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Friedman et al.

[11] **3,788,744**

[45] **Jan. 29, 1974**

[54] **METHOD AND APPARATUS FOR PHOTOANALYSIS**

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Related U.S. Application Data

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[52] **U.S. Cl.** **356/39,** 356/102, 356/104, 250/458

[51] **Int. Cl.** **G01n 33/16,** G01n 21/00

[58] **Field of Search** ..... 356/39, 102, 103, 173, 186, 356/201, 104; 250/71 R

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Assistant Examiner—Conrad Clark
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[57] **ABSTRACT**

A narrow beam of monochromatic light is directed through an optical chamber to intersect a thin stream of small particles to be optically analyzed, causing the particles to emit fluorescent radiation in the presence of a significant particle characteristic to be detected. A spectral filtering element is positioned to intercept the fluorescent particle radiation and to pass only a selected wave length to a photoresponsive pick-up element for detection thereby.

13 Claims, 7 Drawing Figures

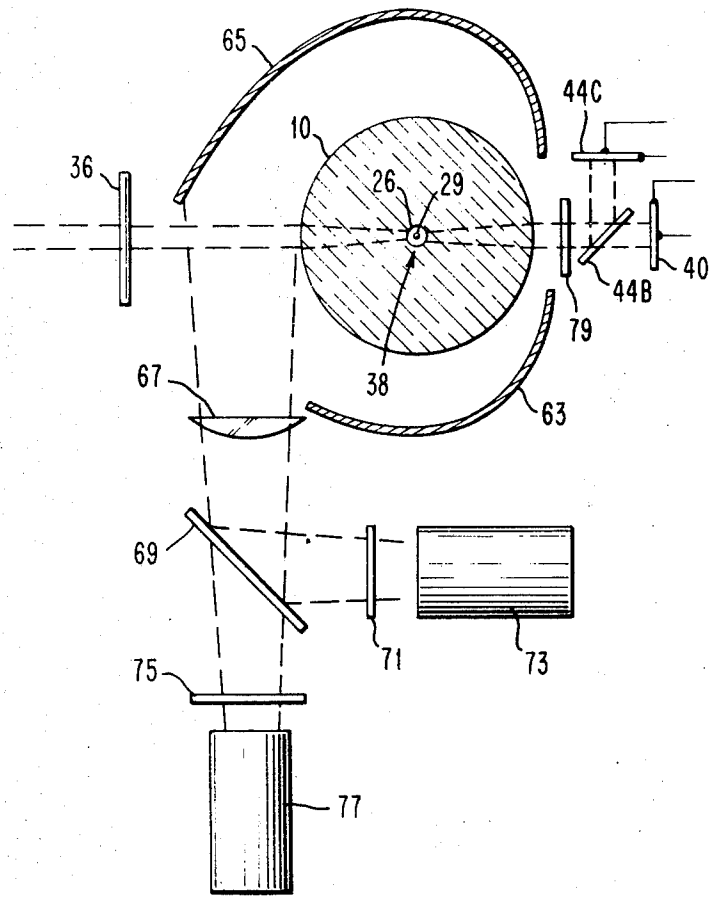


FIG. 1

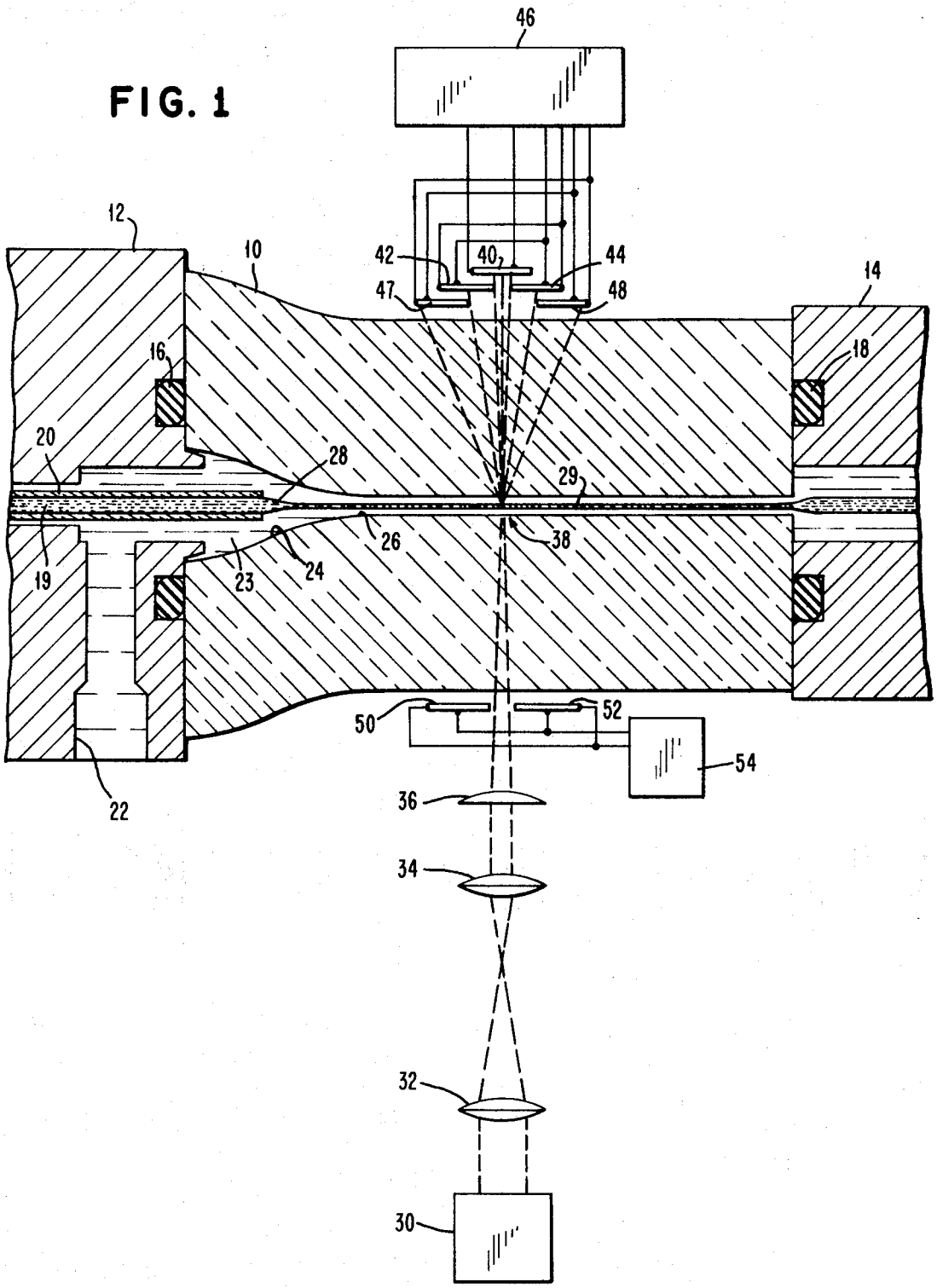

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42
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44
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FIG. 2

