United States Patent [19]

Stiller

[11] **3,790,760**

[45] **Feb. 5, 1974**

[54] **APPARATUS FOR COUNTING, DIFFERENTIATING AND SORTING PARTICLES ACCORDING TO THEIR MICROSTRUCTURE VARIATIONS**

[75] Inventor: **Paul F. Stiller**, Clarence, N.Y.

[73] Assignee: **Cornell Aeronautical Laboratory, Inc.**, Buffalo, N.Y.

[22] Filed: **Sept. 27, 1971**

[21] Appl. No.: **184,183**

Related U.S. Application Data

[63] Continuation of Ser. No. 877,729, Nov. 18, 1969, abandoned.

[52] **U.S. Cl.** ..... **235/92 PC**, 235/92 R, 235/92 V, 356/102

[51] **Int. Cl.** **G06m 11/02**

[58] **Field of Search** ..... 235/92 PC

[56] **References Cited**

UNITED STATES PATENTS

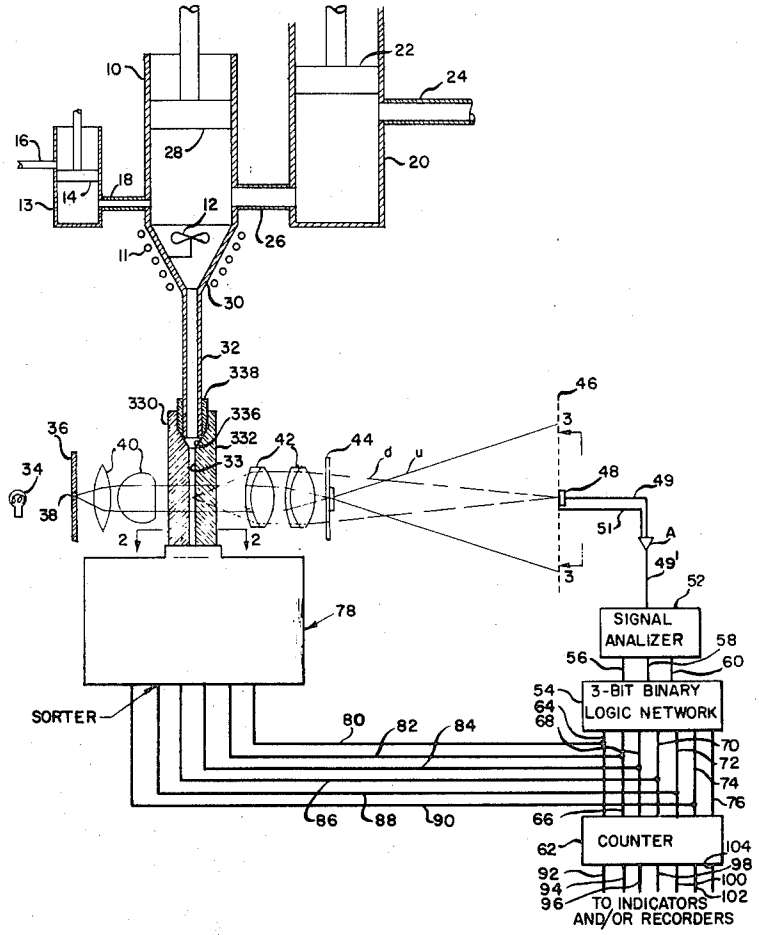
2,875,666	3/1959	Parker	235/92 PC
3,676,647	7/1972	Staffin	235/92 PC
3,515,884	6/1970	Imadate	235/92 PC

Primary Examiner—Maynard R. Wilbur
Assistant Examiner—Robert F. Gnuse
Attorney, Agent, or Firm—Allen J. Jaffe

[57] **ABSTRACT**

An apparatus for counting and sorting the formed elements of blood, comprising the phase illumination of sample flow through a microcapillary passage, photodetectors responsive to the microstructure of the formed elements, a signal analyzer responsive to the photodetectors, a logic network for generating control signals, a sorter responsive to the control signals for separating and collecting the formed elements.

