Sept. 2, 1958 M. L. POLANYI ET AL 2,850,239

COUNTING DEVICE

Filed Aug. 16, 1952 4 Sheets-Sheet 1

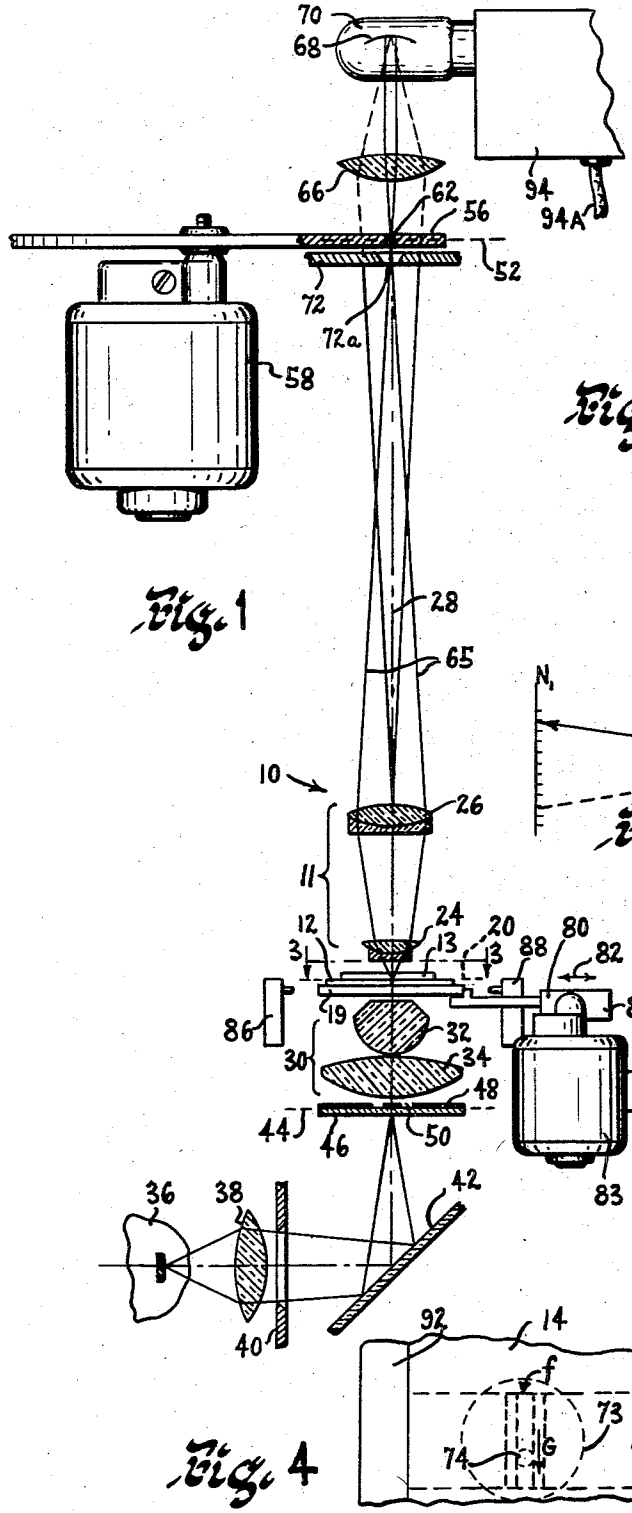

Fig. 1

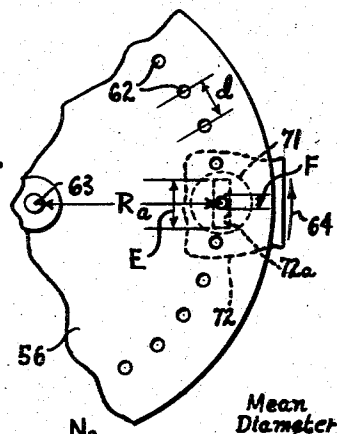

Fig. 2

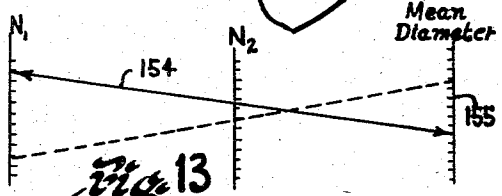

Fig. 13

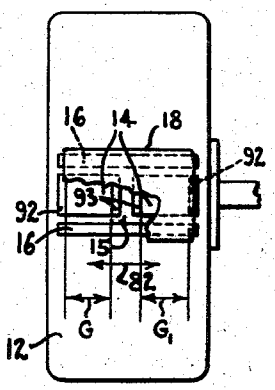

Fig. 3

Fig. 4

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COUNTING DEVICE

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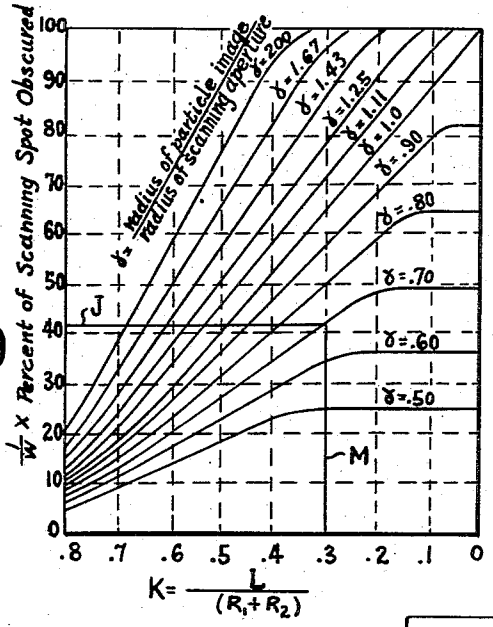