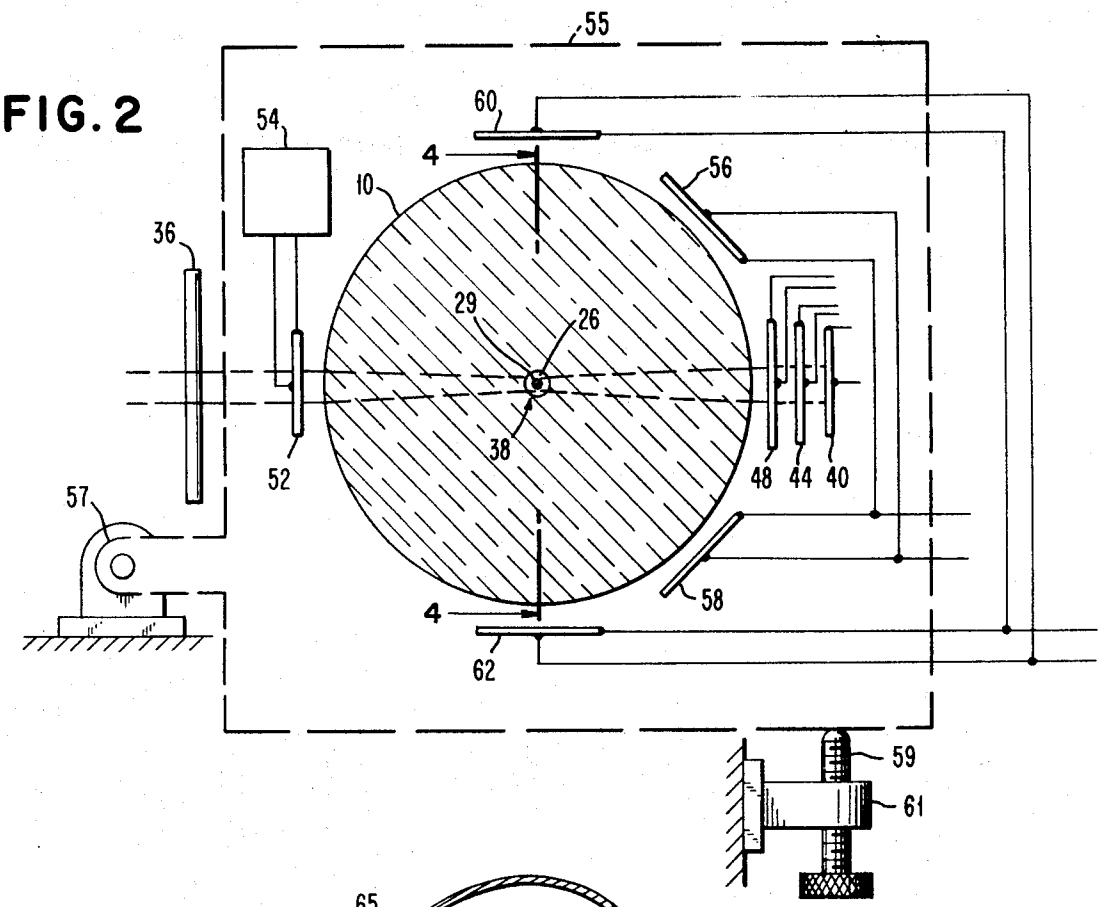

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FIG. 2A

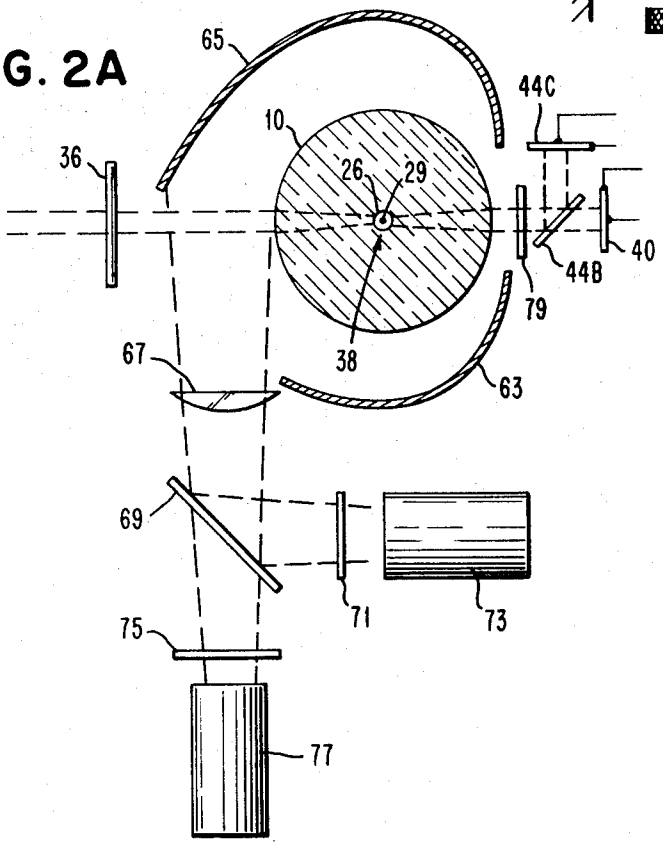

65
44C
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36
26
29
40
44B
79
38
67
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75
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FIG. 3

