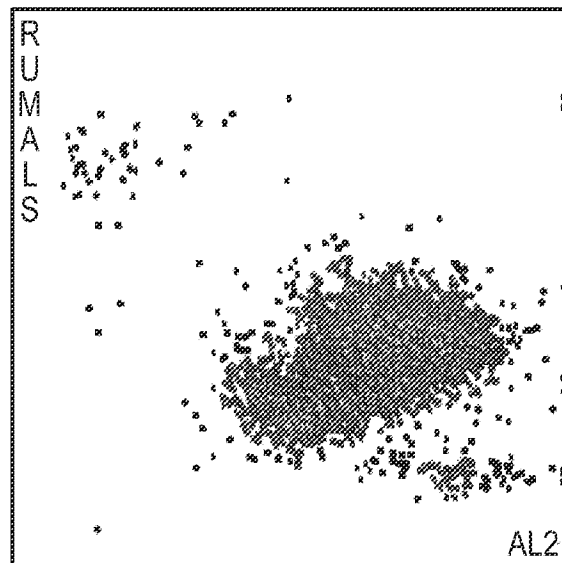


FIG. 3B

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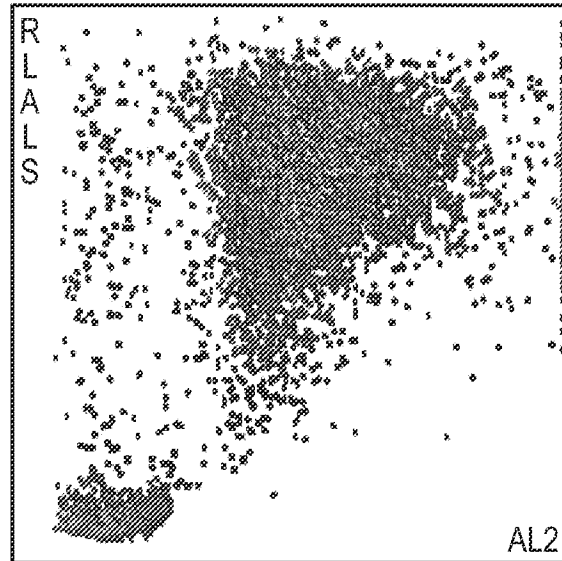


FIG. 4A

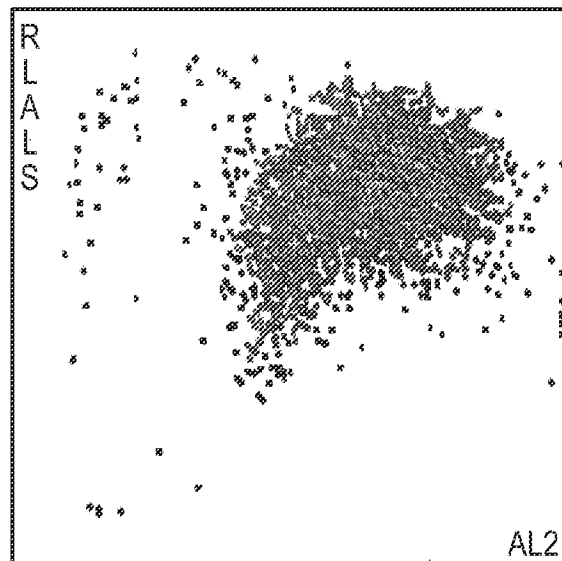


FIG. 4B

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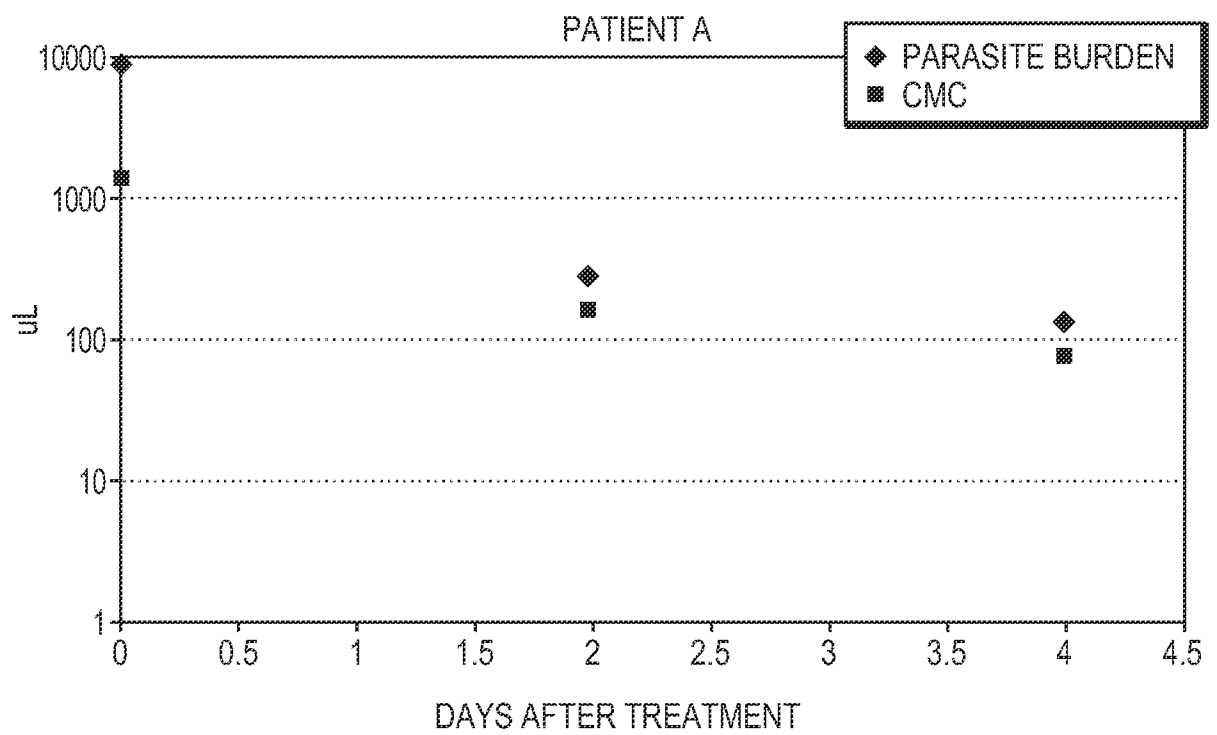


FIG. 5A

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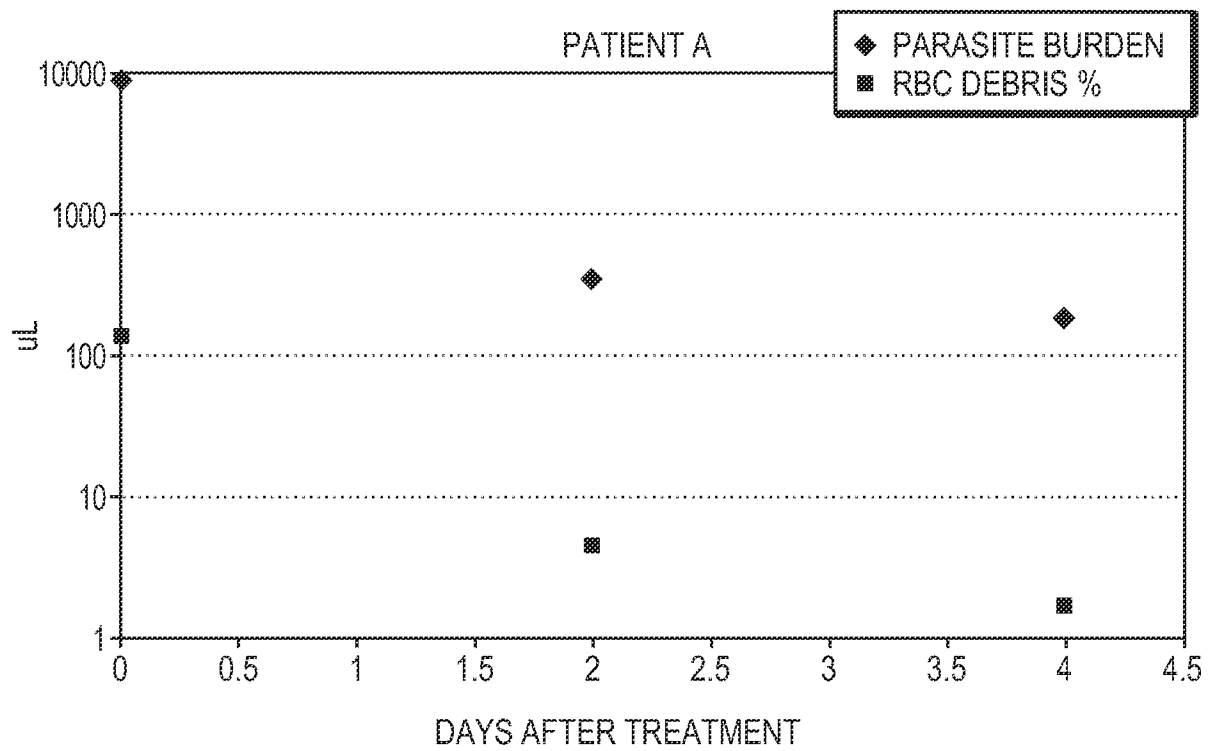


FIG. 5B

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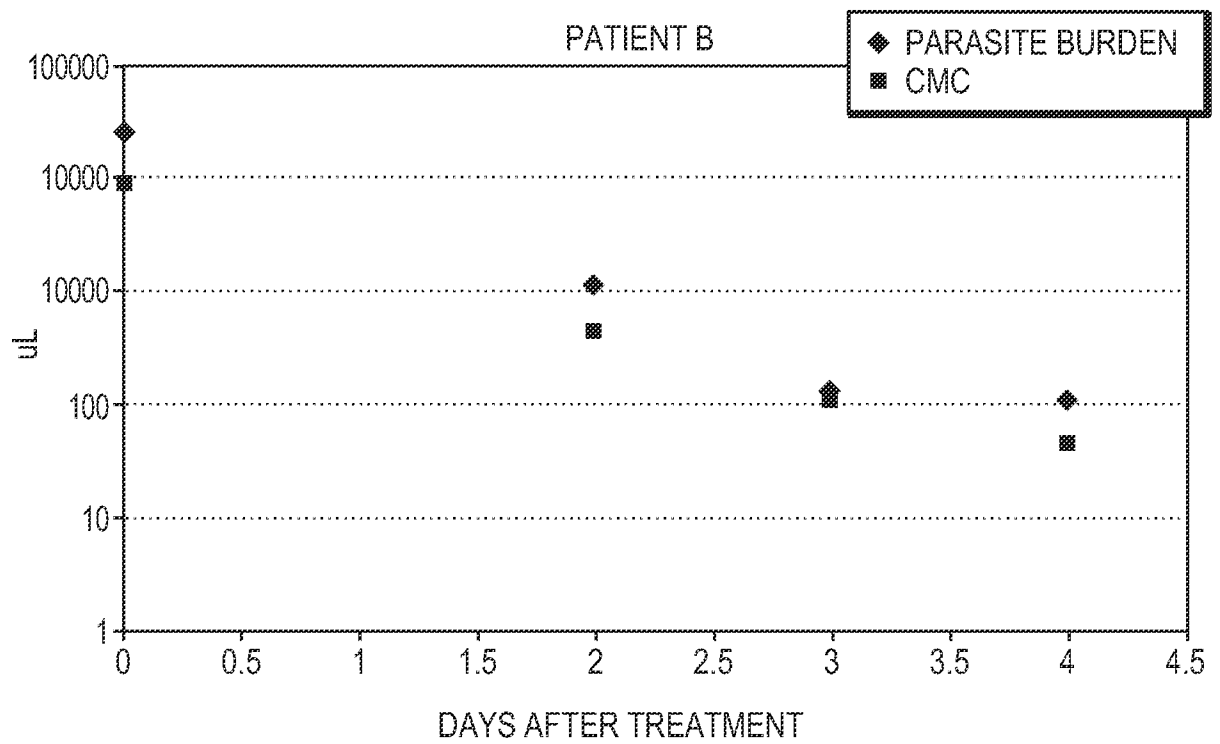