Fig. 9

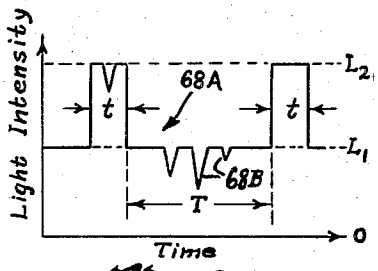

Fig. 2A

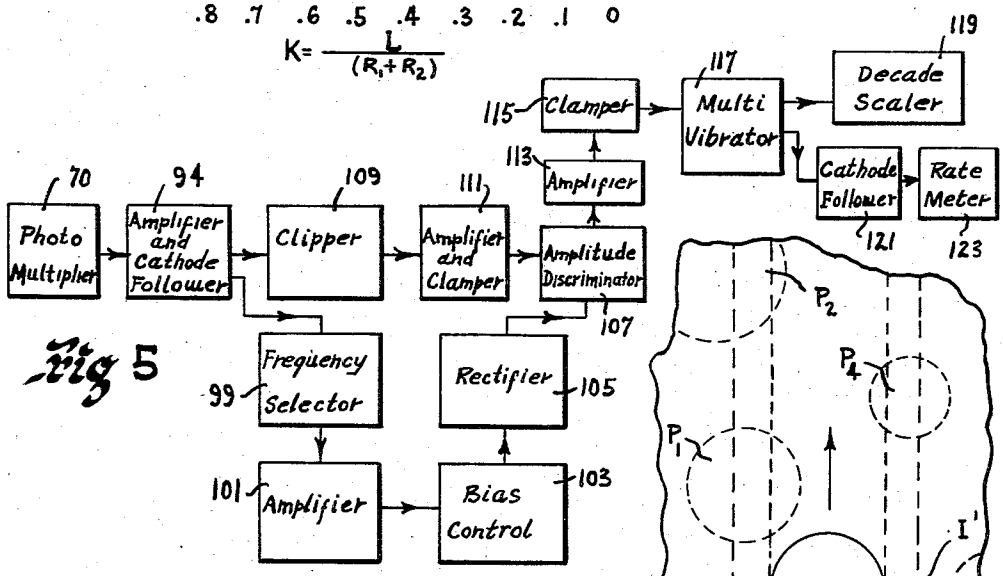

Fig. 5

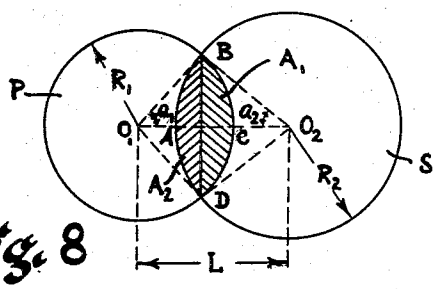

Fig. 8

Fig. 7

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Fig. 6

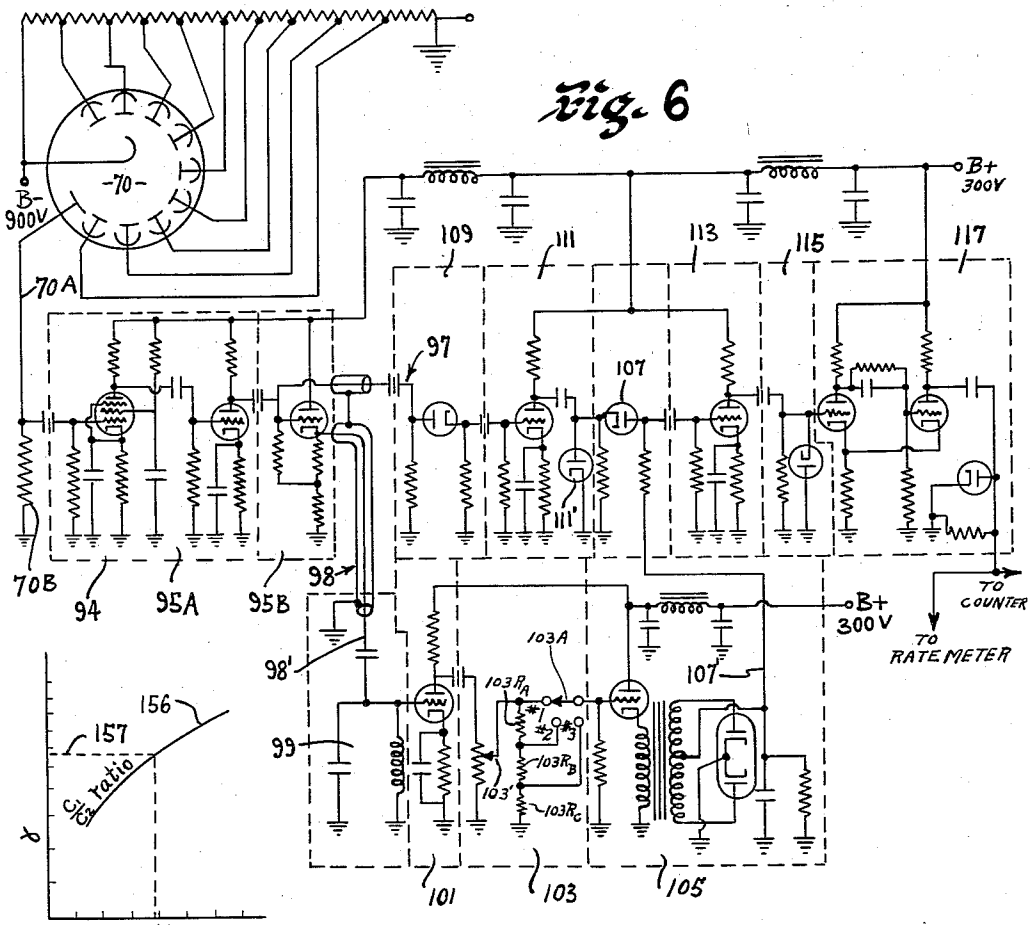

Fig. 14

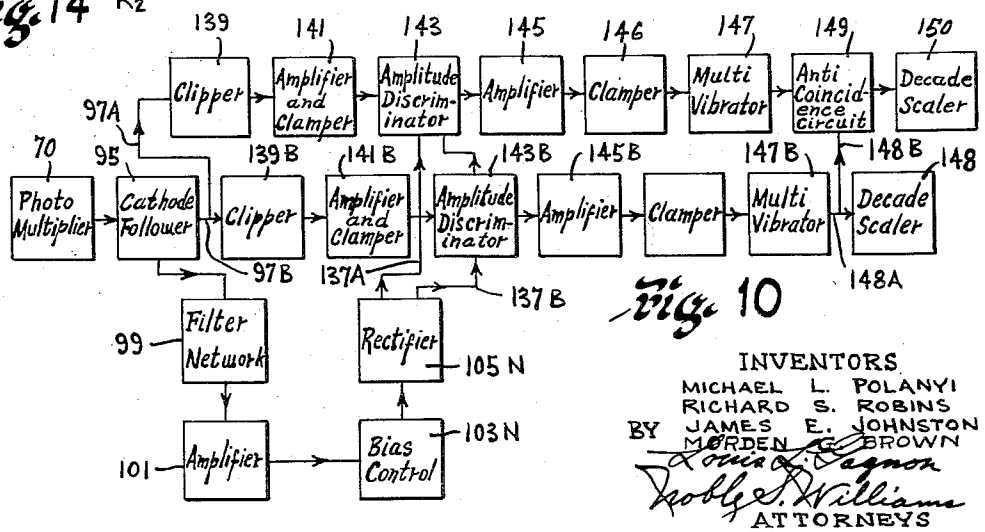

Fig. 10

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COUNTING DEVICE

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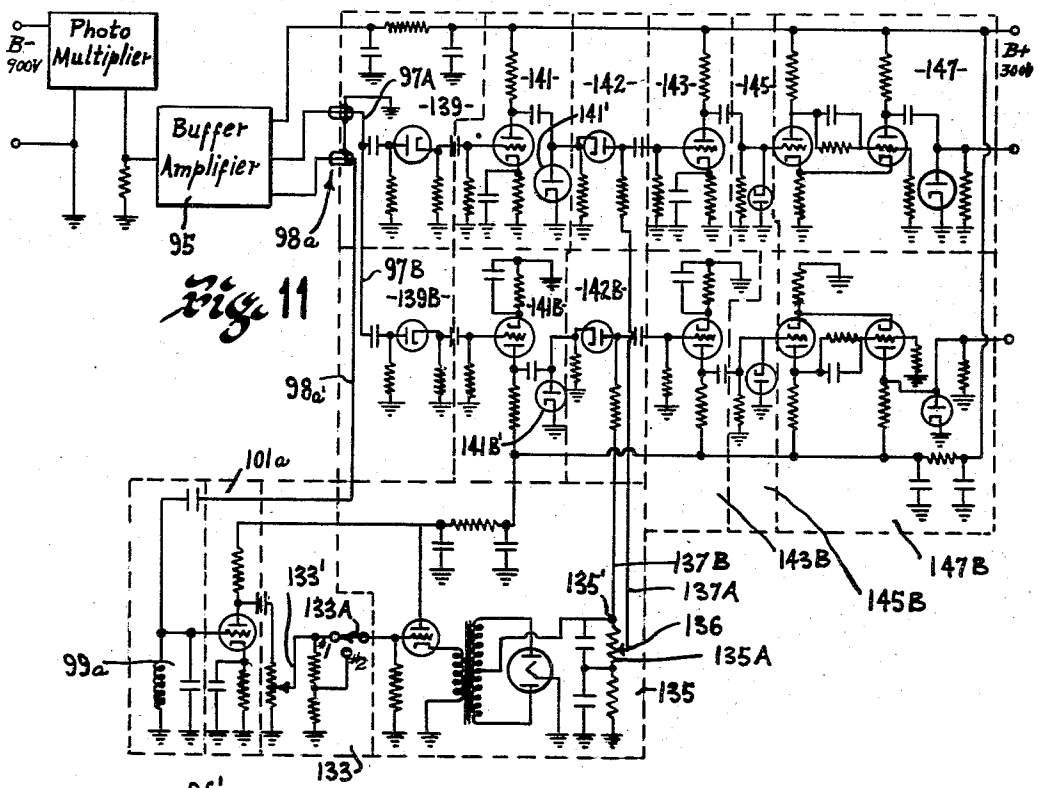

Fig. 11

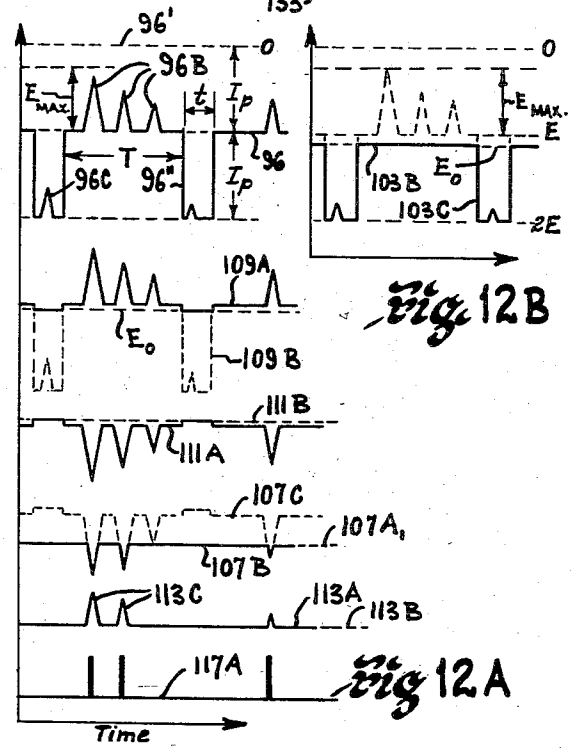

Fig. 12A

Fig. 12B

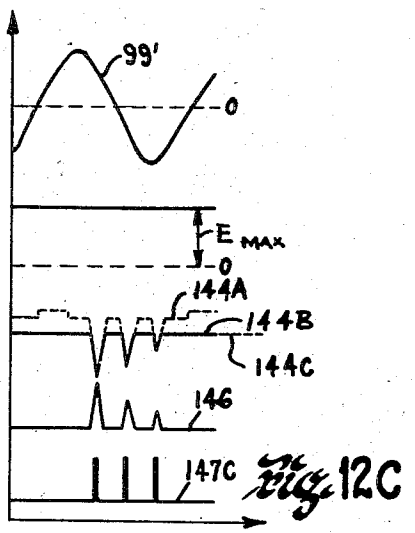

Fig. 12C

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2,850,239

COUNTING DEVICE

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Application August 16, 1952, Serial No. 304,802

8 Claims. (Cl. 235—92)

This invention relates to apparatus for counting and analyzing various kinds of microscopic particles appearing in large numbers and in relatively close relation generally in suspension in solution, such as red corpuscles, particles of yeast, cocci bacteria, mold spores and the like, in an automatic manner and at a relatively high rate of speed. The invention also includes a method for use with such apparatus. While the apparatus of the present invention has broad utility for counting and analyzing purposes, its greatest usefulness at the present time is in the field of blood cell counting and blood analysis.