45 Claims, 9 Drawing Figures



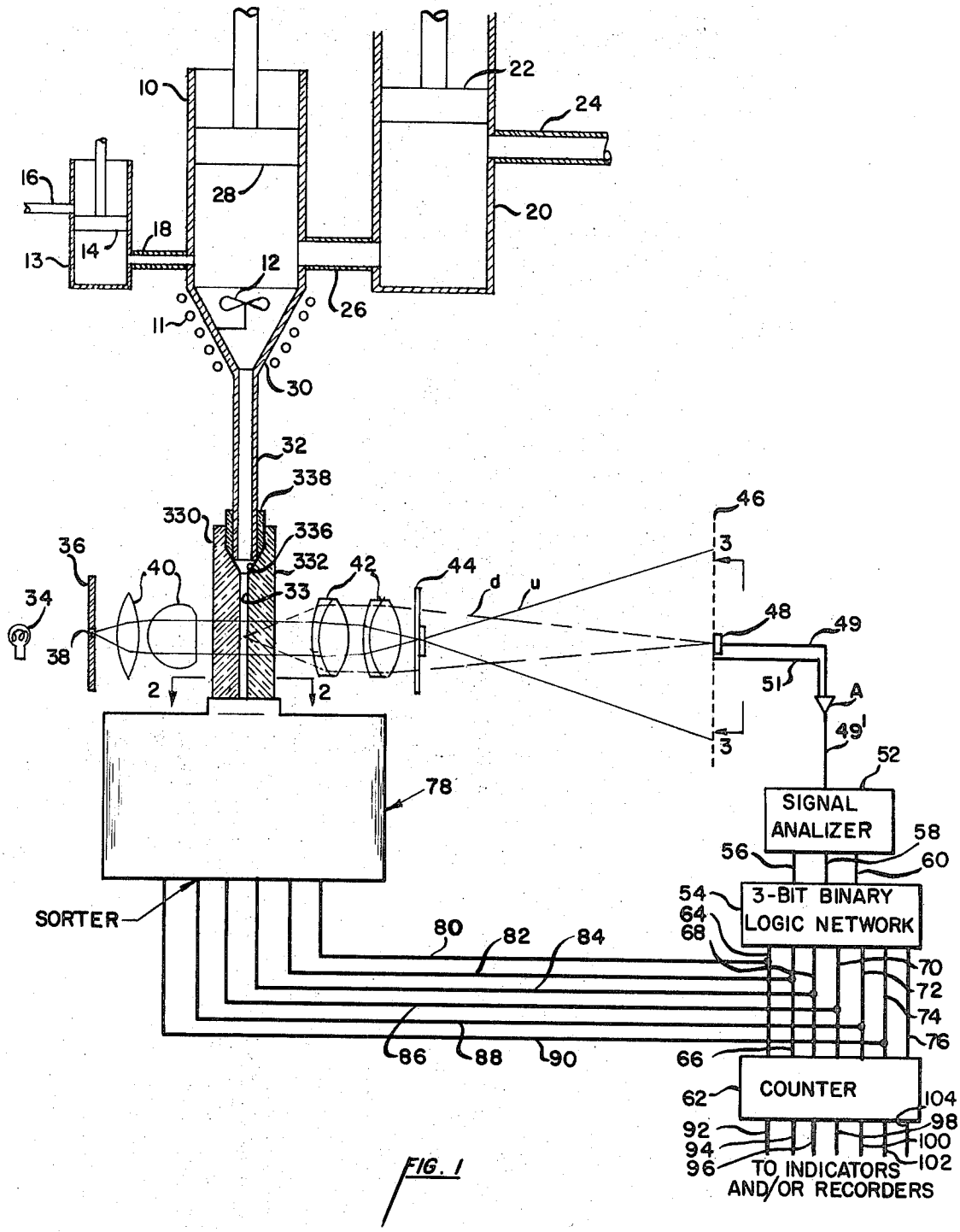


FIG. 1

INVENTOR

PAUL F. STILLER

BY

ATTORNEY

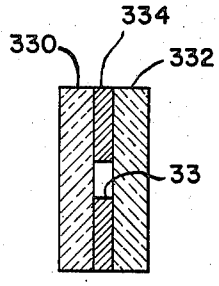


FIG. 2

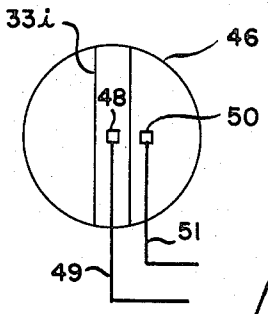


FIG. 3

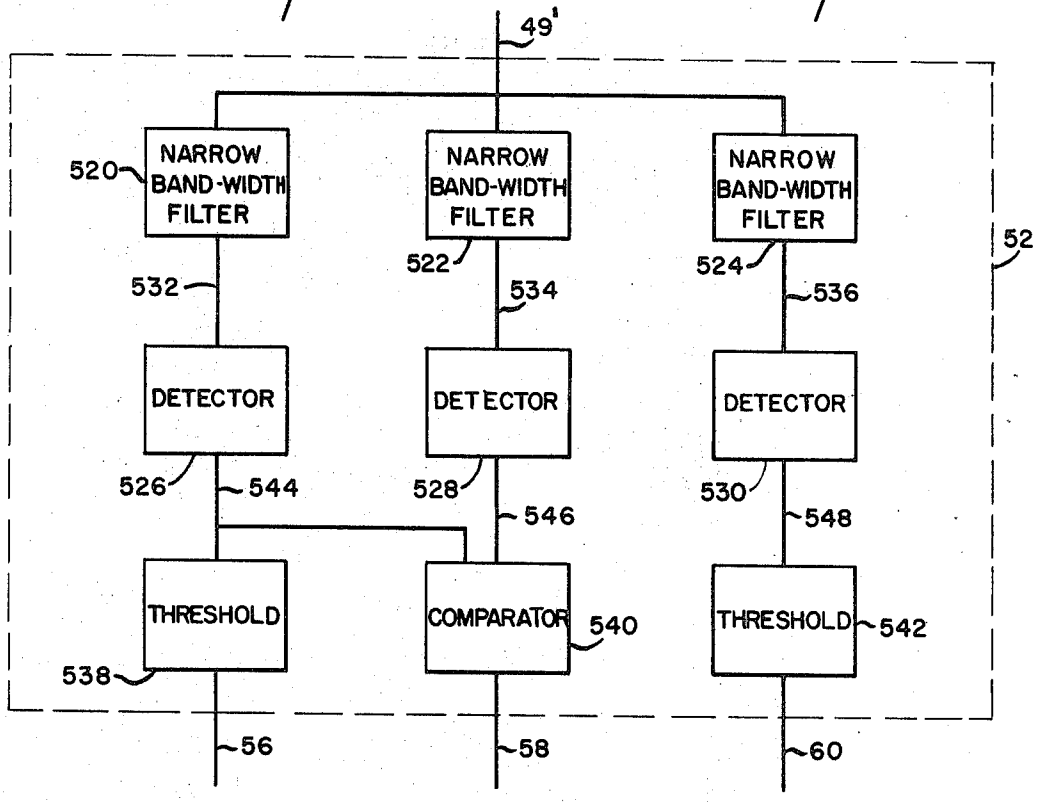


FIG. 4

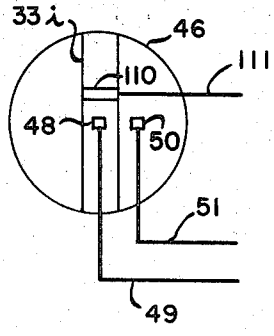


FIG. 9

INVENTOR

PAUL F. STILLER

BY

ATTORNEY

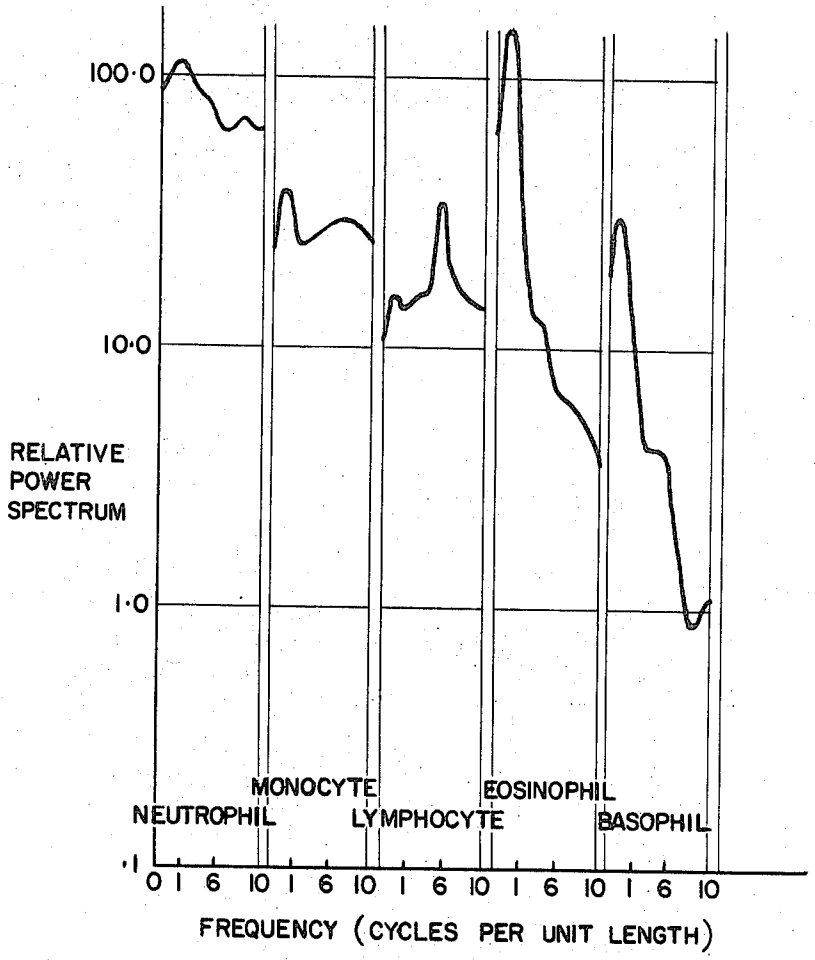


FIG. 5

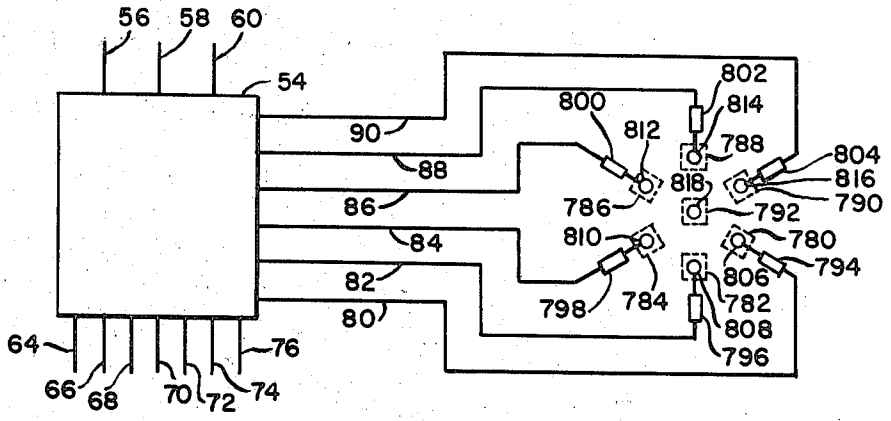


FIG. 6

INVENTOR
PAUL F. STILLER

BY

ATTORNEY

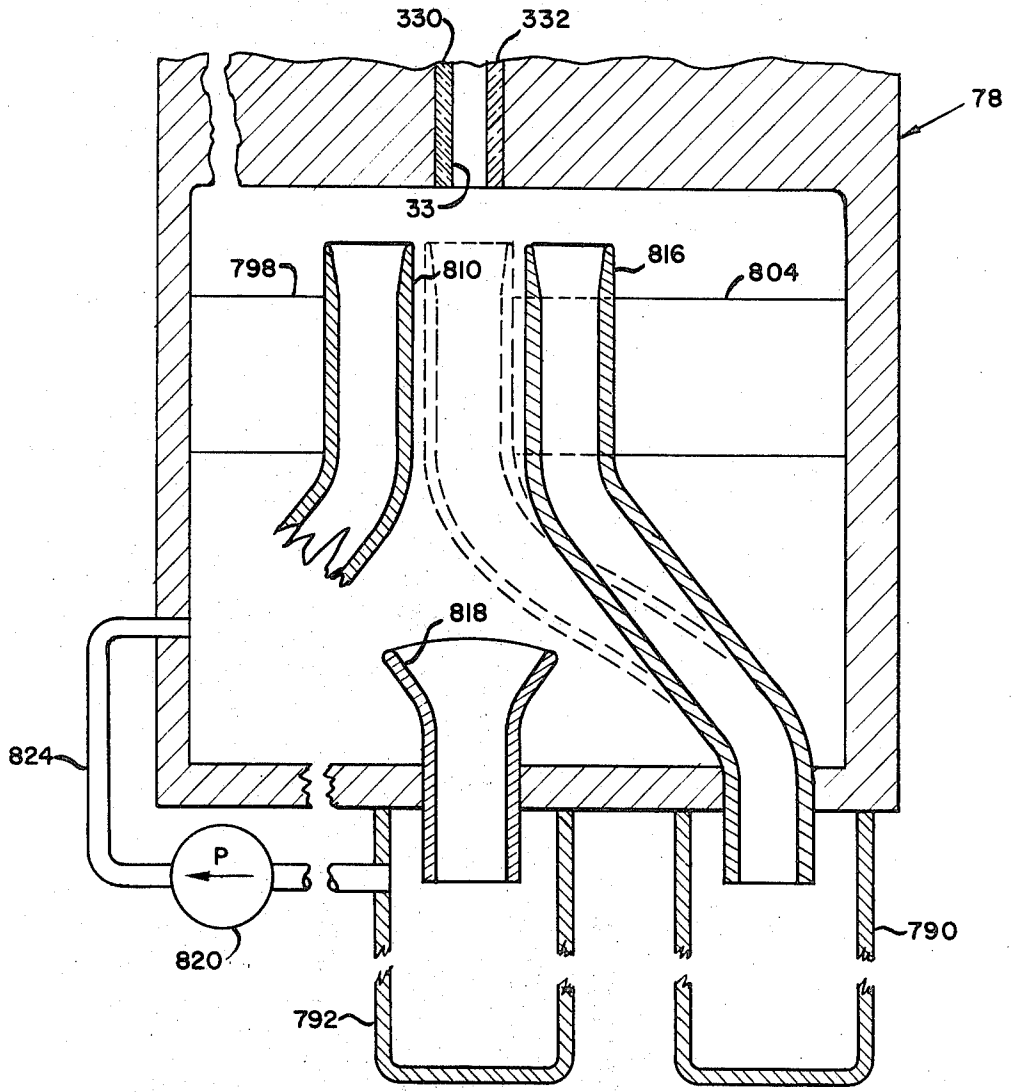


FIG. 7

INVENTOR

PAUL F. STILLER

BY

ATTORNEY

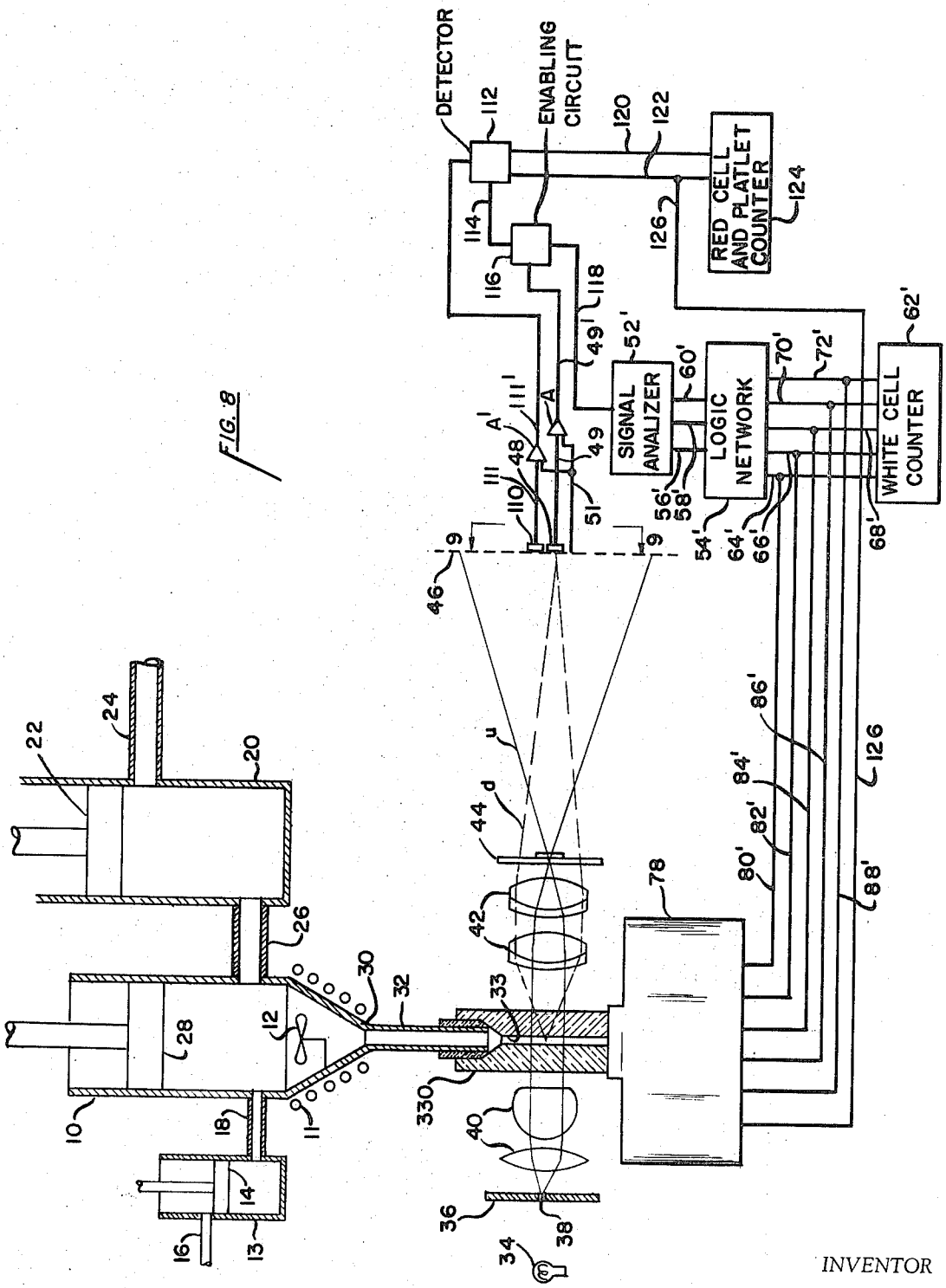


FIG. 8

INVENTOR

PAUL F. STILLER

BY

ATTORNEY

APPARATUS FOR COUNTING, DIFFERENTIATING AND SORTING PARTICLES ACCORDING TO THEIR MICROSTRUCTURE VARIATIONS

This is a continuation of application Ser. No. 877,729, filed Nov. 18, 1969, now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates to the analysis and control of fluids containing particles which have microstructures of varying optical densities. More particularly, the present invention finds specific application in the analysis of fresh whole blood to differentiate, count, sort and collect the formed elements thereof.

Current clinical practice involves the sampling of a patient's blood to determine the type, quantity, and characteristics of the various formed elements thereof; which, as is well known, comprise; red cells, five major varieties of white cells, and blood platelets. The current technique is to smear a small quantity of whole blood on a microscope slide, to stain it, and then, it manually observe, differentiate and count the "blood cells."

Various devices are presently known which attempt the automation of blood counting. These for the most part are capable of counting red cells and, by chemical lysing, total white cells. None of these known devices are capable of differentiating in real time the five basic varieties of white cells, nor can they handle all of the cells, including the platelets, without secondary procedures, and none are capable of sorting and saving the sampled cell.

According to one of the currently known devices, a blood sample is diluted and, to count white cells, a portion of the sample is lysed to destroy the red cells and pumped through a dark field illuminated observation chamber. As each particle passes through the chamber a photo sensor records the forward scattering of light caused by the white cells. To count red cells, the remaining unlysed sample is further diluted and pumped through the chamber. This device cannot distinguish between cell types and the sample is destroyed in the test.

Another known prior device utilizes a conductive fluid to dilute the sample. As the fluid passes through the sampling chamber the conductivity of the fluid changes because of the presence of the cells. Red and white cell differentiation is possible because of the relative size of the two cells and the resulting difference in conductivity. This system is not capable of reliable platelet counting and the sample is also destroyed in the process.