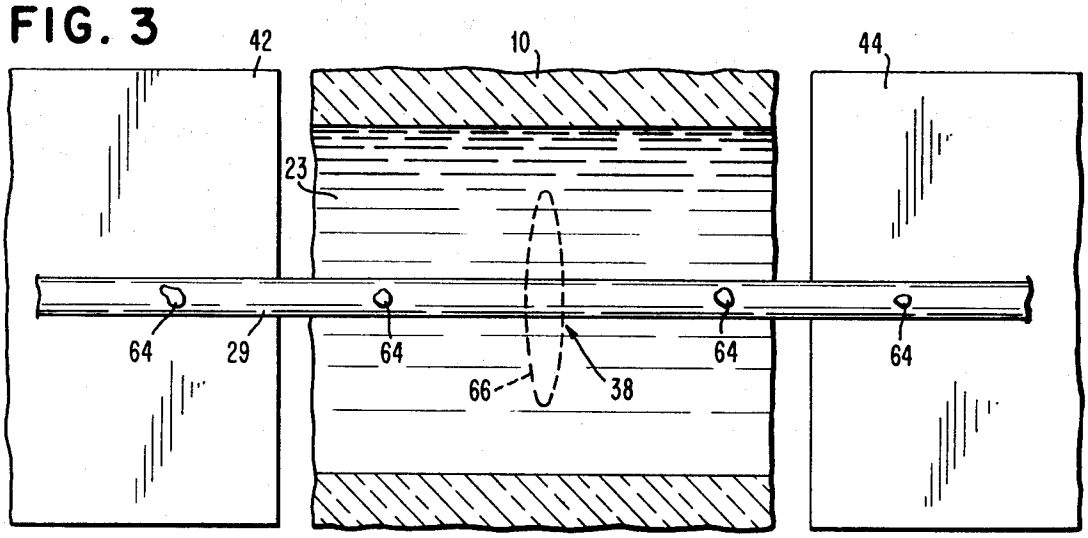


FIG. 4

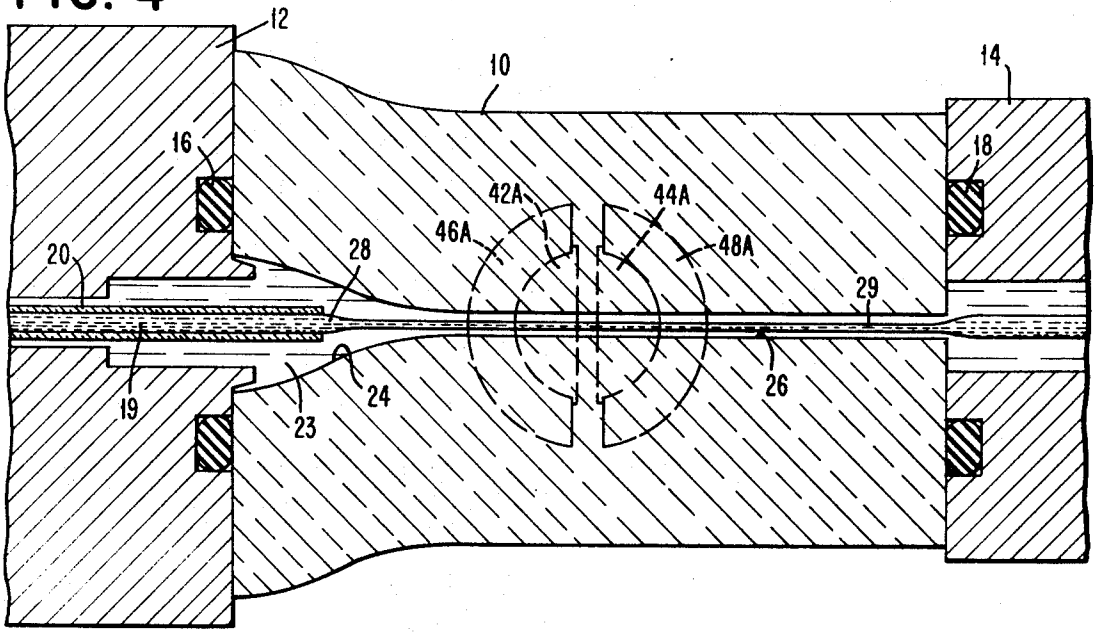


FIG. 6

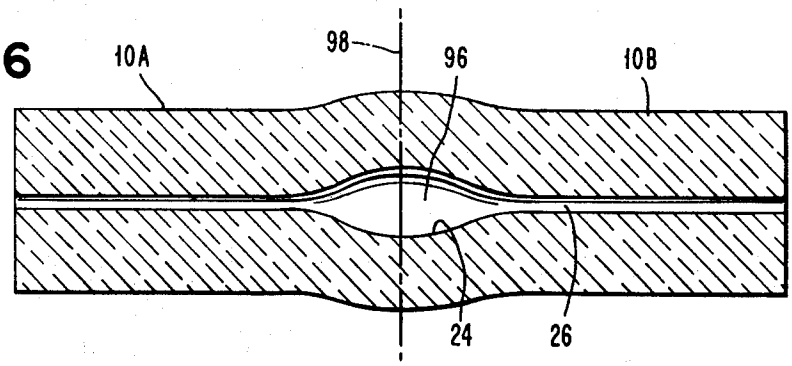
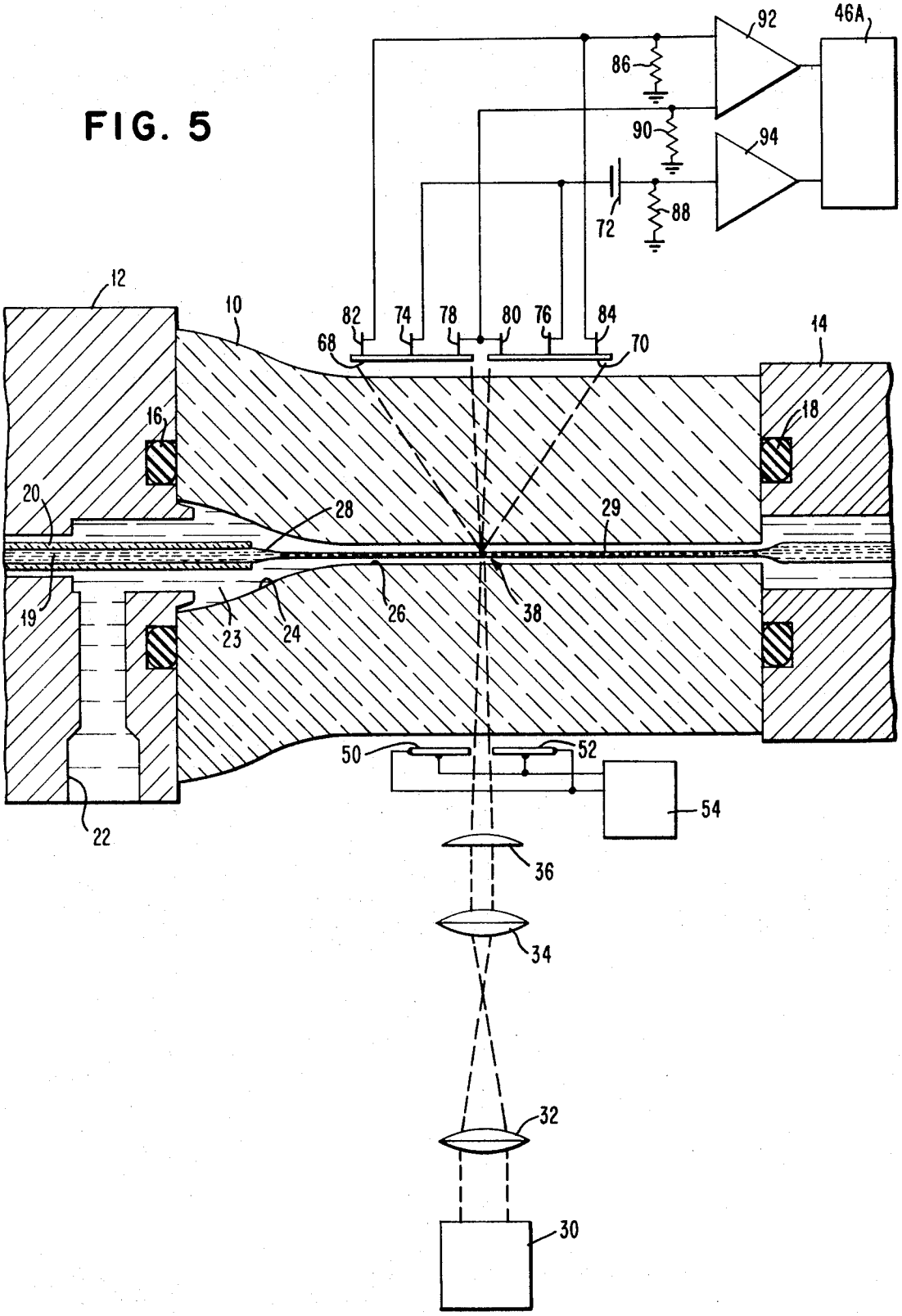


FIG. 5


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46A
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12
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METHOD AND APPARATUS FOR PHOTOANALYSIS

This is a division, of application Ser. No. 2,750 filed Jan. 14, 1970 and now U.S. Pat. No. 3,687,553 issued 8/29/72.

The present invention relates to photoanalysis apparatus, and more particularly to photoresponsive apparatus for detecting various characteristics of small particles such as blood cells.

There is a great need for accurate analysis of the characteristics of groups of small particles such as in the analysis of air pollution and water pollution conditions. A particularly important field for such analysis is in medical research and diagnosis. For this purpose, blood cells and other biological cells must be analyzed.

In the present invention the analysis of small particles is accomplished optically by entraining the particles in a very thin stream of liquid so that the particles pass one by one in the stream through an optical scanning station. A photo-optical detecting device is arranged to detect the optical reaction of each particle to illumination from a beam of light. This method has been shown to impart very important information about the particles which have been scanned. The information derived is particularly valuable when special procedures are followed for preliminary preparation of the particles, such as the prior application of dyes which are taken up by the particles in different ways related to the differences in particles which are to be detected. The particles having these differences may be differentially counted.

In accordance with one aspect of the present invention, it has been determined that in an apparatus of the above description, the value of the information which can be derived photo-optically is tremendously increased if at least two different optical reactions of the particles can be detected simultaneously by means of optical pick-up elements arranged at different angular positions with respect to the direction of the beam of light directed at the particle.