FIG. 6A

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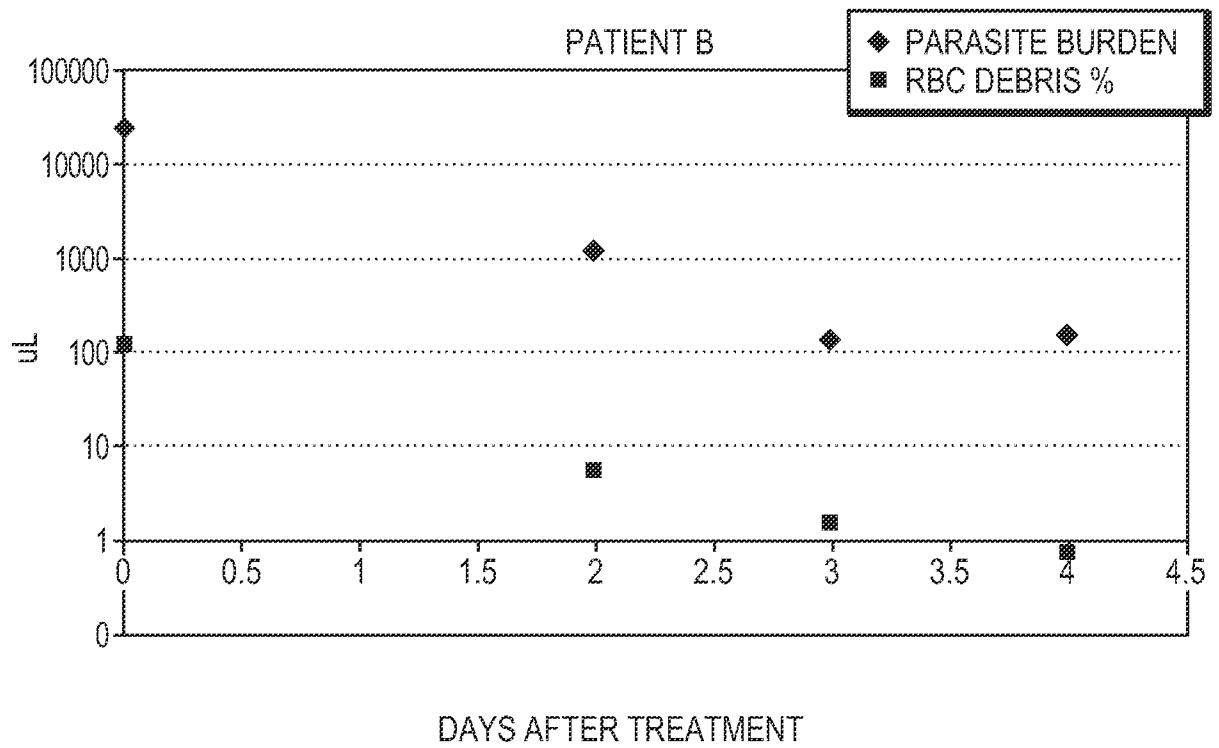


FIG. 6B

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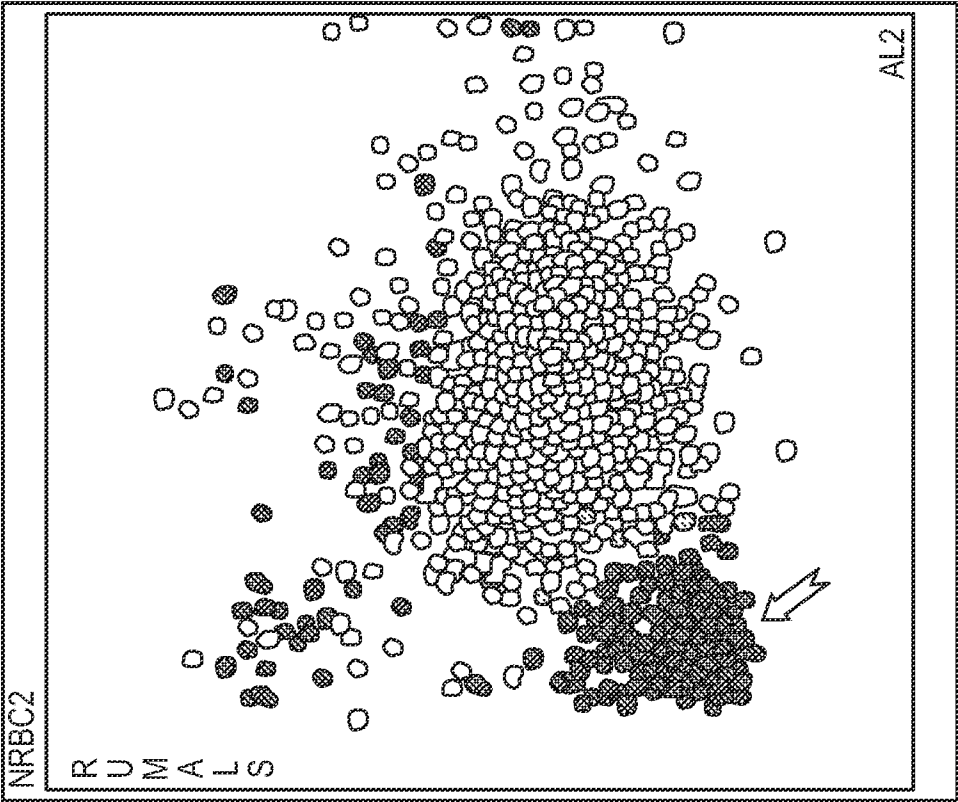


FIG. 7B

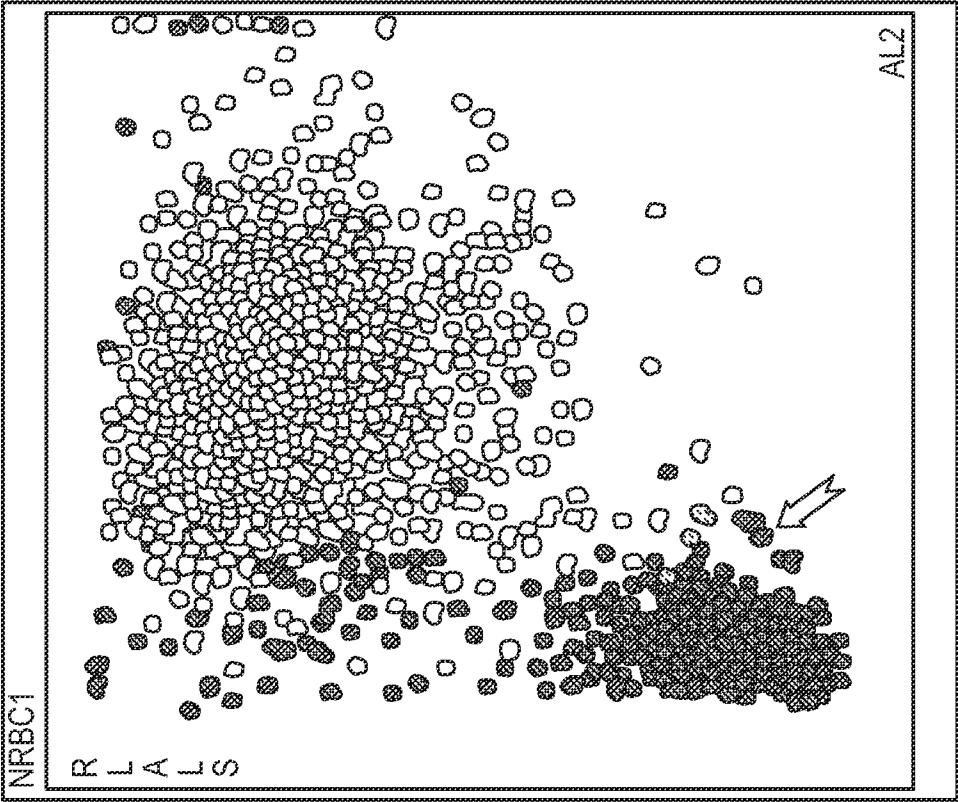


FIG. 7A

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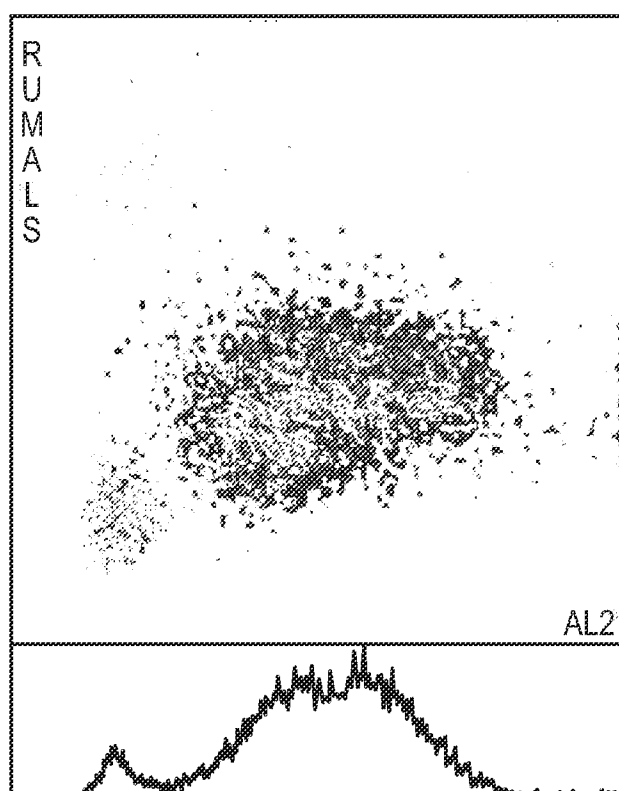


FIG. 7C

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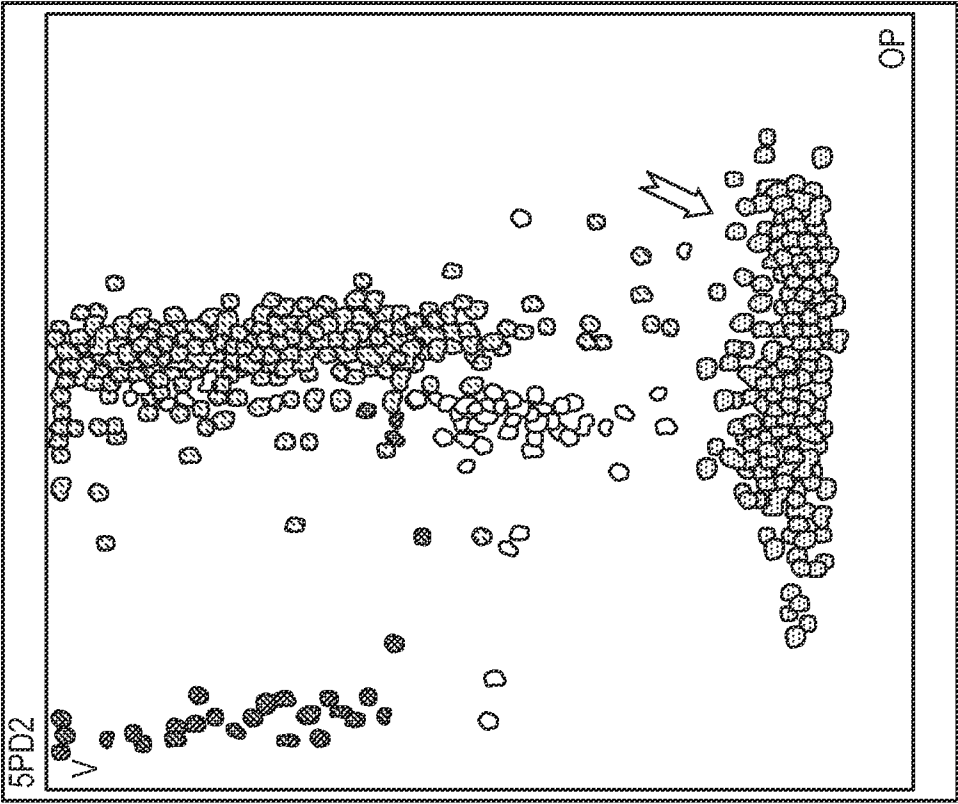


FIG. 7E

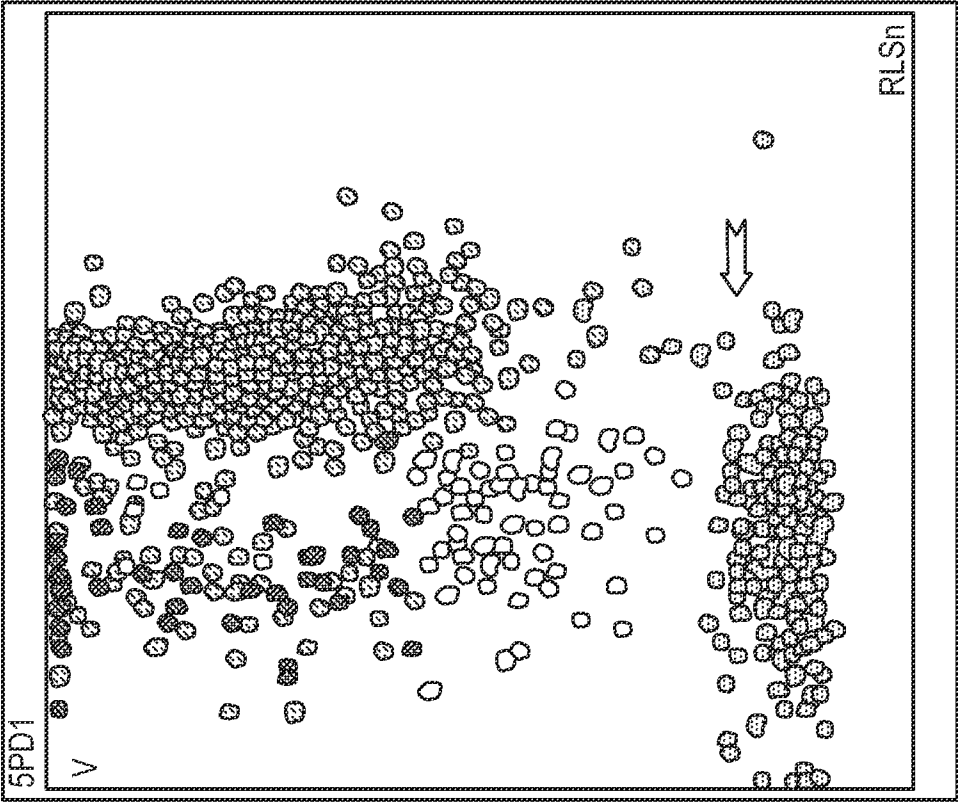


FIG. 7D

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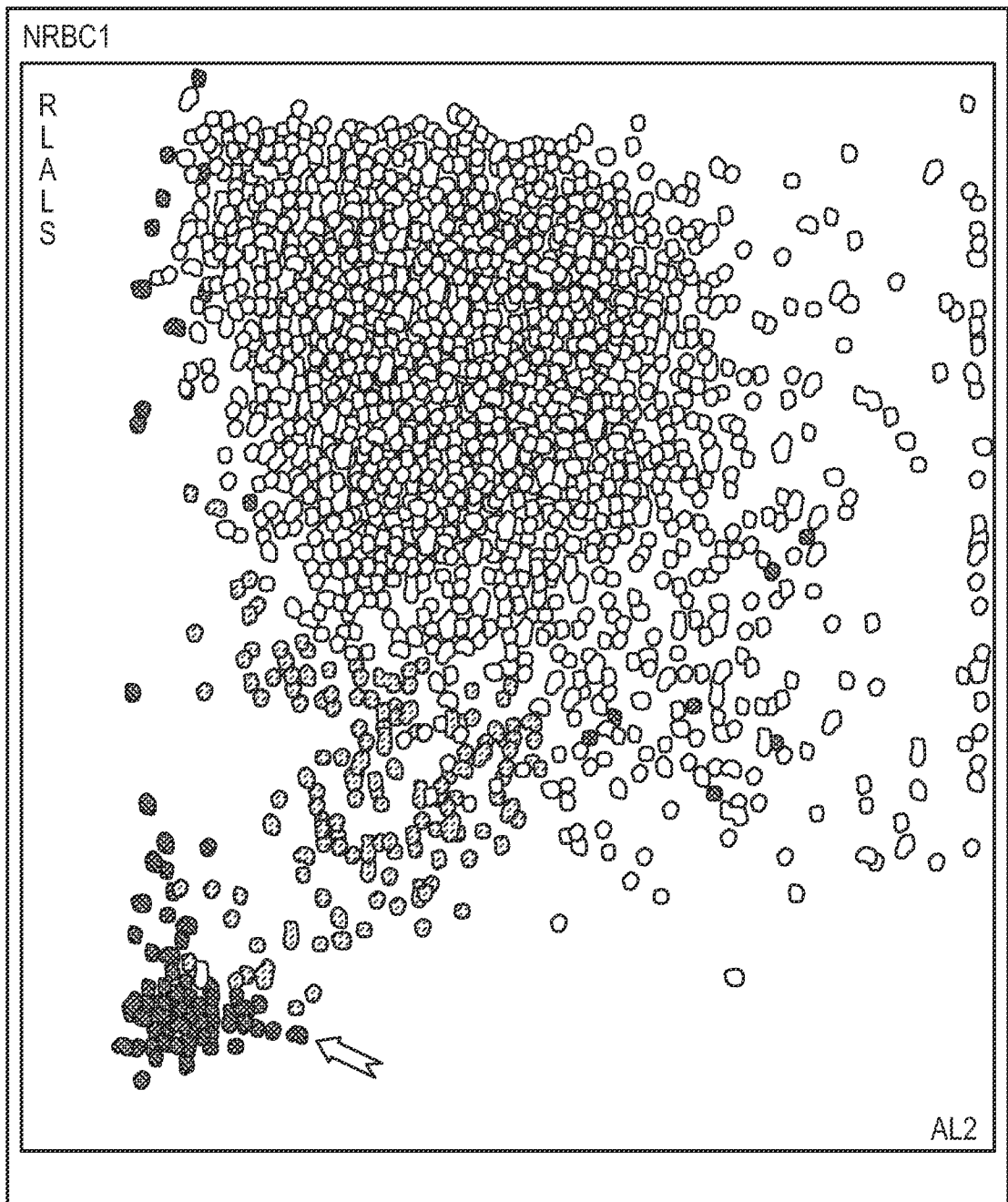


FIG. 8A

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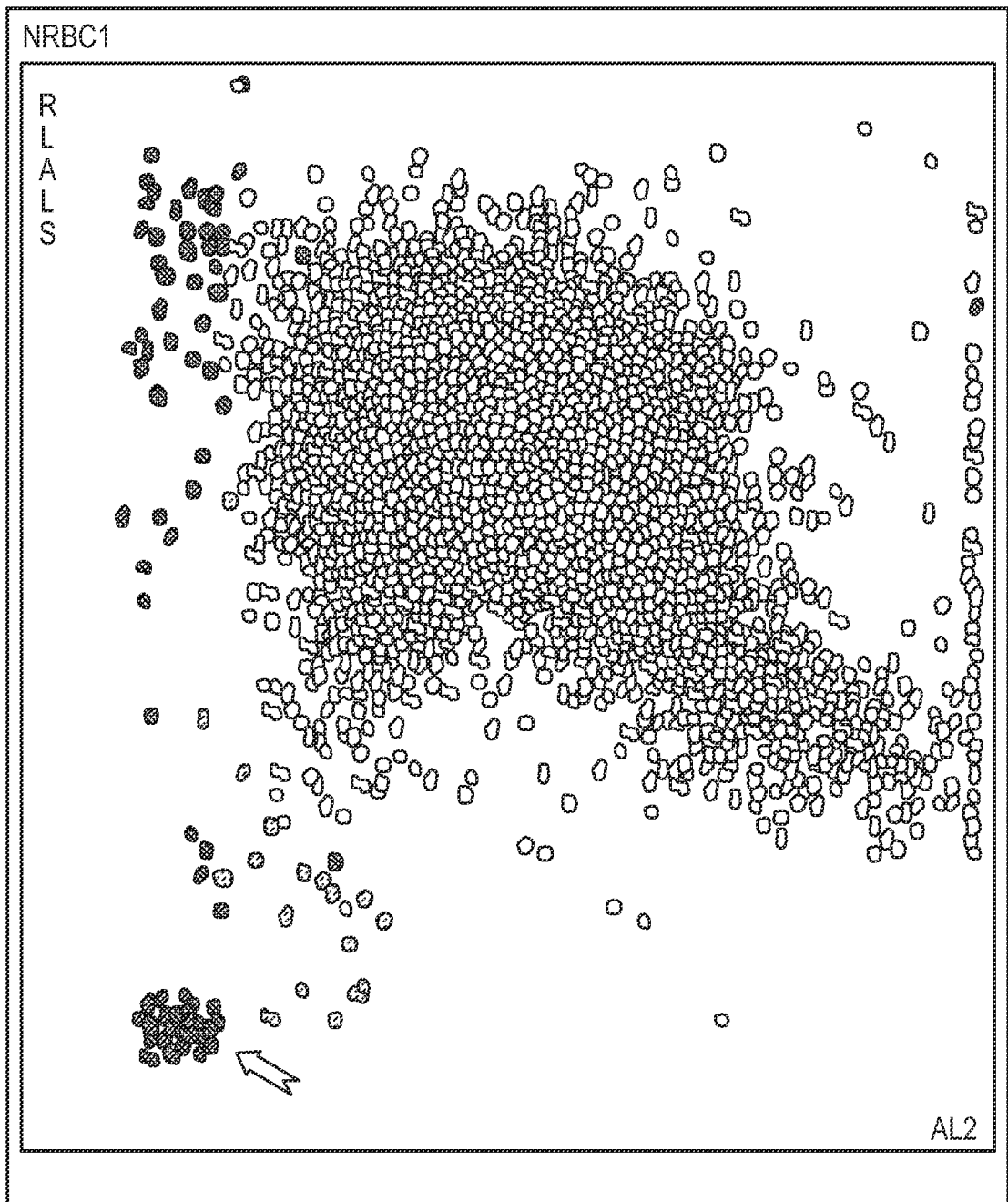


FIG. 8B

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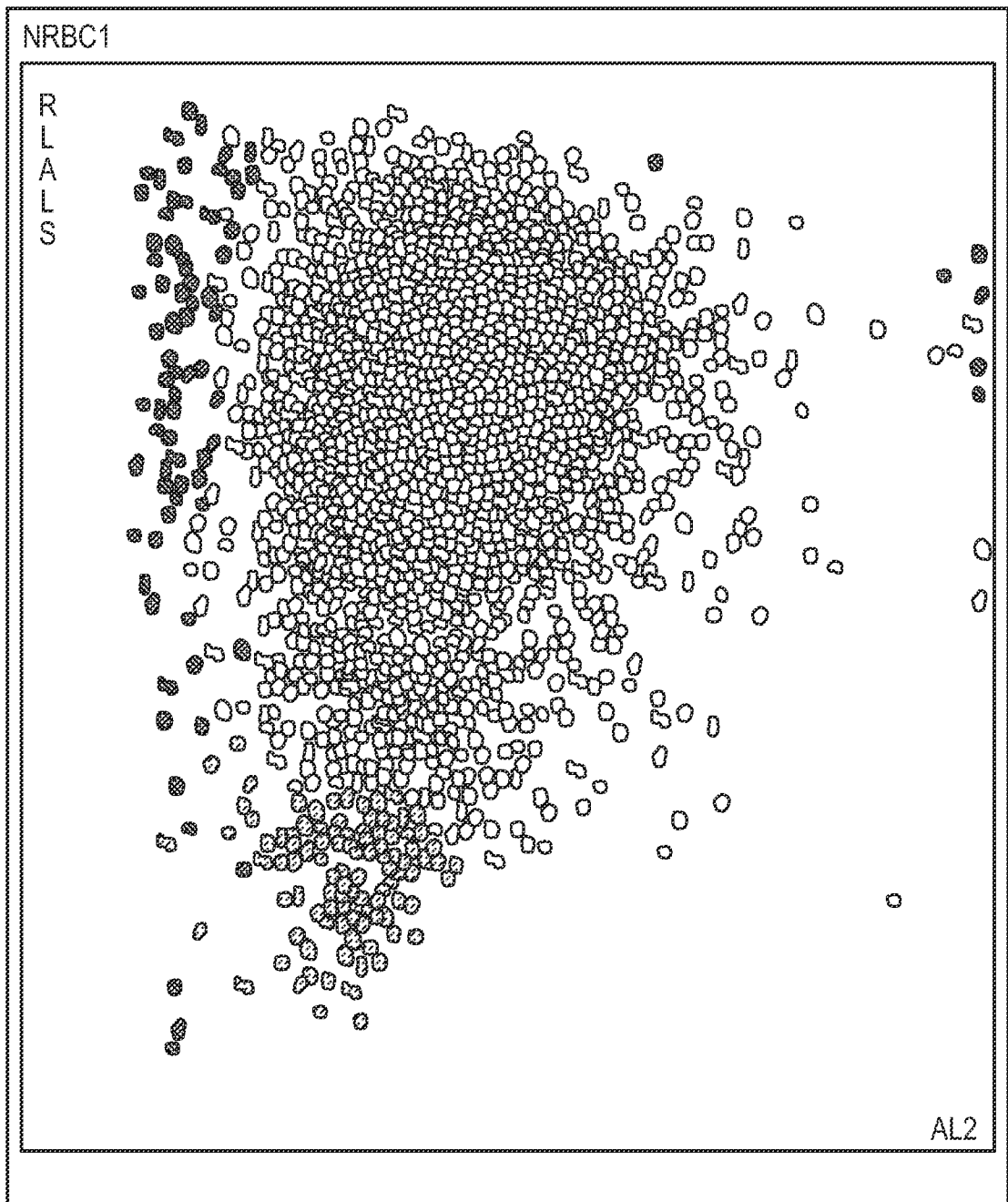


FIG. 8C

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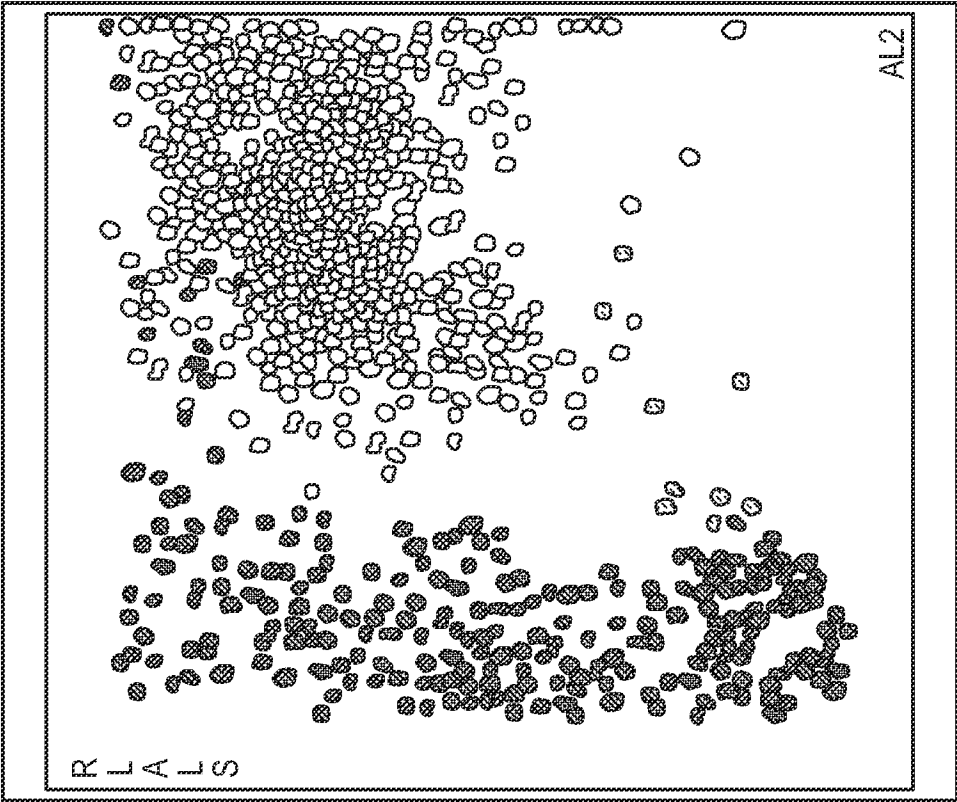


FIG. 9B

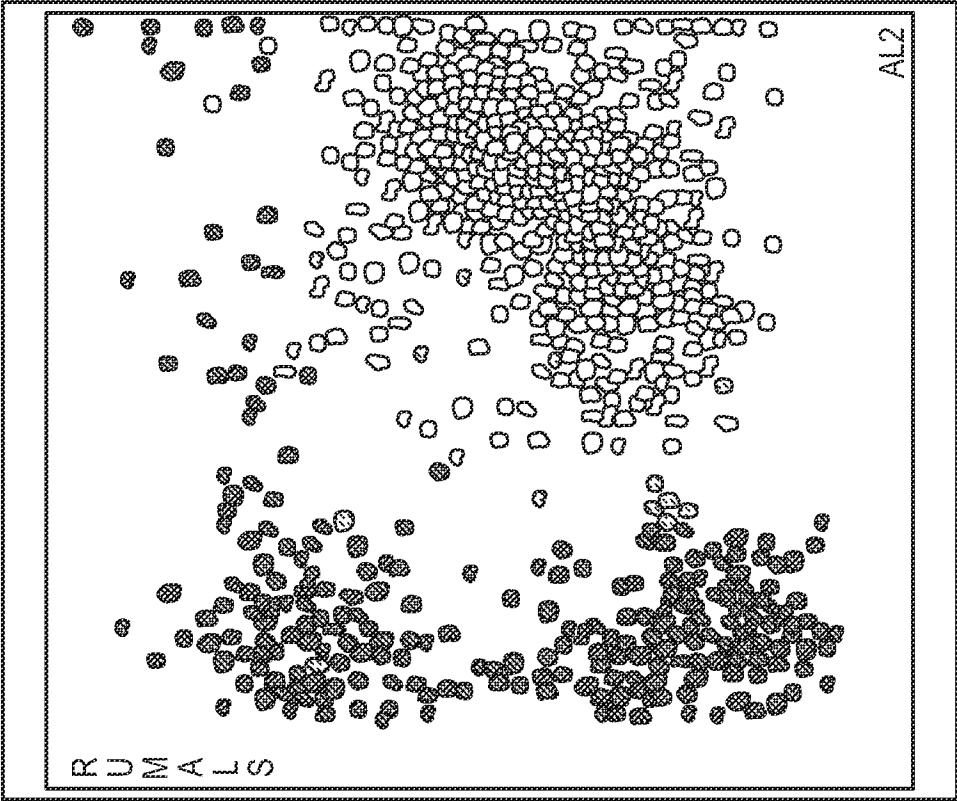


FIG. 9A

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/037830

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N21/53 G01N15/00 G01N33/569
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 746 407 A2 (SYSMEX CORP [JP]) 24 January 2007 (2007-01-24)	1-9, 12-25
Y	page 2, line 5 - line 6 page 3, line 14 - line 21 page 3, line 32 - line 34 page 5, line 39 - line 52 page 7, line 2 - line 6 page 8, line 53 - line 54 page 10, line 27 - line 34 page 11, line 36 - line 44 page 13, line 29 - line 36 page 14, line 5 - line 29 figures 1,4,11,12 ----- -/--	1-26



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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

"&" document member of the same patent family

Date of the actual completion of the international search

22 August 2012

Date of mailing of the international search report

05/09/2012

Name and mailing address of the ISA/

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Authorized officer

D'Alessandro, Davide

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/037830

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	column 2, line 29 - line 50 column 5, line 13 - line 22 column 6, line 16 - line 30 -----	1-26
Y	JP 2005 333868 A (SYSMEX CORP) 8 December 2005 (2005-12-08) paragraphs [0019], [0020], [0030], [0033], [0045] figures 9,10 -----	1-26
A	GB 2 270 752 A (FAZAL AZIM [GB]) 23 March 1994 (1994-03-23) page 2 - page 3 -----	1-26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/037830

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			JP 2007024844 A	01-02-2007
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