SUMMARY OF THE INVENTION

The foregoing, as well as other, disadvantages of the prior art devices are overcome by the principles of the present invention which provides an apparatus which is capable of differentiating all of the blood cell types without the necessity of chemical lysing or dying. In addition the cells are not destroyed during the test and can be sorted and collected for further analysis.

The present invention generally relates to a method and apparatus for the analysis and control of fluids containing particles whch have microstructures of varying optical path lengths. A sample of the fluid is diluted and pumped through a microcapillary observation chamber for substantially sequential flow of the individual particles within the fluid. A portion of the microstructure of each particle is observed by transilluminated phase optics. Photosensitive devices respond to the optical path length variations within each observed particle and generate a varying output signal indicative thereof. The output signal is analyzed by a suitably programmed logic network which develops control signals that are applied to flow controllers for sorting and collecting the different particles based on the varying microstructures thereof.

With respect to the formed elements of whole blood, it has been found that each blood cell type exhibits a microstructure having its own special random pattern which does not vary with respect to cell size or maturity. That is to say, the spatial distribution of the optical densities for each of the microstructure patterns are specific to each cell type. Thus, the individual cell types can be identified by differences in their microstructures. It is not necessary, however, to observe or scan the complete microstructure of any given cell before an identification can be made. It has been found, according to the present invention, that sufficient information for cell identification can be obtained by observing only that portion of the microstructure contained within a narrow slice across a randomly oriented cell, as it passes through the microcapillary tube.

Since the cell and its microstructure exhibit substantially the same color and contrast, ordinarily incoherent illumination will not permit visualization of the microstructure. However small optical path length differences do exist within the cell microstructure and, according to the present invention, detection means are provided that are responsive to these path length differences as the cells flow sequentially through the microcapillary tube. The signal developed by such detection means, suitably processed will be indicative of the identity of the particular cell type that generated such signal. In this manner, the apparatus can function in real time to differentiate, count, sort and collect the formed elements of whole blood.