One of the most useful optical reactions to the illumination is a measurement of the optical absorption of the particles. The amount of optical absorption of the particles may be directly related to the amount of dye which has been taken into each particle in a preliminary conditioning step. Since the dye uptake by the particles may be different based upon different characteristics, the optical absorption may thus be used to distinguish these characteristics. The combined detection of absorption and another optical reaction, such as scatter, therefore provides a very useful combination of information about the particles under observation. For instance, in the analysis of biological cell samples, such as blood cells, it is recognized that dead cells absorb the dye Trypan blue while live cells do not. Accordingly, it is possible to analyze a sample of such cells by first exposing the sample to such a dye and then optically measuring both absorption and scatter. The dead cells which take up the stain are then optically detected by the absorption signal, and the live cells are detected by the optical scatter signal, and the apparatus may be used to count and record the numbers of dead and live cells in the sample.

Accordingly, it is an object of the present invention to provide a method and an apparatus in which at least two different optical reactions of particles can be detected, and more specifically in which sidderent conditions of individual cells, such as the differentiation of live and dead cells may be accomplished reliably and quickly.

In photoanalysis apparatus of the above description, it has been recognized that the light scattering effect produced by particles under observation varies according to different characteristics of the particles, including such factors as particle size, refractive index, and the presence of refractive and absorbent substances in the particles. Accordingly, the detection of scattered light from the particles provides an extremely valuable method for determining the characteristics of the particles. Furthermore, the magnitude of the scatter radiation as a function of the angles or the ranges of angles over which the scattering of light occurs provide distinctive information. In apparatus heretofore available, it is generally been possible to detect light scattered by particles under observation only for a very small fixed range of scatter angles. This has been accomplished by projecting illumination to the particle by means of a lens having a mask over the central portion thereof to create a "cone of darkness" beyond the particle positioned at the focal point of the lens. The scatter illumination is detected within this cone of darkness.

In accordance with another aspect of the present invention, it is another object of the present invention to provide a photoanalysis apparatus for optical analysis of particles in which very accurate measurements of scatter illumination can be made at any desired angle, or over any desired range of angles, without specific limitation to a particular cone of darkness.

In carrying out the above object, a structure is employed in which there is produced an extremely narrow beam of illumination directed at the particles, and photosensing devices are arranged at positions displaced from the beam to receive illumination scattered at predetermined angles from the beam by the particles.

Another problem in photoanalysis apparatus of the above description has always been the economical production of a suitable optical chamber through which the particles to be examined are passed and subjected to illumination. It has been previously thought to be absolutely essential to provide flat sides of this optical chamber in order to avoid distortion of the light beam directed through the chamber by the material of the side walls of the chamber.

Accordingly, it is another object of the present invention to provide a very satisfactory and easily produced optical chamber in a photoanalysis apparatus which is extremely economical to produce because of the manner in which provision is made for satisfactory optical properties of the side walls.

In carrying out the above object of the invention, photoanalysis apparatus is provided having an optical chamber in the form of a cylindrical tube.

Further objects and advantages of the invention will be apparent from the following description and the accompanying drawings.

In carrying out the invention in one preferred form thereof, there is provided an apparatus for simultaneous optical measurement of several characteristics of each particle of a group of small particles such as blood cells while the particles are suspended in a liquid, including a source of light, a housing comprised of a material which transmits light from the source and defining an optical chamber. A means is provided for moving the particle suspending liquid through the housing

in a thin stream to convey the particles in sequence through the stream one by one. Another means is provided for directing light from the light source into one side of the housing to intersect with the thin stream of particles in a narrow beam, and at least two photoresponsive pick-up elements are positioned outside of said housing at different angular positions with respect to the direction of the beam when measured from the intersection of the beam with the stream of particles, the photoresponsive pick-up elements being effective to simultaneously detect different optical reactions of each particle to illumination from the beam.

In the accompanying drawings:

FIG. 1 is a schematic top view, partly in section, of the most essential elements of a photoanalysis apparatus in accordance with the present invention.

FIG. 2 is a sectional side view of a portion of the apparatus shown in FIG. 1 and illustrating additional photooptical pick-up elements for detecting scatter at additional angles.

FIG. 2A is a sectional side view corresponding to FIG. 2 and illustrating, on a reduced scale, an alternative arrangement of the apparatus including means for picking up and detecting fluorescence of the particles.

FIG. 3 is an enlarged sectional front view taken through a section at the center of the optical chamber of the apparatus of FIG. 1, and illustrating the intersection of the beam of light with the particles being measured.

FIG. 4 is a partial front view of a photoanalysis apparatus in accordance with the invention and having modified photoelectric pick-up elements for the detection of scattered illumination.

FIG. 5 is a top view, corresponding to FIG. 1, but illustrating a modification of the photoresponsive pick-up elements in which separate signals are derived to indicate the intensity of scattered radiation an the angle of the scattered radiation.

And FIG. 6 is a top view of two optical chamber tube members for a photoanalysis apparatus in the course of production, in accordance with a preferred method of production.

Referring more particularly to FIG. 1, there is shown an optical chamber formed by a glass tube member 10 clamped between metal members 12, 14, which respectively include liquid tight annular seals 16 and 18. The liquid 19 containing the particles to be observed enters the apparatus through a tube 20 centrally disposed within member 12. Another liquid 23, which forms a sheath for the liquid 19 containing the particles enters the member 12 through an entrance opening 22. The liquids come together in the cone or funnel-shaped entrance portion 24 of the central bore 26 of the cylindrical member 10.

The velocity and volume of flow of the particle-bearing liquid 19 entering through tube 20 and the other liquid 23 entering through entrance 22 are such as to cause the stream of particle-bearing liquid to be narrowed down at the end of the tube 20, as shown at 28, into a very narrow stream 29 having a maximum dimension of the same order of magnitude as the maximum dimension of the particles being carried by the stream. For instance, this dimension may be in the order of a 25 micron stream diameter. The particle of greatest interest are often somewhat smaller than this, being in the range from 1 to 10 microns in diameter. The liquid 23 may be referred to hereinafter as the "sheath flow" liquid since it forms a liquid sheath about the narrowed stream 29. In order to provide a smooth and non-turbulent flow of the sheath liquid 23, two or more radial inlet openings 22 may be provided to the central bore of the member 12. The funnel-shaped entrance portion 24 of the cylindrical member 10 is preferably provided with a special exponential function shape, as described more fully below, particularly in connection with FIG. 6, in order to provide for smooth non-turbulent flow of the liquids at the critical position 28 where the particle-carrying liquid is narrowed down. Typically, the particle-carrying liquid may be an aqueous solution and the sheath liquid 23 may be water.

The stream 29 of particles is illuminated by a beam of light emitted by a light source 30 which preferably consists of a laser. One satisfactory laser, for instance, is a helium-neon laser. The beam of light from the laser is reduced in diameter by a combination of spherical lenses 32 and 34. The resultant reduced diameter beam is collimated. This concentrated beam is narrowed by a lens 36 to provide a very narrow beam at the point 38 where the beam intersects with the stream 29 of particles under observation. For this purpose, the lens 36 is preferably a cylindrical lens having its cylinder axis arranged in a plane perpendicular to the axis of the chamber cylinder 10. Thus, the pattern of the illumination of the beam at the point 38 where it strikes the stream of particles is a very narrow ellipse which appears to be a thin line of light transverse to the stream of particles. This will be described more fully below in connection with FIG. 3.