In the medical profession, for example, it is well known that analysis and evaluation of various characteristics of a single small blood sample may be advantageously employed in many ways. For such reasons it is most desirable to be able to accurately determine the red cell population, and the average red cell size of a sample of blood in a rapid and accurate manner. The red corpuscle count in a sample of human blood may vary over wide ranges depending upon the condition of the patient from whom it was extracted. Also, the size of individual cells within a selected specimen and the degree of opacity thereof may vary appreciably. When an accurate evaluation of cell population, cell size and hemoglobin concentration can be accurately and rapidly determined, hospital technicians, medical researchers and the like, will be greatly aided. The device of the present invention greatly facilitates this work and provides information of extremely high accuracy.

It must be kept in mind while considering the present invention, that the very small size of the red corpuscles in a blood sample or other microscopic particles being analyzed increases the difficulties which must be overcome when accurately and rapidly providing the above mentioned information. The structure of the present invention, however, is capable of providing the desired information accurately and in a matter of seconds and accordingly has been found in practice to be of great utility.

It is, accordingly, an object of the present invention to provide a combined optical and electrical apparatus capable of receiving a sample of fluid containing microscopic particles and rapidly counting the particle population thereof for unit volume as well as analyzing same for mean particle size thereof.

It is a further object of the present invention to provide an apparatus of the above character employing an improved electronic differentiating means whereby such evaluation of sample containing microscopic particle may be rapidly, accurately and automatically carried out.

It is a particular object to provide a combined optical and electronic device constructed and arranged to rapidly and accurately count a large number of blood cells in a unit volume blood sample and to accurately compensate according to mean cell size to provide an accurate indication of the true cell count of the unit volume.

It is a particular object to provide a combined optical and electronic device constructed and arranged to rapidly and accurately count a large number of blood cells in a unit volume of blood sample and to accurately compensate according to absorptive properties of the cells to provide an accurate indication of the true cell count of the unit volume. The method of use of the apparatus is also being claimed.

It is an additional object to provide a combined optical and electronic device of the above character which will rapidly and accurately count a large number of blood cells in a unit volume and to accurately compensate according to mean cell size and absorptive properties thereof to provide an accurate indication of the true cell count of the unit volume.

Other objects and advantages of the invention will become apparent from the detailed description which follows when taken in conjunction with the accompanying drawings in which:

Fig. 1 is a diagrammatic view of apparatus embodying optical, electrical and mechanical means which may be used in carrying out the present invention;

Fig. 2 is a fragmentary view of a scanning disc which may be embodied in the apparatus of Fig. 1;

Fig. 2A is a graph showing light intensity transmitted by the scanning disc plotted against time;

Fig. 3 is a sectional view taken substantially upon section line 3—3 of Fig. 1 and showing a specimen carrying slide thereof;

Fig. 4 is an enlarged fragmentary view of a portion of the structure of Fig. 3;

Fig. 5 is a block diagram of an electronic system which may be used with the apparatus of Fig. 1;

Fig. 6 is a schematic wiring diagram of the block diagram of Fig. 5;

Fig. 7 is an enlarged fragmentary view of a portion of structure of Fig. 2;

Fig. 8 is a diagram for use in analytically comparing the image of a mean size particle with the size of the apertures employing in the scanning disc of Fig. 1;

Fig. 9 is an interpolation chart for use in obtaining true count values from the actual count values obtained by the apparatus;

Fig. 10 is a block diagram of a modified form of electronic system which may be used with the apparatus of Fig. 1;

Fig. 11 is a schematic wiring diagram of the block diagram of Fig. 9;

Fig. 12A, 12B and 12C are graphs showing wave shapes and pulses which may be obtained at different locations in the electronics block diagram of Figs. 5 and 6;

Fig. 13 is a nomograph for use in interpretation of the information obtained from the counting units of the device; and,

Fig. 14 is a graph which may be used in place of the nomograph for such purposes.

Optical section

As stated previously, the apparatus of the present invention may be used for counting and analyzing microscopic particles of different kinds. However, since one of the greatest needs for such an apparatus at the present time is in the analysis of human blood, the detailed description which follows will be first directed primarily to the apparatus as adapted to such use.

Referring to Fig. 1 in detail, it will be seen that a microscope optical system **10** comprises an objective **11** optically aligned with a microscope slide **12** for supporting a solution containing light absorbing microscopic particles or the like, to be counted and analyzed. The specimen slide **12** is positioned at the front focal plane of said objective and a cover glass **13** is ordinarily used with the slide. It has been found convenient in carrying

out the present invention to employ as the slide **12** one somewhat like a conventional haemacytometer commonly provided for blood count purposes. The slide **12**, as will be seen in Fig. 3, comprises a pair of accurately finished slightly elevated specimen supporting plateaus **14** in side-by-side spaced relation, with these plateaus being separated and partially surrounded by a moat **15** for receiving excess fluid.

The slide **12** is provided with a pair of accurately finished spaced supporting ribs **16** which extend along opposite sides of said plateaus and in spaced relation thereto to accommodate the moat therebetween. The ribs are a carefully controlled distance above the common plane or level of the plateaus so as to accurately position the flat lower surface of the cover plate, when placed thereon, an exact known minute distance (preferably substantially 0.1 mm.) above and parallel to the plateaus. In this manner an exact depth of solution enclosed between the plateaus and the cover plate will be obtained.

The microscope slide **12** during use in the apparatus is located upon a horizontally movable stage **19** and in the object plane **20** of the objective. In the present instance the objective **11** is shown as comprising front and rear doublet components **24** and **26**, and aligned with the objective upon the optical axis **28** of the optical system is a sub-stage condenser assembly **30** comprising lens components **32** and **34**. A light source, preferably of the relatively heavy ribbon filament type, arranged to operate on D. C. current is indicated at **36**. Arranged to function with this light source is a lamp condenser **38** having a field stop **40** adjacent thereto.

The light source **36** and lamp condenser **38** are so positioned relative to a reflector **42** and so aligned with the condenser assembly **30** and objective **11** that the filament of the light source **36** will be re-imaged substantially at the plane of the entrance pupil **44** of the condenser and objective combined. At this entrance pupil **44** may be disposed an aperture plate **46** having an opaque coating **48** thereon and so arranged as to provide an annular light aperture **50** therein. Light rays passing through the aperture **50** will provide a cone of oblique light rays for illuminating a specimen upon the slide **12** at the plane **20**.

The light illuminating the specimen upon the slide **12** will be in part diffracted and in part absorbed by the red corpuscle or erythrocyte population in the solution thereon, with the result that improved contrast between each partially light absorptive particle and the lighter background will be produced. While this type of illumination is preferred for electronic counting purposes, particularly for small partially transmitting particles because best operating results have been obtained therewith, it is possible to omit the aperture plate **46** and obtain usable results with ordinary transmitted or direct illumination. However, when more direct light which passes through the particle and through the objective, as is the case when the aperture plate **46** is omitted, the effective ratio of the light intensity when a patricle is in the microscope field area being used at any instant relative to the total unobstructed light of this field area is lessened.

Particles in fluid on the slide at the object plane **20** will be imaged by the objective **11** substantially at a conjugate image plane **52**. At this image plane **52**, however, there is provided a relatively thin circular disc **56** for restricting the area of the image field which is transmitting energy at any instant, as will be described more fully hereafter. The disc **56** is rotatably carried by a synchronous motor **58** and this motor is arranged to rotate disc **56** at a predetermined uniform speed. The motor **58** will normally be energized by an alternating current source, such as a conventional 60 cycle, 120 volt supply line and an operating speed of 30 revolutions per second has been found to be satisfactory, although the apparatus could be arranged to operate at any other constant speed within a wide range. The disc **56**, as better shown in Fig. 2, is provided with a large predetermined number of light apertures **62** substantially equally peripherally spaced from each other a predetermined distance *d*. These apertures are also equidistantly spaced a radial amount R_a from the center of rotation **63** of the disc **56**. Each aperture is circular in shape and of an exact predetermined diameter F which, as will be later more fully explained, closely approximates a predetermined numerical relationship to the "normal size" of blood cells which is substantially **7.2** microns. (This numerical value will differ for other kinds of particles to be counted.)

When the disc **56** is caused to rotate, as indicated by the arrow **64**, the light apertures **62** will be caused to successively sweep across an image field **71** (indicated by dotted lines in Fig. 2) of the objective located at the conjugate image plane **52**. Rays **65** coming from the specimen on the slide **12** are imaged at **52** and will pass through an aperture **62** and will be intercepted by a lens **66** which is arranged to receive these rays and direct same substantially upon a fixed area of a photo-sensitive cathode **68** of a photoelectric detector such as a photomultiplier or photocell **70** which is connected to electronic counting and analyzing means to be presently described. The lens **66** is focused substantially upon the back surface of the objective **11** and has its conjugate plane at cathode **68**. In this manner there is provided a concentrated spot of light at a substantially fixed location upon the cathode **68** regardless of the various positions of an aperture **62** transmitting light as it moves across the image field **71**. The extent of useful travel of the aperture **62** while transmitting light will be controlled by the longitudinal dimension E of an aperture **72***a* in a field stop **72**. The field stop **72** is disposed closely adjacent the disc **56**. The length of the scanned image field E is so chosen relative to the size of the apertures **62** and the space between apertures that distance E will be somewhat greater than the diameter F of apertures **62** plus the distance *d* between apertures **62**. The arrangement will then provide, as shown by the graph of Fig. 2A, during each single scanning increment of the device, a time period T during which one aperture transmits light at a certain intensity L_1 followed by a shorter period *t* during which two apertures transmit substantially double this amount of light L. Thus, a rectangular wave form **68**A may be plotted as shown by Fig. 2A.

It will be readily appreciated that as each successive light aperture **62** is caused, during rotation of the scanning disc **56**, to sweep across the aperture **72***a* in field stop **72**, the greater part of the object field **73** on the plateau **14** of the slide **12** (see Fig. 4) will be, in effect, scanned by a small area **74** corresponding to the aperture **62** but which area **74** is of a reduced size so that its diameter will be in inverse proportion to the magnification of the objective **11**, times the diameter F of the aperture **62**. Shown impressed upon this rectangular wave **68**A are small depressions **68**B indicative of microscopic particles in or partly in the object field scanned by spot **74** and absorbing various amounts of light. In other words, if the objective **11** has a **44**X magnification, the lonigtudinal distance *e* of the object field being scanned upon the plateau **14** will be only one forty-fourth of the distance E in the image field **71** being scanned and the small area **74** which is scanning the plateau **14** as the disc **56** rotates will have a diamter *f* which is only one forty-fourth of the diameter F of the aperture **62**. The size of the apertures **62** should be chosen so as to fairly closely bear a definite relation to the "normal" size of particles which are to be counted as will be hereinafter described. It should be here noted that, in order to obtain as uniform object field illumination as possible at slide **12**, the bulb **36** will be positioned in the optical system so the image of its flat front surface formed at the plane **44** will have its longer dimension

extending in the same direction as the longer dimension of aperture 72a.

Since it is also desirable to scan an area of appreciable size, in order to obtain a particle count value which will be indicative of the average condition throughout of the fluid being analyzed, the device of Fig. 1 is provided with an actuating mechanism 80 of suitable construction for moving the stage 19 and the slide 12 thereon horizontally back and forth across the object field of the objective 11. This travel of the slide 12 is indicated by the double headed arrow 82. The actuating mechanism 80 may comprise a suitable reversible synchronous electric motor 83 having a reduction gearing 84 of high reduction ratio. In the present preferred embodiment of the invention the actuating mechanism is arranged to perform one complete fore and aft operating cycle each time a specimen is to be analyzed.

The motor 83 and gearing 84 are arranged to cause the plateaus 14 on the slide 12 to move entirely across the object field, after which a sensitive snap action switch 86 adjacent a side of the stage and engaged by the stage 19 will cause the motor 83 to reverse the direction of movement of the slide. The reverse movement or travel of the slide 12 will continue until a second normally closed snap action switch 88 adjacent the opposite side of the stage is engaged thereby for interrupting the supply of current to the motor. A push button 90 for initiating such an operating cycle of the motor 82 may be connected in parallel relation with the switch 88 so that it will only be necessary to manually momentarily actuate the push button 90 in order to start each operating cycle. As soon as switch 88 is free a cycle will be completed automatically. Of course the switch 90 could also serve to start motor 58.

By the use of thin opaque coated areas 92 upon opposite ends of the plateaus 14 and coated areas 93 upon adjacent ends thereof as well as an opaque material in the moat between areas 93 an exact predetermined clear distance, which is represented by the letter G plus letter G_1 (see Fig. 3), may be easily controlled. Areas 92 and 93 may be easily increased or decreased during coating to provide this exact clear distance, and thus the desired total length of the area to be scanned may be accurately established. Since the depth of fluid chamber between the plateaus and cover glass may vary slightly, this variation may be readily compensated for by the control of the total distance (G plus G_1). This method is used to keep the volume of sample being scanned constant. Since the depth of the solution on the slide is established by the height of the cover plate 18 above the plateaus 14, and since the distances G and G_1 between opaque areas 92 and 93, and the width of the scanned area is controlled by the distance between centers of adjacent apertures 62, the volume of the solution being analyzed may always be maintained constant.

During operation of the synchronous motor 58 successive light apertures 62 will be moved lengthwise of the field stop 72 at a constant predetermined rapid speed (in a preferred construction 900 sweeps per second) and by the correct approximately equal spacings *d* of these apertures circumferentially in the disc 56, one relative to the next, it will be possible to arrange the structure so that two successive light apertures 62 will be completely in the field being scanned simultaneously for a time equal to approximately *t* (see Fig. 2A). The result of this arrangement will be that "background" light (through the solution when no particle or particles are in the field) will be transmitted through one aperture 62 to the photo tube 70 for a predetermined interval of time T and thereafter approximately double this amount of light will be transmitted therethrough for a shorter period of time *t* while two adjacent apertures are simultaneously in the field. Since the motors 58 and 83 always operate in fixed relative relation to each other (regardless of what speeds they are arranged to operate at) and since the total volume being scanned is constant, the same volume of material containing particles will be explored during each complete operating cycle.

There has been described above a method by which a volume of solution may be synchronously scanned in two directions for determining the total particle count of this volume. It should be clearly understood, however, that other methods may be employed for count determination. The above method, in fact, causes the traces 74 of the individual small aperture 62 in the scanning disc to collectively traverse a certain total distance upon slide 12, which taken in consideration with the depth of cell being scanned, is counting a definite volume of solution each time a specimen is being analyzed by the apparatus. A different method which might be employed which would provide as accurate a final count value might be provided by an accurate control of the number of revolutions of the scanning disc (and thus the total number of individual apertures 62 traversing the image field) and without regard to the total area along the slide which is being traversed by the traces 74 of the apertures at the object field. It would be, in fact, possible to move the slide 12 at random to expose various parts of the specimen in the object field and be able to obtain accurate results. Thus, the total count would depend upon the total length of all of the traces effected by the aperture 62 times the depth of chamber between slide 12 and cover plate 18. Such measurement of total scanning effected by the aperture disc 56 could be accomplished by allowing a synchronous motor to operate for a certain predetermined period, or alternatively, it might be possible to rotate the motor at any suitable speed and accurately count by suitable means the number of revolutions of the disc to be used.

The problem of counting opaque microscopic particles of a known size on the slide 12 electronically would not be too difficult a problem (when the particles are sufficiently dispersed thereon) if they were all of the same size and if the number of particles were the only information sought for. In such a case the slide could be, in effect, swept successively by the apertures 62 of the rotating disc while the slide is simultaneously moved across the object field of the microscope by the motor 83. Only electrical signals or pulses above a selected value indicating when one half of a particle or more occludes the light of the scanning area 74 would be counted. However, in attempting to accurately count and analyze materials with particles which vary appreciably in size, which vary appreciably in transmission characteristics and which vary appreciably in an unpredictable way between not definitely known or commonly accepted limits for sample to sample, such as in human blood analysis work, the problem of obtaining an accurate or true count value as well as additional usable information, such as mean particle size and light absorption properties, is not a simple one.

It will be shown, however, that the size for the aperture 62 in the scanning disc should be carefully chosen in accordance with the normal size of the kind of particles to be counted so that the apertures 62 will closely approximate the size of the image of such normal particles. By careful consideration of the preselected size for the scanning apertures 62 and careful treatment of the information obtained from the electrical signals derived from the phototube 70 due to "background lighting" when no cell is present in the field to obscure light and due to different amounts of light flux modulation being produced as the individual particles or blood cells in effect traverse the area 74 being scanned and superimpose pips or minute pulses upon this background signal, accurate blood cell counts may be obtained. The electrical apparatus presently to be described may be arranged to simultaneously or successively count at two different known electrical pulse magnitude discrimination levels for obtaining this accurate information,

It may be assumed of course that a large number of particles of varying size will be dispersed at random within the sample to be analyzed but will be free from clumping or undesirable "overlapping" so as to be in the main individually distinguishable and that the individual particles will appear as disc-like objects of nearly uniform optical density. The solution should be diluted to such an extent that ordinarily only a single particle will be traversed by the scanning aperture 62 or partially traversed thereby at a single instant. Consideration should be given to the size of the scanning area or spot in the object field relative to the size of particles therein, or alternatively, and more conveniently, the size of the apertures 62 in the disc 56 relative to the size fo the images of the particles at the image field 71, particularly when it is recalled that the size of particles obscuring the light vary and also that such particle images may be substantially totally or only partially intercepted by the scanning apertures.

In Fig. 7 a small portion of the disc 56 containing a single aperture 62 is shown and dotted parallel lines H and I are intended to represent the width of the path normally traveled by this aperture during rotation of the disc. The dotted circles P_1, P_2, P_3 and P_4 represent the images of a plurality of particles of a single sample at the image plane 52. It will be readily seen that the images are of different sizes, the image P_1 representing an "average" size or "mean" size particle for the sample. For simplifying this discussion this average size particle will be taken as equal to a normal size particle. (The size of a normal particle is well known.) The image P_2 represents a particle which is greater than average size. Image P_3 also represents an average size particle while image P_4, on the other hand, represents a particle which is less than an average size particle. The sizes of images P_2 and P_4 have been assumed to represent approximately the largest and smallest size particles present in appreciable numbers in the blood sample to be counted and for which the apparatus is arranged to operate. It may be assumed that substantially all particles in the sample will fall within these two limits. Obviously, all such particles with their image centers between lines H and I will be so positioned as to momentarily block off sufficient "background light" which ordinarily passes through the aperture 62 when no particle is present in the field as to be counted.

Images P_1, P_2, P_3 and P_4, however, have their centers outside line H and I. P_1, P_4 do, however, have their centers within line H' and I'. These lines H' and I' represent the limits of the region within which all normal cells will be counted provided their centers are located therebetween. The relative locations of lines H' and I' must be determined. It will be seen in Fig. 7, that the image P_1 of a normal cell has its center inwardly of line H' and this will be counted. Image P_2, has its center just outside line H' and ordinarily should not be counted. But since P_2 is sufficiently larger than normal to make up for its displacement from line H', it will be counted. Image P_3 is outside line H' by an amount equal to the displacement of P_2 but since image P_3 is of a normal size cell it will not be counted. Image P_4 is located inwardly of line I', as is image P_1, but since P_4 is somewhat smaller than normal it will not be counted. Notwithstanding this spread or variation in size of cells within a single sample the total count from Fig. 7 will always be two. The larger and smaller cells together will provide the same number of counts as an equal number of normal cells will provide when located in equivalent positions. Since in the example just given the spread or variation in cell size within a single sample does not introduce any appreciable error hereinafter reference will be made to the mean cell size of any sample being considered and it should be appreciated that in each case the mean size may be equal to the normal cell size or may be various amounts less than or greater than this normal cell size within well recognized limits. While Fig. 7 has been used to physically show the problem with which the invention deals, it will be hereinafter more fully shown mathematically and graphically how the amount of variations in cell size within a sample does not materially effect the count obtained for such sample. For this reason hereinafter consideration will deal with mean cell size for each sample and proper use of this value provides a correction factor which may be used together with the actual count obtained in arriving at a closer approximation of the true count of the sample being considered.

In the example just mentioned the average size and normal size were considered to be the same. In most cases, however, the mean cell size will deviate somewhat above or below this normal cell size, and accordingly, a correction factor in every case of this type must be obtained and correctly applied. The reason for such a correction is that the apparatus will be constructed and arranged and calibrated to give a correct count when a sample having a mean cell size equal to the normal cell size is being counted. However, when a sample has a mean cell size greater than normal too many cells will be counted and a negative correction must be applied. Conversely, when a sample has a mean cell size less than normal a positive correction is needed. The electronic counting means previously mentioned can be arranged to transmit an electrical pulse or signal each time a predetermined amount of light is blocked off from the phototube 70 by a particle.

It will now be shown that a correction for mean cell size can be obtained in the following manner. The mean size particle for nearly normal blood may be, according to well recognized authorities assumed to be 7.2 microns while other not uncommon sizes may vary between approximately 5.5 and 9.5 microns and the best size of aperture to function therewith and the proper correction factor to be used therewith must be determined. In Fig. 8 is represented at P an absorbing particle of a particular type or class having a radius R_1 and at S is a circle which may represent the size of the image of an aperture best suited for counting particles of this particular type or class. Circles P and S are shown at their nearest approach to each other and as being nearly the same size. Circle S has a radius R_2. The letter O_1 is the center of circle P and O_2 is the center of circle S. L represents this smallest distance between centers O_1 and O_2. The particle may completely obscure or only partially obscure the aperture image, and accordingly, it must be determined when the partially obscuring particle will be counted and when it will not be counted and also what size image or scanning spot S and thus what size aperture 62 should be used therewith.

In Fig. 8 the particle P partially obscures the scanning spot or circle S as indicated by the shaded common areas ABD and DBC. The combined area ABCD common to both circles P and S in terms of percentage of the scanning spot or scanning area being obscured will be proportional to the percent modulation from the light beam intensity at the phototube 70 and consequently directly affect the phototube current or signal being transmitted to amplifier unit 95A and cathode follower 95B (see Figs. 1 and 6).

The particle will be counted if the distance of nearest approach L is such that the inequality

$$c_A=\frac{(\text{area } ABCD)\ W}{\pi R_2^2}\geq\beta \qquad (1)$$

$$c_B=\frac{(\text{area } ABCD)}{\pi R_2^2}\geq\frac{\beta}{W} \qquad (2)$$

holds, wherein c_A represents the relative signal height of a particle transmitting a part of the incident light thereon to the maximum possible signal height, c_B represents the same relative value as c_A but in the case wherein the particle is completely opaque and thus does not transmit,

wherein W is the fractional portion of the incident light on the particle and being absorbed by the particle, and β is the arbitrarily set minimum relative signal strength or bias not passed to a scaler unit of the electronic system (to be later described). For fully opaque particles the factor W will equal unity.

To calculate the maximum percentage of modulation due to the particle or cell P which at a given moment is located at a minimum distance of L measured between the centers O_1 and O_2 of the circles P and S respectively, BD may indicate the common cord, α_1 may indicate the semi-angle in the particle subtended by the cord BD, α_2 may indicate the corresponding semi-angle in the scanning spot S, and A_1 and A_2 may indicate the areas of particle P and of the aperture S, respectively, determined by the segments BCD and BAD. These two segments, it will be noted will be common to both the particle P and the spot S. For convenience, we may let letter

$$K=\frac{L}{R_1+R_2} \quad (3)$$

and let gamma (γ) equal the ratio of particle radius to spot radius. Thus

$$\frac{R_1}{R_2}=\gamma \quad (4)$$

By substituting in Equation 2 for area ABCD its value in terms of α_1 and α_2 and K and γ we may obtain by simple trigonometric manipulation the following equation:

$$\pi C_A=\gamma^2(\alpha_1-\cos\alpha_1\sin\alpha_1)+(\alpha_2-\cos\alpha_2\sin\alpha_2) \quad (5)$$

Fig. 9 represents graphically the relations stated by the above equations. In Fig. 9 is shown a series of γ curves for values from 0.50 to 2.00 plotted horizontally against abscissa K values from 0.8 to 0 and vertically against ordinate percentages of scanning spot S obscured over W from 0 to 100 percent, the symbol γ represents the ratio of particle diameter or blood cell diameter to spot diameter. K represents the minimum distance between particle or cell center and scanning spot center divided by the sum of the cell radius and half of the spot diameter. Those particles whose distance of nearest approach L in terms of K and γ is smaller than the value where

$$c_A=\frac{\beta}{W}$$

(see Equation 2) will be counted, while all others touched by the scanning spot will not.

Thus given a value for γ, the ratio of the particle radius R_1 to the scanning spot radius R_2, only those cells closer to the center of the scanning aperture than the value of K at which the curve therefor intersects the bias level line for which the electronic means is set will pass a signal to the counting unit of the associated electronic equipment. In other words, for example, for a value of γ equal to .7 and a bias level value set at .425 or 42.5% as indicated by the solid line J, only those particles closer to the center of the scanning spot than $K=.3$, indicated by solid line M, will be counted. But if the bias value is re-set at the .50 level no such particle will be counted regardless of its position relative to the center of the scanning spot.

Since the particles on the slide **12** are arbitrarily distributed, K statistically assumes all values between zero and one the same number of times during a single count involving a large number of particles. (Of course L assumes values which are larger than one but these values are of no particular interest in the present analysis.) Since only those particles will be counted for which K is .30 or smaller in the previous example in Fig. 9, this means that only 30% of all the particles touched by the scanning spot S (**74** in Fig. 1) will be counted. If now the number counted is multiplied by 100 over 30 there will be obtained the correct number of particle centers located between the parallel lines H' and I' indicating an unknown distance equal to the diameter of the scanning spot plus the particle under investigation. It is obvious therefore that with the additional knowledge of the "mean" or "average" particle size being counted, it is possible to determine the exact count. This is so because the total length of the scan is known, the depth of solution in the specimen chamber is known and the width of the scanning spot is also known.

From Fig. 9, which comprises a series of curves plotted from tabulated values previously obtained, it is possible to plot a curve which will give K_1 over K_2 values as a function of γ for a given ratio of C_A over C_B; wherein K_1 is proportional to the count which would be obtained from a sample at the C_A bias level, while K_2 is proportional to the count which would be obtained from the same sample under identical conditions but at a C_B bias level. It follows that in order to obtain the mean or average size of blood particle under examination, it is possible to take two counts at two different known bias levels, (for example, .30 and .45) and under otherwise identical conditions and then take the ratio of these two readings and refer to a graph substantially like that in Fig. 14, in which the ratio of

$$\frac{K_1}{K_2}$$

is identical with the ratio of the two readings made with the two different biases.

From the knowledge of the ratio (and for the C_A and C_B values used) it is possible to determine gamma (γ) for the particle being counted. Since γ is equal to the average cell diameter being counted divided by the scanning spot diameter which is known, the average cell diameter is obtained. Once the γ value for the particular count is known reference must be made to Fig. 9 and the value of K read from same. This value of K in combination with the right value of *c* (the proper *c* setting which takes into account a suitable correction for the transmission characteristic of the particle will be explained later) will give the value of K which is the correction to be used for the particular count.

Electronic section

In a preferred embodiment of an electronic system which may be used, the photo-multiplier **70** (Fig. 1) will be carried by a housing **94** containing an amplifier unit **95A** and a cathode follower unit **95B** coupled through lead **70A** to the multiplier (see Figs. 5 and 6). The light intensity when a single aperture **62** is transmitting is arranged to produce an electrical current through a load resistor **70B** proportional to the light incident upon the photo-cathode **68** and thus a potential across this load resistor for the photo-multiplier. The current wave form is grahically indicated at **96** in Fig. 12A and relative to a "zero" dotted line **96'**. Its magnitude is equal to I_p when one aperture **62** is transmitting and 2 I_p when two apertures are in the field as indicated by substantially uniformly spaced pulses **96''**, and these amounts are lessened when particles are momentarily in or partly in the field and creating signal pulses shown by pips **96B** and **96C**. The amplitude and width of each of these pips will depend upon the amount of light blocked off by each of the particles. The instantaneous decrease in light creating each pip, it should be noted, is due to the size of particle being totally scanned or part of particle being scanned as well as the light absorbing characteristics of this particle.

The housing **94** is of relatively small size and, accordingly, may be attached directly to the supporting housing or equivalent means (not shown) for positioning the optical and mechanical components of the apparatus. The amplifier unit **95A** and the cathode follower **95B** offer very low output impedance so that the signal produced by the photo-cathode may be transmitted without material wave form distortion by means of a flexible

shielded cable 94A to the other electronic parts of the equipment and counting or scaler units (presently to be described) which may be disposed, for convenience and such, at a distance from the microscope.

The signal shown at 96 in Fig. 12A and somewhat amplified is fed simultaneously through two channels which may be conveniently termed a pip channel 97 and a compensation control channel 98. In the channel 98 the voltage wave form is passed by lead 98' to a high Q resonant frequency selector of filter network 99 which in the present preferred construction is tuned to 900 cycles per second. This network is arranged to give a pure sine wave with all harmonics thereof removed and the pips 96B and 96C and pulses 96'' removed as shown at 99' in Fig. 12C. The sine wave is then further amplified by stage 101 and the output of this amplifier stage is fed to a bias control unit 103 which has a manual control 103' adjustable before each run to give an output amplitude equal to or a little greater than the maximum signal or pip to be encountered in the pip channel 97. Unit 103 also has a readily adjustable control 103A which may be moved from its #1 position to its #2 or #3 positions for different bias levels. It will be appreciated that if position #1 gives a 100% voltage drop across resistors $103R_A$, $103R_B$ and $103R_C$ to ground, positions #2 and #3 will give fractional parts thereof proportional to the resistances $103R_B$ and $103R_C$ in the one case and proportional to resistance $103R_C$ in the other. The output from the bias control stage 103 is amplified and passed through a full wave rectifier unit 105 and the average D. C. voltage derived therefrom and used as a negative bias is passed through a conductor 107' onto the plate of an amplitude discriminator diode 107 in the compensation control channel 97. The reason for this negative bias is to maintain the discrimination level of the diode 107 constant notwithstanding small long term changes in light level or output from the light source 36 or response characteristics of photo-multiplier or power supply voltage for the multiplier should such occur.

The signal 96 in the pip channel 97 coming from the grid of the cathode follower 95B is fed to a diode clipper 109, which clips off the negative portion of the wave form 96 below dotted line E_o and results in a wave form 109A. Clipped portions 109B are indicated by dotted lines. This clipped off portion at 109B is due to the double aperture scanning during the shorter period *t*. The signal 109A is passed to an amplifier 111 and amplified to produce an inverted wave form 111A. This wave form is clamped by diode clamper 111' at a D. C. level 111B. This clamped wave shape 111A is fed to the amplitude discriminator 107 for pulse height selection. This diode 107 passes only positive pulses representing blood cells larger than a predetermined minimum amplitude shown by dotted line 107A thereby producing a wave form 107B containing fewer pulses than contained in the partly dotted and partly full line wave form 107C which it received.

The wave shape from the discriminator stage 107 is shown at 107B and further amplified by an amplifier 113 as shown by wave form 113A. This wave form 113A is clamped by a diode clamper 115 at the base line 113B. The pips 113C of this wave form are used to trigger a "one-shot" multivibrator 117 whose output signal containing pulses of equal amplitude and width is shown at 117A. This signal is fed in turn directly to an electronic decade scaler unit of known construction 119. From this scaler unit 119 a numerical count will be obtainable and this count may be at times used directly as an approximate indication of red blood cell count conditions or used with a variable corrective factor to obtain a true red blood cell count as will be later described.

By the use of control 103A, a plurality of bias levels may be used to obtain counts of different magnitudes for a single specimen during successive runs. In fact, a pair of counts at different known bias levels, say 80% and 35%, as set by the control 103A may be used to obtain counts N_1 and N_2 on the decade scaler 119, and it might be convenient to arrange the support 19 of Fig. 1 to actuate the control 103A so as to count at one bias level while the support is moving in one direction and so as to count at the other bias level during its reverse travel. The decade scaler could be equipped, if desired, with means arranged to record both counts. Or alternatively a pair of decade scalers could be alternately used for the successive counts.

At times it may be desirable to additionally provide a rate meter unit for indicating approximate red blood cell population and this may be accomplished by also taking signal 117A and feeding it through a cathode follower 121 to a rate meter 123. The rate meter is essentially a voltmeter but differently calibrated and arranged to read the average voltage being applied thereto by the cathode follower 121 and signal 117A applied thereto. The pulses from the multivibrator 117 are all of a constant amplitude and pulse width and thus the reading on the rate meter 123 is dependent only upon the number of pulses per given unit of time. Of course, when desired a scaler unit 119 might be omitted and only readings from the meter 123 used but a lower degree of accuracy would result.

A modified form of electronic system is shown by Figs. 10 and 11 and while the photo-multiplier 70, amplifier unit 95A and cathode follower 95B are the same as used in the system of Figs. 5 and 6, the modified system includes additional integrating electronic components for obtaining other information from the specimen containing microscopic particles being counted and analyzed. The signal pulse 96 from the grid of the cathode follower 95B may be divided (see Fig. 11) and used in the two pip channels 97A and 97B. In the pip channel 97A, a diode clipper unit 139, amplifier unit 141, diode clamper 141', amplitude discrimination 142, amplifier 142, diode clamper 145 and multivibrator 147 are like similar units in pip channel 97 of Fig. 6.

As previously, a signal may be taken from cathode of the cathode follower unit, through a lead 98_a, for use in a compensating control channel 98_a. The compensation control channel 98_a, however, passes a signal to the filter network 99_a and then to amplifier unit 101_a and to a bias control stage 133 which has a readily controllable member 133A which may be set at its #1 position while the control 133' is adjusted to its 100% level (similar to control 103' in Fig. 6). Thereafter control 133A may be set at its #2 position to give a preselected percent level such as an 80% bias level. This signal is amplified and rectified by unit 135 to provide a D. C. bias E_b between point 135' and ground.

The rectifier unit 135 is provided with an adjustable control 136 so that a different D. C. voltage derived from a voltage divider 135A may be passed by conductors 137A and 137B, respectively, to pip channels 97A and 97B. Substantially, similar units (139B, 141B, 142B, 141B', 143B, 145B and 147B) are contained in channel 97B as those already mentioned in channel 97A. The two D. C. voltages from divider 135A are for controlling the amplitude discrimination levels of the two amplitude discriminator diodes 142 and 142B. The remainder of the two pip channels are like that of Fig. 6 and thus it is possible to develop two signals at different bias levels in these two channels as shown at 107A, Fig. 12A, and 144C on wave 144B in Fig. 12C, and thus control 136 simultaneously allows relative adjustment between the wave shapes 113A and 146. Thus the resulting pulse waves 117A and 147C will be different although obtained simultaneously from the same sample. A decade counter 148 may be adjusted to count the pulses N_1 received through connector 148A at a preselected level and this pulse signal N_1 is also taken off at 148B and used in an anti-coincidence circuit 149 of known type. The signal pulses in the two pip channels 97A and 97B are fed into the anti-coincidence circuit which will only give an out-

put pulse when either signal appears but not when both appear simultaneously. They cancel each other. The output from this unit **149** is supplied to a second decade counter **150** which will then give a count N_3 which is in effect the difference between N_1 count and N_2 count obtained simultaneously.

From the N_1 and N_1-N_2 values from either electronic system there is obtained the ratio of

$$\frac{N_1}{N_2}$$

needed for average size determination.

Selection of scanning aperture size

The optimum scanning aperture size may be determined from inspection of Fig. 9, keeping in mind that the operating region selected must be such that the distance as a function of K between the γ lines should be as constant as possible. This is important because by so doing the spread of size distribution effect on count is best minimized. The other requirement is that the changes of γ in functions of the ratio of

$$\frac{K_1}{K_2}$$

needed for a size determination should be the maximum possible within size range in which the instrument is intended to operate. Keeping these two factors in mind it appears that the best value for γ is 1.11. This means that a preferred size for the scanning spot for blood cell count is

$$\frac{7.2}{1.11}$$

microns or 6.5 microns when one bias level c_A is equal to .45 and the other bias level c_B is equal to .30.

Use of device

When the device is to be placed in service in the counting and analyzing of blood the following technique may be used: A sample of blood may be obtained from a puncturing of a finger or ear lobe in the normal clinical fashion and diluted in a standard pipette with a conventional fluid (such as Hayem's solution) to the correct proportions, preferably 1 part blood to 200 parts of solution. A portion of the prepared sample is applied to the slide **12** and the cover plate **18** placed thereover. This assembly is then placed upon the movable support or stage **19** with the coated areas **92** and **93** spaced from each other in the direction of movement of the stages as indicated at **82**.

Since two different counts at different known percentage bias levels are to be made and are to be based upon the total number of pulses exceeding these known percentages of the maximum signal height obtainable, it is essential to adjust the control **103'** while the disc **56** is rotating to the position where the decade scaler just ceases to count pulses with control **103A** set in its #1 position. This establishes a 100% reference or bias level. The control **103'** may be set at a bias level so that pulses pass to the scaler units and then gradually adjusted until no pulse passes to the scaler. Once the maximum pulse height or 100% level is established, it is a simple matter to set the bias control **103A** to its #2 position, start the stage motor **83** and make a run and then set the control at its #3 position and make a second run. These runs would be at known percentages of the total resistance $103R_A$, $103R_B$ and $103R_3$ being used. As previously stated, two levels may be successively set manually or automatically when the single pulse channel system of Fig. 6 is being used to obtain counts N_1 and N_2.

The slide **12** will be moved to its extreme right hand position when a count or run is to be made. And, of course, at this time the decade scaler **119** will be set at zero. The bias control **103A** is switched to its #2 position (for example its 45% bias position), a run is made

and a reading taken on the scaler **119**. Switch **103A** is then shifted to its #3 position (for example its 30% bias setting) and a run of the slide **12** across the object field of the instrument in the opposite direction made. A second count is taken. The ratio of one reading to the other reading is now to be used. For this discussion, a ratio of the larger reading N_1 (at the lower bias level) to the smaller reading N_2 (at the higher bias level) will be used.

On the diagram of Fig. 14 (which has the

$$\frac{K_1}{K_2}$$

ratio values

$$\left(\frac{K_1}{K_2} \text{ being equal to } \frac{N_1}{N_2}\right)$$

for the two readings on the horizontal scale and gamma (γ) values

$$\left(\text{equal to ratio } \frac{R_1}{R_2}\right)$$

on the other scale in function of bias level C_1 and bias level C_2), take a γ value as indicated by line **157** corresponding to the average or mean size cell counted, and with this value and the value of the bias used obtain from the diagram of Fig. 9 a value for K for the particular count under consideration.

From the basic calibration curve for the instrument (the instrument being calibrated for normal size cells) read the normal count corresponding to normal size particles. Multiply the normal count by correction factor

$$\frac{1}{K}$$

obtained from Fig. 9 and by an additional correction factor

$$\frac{\text{spot radius}+\text{normal radius}}{\text{spot radius}+\text{average radius}}$$

to obtain the correct number of cells per unit volume.

$$\frac{(1}{K}$$

correction is a detection error while the additional factor is a volume error correction). These two factors together should correct the actual count so as to give a true count for the solution being counted. A nomograph such as shown in Fig. 13 having N_1 and N_2 values and mean diameter values may be used for any preselected pair of bias levels and preselected scanning aperture in place of the above mentioned procedure and curves. In order to obtain the average size from the readings N_1 and N_2. A straight line **154** extending through obtained values for N_1 and N_2 will give a mean diameter value on scale **155**. If either the scanning aperture size or the bias levels were changed, obviously a different nomograph would be required.

When using the two pip channel systems of Figs. 10 and 11 with anti-coincidence circuit **149**, values N_1 and N_3 may be obtained and used; N_3 being in effect equal to the N_1 count minus the N_2 count. These values may be used in a similar manner, or a nomograph (not shown) using N_1 and N_3 drawn to provide mean diameter values.

While particles in solutions or fluids have been discussed in the preceding disclosure it should be understood that dust particles and the like not in solution also may be counted and analyzed in like manner. While red cells have been discussed primarily in connection with this equipment, white cells may also be counted. For this count the red cells would be removed by hemolysis and the nuclei of the white cells, which fortunately are close to the same size as the red cells, would be stained or alternatively the illumination system changed to give high contrast between nuclei and the rest of the cell. For some specimens a true phase microscope optical system would be used in place of the system shown in Fig. 1.

Suitable mechanical recording means could be provided to respond to the number of pulses (N_1 and N_2) being obtained during successive runs or during simultaneous runs and to automatically indicate (in place of the nomograph of Fig. 13) the true count value for the fluid being counted or the mean diameter of the particles thereof.

Having described our invention, we claim:

1. An optical system for use with electronic counting means for rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said optical system comprising an objective having an object field and an image field conjugate thereto, a field stop in fixed position adjacent said image field and optically aligned with said objective, a support for positioning a specimen in said object field, a light source and condenser means for illuminating the specimen when positioned on said support in said object field, means for moving said support transversely with respect to said optical axis of said optical system, opaque scanning means having a series of substantially equally spaced scanning apertures of predetermined size, the predetermined size of each scanning aperture being closely related in size to the size of the image of an average size particle of said preselected type of specimens, means for actuating said scanning means for moving said scanning apertures in rapid succession and at a predetermined uniform speed through said image field and into and out of optical alignment with said field to stop and objective, said scanning means being arranged to operate concurrently with the transverse movement of said support and in a direction angularly disposed relative to the direction of movement of said support, the spacing between centers of adjacent scanning apertures in said scanning means being no greater than the dimension of the light aperture in said field stop taken in the direction of movement of said scanning apertures and no less than one half a specimen on said support, whereby light from said specimen at said object field will be passed through at least one and no more than two scanning apertures at any time during use of the optical system for reception by said electronic counting means.

2. An optical system for use with electronic counting means for rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said optical system comprising an objective having an object field and an image field conjugate thereto, a field stop in fixed position adjacent said image field and optically aligned with said objective, a support for positioning a specimen in said object field, means forming a light source of substantially annular contour and condenser means aligned therewith for conically illuminating the specimen when positioned on said support in said object field, means for moving said support transversely with respect to the optical axis of said optical system, opaque scanning means having a series of substantially equally spaced scanning apertures of predetermined size therein, said predetermined size of each scanning aperture being closely related in size to the size of the image of an average size particle of said preselected type of specimens, means for actuating said scanning means for moving said scanning apertures in rapid succession and at a predetermined uniform speed through said image field and into and out of optical alignment with said field stop and objective, said scanning means being arranged to operate concurrently with the transverse movement of said support and in a direction angularly disposed relative to the direction of movement of said support, the spacing between centers of adjacent scanning apertures in said scanning means being no greater than the dimension of the light aperture in said field stop taken in the direction of movement of said scanning apertures and no less than one half said dimension, whereby light from a specimen on said support at said object field will be passed through at least one and no more than two scanning apertures at all times during use of the optical system for reception by said electronic counting means.

3. An optical system for use with electronic counting means for rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said optical system comprising an objective having an object field and an image field conjugate thereto, a field stop in fixed position adjacent said image field and optically aligned with said objective, a support for positioning a specimen in said object field, a light source and condenser means for illuminating the specimen when positioned on said support in said object field, means for moving said support transversely with respect to the optical axis of said optical system, opaque scanning means having a series of substantially equally spaced scanning apertures of predetermined size therein, the ratio of the diameter of the image of an average size particle of said preselected type of specimens to the diameter of each scanning aperture being within a range of ratios between approximately 1.25 and approximately 0.8, means for actuating said scanning means for moving said scanning apertures in rapid succession and at a predetermined uniform speed through said image field and into and out of optical alignment with said field stop and objective, said scanning means being arranged to operate in predetermined timed relation to the transverse movement of said support and in a direction angularly disposed relative to the direction of movement of said support, the spacing between centers of adjacent scanning apertures in said scanning means being no greater than the dimension of the light aperture in said field stop taken in the direction of movement of said scanning apertures and no less than one half said dimension, whereby light from a specimen on said support at said object field will be passed through at least one and no more than two scanning apertures at all times during use of the optical system for reception by said electronic counting means.

4. A device for use in rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said device comprising an optical system including an objective having an object field and an image field conjugate thereto, a support for positioning a specimen in said object field, light source means for illuminating said specimen when positioned on said support in said object field, means for moving said support transversely with respect to the optical axis of said optical system, scanning means having a series of scanning apertures of predetermined size arranged to be moved in rapid succession through said image filed, said scanning means being arranged to operate concurrently with the movement of said support and in a direction angularly disposed relative to the direction of movement of said support, said predetermined size of each scanning aperture being closely related in size to the size of the image of an average size particle of said preselected type of specimen, whereby light flux modulation of appreciable value will be produced in the light passing beyond said scanning means when microscopic particles in said specimen on said support are being scanned, photoelectric means optically aligned with said objective so as to receive the modulated light and provide an electrical signal the instantaneous magnitude of which is substantially proportional to the amount of light being received thereby, an electronic network for amplifying said signal, first electrical means in said electronic network for passing only those electrical pulses due to particles being scanned which have electrical amplitudes greater than a first preselected value, second electrical means in said network for passing only those electrical pulses due to said particles being scanned which have electrical amplitudes greater than a second different preselected value,

and first and second counting means connected to said first and second electrical means, respectively, for separately counting the total number of pulses passed by said first electrical means and the total number of pulses passed by said second electrical means during a single complete cycle of operation of said device.

5. A device for use in rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said device comprising an optical system including an objective having an object field and a image field conjugate thereto, a support for positioning a specimen in said object field, light source means for illuminating said specimen when positioned on said support in said object field, means for moving said support transversely with respect to the optical axis of said optical system, scanning means having a series of scanning apertures of predetermined size arranged to be moved in rapid succession through said image field, said scanning means being arranged to operate concurrently with the movement of said support and in a direction angularly disposed relative to the direction of movement of said support, said predetermined size of each scanning aperture being closely related in size to the size of the image of an average size particle of said preselected type of specimen, whereby light flux modulation of appreciable value will be produced in the light passing beyond said scanning means when microscopic particles in said specimen on said support are being scanned, photoelectric means optically aligned with said objective so as to receive the modulated light and provide an electrical signal the instantaneous magnitude of which is substantially proportional to the amount of light being received thereby, an electronic network for amplifying said signal, adjustable bias control means in said electronic network for establishing a maximum bias level which is substantially equal to the maximum pulse amplitude due to particles being scanned, first and second fractional bias selecting means in said network for selecting first and second biases equal to two different known fractional portions respectively of said maximum bias, and for passing respectively only those electrical pulses of amplitudes greater than said selected biases, and first and second counting means connected to said first and second fractional bias selecting means for separately counting the number of pulses, representative of the particles, passed by said last-mentioned means at said first fractional bias level and at said second fractional bias level respectively.

6. A device for use in rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said device comprising an optical system including an objective having an object field and an image field conjugate thereto, a support for positioning a specimen in said object field, light source means for illuminating said specimen when positioned on said support in said object field, means for moving said support transversely with respect to the optical axis of said optical system, scanning means having a series of scanning apertures of predetermined size arranged to be moved in rapid succession through said image field, said scanning means being arranged to operate concurrently with the movement of said support and in a direction angularly disposed relative to the direction of movement of said support, said predetermined size of each scanning aperture being closely related in size to the size of the image of an average size particle of said preselected type of specimen, whereby light flux modulation of appreciable value will be produced in the light passing beyond said scanning means when microscopic particles in said specimen on said support are being scanned, photoelectric means optically aligned with said objective so as to receive the modulated light and provide an electrical signal the instantaneous magnitude of which is substantially proportional to the amount of light being received thereby, an electronic network for amplifying said signal, first electrical means in said electronic network for passing only those electrical pulses due to particles being scanned which have electrical amplitudes greater than a first preselected value, second electrical means in said network for passing only those electrical pulses due to said particles being scanned which have electrical amplitudes greater than a second different preselected value, first and second counting means connected to said first and second electrical means, respectively, for separately counting the total number of pulses passed by said first electrical means and the total number of pulses passed by said second electrical means during a single complete cycle of operation of said device, and additional counting means connected to said first and second counting means for counting only the number of pulses which are counted by one of said counting means and not counted by the other of said counting means.

7. A device for use in rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said device comprising an optical system including an objective having an object field and an image field conjugate thereto, a support for positioning a specimen in said object field, light source means for illuminating said specimen when positioned on said support in said object field, means for moving said support transversely with respect to the optical axis of said optical system, scanning means having a series of scanning apertures of predetermined size arranged to be moved in rapid succession through said image field, said scanning means being arranged to operate concurrently with the movement of said support and in a direction angularly disposed relative to the direction of movement of said support, said predetermined size of each scanning aperture being closely related in size to the size of the image of an average size particle of said preselected type of specimen, whereby light flux modulation of appreciable value will be produced in the light passing beyond said scanning means when microscopic particles in said specimen on said support are being scanned, photoelectric means optically aligned with said objective so as to receive the modulated light and provide an electrical signal the instantaneous magnitude of which is substantially proportional to the amount of light being received thereby, the spacing between centers of adjacent scanning apertures being such as to present to the light transmitted by said objective at all times during operation of the device an effective scanning area which is equal to the area of at least one scanning aperture, an electronic network for amplifying said signal, adjustable bias control means in said electronic network for establishing a maximum bias level which is substantially equal to the maximum pulse amplitude due to particles being scanned, first and second fractional bias selecting means in said network for selecting first and second biases equal to two different known fractional portions respectively of said maximum bias, and for passing respectively only those electrical pulses of amplitude greater than said selected biases, and first and second counting means connected to said first and second fractional bias selecting means for separately counting the number of pulses, representative of the particles passed by said last-mentioned means at said first fractional bias level and at said second fractional bias level respectively.

8. A device for use in rapidly and accurately determining the number of microscopic particles dispersed within a predetermined unit volume of a specimen of a preselected type, said device comprising an optical system including an objective having an object field and an image field conjugate thereto, a support for positioning a specimen in said object field, light source means for illuminating said specimen when positioned on said sup-

port in said object field, means for moving said support transversely with respect to the optical axis of said optical system, scanning means having a series of scanning apertures of predetermined size arranged to be moved in rapid succession through said image field, said scanning means being arranged to operate concurrently with the movement of said support and in a direction angularly disposed relative to the direction of movement of said support, said predetermined size of each scanning aperture being closely related in size to the size of the image of an average size particle of said preselected type of specimen, whereby light flux modulation of appreciable value will be produced in the light passing beyond said scanning means when microscopic particles in said specimen on said support are being scanned, photoelectric means optically aligned with said objective so as to receive the modulated light and provide an electrical signal the instantaneous magnitude of which is substantially proportional to the amount of light being received thereby, the spacing between centers of adjacent scanning apertures being such as to present to the light transmitted by said objective at all times during operation of the device an effective scanning aperture which is alternately equal to the area of one scanning aperture and an area appreciably greater than the area of one scanning aperture, an electronic network for amplifying said signal, adjustable bias control means in said electronic network for establishing a maximum bias level which is substantially equal to the maximum pulse amplitude due to particles being scanned, first and second fractional bias selecting means in said network for selecting first and second biases equal to two different known fractional portions respectively of said maximum bias, and for passing respectively only those electrical pulses of amplitudes greater than said selected biases, and first and second counting means connected to said first and second fractional bias selecting means for separately counting the number of pulses, representative of the particles passed by said last-mentioned means at said first fractional bias level and at said second fractional bias level respectively.

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