Once the particle passing through the microcapillary is identified the present invention permits segregation thereof by the provision of suitable means controlling the flow of particles to a plurality of separate collecting vessels or containers. The containers or collecting vessels may be controlled by piezoelectric, electrostrictive or magnetostrictive elements connected thereto which cause movement of preselected containers to align with the efflux from the microcapillary in response to the control signal. Basically, then, the present invention provides means for identifying, counting and sorting fluidized particles according to their microstructures, comprising, conduit means for containing the flow of a sample of fluid, the constituents of which include particles each having a microstructure indicative of the identity thereof; said conduit means having at least a portion thereof that is transparent to electromagnetic radiation; means for illuminating said portion of said conduit means and the sample fluid flowing therethrough; optical means for magnifying the image of said portion of said conduit means and the sample fluid flowing therethrough for converting optical path length or phase differences between the microstructure components of each particulate constitutent of said sample fluid to contrast or intensity differences; detection means responsive to the radiation passing through said conduit means for developing a varying output signal

indicative of the varying intensity of said radiation, and means for analyzing and processing said varying output signal for developing a plurality of output signals each indicative of a specific particulate constituent of said sample fluid.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the present invention; reference should be had to the following detailed description of the same taken in conjunction with the accompanying drawings, wherein;

FIG. 1 is a schematic representation of the apparatus according to the present invention; with parts thereof shown in block form,

FIG. 2 is a sectional view taken across line 2—2 of FIG. 1,

FIG. 3 is a sectional view taken across line 3—3 of FIG. 1,

FIG. 4 is a more detailed schematic of the signal analyzer of FIG. 1 illustrating the conventional components thereof in block form,

FIG. 5 is an exemplary graphical representation of relative power spectrum versus frequency for the various types of white blood cells,

FIG. 6 is a schematic functional diagram of the sorter illustrated in FIG. 1.

FIG. 7 is a sectional view of the sorter illustrated in FIG. 1, and

FIG. 8 is a view similar to FIG. 1 illustrating a modification.

FIG. 9 is a view similar to FIG. 3 illustrating a detail of the FIG. 8 modification.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the forthcoming description of the present invention, examples of its application in the field of blood analysis will be discussed in detail. It is to be emphasized that these examples are for illustrative purposes only and are not necessarily intended to limit the application of the inventive concepts to this particular field.