Electrical photoresponsive pick-up elements are arranged around the outside of cylindrical chamber member 10 to detect different optical reactions of each particle to illumination from the beam through lens 36. For instance, an electrical photo-responsive pick-up element 40 is arranged in direct line with the beam to measure the absorption of illumination by each particle. The resultant electrical signlas are connected to apparatus schematically shown at 46 for amplification and recording or display. In the absence of a particle at the intersection of the beam, or in the absence of any substantial absorption, the beam strikes the element 40 without any substantial diminution.

As illustrated in the drawing, the beam iverges to a certain extent after having been converged at the center of the chamber at 38. The effective divergence in a practical embodiment has been limited to approximately one degree on each side of the center line of the beam as measured from the particle scanning point 38 at the center of the chamber. Thus, photoresponsive pick-up elements 42 and 44 are arranged on opposite sides of the direct beam and can be used to measure illumination scattered out of the direct beam by the particles over a selected range of angles from one degree up to a predetermined angular limit. For instance, this range of angles may be from one to nine degrees. As shown in the drawing, the photoresponsive pick-up elements 42 and 44 may be electrically connected in parallel so that electrical signals resulting from illumination scattered on either side of the beam will be detected and may be recorded by the electrical apparatus schematically illustrated at 46. Additional pairs of photoresponsive pick-up elements for detecting scattered light at other ranges of angles may be provided as shown at 47 and 48. For instance, this additional pair of pick-up elements may detect scatter over the scatter

angle range from 9° to 22°. The wider angle scatter sensors **47** and **48** may be employed for the purpose of providing a measurement of optical absorption of the particles as an alternative to the absorption measurement by pick-up element **40.** It is known that abosrption of the incident beam will decrease the amount of light scattered by the particles. This decrease in scattered illumination is more pronounced for the wider angle scatter, than for the near forward scatter detected by sensors **42** and **44.** Accordingly, it has been found to be advantageous to use a wider angle scatter measurements at **47** and **48** to detect absorption because "noise" signals due to light source intensity fluctuations and flow stream vibrations are much less for the scatter sensors than for the direct measurement absorption sensor **40** which is in the direct path of the beam.

Scattering of illumination from the particles in the reverse direction, called "back-scattering," can also be detected by photoresponsive elements **50** and **52** arranged on the same side of the chamber as the light source **30** and connected in parallel to an electrical pick-up and recording apparatus **54.** It will be understood that the electrical apparatus **54** may be combined with the apparatus **46,** but it is shown separately here to simplify the drawing.

The apparatus **46** and **54** may include amplifiers, logic circuitry, digital counters, and electronic display devices. It is one of the important features of the invention that different optical reactions of each particle to illumination may be detected, processed, and recorded substantially simultaneously. The relationships between these different optical reactions may be processed by analog and digital circuitry, displayed, recorded, and plotted as a basis for making detailed determinations about the particles, differentially classifying the particles, or determining the frequency with which the particular characteristics appear in successive particles. Because of the unique features of this invention, particle analysis and counting rates in the order of ten thousand particles per second may be achieved. It will be understood that this speed is well within the capacity of the electronic and digital portions of the system.

The photoresponsive pick-up elements, such as elements **42** and **44** for the detection of scatter, are illustrated in FIG. **1** as though they were fixed with relation to the cylindrical chamber member **10.** However, suitable means is provided for precisely changing the position of those pick-up elements in relation to the beam from source **30.** This adjustment may be an adjustment from side to side and it may also be an adjustment to place the elements in greater or lesser proximity to the scanning position **38.** By moving the elements away from the scanning position, the inner margins of the elements may be precisely positioned with respect to the outer margins of the direct radiation beam directed to the absorption pick-up element **40.** Thus, the elements **42** and **44** are capable, when so adjusted, of picking up scatter radiation over the narrowest possible angle outside of the direct radiation beam path.

FIG. **2** is a partial sectional side detail view of the apparatus of FIG. **1** including the cylindrical chamber member **10,** the cylindrical lens **36,** the absorption and scattering pick-up elements **40, 44,** and **48,** and the back-scattering pick-up element **52.** As illustrated in FIG. **2,** the cylindrical shape of the chamber member **10** causes a refractive effect upon the light beam supplied through the cylindrical lens **36** which causes the light beam to converge towards the center bore **26** of the chamber member **10.** This effect is shown in an exaggerated form in FIG. **2.** The diamter of the beam as it enters the cylindrical lens **36** is actually selected so as to be approximately equal to the diameter of the center bore **26.** This diameter is of the order of 250 microns. However, the convergence of the beam in the plane of FIG. **2** (perpendicular to the axis of the chamber member **10**) is not a disadvantage since it serves to concentrate the beam in the central portion of the center bore **26** where the particle carrying strea is located. Since the particle carrying stream has a diameter of only about 25 microns, a considerable convergence of the beam is desirable. This provides relatively uniform illumination over the diameter of the particle-carrying stream, even though the original energy distribution from the laser beam is non-uniform. Furthermore, if the beam were not caused to converge upon the center bore **26,** the outer portions of the beam would strike the interface between glass and liquid at the center bore **26** at an angle greater than the critical angle of refraction so that those outer portions of the beam would be reflected away from the center bore, and lost, without passing through the liquid.

FIG. **1** illustrated how pairs of photoresponsive pick-up elements such as **42** and **44,** and **46** and **48,** can be arranged in positions spaced away from the primary beam in directions parallel to the axis of the cylindrical chamber member **10** for detecting small angles of scattered illumination. However, as shown in FIG. **2,** when larger angles of scatter are to be detected, the angular displacement of pick-up elements can be around the circumference of the cylindrical chamber member. Thus, as shown in FIG. **2,** a pair of pick-up elements **56** and **58** may be circumferentially arranged to detect light scattered in a range at about **45** degrees from the particle scanning point **38.** Similarly, pick-up elements **60** and **62** may be provided to detect scatter in a range near 90 degrees. It will be understood that these arrangements of pick-ups are by way of illustration only. Particular analyses will require the detection of scatter for particular ranges of angles. The important principle illustrated by FIGS. **1** and **2** is that light scattered by particles under analysis can be detected for any selected ranges of scatter angles from essentially 1° up to 179° with the analyzer configuration as illustrated.

All of the components illustrated in FIG. **2,** with the exception of the cylindrical lens **36,** are preferably mounted upon and movable with a support block schematically shown as a box **55** pivotally supported on a fixed mounting at **57.** The support block **55** may be vertically adjusted by rotation about the pivot **57** by means of a thumb screw **59** engaging the lower edge of the block **55.** Thumb screw **59** is threadedly engaged within a fixed support **61.** The purpose for this vertical adjustment is to precisely position the chamber **10** with respect to the light supplied from the light source through the cylindrical lens **36.** If the light beam is not vertically centered upon the center bore **26** of the chamber **10,** so that the beam accurately intersects with the stream **29** of particle-carrying liquid, then the device may be inoperative. The accurate positioning of the chamber with respect to the beam is very important because an offset in the positioning of the chamber with respect to the beam causes undue loss of beam en-

ergy through excessive refraction of beam energy at the bore **26.**

In the embodiment of the invention illustrated in FIGS. **1** and **2,** there is unavoidably a certain amount of radiation which is refelcted radially outwardly from the scanning point **38** in a narrow ring which is confined to a longitudinal dimension along the cylinder of the cylindrical chamber member **10** generally corresponding to the width of the entering beam of illumination from source **30.** Thus, the scatter detectors are always longitudinally displaced out of this ring of radiation and preferably arranged in pairs on opposite sides of the direct radiation position as shown by detectors **42** and **44** in FIG. **1.** Similarly, in FIG. **2,** the scatter detectors **56, 58, 60,** and **62** each represent a pair of detectors preferably arranged on opposite sides of the ring of radiation.

