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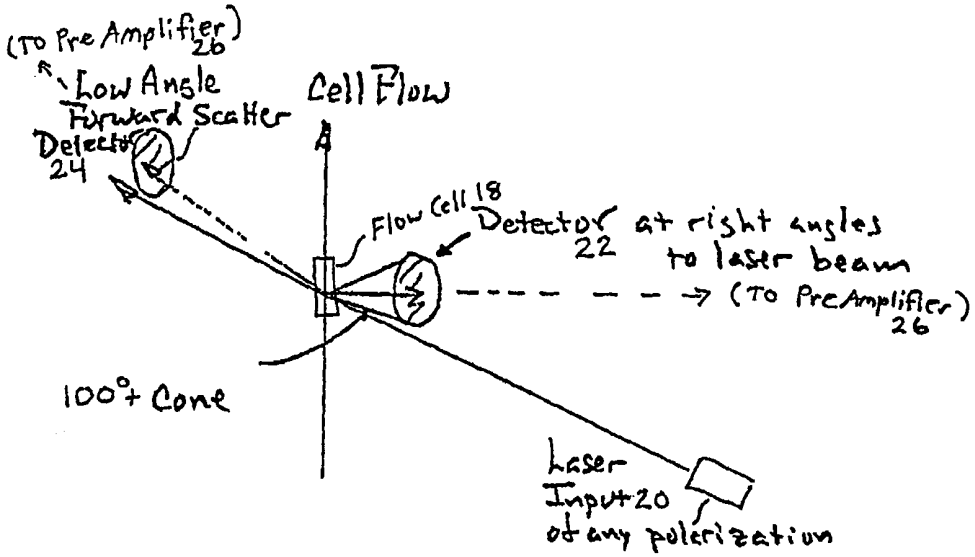

(57) Abstract

The high numerical aperture flow cytometer of the present invention includes a flow cell and a laser input. The laser input emits a beam of light that is oriented substantially orthoganilly to the flow of blood cells through the flow cell such that laser light impinges upon the blood cells as they pass through the flow cell. A portion of the beam from the laser input that impinges upon the blood cells in the flow cell is scattered at a substantially right angle to the beam of laser input ("right angle scatter"). A second portion of the beam from the laser input that impinges upon the cells in the flow cell is scattered at a much lower angle than 90°. This scatter is termed "low angle forward scatter light" and has an angle of from about 2° to about 5° from the orientation of the original beam from laser input. A right angle scatter light detector is oriented to receive the previously mentioned right angle scatter light. A low angle forward scatter light detector is oriented to capture the previously mentioned low angled forward scatter light oriented at about 2° to about 5° beam from laser input.

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HIGH NUMERICAL APERTURE FLOW CYTOMETER AND METHOD OF USING SAME

FIELD OF THE INVENTION

The present invention relates to particle discrimination by light scattering, and more particularly to a flow cytometer and method therefore that discriminates particles employing a high numerical aperture. Numerical aperture is defined as the refractive index of the medium through which light is collected multiplied by the sine value of one-half of the angle of light collection.

BACKGROUND OF THE INVENTION

The discrimination of particles is useful in numerous clinical assays including ascertaining the types and numerical quantity of cells in blood, ascertaining invasive particles in a fluid sample, such as bacteria and virus, and quantifying the density and volume of cells in a fluid sample.

One method of the above is disclosed in U.S. Patent No. 5,017,497 issued to de Grooth et al. Referring to FIG. 1, the '497 Patent discloses a flow cell 2 through which cells from, for example, blood or the like, flow substantially one by one therethrough. A laser input 4 emits a polarized beam of laser light that is oriented substantially orthogonally to the flow of blood cell through flow cell 2 such that the polarized laser light impinges upon the blood cells as they pass through flow cell 2. By "polarized" it is meant that the plane of the electric field oscillization of the laser light is uniform. An optical lens 6 has an aperture which limits the cone of scattered light from the blood cells that can be collected to 72° or less. The central axis of the cone of lens 6 is 90° to both the path of the polarized laser light and the flow of blood cells through flow cell 2. The scattered light eminating from lens 6 is columnated in a matter known in the art. The scattered light now has a mixed polarization that is characteristic of the cell type. The light next passes through a beam splitter 8 that divides the light into two separate beams. A first light beam, substantially concentric with the light beam that originally eminated from lens 6, passes

through first polarization analyzer 10. Polarization analyzer 10 is configured to pass therethrough only polarized light having a vector the same as the original laser light. The second beam eminating from beam splitter 8 is oriented substantially perpendicular to the orientation of the first beam eminating from beam splitter 8. This second beam enters second polarization analyzer 12. Second polarization analyzer 12 is configured to pass therethrough only light having a polarization vector substantially orthogonal to the polarization vector of the other beam from beam splitter 8 that passed through first polarization analyzer 10. The beams that pass through first polarization analyzer 10 and second polarization 12 enter polarized detector 14 and depolarized light detector 16, respectively. The ratio of the outputs of polarized light detector 14 and depolarized light detector 16, based on intensity, provide the depolarization ratio.

As shown in FIG. 4 eosinophils, a subset of leukocytes (white blood cells), depolarize the right angle of scattered light quantified by the above configuration to a greater degree than other glucosites. FIG. 4 is a graphical representation having the output of polarized light detector 14 as one axis and the output of depolarized light detector 16 as the axis. While the above invention does provide some useful data regarding leukocytes, and more specifically eosinophils, as shown in FIGS. 6B, 7B, 8B and 9B, the cluster points within the eosinophil cluster (the cluster points above the angled threshold line on the graphical representation having "DEPOL" as one axis and "ORTHAGONAL" as the other axis) are quite condensed. The dense nature of the points within the eosinophil cluster results in difficulty for the computer software programs that ascertain and identify clusters to accurately identify eosinophil clusters. Additionally, this prior art configuration requires expensive optical devices such as photo multiplier tubes, and lens 6, first polarization amplifier 10 and second polarization amplifier 12.

A need thus exists for a flow cytometer apparatus and related method in which the cell cluster points are less dense for ease of characterization of the different cell clusters. A need also exists for the above apparatus and method which has fewer and less expensive components.

SUMMARY OF THE INVENTION

The high numerical aperture flow cytometer of the present invention includes a flow cell and a laser input. The laser input emits a beam of light that is oriented substantially orthoganilly to the flow of blood cells through the flow cell such that laser light impinges upon the blood cells as they pass through the flow cell. Unlike the prior art, the laser light emitted by the laser input need not be polarized for analysis of the cells according to the present invention. A portion of the beam from the laser input that impinges upon the blood cells in the flow cell is scattered at a substantially right angle to the beam of laser input ("right angle scatter light"). A second portion of the beam from the laser input that impinges upon the cells in the flow cell is scattered at a much lower angle than 90°. This scatter is termed "low angle forward scatter light" and has an angle of from about 2° to about 5° from the orientation of the original beam from laser input. A right angle scatter light detector is oriented to receive the previously mentioned right angle scatter light. The right angle scatter light detector is preferably located about 2 millimeters from the blood cells in the flow cell. An important aspect of the present invention is that, at the distance of about 2 millimeters from the blood cells, the right angle scatter light detector collects a cone of scattered light of at least 100° or greater, and preferably 130° or greater. It is this larger light cone value over the prior art light cone of about 72° that results in the greater cluster separation in the present invention due to the larger signal gathered. In contrast, the smaller 72° cone of the prior art results in missed signals and lesser cluster separation.

A low angle forward scatter light detector is oriented to capture the previously mentioned low angle forward scatter light oriented at about 2° to about 5° from the beam of the laser input.

In one embodiment of the present invention, both right angle scatter light detector and low angle forward scatter light detector are employed in order to produce a 2-dimensional cytrogram. However, it should be noted that in another embodiment of the

present invention, only right angle scatter light detector is employed, low angle forward scatter light detector is not employed, and characterization of eosinophils is possible.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

FIG. 1 is a schematic representation of the electro-optical components of prior art;

FIG. 2 is a schematic representation of the electro-optical components of the present invention;

FIG. 3 is a block diagram of the electronic processing components of the present invention;

FIG. 4 is a graphical representation of the separation of eosinophils and other white blood cell components based on light scatter in the prior art;

FIG. 5 is a graphical representation of the separation of eosinophils and other white blood cell components based on light scatter in the present invention;

FIG. 6A is a graphical representation of 2% canine eosinophil data employing the prior art;

FIG. 6B is a graphical representation of 2% canine eosinophil data employing the present invention;

FIG. 7A is a graphical representation of 8% canine eosinophil data employing the prior art;

FIG. 7B is a graphical representation of 8% canine eosinophil data employing the present invention;

FIG. 8A is a graphical representation of 10% canine eosinophil data employing the prior art;

FIG. 8B is a graphical representation of 10% canine eosinophil data employing the present invention;

FIG. 9A is a graphical representation of human eosinophil data employing the prior art; and

FIG. 9B is a graphical representation of human eosinophil data employing the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring to FIG. 2, the high numerical aperture flow cytometer of the present invention includes a flow cell 18, which is preferably a quartz flow cell manufactured by Opco Laboratories of Fitchburg, Massachusetts. Preferably flow cell 18 has a flow length of about 1 centimeter and a cross section of 4 millimeter by 4 millimeter. Cells from, for example, blood or the like, flow substantially one by one through flow cell 18 during analysis. Laser input 20 emits a beam of light that is oriented substantially orthoganilly to the flow of blood cells through flow cell 18 such that laser light impinges upon the blood cells as they pass through flow cell 18. Unlike the prior art, the laser light emitted by laser input 20 need not be polarized for analysis of the cells according to the present invention. Laser input 20 maybe for example a 635 manometer semiconductor diode laser with an output power of 10 miliwatts, model No. HL6320G manufactured by Hatachi and available from Thor Labs, Inc. of Newton, New Jersey. A portion of the beam from laser input 20 that impinges upon the blood cells in flow cell 18 is scattered at a substantially right angle to the beam of laser input 20 ("right angle scatter light"). A second portion of the beam from laser input 20 that impinges upon the cells in flow cell 18 is scattered at a much lower angle than 90°. This scatter is termed "low angle forward scatter light" and has an angle of from about 2° to about 5° from the orientation of the original beam from laser input 20. Right angle scatter light detector 22 is oriented to receive the previously

mentioned right angle scatter light. Right angle scatter light detector is preferably located about 2 millimeters from the blood cells in the flow cell 18. An important aspect of the present invention is that, at the distance of about 2 millimeters from the blood cells, right angle scatter light detector 22 collects a cone of scattered light of at least 100° or greater, and preferably 130° or greater. It is this larger light cone value over the prior art light cone of about 72° that results in the greater cluster separation in the present invention due to the larger signal gathered. In contrast, the smaller 72° cone of the prior art results in missed signals and lesser cluster separation.

Low angle forward scatter light detector 24 is oriented to capture the previously mentioned low angled forward scatter light oriented at about 2° to about 5° from the beam of laser input 20. Both right angle scatter light detector 22 and low angle forward scatter light detector 24 can be, for example, silicone PIN photodiodes Model No. S5106PIN manufactured by Hamamatsu Corp. of Bridgewater, New Jersey.

In one embodiment of the present invention, both right angle scatter light detector 22 and low angle forward scatter light detector 24 are employed in order to produce a 2-dimensional cytrogram. However, it should be noted that in another embodiment of the present invention, only right angle scatter light detector 22 is employed, low angle forward scatter light detector 24 is not employed, and characterization of eosinophils is possible.

While the electro-optical elements of FIG. 2 have been described above using specific components, it will readily be apparent to one skilled in the art that different components can be employed to achieve the desired results described above. More specifically, reference is made to Practical Flow Cytometry 3rd Ed. 1995 by Howard M. Shapiro, Wiley-Liss Publisher, ISBN No. 0-471-30376-3, which is incorporated herein by reference.

Referring to FIG. 3, the electrical outputs from right angle scatter light detector 22 and low angle forward scatter light detector 24, which may be in voltage or current form, for example, are amplified by preamplifier 26 and then sent to signal processor 28. Signal processor 28 measures the area under the voltage or current curve, or measures the peak of

the voltage or current curve, received from right angle light scatter detector 22 and/or low angle forward scatter light detector 24. The data from signal processor 28 is converted by analog to digital converter 30. The digital data is next processed by central processing unit 32 based on software programs to display the data in graphical representation on display 34. It will be readily apparent to those skilled in the art that the signal amplification, processing, conversion and display can be accomplished by many well known methods, including but not limited to those disclosed in Practical Flow Cytometry 3rd Ed. by Howard M. Shapiro, 1995 Wiley-Liss Publishers, ISBN No. 0-471-30376-3, incorporated herein by reference.

Referring to FIG. 5, the output of the data from the flow cytometer of the present invention is shown. FIG. 5 has the output of right angle scatter light detector 22 as one axis and the output of low angle forward scatter light detector 24 as the other axis. Eosinophils are located to the right of the software threshold line and, as shown in FIGS. 6A, 7A, 8A, and 9A, produce cluster points that are less concentrated than are those of the prior art. Computer software programs employed to identify clusters based on cluster points can thus do so more reliably with the present invention.

Next referring to FIGS. 6A, 6B, 7A, 7A, 7B, 8A, 8B, 9A and 9B, graphical representations of leukocyte identification is shown, with specific reference to eosinophil identification. The data of FIGS. 6A, 7A, 8A, and 9A was employed using the apparatus of the present invention. In FIGS. 6A, 7A, 8A, and 9A the term R2 denotes primarily lymphocytes, R3 denotes primarily monocytes, R4 denotes primarily neutrophils and R5 denotes primarily eosinophils. FIGS. 6B, 7B, 8B, and 9B pertain to data employing an apparatus substantially disclosed in US Patent No. 5,017,497. Whole blood samples of either canine or human blood were prepared as follows before analyzing with the apparatus of present invention or the prior art. The whole blood sample was diluted 10 to 1 in phosphate buffered saline. Then 40 microliters of the phosphate buffered saline treated whole blood sample was mixed with 1,200 microliters of a lysing solution. The lysing solution consisted of 8.3 grams of ammonium chloride, 1 gram of potassium bicarbonate, 0.37 grams tetrasodium EDTA per liter of lysing solution. The whole blood

sample was lysed for 20 minutes to one-half of an hour. It will be readily understood by those skilled in the art that lyse time can readily be reduced to between 30 seconds and one minute.

A good correlation exists between the eosinophil of the present invention of FIGS. 6A, 7A, 8A and 9A with the eosinophil data of the DEPOL/ORTHOGONAL graphical representation of the prior art as shown in FIGS. 6B, 7B, 8B and 9B. More specifically, regarding FIGS. 6A and 6B, the eosinophil value for the present invention is 2.1% and for the prior art is 2.0%. Regarding FIGS. 7A and 7B, the eosinophil data for the present invention is 7.6% and for the prior art is 8.2%. Regarding FIGS. 8A and 8B the eosinophil data for the present invention is 13.1% and for the prior art is 9.8%. Regarding FIGS. 9A and 9B the eosinophil data for the present invention is 10.8% and for the prior art is 14.6%. For all of the above graphical representations of the present invention, FIGS. 6A, 7A, 8A and 9A an eosinophil cluster is present at R5. Regarding the prior art data of FIGS. 6B, 7B and 8B the SIZE/COMPLEXITY graphical representation shows no eosinophil cluster, while the graphical representation of FIG. 9B does show a cluster.

A comparison of the data of the present invention from FIGS. 6A, 7A, 8A and 9A with the prior art data of FIGS. 6B, 7B, 8B and 9B show a marked decreased density or concentration of the cluster points within the eosinophil clusters. The separation of these cluster points allows the software programs that locate and identify different clusters to more readily locate and identify the clusters produced by the apparatus and method of the present invention compared to those of the prior art.

While the invention has been described with reference to particular embodiments and applications, it will be appreciated that various embodiments and applications based on the teaching of the present invention are possible.

1. A high numerical aperture flow cytometer for detecting particles in a fluid sample comprising:

a flow cell comprising a passage through which the fluid sample containing the particles to be detected flows;

a laser input which emits a beam of light oriented substantially orthogonally to the flow of fluid sample through the flow cell such that the laser light impinges upon the particles in the fluid sample as they pass through the flow cell; and

at least one light detector oriented to receive and measure the light after it passes through the flow cell, wherein the light detector collects a cone of scattered light of at least 100°.

2. The flow cytometer of claim 1 wherein the laser light which enters the flow cell is not polarized.

3. The flow cytometer of claim 1 wherein the flow cell is quartz.

4. The flow cytometer of claim 1 wherein the particles are blood cells.

5. The flow cytometer of claim 1 wherein the blood cells are leukocytes.

6. The flow cytometer of claim 1 wherein the leukocytes are eosinophils.

7. The flow cytometer of claim 1 wherein the particles are viruses.

8. The flow cytometer of claim 1 wherein the laser input is a semiconductor diode laser which emits laser light at a wavelength of approximately 635 nm.

9. The flow cytometer of claim 1 wherein a portion of the beam of light from the laser input which impinges upon the particles as they pass through the flow cell is scattered at a substantially right angle to the beam of laser input.

10. The flow cytometer of claim 1 wherein the cone of scattered light is at least 130°.

11. The flow cytometer of claim 9 wherein the light detector is oriented to receive the beam of light scattered at a substantially right angle to the beam of the laser input.

12. The flow cytometer of claim 9 wherein the light detector is positioned approximately 2 mm from the particles in the flow cell.

13. The flow cytometer of claim 9 wherein another portion of the beam from the laser input which impinges upon the particles as they pass through the flow cell is scattered at an angle of less than 90° from the orientation of the beam from the laser input.

14. The flow cytometer of claim 13 wherein the light scattered at an angle of less than 90° is scattered at an angle of from about 2° to about 5°.

15. The flow cytometer of claim 14 wherein a second light detector is oriented to receive the beam of light scattered at a angle of from 2° to 5° from the orientation of the beam of the laser input.

16. The flow cytometer of claim 15 wherein the light detector and the second light detector are employed to produce a two dimensional cytogram.

17. A method of detecting particles in a fluid sample comprising:

providing a high numerical aperture flow cytometer comprising,

a flow cell with a passage through which the fluid sample containing the particles to be detected flow;

a laser input which emits a beam of light oriented substantially orthogonally to the flow of fluid sample through the flow cell such that the laser light impinges upon the particles in the fluid sample as they pass through the flow cell thereby producing scattered light; and

at least one light detector oriented to receive and measure the scattered light after it passes through the flow cell, wherein the light detector collects a cone of scattered light of at least 100°; and

passing the fluid sample through the flow cytometer to obtain readings from the light detector(s);

constructing a graphical illustration of the readings obtained from the light detector(s); and

identifying particles in the sample to be detected.

18. The method of claim 17 wherein the light is scattered at a substantially right angle to the beam of laser input, and wherein the light detector is oriented to collect the cone of scattered light.

19. The method of claim 17 wherein the flow cytometer comprises a second light detector oriented to collect light scattered at an angle different from the scattered light collected by the first light detector.

20. The method of claim 19 wherein the first light detector is oriented to receive a beam of light scattered at a substantially right angle to the beam of the laser light, and the second light detector is oriented to receive the beam of light scattered at an angle less than 90° from the beam of the laser light.

21. The method of claim 20 wherein the beam of light scattered at an angle less than 90° from the beam of the laser light is scattered at an angle of from about 2° to about 5° from the beam of the laser light.

22. The method of claim 20 wherein the graphical illustration comprises one axis recording readings from the first light detector and a second axis recording readings from the second light detector.

23. The method of claim 21 wherein the graphical illustration comprises one axis recording readings from the first light detector and a second axis recording readings from the second light detector.

24. The method of claim 17 wherein the particles are leukocytes.

25. The method of claim 24 wherein the leukocytes are eosinophils.

26. The method of claim 18 wherein the particles are eosinophils.

27. The method of claim 17 wherein the light detector collects a cone of scattered light of at least 130°.

28. The method of claim 17 wherein the laser light emitted by the laser input is not polarized.

Prior Art

FIG. 1

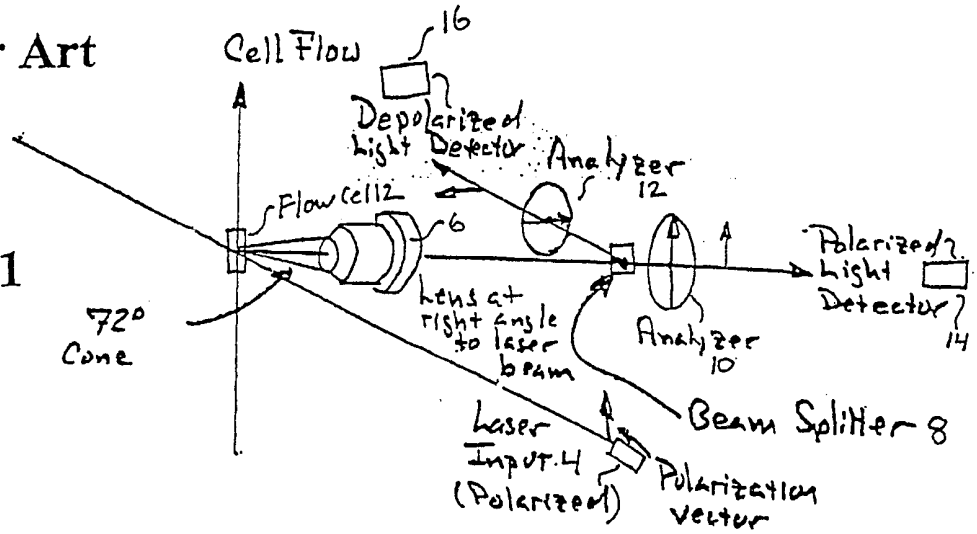
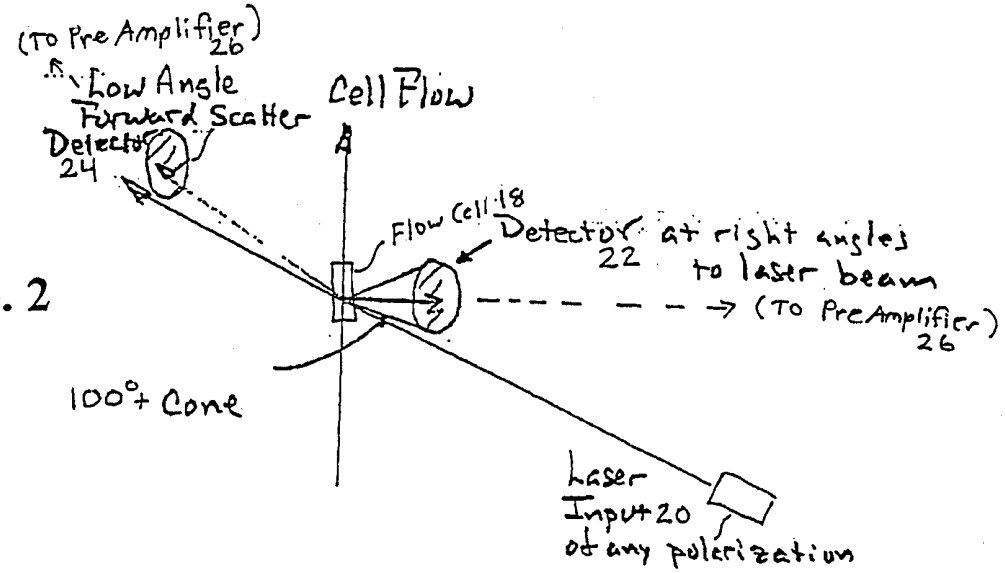
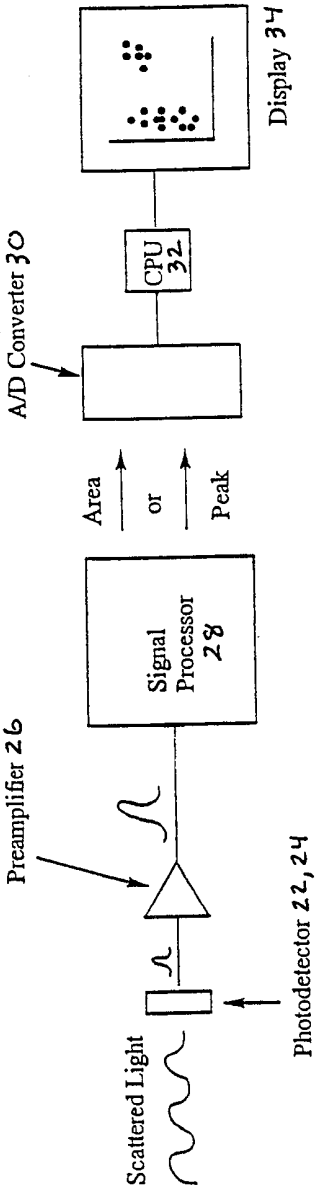


FIG. 2

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Preamplifier 26
Signal Processor 28
Area
or
Peak
A/D Converter 30
CPU 32
Display 34

FIG. 3

Prior Art

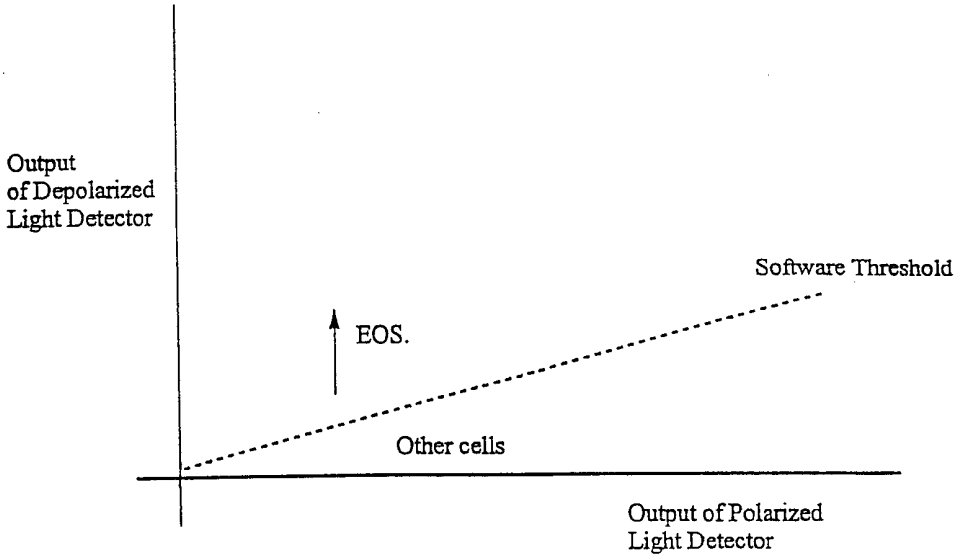
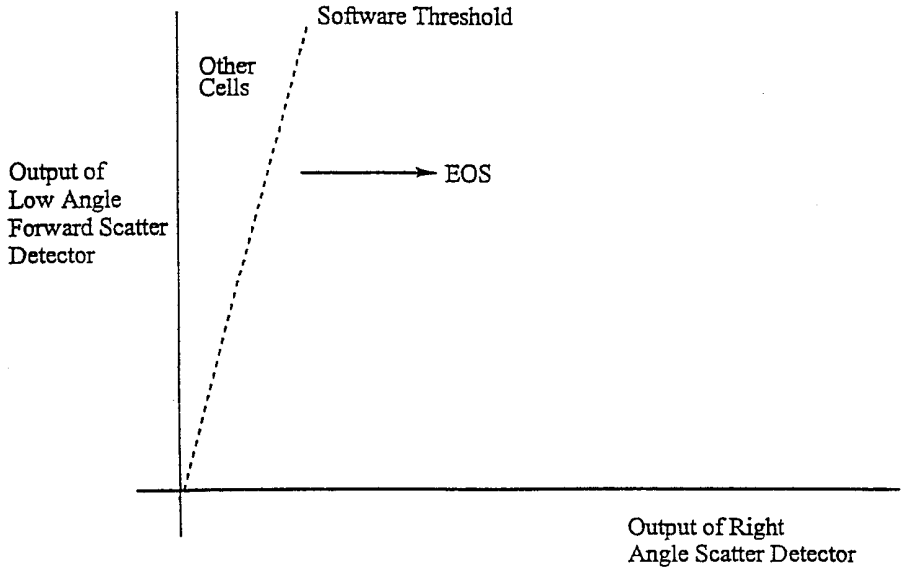


FIG. 4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EOS
Output of
Low Angle
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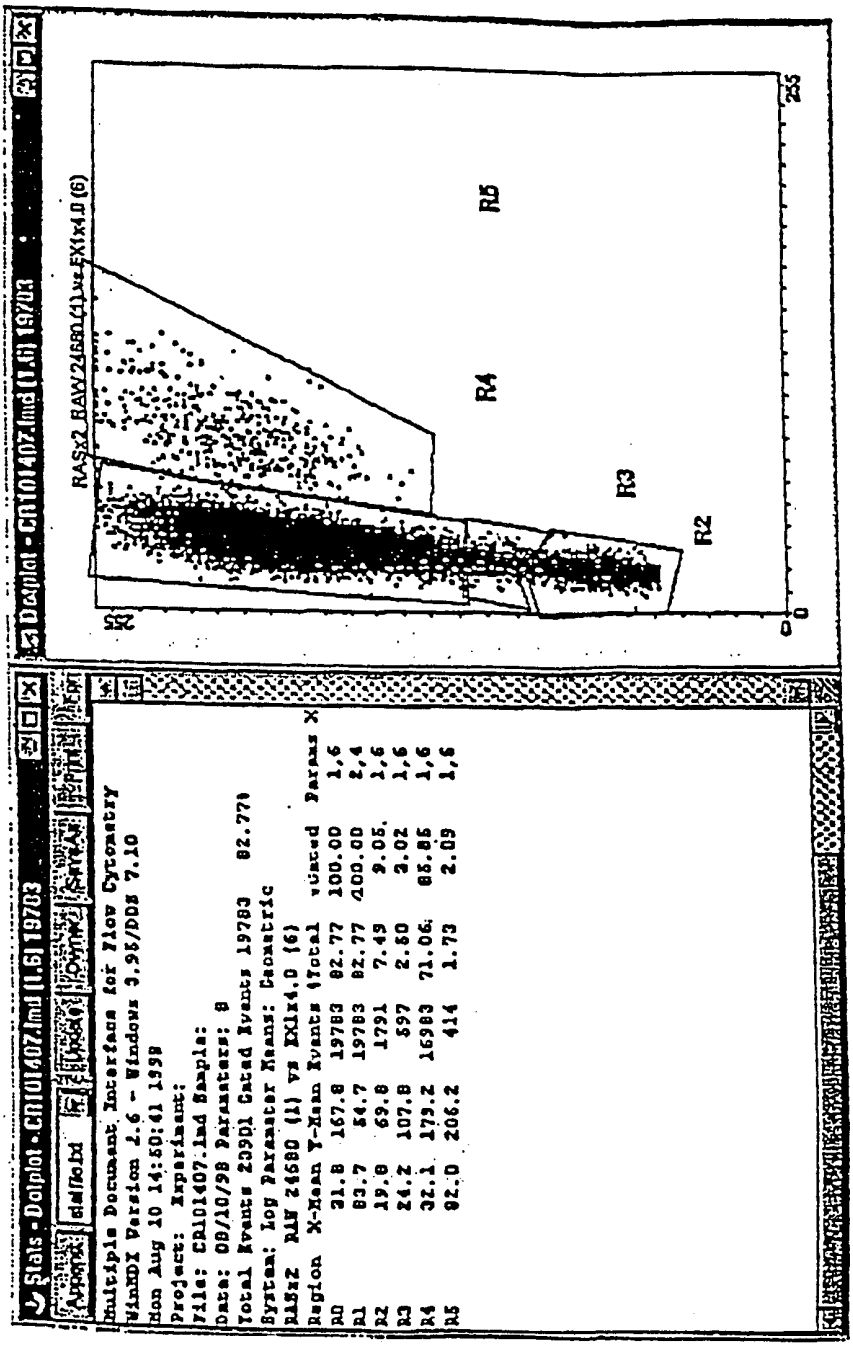
Detector
Output of Right
Angle Scatter Detector

FIG. 5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R3
R4
R5
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0
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WinMDI Version 2.6 - Windows 3.95/DOS 7.10
Mon Aug 10 14:50:41 1998
Project: Experiment:
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Date: 08/10/98 Parameters: 8
Total Events 23901 Gated Events 19783 82.771
System: Log Parameter Means: Geometric
RAS2 RAW 24680 (1) vs EX1x4.0 (6)
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R1 83.7 54.7 19783 82.77 100.00 2,4
R2 19.8 69.8 1791 7.49 9.05 1,6
R3 24.2 107.8 597 2.50 3.02 1,6
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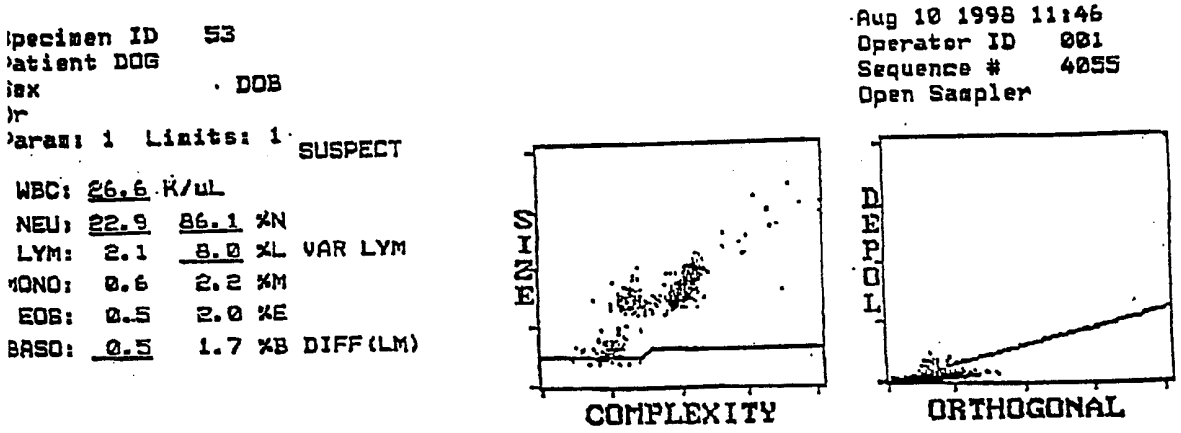
R5 92.0 206.2 414 1.73 2.09 1,6

FIG. 6A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Sex DOB
Dr
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MONO: 0.6 2.2 %M
EOS: 0.5 2.0 %E
BASO: 0.5 1.7 %B DIFF(LM)
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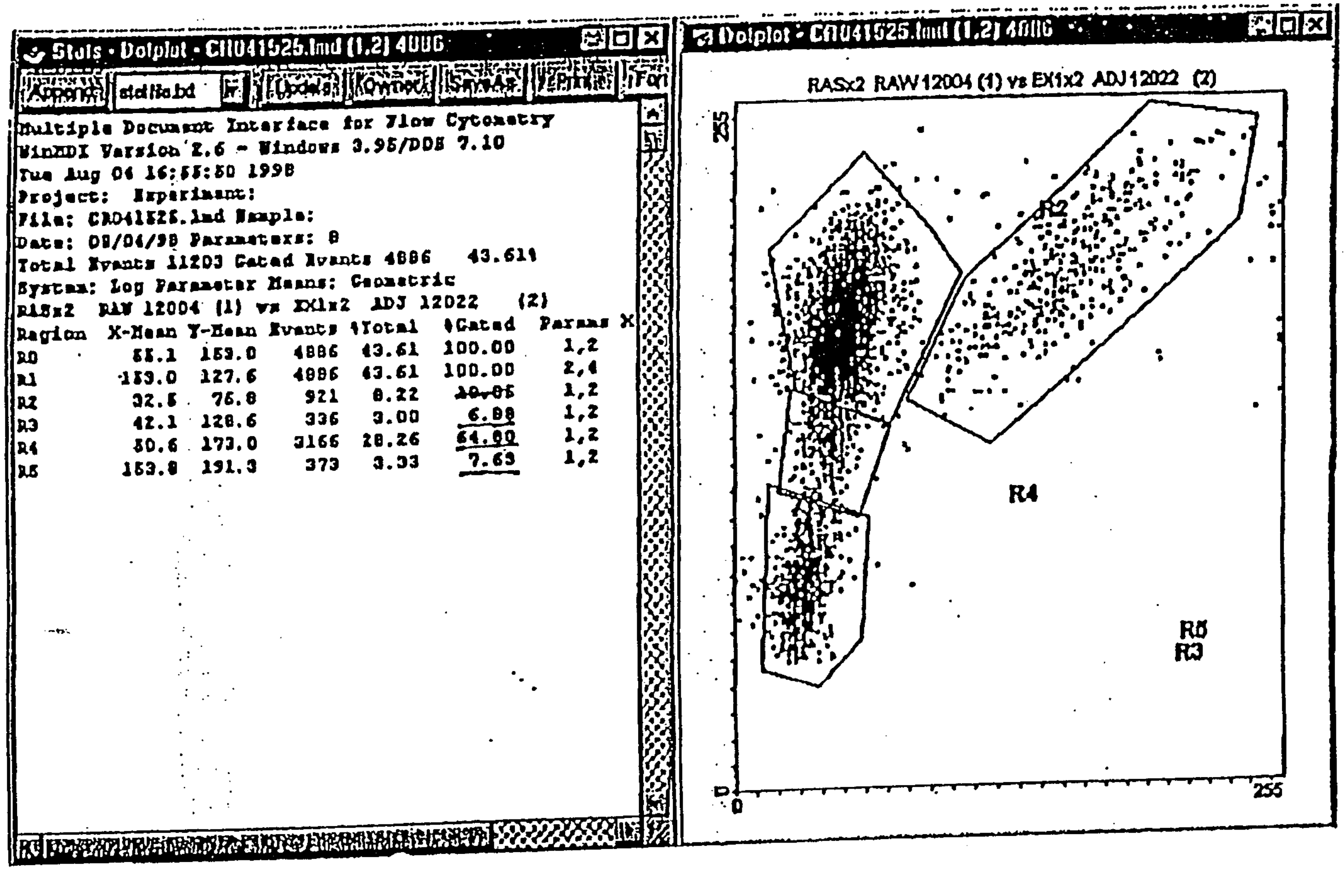
COMPLEXITY
DEPOL
ORTHOGONAL

FIG. 6B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Project: Experiment:
File: CH041525.lmd Sample:
Date: 08/04/98 Parameters: 8
Total Events 11203 Gated Events 4886 43.61%
System: Log Parameter Means: Geometric
RASx2 RAW 12004 (1) vs EX1x2 ADJ 12022 (2)
Region X-Mean Y-Mean Events %Total %Gated Params X
R0 55.1 153.0 4886 43.61 100.00 1,2
R1 153.0 127.6 4886 43.61 100.00 2,4
R2 32.5 76.8 921 8.22 10.05 1,2
R3 42.1 128.6 336 3.00 6.88 1,2
R4 30.6 173.0 3166 28.26 64.80 1,2
R5 153.8 191.3 373 3.33 7.63 1,2
Dotplot - CH041525.lmd (1,2) 4886
RASx2 RAW12004 (1) vs EX1x2 ADJ12022 (2)
255
0
255
R2
R4
R5
R3

FIG. 7A

Specimen ID 35
Patient 814DOG
Sex DOB
Dr
Param: 1 Limits: 1 SUSPECT

WBC:	5.9	K/uL		
NEU:	4.1	69.7	%N	
LYM:	1.2	20.9	%L	VAR LYM
MONO:	0.1	1.1	%M	NRBC
EOS:	0.5	8.2	%E	
BASO:	0.0	0.1	%B	DIFF(LM)

Aug 04 1998 12:17
Operator ID 001
Sequence # 4019
Open Sampler

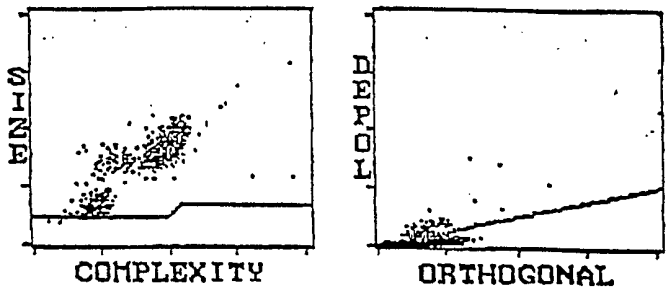

FIG. 7B

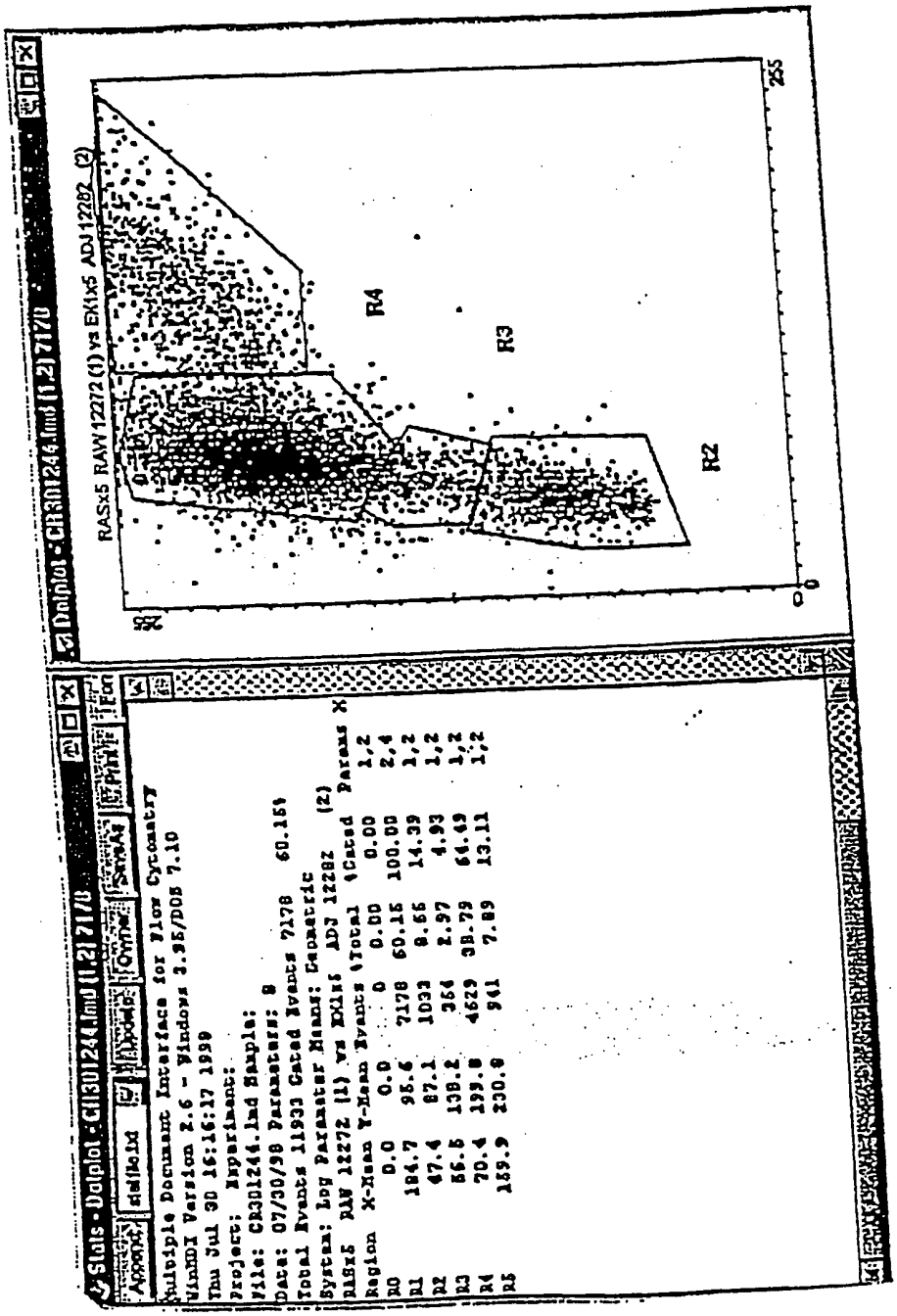
Stats - Dotplot - CB301244.lmd (1,2) 7170
Dotplot - CB301244.lmd (1,2) 7170
RASx5 RAW 12272 (1) vs EK1x5 ADJ 12282 (2)
R4
R3
R2
255
0
Multiple Document Interface for Flow Cytometry
WinMDI Version 2.6 - Windows 2.95/NT5 7.10
Thu Jul 30 16:16:17 1998
Project: Experiment:
File: CB301244.lmd Sample:
Data: 07/30/98 Parameters: 8
Total Events 11933 Gated Events 7178 60.151
System: Log Parameter Means: Geometric
RASx5 RAW 12272 (1) vs EK1x5 ADJ 12282 (2)
Region X-Mean Y-Mean Events %Total %Gated Params X
R0 0.0 0.0 0 0.00 0.00 1,2
R1 184.7 95.6 7178 60.15 100.00 1,2
R2 47.4 87.1 1033 8.66 14.39 1,2
R3 86.5 138.2 354 2.97 4.93 1,2
R4 70.4 193.8 4629 38.79 64.49 1,2
R5 159.9 230.9 941 7.89 13.11 1,2

FIG. 8A

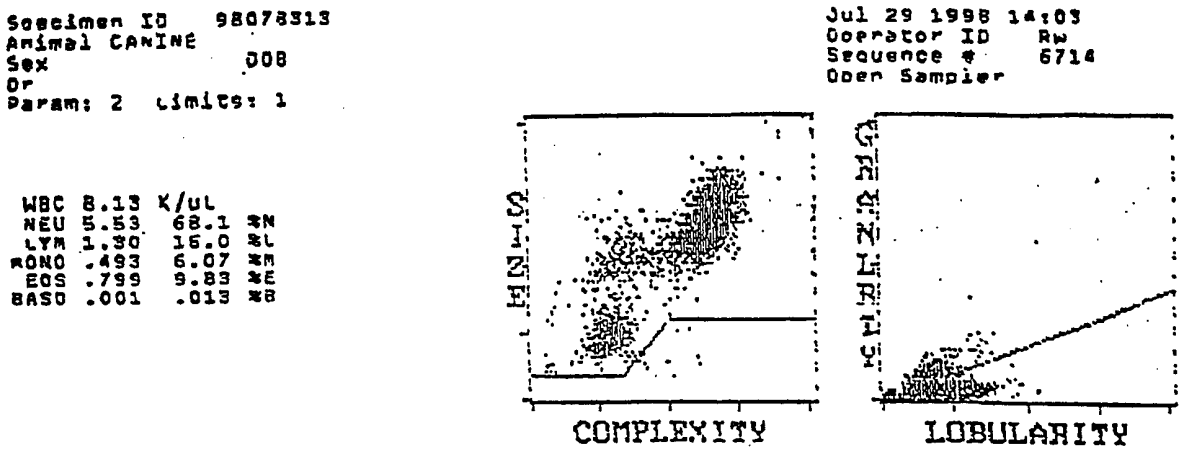
Specimen ID 98078313
Animal CANINE
Sex 008
Dr
Param: 2 Limits: 1
WBC 8.13 K/uL
NEU 5.53 68.1 %N
LYM 1.30 16.0 %L
MONO .493 6.07 %M
EOS .799 9.83 %E
BASO .001 .013 %B
Jul 29 1998 14:03
Operator ID RW
Sequence # 6714
Open Sampler
GRANULARITY
COMPLEXITY
LOBULARITY

FIG. 8B

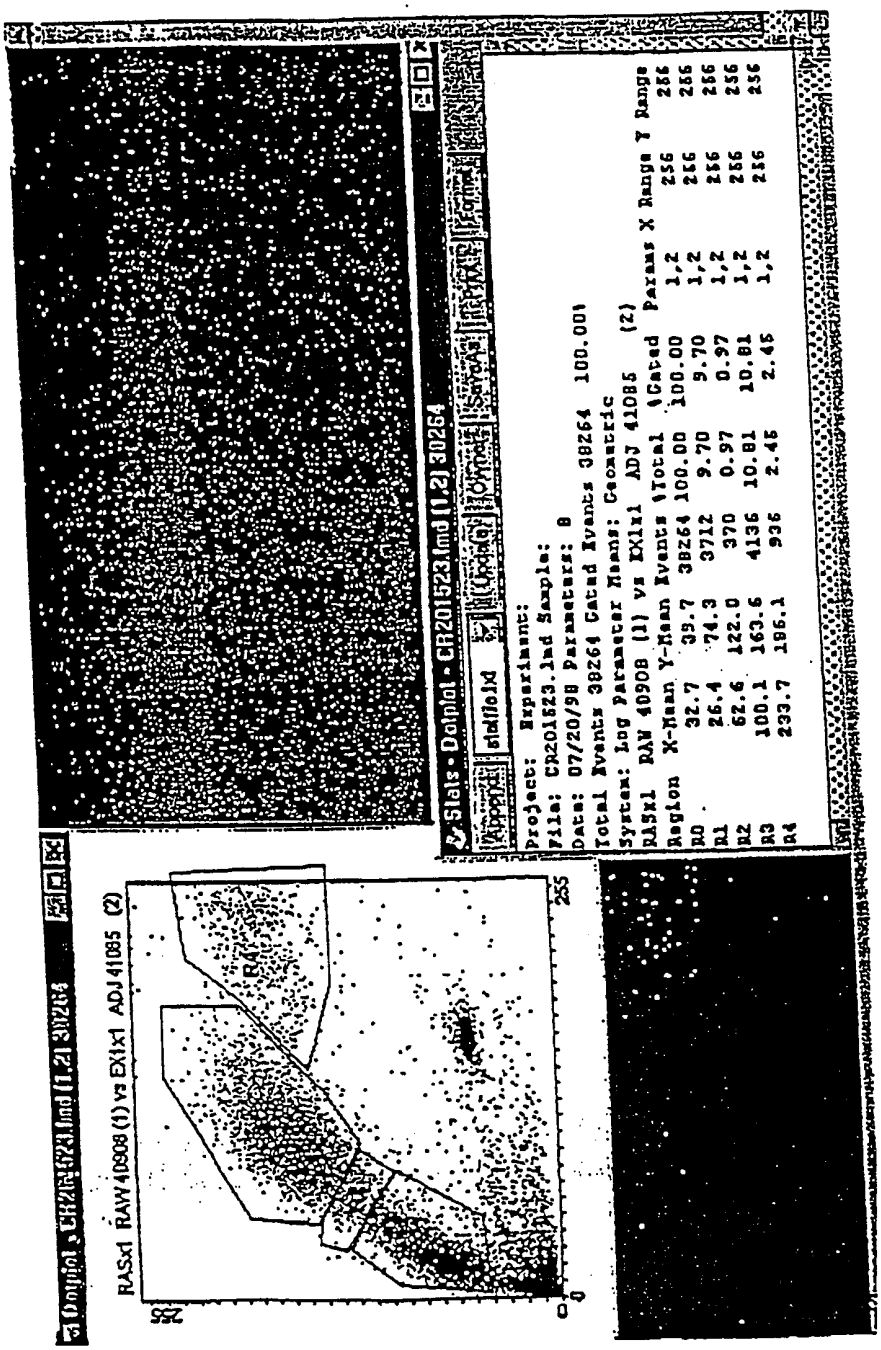
Doublet - CR201523.lmd [1,2] 30264
RAS x1 RAW 40908 (1) vs EX1x1 ADJ 41085 (2)
255
0
Stats - Doublet - CR201523.lmd [1,2] 30264
Project: Experiment:
File: CR201523.lmd Sample:
Date: 07/20/98 Parameters: 8
Total Events 38264 Gated Events 38264 100.001
System: Log Parameter Name: Geometric
RAS x1 RAW 40908 (1) vs EX1x1 ADJ 41085 (2)
Region X-Mean Y-Mean Events %Total %Gated Params X Range Y Range
R0 32.7 39.7 38264 100.00 100.00 1,2 256 256
R1 26.4 74.3 3712 9.70 9.70 1,2 256 256
R2 62.6 122.0 370 0.97 0.97 1,2 256 256
R3 100.1 163.6 4136 10.81 10.81 1,2 256 256
R4 233.7 186.1 936 2.45 2.45 1,2 256 256

FIG. 9A

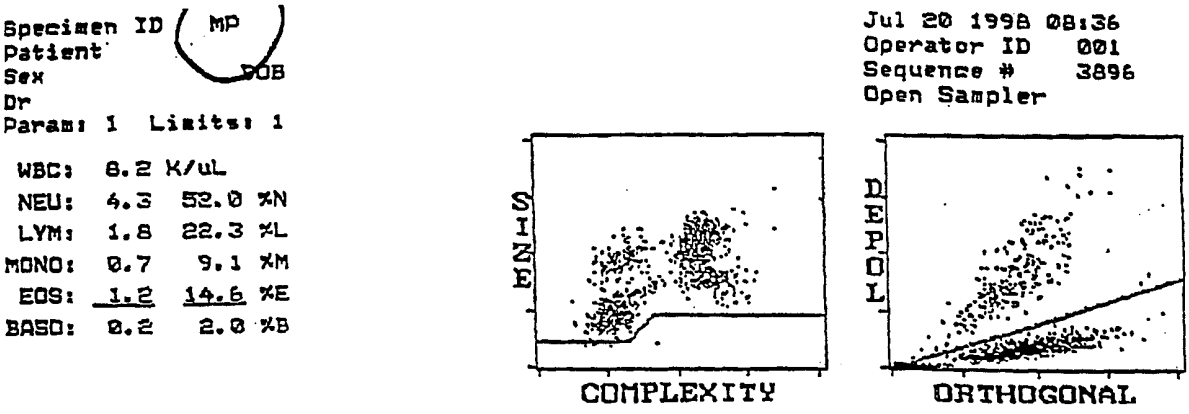
Specimen ID MP
Patient
Sex DOB
Dr
Param: 1 Limits: 1
WBC: 8.2 K/uL
NEU: 4.3 52.0 %N
LYM: 1.8 22.3 %L
MONO: 0.7 9.1 %M
EOS: 1.2 14.6 %E
BASO: 0.2 2.0 %B
Jul 20 1998 08:36
Operator ID 001
Sequence # 3896
Open Sampler
SINE
COMPLEXITY
DEPOL
ORTHOGONAL

FIG. 9B