There are seven formed elements in normal human blood, which are in the form of cells or cell fragments that are released with the circulating blood from the bore marrow and other sources. The formed elements are suspended in a saline plasma solution. The cells occupy approximately 50 percent of the total volume of the circulating blood. The white cells or leukocytes are true cells with nucleii and active motility; they are the largest of the formed elements ranging from 12 microns to 20 microns. There are five types of white cells; viz., neutrophils, eosinophils, basophils, lymphocytes and monocytes. The other formed elements of blood are the erythrocytes (red cells) and the thrombocytes (platelets). The average number of formed elements per cubic millimeter of blood is as follows:

"Cell" Type	Number
Erythrocytes	5.40×10^6
Neutrophils	4.40×10^3
Eosinophils	0.20×10^3
Basophils	0.04×10^3
Lymphocytes	2.50×10^3
Monocytes	0.30×10^3
Thrombocytes	250×10^3

The leukocytes have a neutral optical density and are quite flexible and easily disturbed from their generally spherical shape. The erythrocyte (red cell) is a cell fragment containing no nucleus. All red cells are approximately the same size and shape, in the form of a bi-concave disc 7.2 microns in diameter by 2.4 microns in thickness. They are quite flexible and quite optically dense because of the hemoglobin content. Thrombocytes are the smallest formed elements. They are approximately 2 microns in diameter and contain a rather dense granule.

Referring now to the drawings and, more particularly, to FIG. 1, a sample container is schematically depicted at **10** as having a conically tapered lower portion about which is located a plurality of electrical wires forming coils **11** for coaction with an internally mounted stirring impeller **12.** Coils **11** are connected to a suitable source of electrical power (not illustrated).

Located adjacent sample container **10** is a vessel **13** for dispensing a measured quantity of undiluted blood to container **10.** For this purpose a piston member **14** slidably engages the interior cylindrical wall of vessel **13** for delivering the undiluted blood from an inlet **16** to an outlet **18** which communicates with container **10.**

A dilutent supply vessel **20** is provided adjacent container **10** for supplying a measured quantity of dilutent fluid, such as a saline solution, to sample container **10.** A piston **22** slides within the cylindrical wall of vessel **20** and controls the delivery of dilutent from an inlet **24** to an outlet **26** which is in fluid communication with container **10.**

A piston **28** is slidably received in container **10** for dispensing the mixture of blood and dilutent through the lower conical portion **30** thereof into an integral or suitably attached cylindrical conduit **32.** Pistons **14, 22** and **28** may be operated to provide constant rate of flow by any number of suitable conventional actuators (not illustrated). Alternatively, any suitable structure can be provided for mixing and dispensing the blood and dilutent at a constant rate.

Conduit **32** communicates with a reduced diameter microcapillary passage **33,** which as shown in FIG. 2 is of flattened rectangular cross section. The size of passage **33** must be sufficiently small to allow the particles to pass therethrough in substantially single file. When the particles are the formed elements of whole blood a size on the order of 8 by 10 microns has been found to be appropriate. The properly sized microcapillary passage **33** can be fabricated as illustrated in FIGS. **1** and **2** from a pair of optically transparent plates such as glass **330** and **332** having sandwiched therebetween a layer **334** which may have been vacuumed deposited and processed with an appropriate mask or barrier to prevent a central strip thereof from being deposited on the glass plate. When the plates **330** and **332** are combined this central undeposited strip will then form the capillary passage **33.** It is to be pointed out that any other suitable technique may be employed for achieving a capillary passage of the required dimensions. The conduit **32** is fitted to a funnel-shaped mouth portion **336** of the glass sandwich by means of a resilient plug **338** or the like.

A phase optical system is provided adjacent the microcapillary passage **33** for magnifying and observing the particle flow therethrough, which system comprises; a source of illumination **34,** a diaphragm **36** having a central opening **38,** condensing lenses **40,** objective lenses **42** and a phase or diffraction plate **44.** The optical system images the specimen passing through the microcapillary passage **33** onto an image plane **46** at which is located a suitable photosensitive device **48,** il-

lustrated greatly enlarged for clarity, which may typically comprise an N-P-N silicon planar photodetector. The size of the photosensitive device or detector **48** is such with respect to the size of the imaged specimen, a white blood cell for example, that only a thin cross-sectional slice of the cell image traverses the detector as it passes through the microcapillary **33**, the image of which is depicted at **33i** in FIG. **3**. The signal from detector **48** is delivered via line **49** to and suitably amplified by a signal amplifier A, the output from which is carried by line **49'**.

As shown in FIG. **3** a second photodetector **50** is located adjacent detector **48** and is responsive to the illumination falling outside of the projected image **33i** for compensating for changes in the illumination levels from light source **34**, as is common. The reference illumination signal is carried by line **51** which leads to amplifier A.

The signal from detector **48** is fed into a signal analyzer **52** via line **49'**. As shown in FIG. **4** the signal analyzer comprises a plurality of narrow band-width filters **520, 522** and **524** which severally deliver signals to a plurality of detectors or low pass filters **526, 528** and **530** via lines **532, 534** and **536**. Detectors **526, 528** and **530** deliver signals to circuits **538, 540** and **542**, respectively via lines **544, 546** and **548**. Circuits **538** and **542** are threshold circuits, which might typically comprise schmidt triggers, whereas circuit **540** is a variable threshold circuit which might typically comprise a conventional electronic comparator. The output of detector **526** is also fed into comparator **540** via branch from line **544**, as is illustrated.

Referring again to FIG. **1**, the output signals from circuits **538, 540** and **542** are delivered to a logic network **54** via lines, **56, 58** and **60**, respectively. As will be discussed in greater detail hereinbelow, network **54** might typically comprise a 3-bit binary logic network which can yield up to eight decision outputs in response to three inputs. The output signals from network **54** are delivered to a counter **62** via lines **64, 66, 68, 70, 72, 74** and **76**. Branch signals from lines **64, 66, 68, 70, 72** and **74** are severally delivered for the control of a sorter **78** via lines **80, 82, 84, 86, 88** and **90**. Signals from the counter **62** may be delivered to suitable indicators and/or recorders via lines **92, 94, 96, 98, 100, 102** and **104**.

Sorter **78** is suitably fixedly attached to the glass sandwich **330, 332** such that capillary passage **33** communicates with the interior thereof as shown in FIG. **7**. With reference to FIGS. **6** and **7**, the sorter housing **78** has affixed to the bottom external surface thereof a plurality of circularly arrayed sample collecting vessels **780, 782, 784, 786, 788** and **790** about a central collecting vessel **792**. A plurality of motive devices **794, 796, 798, 800, 802** and **804** are fixed at one end to the interior of the sorter housing **79** and severally at their other end to a plurality of flexible collecting tubes **806, 808, 810, 812, 814** and **816**, which are circularly arrayed at one end about the longitudinal axis of capillary passage **33**. The tubes pass through openings in the base of sorter **78** with the lower ends of each communicating with the interior of their respective collecting vessel **780, 782, 784, 786, 788** and **790**. A central tube **818** having a flared upper end is coaxial with capillary passage **33** and is in communication with central collecting vessel **792** as is illustrated in FIG. **7**. The signals from logic network **54** via lines **80, 82, 84, 86, 88** and **90** are delivered respectively to motive devices **794, 796, 798, 800, 802** and **804** for the actuation thereof.

Motive devices **794, 796, 798, 800, 802** and **804** may either be of the piezoelectric, electrostrictive or magnetostrictive type which, as is well known, elongate in response to an applied signal.