The preferred photoresponsive pick-up elements to be employed in the present invention as thus far described may consist of silicon barrier layer photo diodes. These devices are photo-voltaic devices commonly referred to as silicon solar cells. Suitable devices of this kind are available from many commercial sources.

FIG. **2A** corresponds generally to FIG. **2,** but illustrates alternative arrangements of the compounds, and also additionally illustrates an arrangement for picking up and detecting fluorescence of the particles. The light from the light source is again supplied through the cylindrical lens **36** to the optical chamber **10,** and absorption is detected by photo detector **40.** In this embodiment of the invention, the scatter detector **44** includes an optical reflector which may consist of a mirror **44B,** and a photo electric device **44C** arranged to receive the scatter radiation reflected by the optical reflector **44B.** Since these two elements accomplish the same combined function as the scatter pick-up element **44** of FIG. **2,** the combination of the two elements **44B** and **44C** may be referred to collectively as a photoresponsive pick-up element. As explained above in connection with FIG. **1,** the scatter pick-up element **44** is preferably paired with another scatter pick-up element **42,** the combination of the two pick-up elements being effective to detect scatter on the two sides of the light beam passing through the scanning point. By means of the reflective arrangement shown and described in connection with FIG. **2A,** it is possible to employ two separate reflectors on opposite sides of the beam, both reflectors directing scatter radiation to a single photoelectric device such as **44C.** By this means, the scatter radiation signals from the two sides of the beam need not be electrically combined. They are, instead, optically combined by being directed by separate reflectors to the single photoelectric device.

The particular arrangement thus far described, with the reflector **44B,** and a possible additional reflector, reflecting scatter signals to a single photoelectric device **44C** may be employed in the embodiment previously shown and described in FIG. **2,** and is not necessarily limited to combination with other features of FIG. **2A** described immediately below.

One of the most useful categories of optical measurement available in the photoanalysis of particles is the measurement of fluorescence of the particles in response to the primary radiation by the light beam directed through the cylindrical lens **36.** The particles may be stained with dyes so that when excited with light they are caused to emit fluorescent radiation at one or more wave lengths different from the wave length of the primary light beam. The intensity of fluorescent radiation at various wave lengths is an extremely useful indication of the properties and characteristics of the particles. Since the fluorescent radiation is emitted in virtually all directions from the particle, the fluorescent radiation is gathered by the reflectors **63** and **65** and generally directed, through a cylindrical lens **67,** to a dichroic mirror **69.** As is well known, the dichroic mirror **69** is designed to reflect radiation having a wave length shorter than a predetermined limit such as 5,500 angstroms and to transmit radiation having a longer wave length. The reflected radiation is directed through a filter element **71** to a first photo multiplier tube **73.** The transmitted radiation is directed through a second optical filter **75** to a second photomultiplier tube **77.** By means of the combination of the dichroic mirror **69** and the optical filters **71** and **75,** each of the photo multiplier tubes **73** and **77** receives only that light at the wave length predetermined by the respective filters. If desired, additional dichroic mirrors may be provided to split up the fluorescent radiation into additional spectral components in order to obtain additional optical analysis information.

The reflective elements **63** and **65** generally extend axially along the tubular chamber member **10.** However, these reflective elements are preferably split similarly to the scatter-sensing devices as pictured in FIG. **1,** so as to avoid reflecting and transmitting the ring of radiation which is discussed immediately above. Avoiding the reflection and transmission of the ring of radiation is particularly important when dealing with radiation at the green end of the visible spectrum because it is difficult to provide efficient optical filters **71** and **75** at that end of the spectrum. However, when dealing with fluorescence at the red end of the visible spectrum, efficient filtering is available and it is then preferably not to provide split reflective elements **63** and **65** and to gather the ring of radiation, which includes fluorescence signals at the desired wave length.

The reflector **63** is preferably arranged so that fluorescent radiation emitted through the lower surface of the chamber member **10** is reflected up to the upper reflector **65.** The upper reflector **65** is preferably arranged so that all of the radiation directed to that reflector is ultimately reflected to and gathered by the left portion of that reflector and directed through lens **67,** down to the dichroic mirror **69.**

When making fluorescence measurements, it has been found quite effective to employ for the light surface **30** an argon laser which emits light in the blue part of the spectrum. Light of this wave length has been found to provide a high degeee of fluorescence emission by biological particles which are often under oabservation. Since the particles fluoresce in the "forward" direction, it has been found to be quite desirable to provide a filter **79** which permits the passage only of the argon blue direct radition to the scatter sensor **44B–44C,** and to the absorption sensor **40.**

It will be appreciated that the arrangement shown in FIG. **2A** is particularly useful because it permits simultaneous detection of at least four different optical reactions of a particular particle simultaneously. Thus, absorption is detected at **40,** scatter is detected at **44B–44C,** and two different wave lengths of fluorescent radiation are detected at photo multipliers **73** and

77. Metachromatic, or combinations of fluorescent dyes are available which, when used to stain cells, will fluoresce at two wave lengths with each fluorescent intensity proportional to a given cellular constituent.

FIG. 3 is an enlarged detail sectional front view of that portion of the apparatus of FIG. 1 where the light beam actually strikes the stream of particles at point 38 in the chamber 10. The stream of particles 29 is shown to contain actual entrained particles 64 which are transported one at a time through the elliptically shaped beam of light at 66. Because of the elliptical shape of the beam of light, the stream 29 carrying the particles 64 may vary in its position within the chamber without having the stream actually move out of the path of the beam. Thus, the beam always intercepts the entire stream of particles. On the other hand, the narrow elliptical shape of the beam, in which the width of the beam is of substantially the same order of magnitude as the diameter of the particles, means that each particle causes an optimum optical reaction with the beam. Furthermore, even if the particles are closely spaced together, it is virtually impossible for more than one particle to be acted upon by the beam at any particular instant.

FIG. 4 is a sectional front view illustrating a modification of the apparatus of FIG. 1 in which arcuate shaped pick-up elements 42A, 44A, and 46A, 48A are employed. This view is taken at a section corresponding to the section line indicated at "4—4" in FIG. 2, although it will be understood that the actual modified structure of FIG. 4 is not illustrated in FIG. 2. The scatter pick-up elements 42 and 44 and 46 and 48 of FIG. 1 are arranged to intercept scatter over selected ranges of angles simply by virtue of a displacement in position along the axis of the cylindrical chamber member 10. As illustrated in FIG. 2, for larger scatter angles, the measurements can be made by circumferential displacement of the pick-up elements about the cylindrical chamber. However, a combination of these techniques is possible in which a high efficiency is achieved by means of the semicircular pick-ups illustrated at 42A, 44A, and 46A, 48A in FIG. 4. Thus, the illumination scattered over a particular range of angles may be scattered anywhere within a possible cone of scatter radiation terminating in a substantially circular radiation area such as the area defined by the pick-up elements 46A and 48A. Thus, the semicircular pick-up elements are particularly efficient in intercepting substantially all of the scatter radiation over the range of scatter angles for which they are designed.

FIG. 5 is a top view corresponding to FIG. 1 and illustrating a modification of the apparatus of FIG. 1 employing special light position-sensing photo-detectors 68 and 70 which are capable of determining both the intensity and the angular position of scatter radiation. These are silicon barrier photo diodes of the type which are commercially available for instance from United Detector Technology of Santa Monica, Calif., the name of the product being designated as Light Position Sensing Photo-Detectors. The photo detectors 68 and 70 are driven by a direct current voltage source indicated at 72. The current from source 72 enters the detectors 68 and 70 at the center detector terminals 74 and 76. The current passes through either or both of the detectors and is emitted at the end terminals 78 and 80, or at the end terminals 82 and 84, the ratio of the current through the different end terminals of each detector device is determined by the position of the radiation striking the device. For instance, if the radiation strikes the device 70, and if the radiation position is closer to the terminal 84 than it is to the terminal 80, there will be a greater current from the terminal 84 than there is from the terminal 80. The currents from terminals 82 and 84 are returned to the source 72 through a load resistor 86 to ground and from ground through a load resistor 88. Similarly, the currents from terminals 78 and 80 are returned through a load resistor 90 to ground and thus through load resistor 88 to the source 72. The resistance values of load resistors 86 and 90 are preferably equal and the relative values of the load currents through those resistors are measured in terms of voltage drops in a differential amplifier 92 to provide a scatter angle signal fed to a detection and recording circuit 46A.

Similarly, the intensity of the scatter illumination is detected in terms of the voltage drop across the common load resistor 88 by an amplifier 94, the output of which is also supplied to the circuit 46A. Thus, the arrangement of FIG. 5 does not simply record scatter radiation in specified ranges of scatter angles, but rather it records the intensity of the scatter radiation and indicates the angle at which that radiation occurs. This provides a particularly valuable analysis tool where the significant range of scatter angle is not previously known.

In accordance with the present invention it has been determined that a new method may be followed, employing the apparatus described above, for rapidly analyzing samples of particles, and particularly biological samples of cells such as blood cells. As mentioned above, different conditions of biological cells may control the amount of dye which individual cells will pick up when the cell sample is exposed to a dye. For instance, when a mixture of live cells and dead cells is exposed to Trypan blue dye, the dead cells take up the dye to a significant extent, while the live cells do not.

In a specific example of the practice of this method, the method may be employed to determine the feasibility of an organ transplant from one individual to another by checking for compatibility of blood cells. For such a compatibility test, lymphocytes may be extracted from the blood of a donor to be matched with the sera of potential recipients. To effect this match the lymphocytes are diluted in 0.85 percent sodium chloride solution or an appropriate nutrient medium to a concentration of 5 million cells per milliliter and added to the diluted serum of the potential donor. The mixture is then incubated at a temperature in the range from 35° to 40° Centrigrade for approximately 45 minutes. The incubation is then stopped by placing the samples on ice. Immediately before examination a freshly prepared Trypan blue aqueous solution containing 0.24 percent Trypan blue and 0.85 percent sodium chloride is added to the sample in a volume equal to 50 percent of the sample volume so as to increase the sample volume by about 50 percent. The final suspension should have a cell concentration in the range of one million cells, or less, per milliliter of solution to provide reasonable assurance that the cells will pass through the apparatus one at a time. The sample is then caused to pass through the apparatus as described above in connection with FIG. 1, and the optical absorption of the cells is detected concurrently with the optical scatter over a range from about one degree to about thirty degrees away from the axis of the beam. The stained

cells, which provide a large absorption signal, are indicated as dead, and the unstained cells, which provide a high scatter signal, and a low absorption signal are indicated as live. A high count of dead cells in this compatibility analysis indicates a lack of compatibility between donor and recipient for the purpose of transplants.

For this analysis, a helium neon laser emitting illumination at 6300 angstroms may be employed. Other dyes may be alternatively used for this live-dead cell analysis method. For instance, nigrosine dyes are useful for this purpose.

FIG. **6** illustrates an essential step in the preferred method of the production of the optical chamber member **10** of the apparatus of the present invention. In practicing this method, a piece of glass tubing having inside and outside diameters of the desired size for the finished optical measurement portion of the tubular optical chamber is cut to a length which is somewhat greater than twice the desired length of the finished optical chamber. The tubing must be substantially free of any cracks, scratches or blemishes. The glass tubing is then heated uniformly around the central portion of the axial length thereof to the softening temperature of the glass. This may be simply accomplished by rotating the tubing while the central portion of the tubing is held in a gas flame.

Next, the central bore of the tubing is placed under pressure. This causes enlargement, or blowing out, of the central bore of the tubing at the heated central portion thereof, as shown at **96** in FIG. **6.**

When the proper degree of enlargement has been achieved, the pressure is released and the tubing is allowed to cool and harden. It is then cut apart through the centerline as indicated at **98.** The two resultant separate pieces **10**A and **10**B can each be used as optical chambers in an apparatus such as previously described in connection with FIG. **1** after the next step. As the final step, in order to provide a liquid-tight seal at the ends of each chamber, both ends of each of the glass parts **10**A and **10**B are preferably ground flat and mutually parallel to eliminate the slight inaccuracies of the glass cutting procedure.

There are a number of useful variations in the process described just above. For instance, the central bore of the tubing may be placed under pressure while the tubing is being heated so that the enlargement of the tubing occurs at the earliest possible moment when the heating has progressed sufficiently to soften the glass. Also, it has been found to be quite practical and desirable to perform the heating and pressurizing steps at spaced axial positions along an extended length of glass tubing. All of the tube cutting operations are then done after the enlargements have been formed, cutting at each enlargement and midway between adjacent enlargements to thereby obtain a yield of two optical chambers for each tubing enlargement.

It has been found that the above method of production of the optical chambers is very simple and inexpensive and also very satisfactory. It has been determined that the changes in the diameter in the resultant enlarged funnel-shaped end **24** of the central bore **26** is substantially an exponential function. That is, the change in the diameter of the central bore is substantially an exponential function of the displacement along the axis of the tube at which the diameter is measured. This provides a graded change in the bore diameter which is very valuable and useful in promoting smooth and non-turbulent liquid flow in the critical bore narrowing region where the sheath liquid is reducing the diameter of the particle carrying liquid stream.

While this invention has been shown and described in connection with particular preferred embodiments, various alterations and modifications will occur to those skilled in the art. Accordingly, the following claims are intended to define the valid scope of this invention over the prior art, and to cover all changes and modifications falling within the true spirit and valid scope of this invention.

We claim:

1. An apparatus for rapid optical measurement of the characteristics of small particles such as blood cells while the particles are suspended in a liquid,

comprising a source of light,

a cylindrical tube member defining an optical chamber,

said tube member being comprised of a material which transmits light from said source,

means for directing said light into one side of said tube member in a beam substantially converging at the center of said optical chamber when viewed in a direction perpendicular to the axis of said tube member defining said chamber,

means for moving the particle suspending liquid through said tube member in a thin stream to cause the particles therein to pass in sequence through said light beam one by one,

at least one photoresponsive pick-up element for detecting light scattered by said particles positioned on the side of said tube member opposite to said light source and displaced away from the direct path of said light beam through said tube member in a direction parallel to the axis of said tube member,

said light source comprising a source of monochromatic light,

a second photoresponsive pick-up element in addition to said scattered light detecting photoresponsive pick-up element,

a spectral filtering element positioned to filter the light received by said second photoresponsive pick-up element,

said spectral filtering element being selected to exclude light at the wave length of said light source and to pass light to the associated photoresponsive pick-up element at a wave length for which the particles under investigation emit fluorescent radiation in the presence of a significant particle characteristic to be detected,

and optical means substantially surrounding the circumference of said cylindrical tube member optical chamber for gathering fluorescent radiation directed radially outwardly from the particles and for directing the fluorescent radiation through said spectral filtering element to said second photoresponsive pick-up element.