A circulating pump **820** is provided for maintaining a flow of inert gas through the collecting tubes to the collecting vessels. To this end, a plurality of inlet passages are provided (only one of which is shown) between each of the collecting vessels and the pump inlet.

In the operation of the above described apparatus, as applied to the counting, identification and sorting of the formed elements of blood, the blood sample is mixed with the dilutent in container **10** by means of the magnetic or other suitable stirrer **12**. The mixture is preferably treated with an anti-coagulant to prevent clotting and may be maintained at a constant temperature by suitable means, not illustrated. The dilutent functions to provide adequate separation of the cells to insure that individual cells will pass through the system in serial fashion. Since the response time of the apparatus will depend upon the spacing between cells as they pass through the microcapillary **33**, which in turn is dependent upon the amount of dilutent, the proportion of dilutent employed can function to keep the flow of cells within a reasonable response time for the apparatus. For example, at a 10:1 dilution rate in order to observe 1,000 cells per second a velocity of cells through the microcapillary of 0.024 meters per second would be required.

If the cells are observed under ordinary incoherent optical illumination their microstructures would not be descernable. This is because there is only a very small difference in optical path length between the microstructure and the surrounding portions of the cells, with essentially no difference in color or contrast. There exists only a phase difference which is not visible under ordinary illumination. However, with the phase illumination of the present invention, the substantially invisible grain structure or microstructure of the cells, as they file through capillary **33** in serial fashion, is made visible in the image plane **46**.

The opening **38** is diaphragm **36** creates substantially a point source of monochromatic light which falls upon the microstructures of the cell as well as the surrounding medium therebetween. Since the microstructures have refractive indicies which result in optical path lengths that differ from that of the surrounding medium, the radiation that passes therethrough experiences a shift in phase with respect to the remainder of the radiation. In other words, the microstructures function to diffract or deviate the portion of the rays impinging thereupon, whereas the remainder of the beam remains undeviated or undiffracted. The deviated beam is shown by the dashed lines *d*, whereas the undeviated beam is shown by the solid lines u in FIG. **1**. As is well known, since the difference in path length between the microstructures and the surrounding is very small, the phase shift introduced by the microstructures will equal one quarter of a wavelength. The diffraction or phase plate **44** functions to introduce an additional quarter wave shift between the deviated and the undeviated beam. In this manner, when the beams *d* and *u* combine or interfere in the image plane **46** there will now be a difference in contrast between the specimen microstructures and the surrounding media, which difference can be seen and detected. For a more detailed

discussion of the phenomena of phase optics or phase illumination reference should be had to "Phase Microscopy Principles and Applications" by Bennett, Jupnik, Osterberg and Richards, John Wiley & Sons, Inc. 1951. The microcapillary passage **33** is preferably of flattened rectangular cross-section as illustrated to avoid the necessity of special corrections that would be required with circular cross-section passages.

The image of the cell passing through the transilluminated observation chamber portion of passage **33** will be magnified many times, 1,000 times for example, and projected onto the surface of the detector **48.** In FIG. **3,** the size of the photodetector **48** with respect to the image of the capillary passage **33**i is shown greatly enlarged in the interests of clarity. Actually, the sensitive area of the detector is quite small compared to the size of the projected image of the cell, and many typically be on the order of 0.10 by 0.10 microns. In this manner, the individual components of the cell microstructure will cause a continuously changing value of light intensity falling upon detector **48** as the cell travels past the sensitive area thereof.

The variations in the intensity of radiation incident on detector **48** may cause a resistance or other suitable change within the detector, depending upon the particular choice of detector, which creates a voltage fluctuation in output line **49.** This voltage is amplified at A, with suitable modulation if necessary from reference detector **50** via line **51,** and delivered as an input signal in line **49'** to signal analyzer **52.** A portion of each magnified cell image as wide as the detector makes only one pass across the detector and only the microstructure lying in a plane perpendicular to the optical axis at the focal point are detected; yet, as will be discussed hereinbelow, the information contained in such portion of the cell image, properly analyzed is sufficient to identify and distinguish the type of cell.

It has been found that Fourier or spectral analysis of the raw signals developed by the detector will yield a distinct power spectrum for each cell type; this being true regardless of the cell size, maturity and orientation. In other words, an analysis of a large number of cells of the same type will yield substantially identical power spectrums.

The data illustrated by the curves in FIG. **5** is exemplary of the power spectrum for the five types of white cells, as determined by a Fourier analysis of a larger number of raw signals of each cell type. As shown in FIG. **5,** the curves are expressed in terms of relative spectral power levels versus frequency, in cycles per unit length. It is to be noted that other signal analysis techniques may yield curves that differ from those shown in FIG. **5.** These curves, however, would also be distinct for each cell type.

Once having determined these distinctive power spectrums, it becomes a relatively simple matter to choose those relative power spectrum values within particular frequency bands that distinguish one cell type from another. In as much as there may be many distinguishing relative power level/frequency band values as well as many different combinations thereof that can be incorporated separately or jointly in a particular design, the examples to be given hereinbelow are to be taken as only illustrative of one set of values that perform satisfactory; others can be used which would perform equally as well.

Thus, in order to identify the white cells, the signals emanating from the amplifier A and applied to the input of the signal analyzer at **49'** are analyzed thereby to determine the following:

1. At one cycle per unit length is the relative power greater than 100?

2. At six cycles per unit length is the relative power greater than or equal to the power at one cycle per unit length?

3. At 10 cycles per unit length is the relative power less than 10?

From an inspection of FIG. **5** it can be seen that; an affirmative answer to question (1.) coupled with negative answers to questions (2.) and (3.) will identify a neutrophil; a negative answer to all three questions will identify a monocyte; a negative answer to questions (1.) and (3.) coupled with an affirmative answer to question (2.) will identify a lymphocyte; an affirmative answer to questions (1.) and (3.) coupled with a negative answer to question (2.) will identify an eosinophil; and negative answers to questions (1.) and (2.) coupled with an affirmative answer to question (3.) will identify a basophil.

Due to the large hemoglobin content of red cells and because, unlike the white cell, the red cell does not contain a nucleus or granules, it has a power spectrum completely different from the white cells. Although not shown in FIG. **5,** it has been found that the relative power of the red cell is over 100 at 1, 6 and 10 cycles per unit length. Therefore an affirmative answer to question (1.) and (2.) coupled with a negative answer to question (3.) will identify a red cell. The platelets, being the only formed element left, can be identified when there is a signal that does not fall within any of the values given above.

In accordance with the above described technique for identification, the narrow band-width filters **520, 522** and **524** function to allow those signals centered about three frequencies in the ratio of 1, 6 and 10 to be passed respectively to detectors **526, 528** and **530** which function to remove the signal transients in a known manner. Threshold device **538** will develop a signal in line **56** only if the power of the signal in line **544** is greater than a predetermined value (greater than 100 in the example given). Thus, a signal at line **56** would be equivalent to an affirmative answer to question (1.). Electronic comparator **540** compares the power of the signal from detector **528** (at six cycles per unit length in the example given) with that from detector **526** (at one cycle per unit length in the example given), developing a signal in line **58** only if the power in line **546** is greater than or equal to that in line **544,** which would be equivalent to an affirmative to question (2.), supra. Threshold unit **542** will develop a signal in line **60** only if the power of the signal in line **548** is less than a predetermined value (less than 10 in the example given). Thus, a signal in line **60** would be equivalent to an affirmative answer to question (3.) supra. It therefore should be apparent that the presence or absence of signals in lines **56, 58** and **60** in particular predetermined combinations will be indicative of the five types of white cells and the red cell. Signals not falling in these combinations, by process of elimination, will be indicative of the platelets.

The signals from lines **56, 58** and **60** are fed into logic network **54,** which could typically comprise a conventional three-bit binary decoder which functions in re-

sponse to three inputs to deliver up to eight decision outputs. only seven of which are used as indicative of the identity of each of the five white cell types, the red cells and the platelets. Thus, a signal in output line 64 could indicate a neutrophil; that in line 66 could indicate a monocyte; that in line 68 could indicate a lymphocyte; that in line 70 could indicate an eosinophil; that in line 72 could indicate a basophil; that in line 74 could indicate a platelet; and that in line 76 could indicate a red cell. These signals are applied to a conventional counter or the like 62 which tallies the number of times each cell type is identified in real time as the images thereof flow past the detector. Signals from the counters via lines 92, 94, 96, 98, 100, 102 and 104 can be delivered to suitable indicators and/or recorders which could indicate or print out the following data:

1. Number or red cells per cubic millimeter of whole blood.
2. Number of platelets per cubic millimeter of whole blood.
3. Number of white cells per cubic millimeter of whole blood.
4. Number of each type of white cell.
5. Percent of total of each type of white cell.

Referring again to FIGS. 1, 6 and 7, the output signals indicative of the cell type from logic network 54 can be employed as control signals via lines 80, 82, 84, 86, 88 and 90 leading to sorter 78. A signal in any one of these lines will actuate its corresponding motive device 794, 796, 798, 800, 802 or 804 which will cause the same to elongate to thereby move one of the circularly arrayed tubes 806, 808, 810, 812, 814 or 816 directly under the exit portion of capillary passage 33 to receive the particular cell type associated with the control signal. As illustrated by the dashed lines in FIG. 7, tube 816 has been moved by motive device 804 to a position directly under the capillary passage 33. The particular cell type samples are delivered via the tubes to collecting vessels 780, 782, 784, 786, 788 and 790. The pump 820 functions to maintain a positive flow of inert gas, such as nitrogen, from the mouth of each tube to its associated collecting vessel. In this manner cells deposited into the collecting tubes from the microcapillary will be drawn into the collecting vessels. When there is no signal to actuate any of the motive devices the effluent stream from capillary 33 will be received by central tube 818 leading to the central collecting vessel 792, which can be used to collect the red blood cells since these have the greatest population. To this end, line 76 from logic network 54 which is indicative of the red blood cell need have no control connection to the sorter. Thus, in the absence of a control signal the sorter will function to normally collect the red blood cells.

The size of the white cells is generally greater than the size of microcapillary passage 33 and some elongation of the white cells will take place upon passage therethrough. Therefore, the detector 48 will always "see" the image of a portion of the white cells, which, as pointed out above, is sufficient to make an identification. As regards the red cells and platelete however, it is possible that a small number of these might not be "seen" by the detector. The platelets, being smaller than the capillary opening, could flow outside the area of the detector. The red cells, being very thin, could avoid the detector when flowing edgewise against the microcapillary passage walls.

To insure that all of the cells, especially all of the red cells and platelets, are counted, the FIG. 1 apparatus can be modified as illustrated in FIGS. 8 and 9, wherein similar numerals are employed to depict similar parts. A second detector 110, which may be a strip photosensor, is located to span across the entire microcapillary and is upstream of the detector 48 as illustrated in FIG. 9. Since the strip sensor spans the entire microcapillary, the small platelets and edgewise red cells are still observed. The strip sensor could be quite narrow, about 0.5 by 10 microns in sensitive area. The signal from strip photosensor 110 via line 111 is suitable amplified by amplifier A' and delivered via line 111' to a detector 112. Amplifier A' may be modulated from the reference illumination detector signal via line 51 as in the previous embodiment.

Detector 112 might typically comprise an electronic integrator with threshold circuits which function to directly classify red cells and platelets on the basis of their distinctly different characteristics both with respect to each other and with respect to the white cells. Thus, the signal from a platelet would be a sharp spike in response to the small 2 micron diameter thereof. On the other hand, the larger red cell signal would last somewhat longer and produce a greater voltage change. The detector 112 could suitably have two thresholds, one for the platelets and one for the red cell. If neither a platelet nor a red cell is observed, a signal is delivered via line 114 to a conventional enabling circuit 116 which functions to render operative the detector 48 for the observation of the white cells. The signals for the platelets and the red cells are delivered respectively via lines 120 and 122 to a red cell and platelet counter 124 which may be separate from the white cell counter 62'. The platelet signal via line 122 is delivered to the sorter 78 via 126 for the control of the platelet sample collecting vessel as in the previous embodiment.

Although the foregoing description has described two preferred embodiments of the invention, other modifications will occur to those skilled in the art without departing from the spirit thereof. It is therefore intended that the present invention is to be limited only by the scope of the appended claims.

I claim:

1. Apparatus of the character described, comprising;
   a. first means for containing a sample of fluid, the constituents of which include particles each having a microstructure indicative of the identity thereof,
   b. said first means having at least a portion thereof that is transparent to electromagnetic radiation,
   c. means for irradiating said portion of said first means and the sample fluid therein with electromagnetic radiation,
   d. optical means for a magnifying the image of said portion of said first means and the sample fluid therein and for converting small invisible optical phase differences between the microstructure components of each particulate constituent of said sample fluid to visible intensity differences,
   e. detection means responsive to the electromagnetic radiation passing through said first means for developing a varying output signal indicative of the varying intensity of said electromagnetic radiation,
   f. means for analyzing and processing said varying output signal for developing a plurality of control

signals each indicative of a specific particulate constituent of said sample fluid,

g. at least two collecting means adapted for fluid communication with said first means downstream of said portion thereof, and

h. control means responsive to said control signals for placing predetermined individual collecting means in fluid communication with said first meanst.

2. The apparatus according to claim **1**, wherein;

i. the number of said collecting means corresponds to the number of different particulate constituents of said sample fluid.

3. The apparatus according to claim **1**, wherein;

i. said first means is so sized relative to said particulate sizes that the particles are substantially sequentially arranged in said portion thereof.

4. The apparatus according to claim **1**, wherein;

i. said detection means has a radiation sensitive area that is much smaller than the image of the particle impinging thereupon whereby only a thin cross-sectional slice of the particle image traverses said sensitive area as said particle moves with respect to said detection means.

5. The apparatus according to claim **4**, wherein;

j. said first means is so sized relative to said particulate sizes that the particles are substantially sequentially arranged in said portion thereof.

6. The apparatus according to claim **5**, wherein;

k. said optical means includes means for shifting the phase of the radiation passing through said microstructure components with respect to the radiation passing therebetween by substantially a quarter of a wavelength.

7. The apparatus according to claim **6**, wherein;

l. said means for illuminating comprises a substantially monochromatic source of radiation.

8. The apparatus according to claim **7**, wherein;

m. said means for analyzing and processing said varying output signal comprises a plurality of narrow band-width filters and a binary logic network.

9. The apparatus according to claim **8**, wherein;

n. said collecting means comprises a plurality of movable tubes, each spaced from an end of said conduit means, and

o. said control means comprises motive devices for moving said tubes into axially alignment with said first means.

10. The apparatus according to claim **9**, wherein;

p. said motive devices elongate in response to said control signals.

11. The apparatus according to claim **1**, wherin;

i. said optical means includes means for shifting the phase of the radiation passing through said microstructure components with respect to the radiation passing therebetween by substantially a quarter of a wavelength.

12. The apparatus according to claim **11**, wherein;

j. said means for illuminating comprises a substantially monochromatic source of radiation.

13. The apparatus according to claim **1**, wherein;

i. said collecting means comprises a plurality of movable tubes, each spaced from an end of said conduit means, and

j. said control means comprises motive devices for moving said tubes in axial alignment with said conduit means.

14. The apparatus according to claim **13**, wherein;

k. said motive devices elongate in response to said control signals.

15. The apparatus according to claim **1**, wherein;

i. said means for analyzing and processing said varying output signal comprises a plurality of narrow band-width filters responsive to said varying output signal.

16. The apparatus according to claim **15**, wherein;

j. said means for analyzing and processing further comprises a plurality of thresholding circuits responsive to the output from said narrow band-width filters.

17. The apparatus according to claim **16**, wherein;

k. said means for analyzing and processing further comprises a binary logic network responsive to the output from said thresholding circuits.

18. The apparatus according to claim **17**, wherein;

l. said narrow band-width filters respond to frequencies contained within said varying output signal from said detection means substantially in the ratio of one, six and ten, and

m. said binary logic network is of the three-bit type.

19. The apparatus according to claim **1**, further comprising;

i. a quantity of sample fluid in said first means, the constituents of which include particles each having a microstructure indicative of the identity thereof.

20. The apparatus according to claim **19**, wherein;

j. said sample fluid comprises blood and said particles comprise the formed elements thereof.

21. The apparatus according to claim **1**, further comprising;

i. counting means responsive to said control signals.

22. The apparatus according to claim **1**, wherein;

i. said detection means has a radiation sensitive area that is much smaller than the image of the particle impinging thereupon whereby only a thin cross-sectional slice of the particle image traverses said sensitive area as said particle moves with respect to said detection means, and there is further provided;

j. second detection means located adjacent said first mentioned detection means, said second detection means has a radiation sensitive area so sized to span a slice of the image of said first means that is substantially perpendicular to the longitudinal axis thereof.

23. The apparatus according to claim **22**, wherein;

k. said second detection means is located upstream of said first mentioned detection means with respect to the direction relative of sample fluid flow.

24. The apparatus according to claim **23**, further comprising;

l. means responsive to predetermined output signals from said second detection means for delivering outputs indicative thereof.

25. The apparatus according to claim **24**, further comprising;

m. counting means responsive to said control signals and to said outputs from said last mentioned means.

26. The apparatus according to claim **24**, further comprising;

m. enabling means for rendering said first mentioned detection means operative in response to a signal derived from said second detection means that differs from said predetermined output signals.

27. The apparatus according to claim 26, further comprising;

n. a quantity of sample fluid in said first means, the constituents of which include particles each having a microstructure indicative of the identity thereof.

28. The apparatus according to claim 27, wherein;

o. said sample fluid comprises blood and said particles comprise the formed elements thereof, and wherein;

p. said predetermined output signals are indicative of the erythrocytes and the platelets, respectively.

29. Apparatus of the character described, comprising;

a. first means for containing a sample of fluid, the constituents of which include particles each having a microstructure indicative of the identity thereof,

b. said first means having at least a portion thereof that is transparent to electromagnetic radiation,

c. means for irradiating said portion of said first means and the sample fluid therein with electromagnetic radiation,

d. optical means for magnifying the image of said portion of said first means and the sample fluid therein for converting small invisible phase differences between the microstructure components of each particulate constitutent of said sample fluid visible to intensity differences,

e. detection means responsive to the radiation passing through said first means for developing a varying output signal indicative of the varying intensity of said electromagnetic radiation, and

f. means for analyzing and processing said varying output signal for developing a plurality of output signals each indicative of a specific particulate constituent of said sample fluid.

30. The apparatus according to claim 29, wherein;

g. said first means is so sized relative to said particulate sizes that the particles are substantially sequentially arranged in said portion thereof.

31. The apparatus according to claim 30, wherein;

h. said detection means has a radiation sensitive area that is much smaller than the image of the particle impinging thereupon whereby only a thin cross-sectional slice of the particle image traverses said sensitive area as said particle moves with respect to said detection means.

32. The apparatus according to claim 31, wherein;

i. said optical means includes means for shifting the phase of the radiation passing through said microstructure components with respect to the radiation passing therebetween by substantially a quarter of a wavelength.

33. The apparatus according to claim 32, wherein;

j. said means for illuminating comprises a substantially monochromatic source of radiation.

34. The apparatus according to claim 33, wherein;

k. said means for anlayzing and processing said varying output signal comprises a plurality of a narrow band-width electronic filters and a binary logic network.

35. The apparatus according to claim 29, wherein;

g. said detection means has a radiation sensitive area that is much smaller than the image of the particle impinging thereupon whereby only a thin cross-sectional slice of the particle image traverses said sensitive area as said particle moves with respect to said detection means.

36. The apparatus according to claim 29, wherein;

g. said optical means includes means for shifting the phase of the radiation passing through said microstructure components with respect to the radiation passing therebetween by substantially a quarter of a wavelength.

37. The apparatus according to claim 36, wherein;

h. said means for illuminating comprises a substantially monochromatic source of radiation.

38. The apparatus according to claim 29, further comprising;

g. counting means responsive to said output signals.

39. The apparatus according to claim 29, wherein;

g. said detection means has a radiation sensitive area that is much smaller than the image of the particle impinging thereupon whereby only a thin cross-sectional slice of the particle image traverses said sensitive area as said particle moves with respect to said detection means, and there is further provided;

h. second detection means located adjacent said first mentioned detection means, said second detection means has an electromagnetic radiation sensitive area so sized to span a slice of the image of said first means that is substantially perpendicular to the longitudinal axis thereof.

40. The apparatus according to claim 39, further comprising:

i. means responsive to predetermined output signals from said second detection means for delivering outputs indicative thereof.

41. The apparatus according to claim 40, further comprising;

j. counting means responsive to said control signals and to said outputs from said last mentioned means.

42. The apparatus according to claim 40, further comprising;

j. enabling means for rendering said first mentioned detection means operative in response to a signal derived from said second detection means that differs from said predetermined output signals.

43. The apparatus according to claim 29, wherein;

g. said optical means include phase illumination optics.

44. The apparatus according to claim 29, wherein;

g. said means for analyzing performs a spectrum analysis of said varying output signal.

45. Apparatus of the character described, comprising;

a. first means for containing a sample of fluid, the constituents of which include particles each having a microstructure indicative of the identity thereof,

b . said first means having at least a portion thereof that is transparent to electromagnetic radiation,

c. means for irradiating said portion of said first means and the sample fluid therein with electromagnetic radiation,

d. optical means for magnifying the image of said portion of said first means,

e. detection means responsive to the electromagnetic radiation passing through said first means for developing a varying output signal indicative of the varying intensity of said electromagnetic radiation, said detection means having an electromagnetic radiation sensitive area that is much smaller than the image of the particles impinging thereupon whereby only a thin cross-sectional slice of the par-

ticle image traverses said sensitive area as said particle moves with respect to said detection means, and

f. means for analyzing and processing said varying output signal for developing a plurality of output signals each indicative of a specific particulate constituent of said sample fluid.

* * * * *