2. Apparatus as claimed in claim **1** wherein

there is provided a third photoresponsive pick-up element positioned outside of said chamber and a second spectral filtering element positioned to filter the light received by said third photoresponsive pick-up element,

said second spectral filtering element being selected to exclude light at the wave length of said light

source and at the wave length passed by said first-mentioned spectral filtering element and operable to pass light to the associated photoresponsive pick-up element at a wave length for which the particles under investigation emit fluorescent radiation in the presence of a second significant particle characteristic to be detected.

3. Apparatus as claimed in claim **2** wherein

said optical means for gathering and directing fluorescent radiation includes a dichroic mirror operable to reflect fluorescent radiation to one of said spectral filtering elements and the photoresponsive pick-up element associated therewith,

and operable to transmit fluorescent radiation to the other one of said spectral filtering elements and the photoresponsive pick-up element associated therewith.

4. An apparatus for rapid optical measurement of the characteristics of small particles such as blood cells while the particles are suspended in a liquid,

comprising a source of substantially monochromatic light,

a cylindrical tube member defining an optical chamber,

said tube member being comprised of a material which transmits light from said source,

means for directing said light into one side of said tube member in a beam substantially converging at the center of said optical chamber when viewed in a direction perpendicular to the axis of said tube member defining said chamber,

means for moving the particle suspending liquid through said tube member in a thin stream to cause the particles therein to pass in sequence through said light beam one by one,

at least one photoresponsive pick-up element positioned outside of said tube member and displaced away from the direct path of said light beam through said tube member,

said pick-up element being operable to respond to fluorescent radiation emitted by said particles in the presence of said light beam,

a spectral filtering element positioned to filter the light received by said pick-up element,

said spectral filtering element being selected to exclude light at the wave length of said light source and to pass light to said pick-up element at a wave length for which the particles under investigation emit fluorescent radiation in the presence of a significant particle characteristic to be detected,

and optical means substantially surrounding the circumference of said optical chamber and operable to gather fluorescent radiation directed radially outwardly through the walls of said optical chamber from the particles and for directing the fluorescent radiation through said spectral filtering element to said photoresponsive pick-up element.

5. Apparatus as claimed in claim **4** wherein there is provided

a second photoresponsive pick-up element positioned in alignment with the light beam on the side of said housing opposite to said light source to receive the unscattered illumination from the light beam.

6. Apparatus as claimed in claim **4** wherein

a second photoresponsive pick-up element is positioned outside of said tube member to receive fluorescent radiation from the particles gathered by said optical means for gathering fluorescent radiation,

a second spectral filtering element positioned to filter the light received by said second pick-up element,

said second spectral filtering element being selected to exclude light at the wave length of said light source and to pass light to said second pick-up element at a wave length for which the particles under investigation emit fluorescent radiation in the presence of a significant particle characteristic to be detected which is different from the characteristic detected by fluorescent radiation received by said previously mentioned pick-up element.

7. Apparatus as claimed in claim **6** wherein

a dichroic mirror is provided and positioned between said optical means for gathering fluorescent radiation from the particles and said spectral filtering elements,

said dichroic mirror being operable to reflect fluorescent radiation to one of said spectral filtering elements and the photoresponsive pick-up element associated therewith,

and said dichroic mirror being operable to transmit fluorescent radiation to the other one of said spectral filtering elements and the photoresponsive pick-up element associated therewith.

8. Apparatus as claimed in claim **4** wherein

said means for moving the particle suspending liquid through said tube member in a thin stream includes means for providing a sheath of liquid flowing in an annular configuration through said tube member and surrounding said thin stream to thereby confine the thin stream of particle suspending liquid to a dimension smaller than the interior diameter of said tube member.

9. Apparatus as claimed in claim **4** wherein said light source is a laser.

10. Apparatus as claimed in claim **9** wherein said laser is an argon ion laser.

11. An apparatus for rapid optical measurement of the characteristics of small particles such as blood cells while the particles are suspended in a liquid comprising

a cylindrical tubular housing defining an optical chamber,

a source of substantially monochromatic light,

said optical chamber being comprised of a material which transmits light from said source,

means for moving the particle suspending liquid through said optical chamber in a thin narrow stream to thereby convey the particles in sequence through the light beam one by one,

means for directing light from said light source into one side of said optical chamber to intersect with the thin stream carrying the particles therein,

said means for directing said light being operable in cooperation with said side of said optical chamber to converge said light beam into a substantially elliptical shape in a plane transverse to the light beam and at the point of intersection with the position of the thin stream of particles,

the major axis of the elliptical shape of said beam being substantially perpendicular to the direction of the stream of particles and the dimension of said beam at said major axis being substantially greater than the transverse dimension of said particle stream,

at least one photoresponsive pick-up element positioned outside of said optical chamber,

said pick-up element being operable to respond to fluorescent radiation emitted by said particles in the presence of said light beam,

a spectral filtering element positioned to filter the light received by said pick-up element,

said spectral filtering element being selected to exclude light at the wave length of said light source and to pass light to said pick-up element at a wave length for which the particles under investigation emit fluorescent radiation in the presence of a significant particle characteristic to be detected,

and optical means substantially surrounding the circumference of said optical chamber and operable to gather fluorescent radiation directed radially outwardly through the walls of said optical chamber from the particles and for directing the fluorescent radiation through said spectral filtering element to said photoresponsive pick-up element.

12. Apparatus as claimed in claim **11** wherein

said means for directing light from said light source comprises a substantially cylindrical lens device.

13. A method for rapidly detecting biological cells having different properties including the steps of exposing a solution containing the cells to be analyzed to a dye which is taken up differently by cells having different characteristics,

moving the particle suspending liquid solution in a thin stream to convey the particles one by one through a cylindrical tubular optical chamber while directing light from a substantially monochromatic light source into one side of the chamber to intersect with the thin stream of particles in a narrow beam,

the narrow beam converging at the stream of particles,

gathering fluorescence radiation signals emitted by the stained cells when excited by the light beam and radiated radially outwardly from the circumference of the housing,

detecting said radiation signals by means of at least one photoresponsive pick-up element positioned outside of the housing,

and filtering the fluorescence radiation signals directed to the pick-up element to exclude light at the wave length of said light source and to pass light to the pick-up element at a wave length for which the particles under investigation emit fluorescent radiation in the presence of a significant particle characteristic to be detected.

* * * * *

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UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,788,744 Dated January 29, 1974

Inventor(s) MITCHELL FRIEDMAN, LOUIS A. KAMENTSKY and ISAAC KLINGEI

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Column 1, lines 4 and 5, "U.S. Pat. No. 3,687,553 issued 8/29/72" should read --U.S. Pat. No. 3,705,771 issued 12/12/72--;
last line, "sidderent" should read --different--.
Column 2, line 17, "is" should read --has--;
line 43, "of" should read --on--.
Column 3, line 37, "an" should read --and--;
line 64, "particle" should read --particles--.
Column 4, line 39, "signlas" should read --signals--;
line 45, "iverges" should read --diverges--.
Column 5, lines 5 and 6, "abosrption" should read --absorption--;
line 11, "a" should read --the--.
Column 6, line 5, "diamter" should read --diameter--;
line 13, "strea" should read --stream--.
Column 7, line 5, "refelcted" should read --reflected--;
line 26, "compounds" should read --components--.
Column 8, lines 38 and 39, "preferably" should read --preferable-
lines 51 and 52, "surface 30" should read --source 30--
lines 55 and 56, "oabservation" should read --observation--;
line 59, "radition" should read --radiation--.

Signed and sealed this 11th day of June 1974.

(SEAL)
Attest:

EDWARD M.FLETCHER,JR.
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents