Nov. 19, 1968 H. MEYER-DÖERING ET AL 3,412,254

APPARATUS FOR COUNTING PARTICLES SUSPENDED IN TRANSPARENT FLUIDS

Filed June 4, 1965 2 Sheets-Sheet 1

Fig. 1

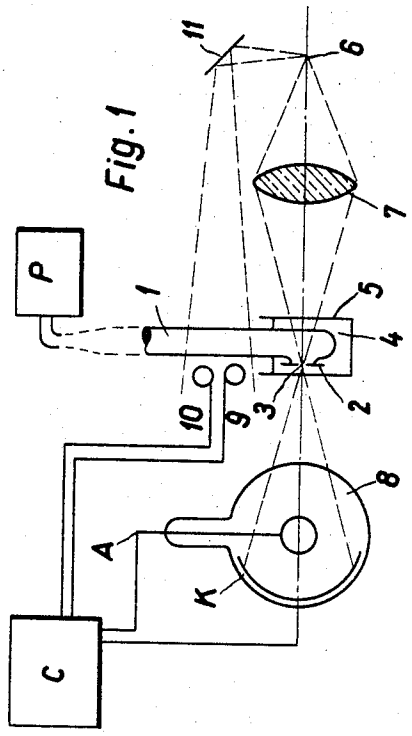



Fig. 4

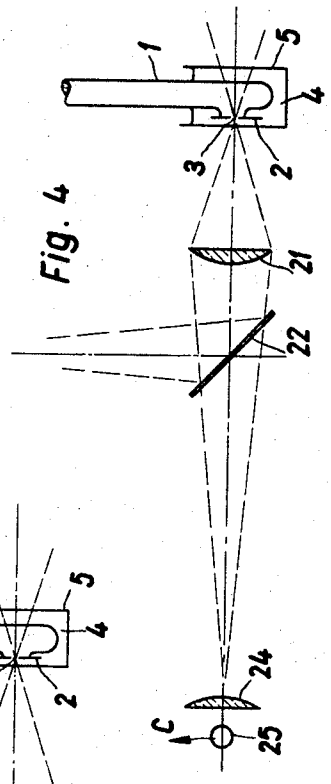
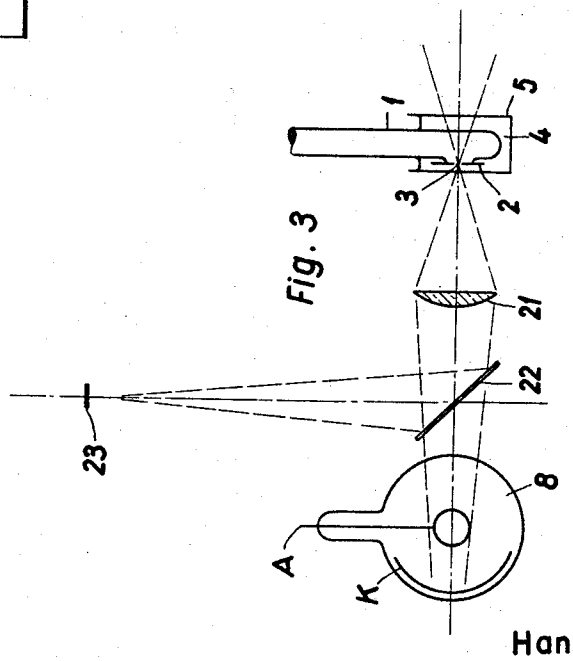



Fig. 3





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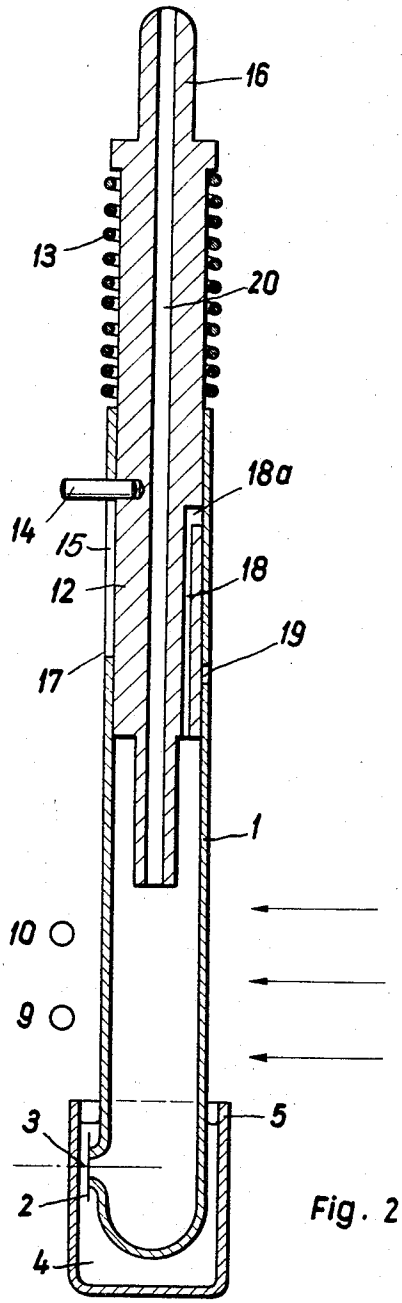



Fig. 2

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# APPARATUS FOR COUNTING PARTICLES SUSPENDED IN TRANSPARENT FLUIDS

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## ABSTRACT OF THE DISCLOSURE

An apparatus for counting particles suspended in a transparent fluid in which the fluid containing the particles to be counted passes through a restricted opening in an opaque element, and a beam of light is directed through the opening and onto a photocell which controls the operation of a counting circuit for advancing a counter or recorder every time a particle passes through the opening.

## Background of the invention

This invention relates to an apparatus for counting particles suspended in a fluid medium. In particular, this invention relates to an apparatus comprising a light source and a photoelectric light sensing means impinged by light from the light source, and means for passing fluid containing suspended particles through the path of the light whereby the intensity of the light impinging upon the light sensing means is influenced by the passage of particles therethrough.

Arrangements of this kind are used for examination of the particles of a suspension or of an aerosol to determine their number and size, especially of erythrocytes and leucocytes, and to determine their size distribution. The counting of the numbers of erythrocytes and leucocytes per cubic millimeter of blood for diagnostic purposes has heretofore been done by introducing a measured volume of blood into a special counting chamber and then counting the number of blood corpuscles directly under a microscope. This method is time-consuming, troublesome and unreliable. The determination of the size distribution of the blood corpuscles (Price-Jones curve) is not possible by the laboratory method using the counting chamber, although it would be desirable under certain conditions to determine this curve.

Various systems have therefore been developed by which the particles which are entrained by a transparent fluid medium can be counted automatically, and which are used especially for counting blood corpuscles. In one known arrangement a thin stream of blood flows through clear water. A short length of the stream is strongly illuminated by a transverse beam of light. The flashes of light that occur whenever the illuminated region is traversed by the blood corpuscle are counted by a photoelectric cell. In the practical form of such a system, the light flashes are detected by a dark-field condenser. Discrimination is obtained with a microscope which is positioned between the blood stream and a slit behind which the electronic light-amplifier is positioned. Such a system is relatively expensive and requires much auxiliary equipment so that it is suitable only for large laboratories.

In another known system the strongly diluted blood flows through a glass capillary tube which is strongly illuminated transversely to the direction of flow and after strong magnification is focussed upon a slit in an opaque plate, behind which a photocell is positioned. If a blood corpuscle passes through the capillary, it will be magnified on the slit and will weaken the illumination of the photocell. It is not known that there has been any extensive practical use of such an arrangement.



It is also old to stain erythrocytes and leucocytes selectively with different dyes, so that the light which is passed therethrough can with the help of differently colored filters and semi-transparent mirrors can be impinged upon two photocells which have different color sensitivities, for the actuation of associated counters. In this method a diluted blood solution is passed through a restricted passageway which is traversed perpendicularly by a beam of light, the latter after magnification being impinged upon a photocell.

There are serious difficulties with this last-mentioned arrangement. Blood corpuscles have indices of refraction which are different from those of the surrounding fluid so that the blood corpuscles will act like spherical lenses and disperse the light. If naturally colored red corpuscles are examined under a microscope with a large aperture, they can hardly be recognized, in any event not because of their coloration. With a smaller aperture there will appear a dark disk with a spot of light in the middle, or a bright disk, depending on whether the focal plane, as measured from the microscope, is in front of or behind the observed blood corpuscles.

Since the restriction or the capillary through which the blood flows must have a minimum diameter so that the resistance to flow will not be too great, the blood corpuscles pass through the capillary partly in front of and partly behind the focal plane of the microscope if it is coincident with the axis of the capillary. Blood corpuscles of equal size are therefore illuminated with different intensities and cause light-weakening impulses of different strengths. This makes it possible to measure the size distribution on the basis of different strengths of light impulses and also makes the simple counting more difficult. This difficulty is not eliminated by staining the particles since no method is known by which blood corpuscles in a solution can be stained sufficiently to effectively make them permanently opaque.

By another method strongly diluted blood is caused to flow through short capillary tubes between two vessels, the tubes measuring about 0.07 millimeter in diameter, and the electric resistance of the liquid in the capillary tube is then measured by means of electrodes dipping into the two vessels. The increases of electric resistance caused by the entrance of blood corpuscles into the capillary are then measured. Such an apparatus is quite expensive and is therefore suitable only for large laboratories.

## Summary of the invention

It is an object of this invention to provide an apparatus for counting particles suspended in a transparent fluid by passing the particles between a light source and a light sensing means which avoids difficulties present in previous devices.

It is another object of this invention to provide an apparatus for counting particles suspended in a transparent fluid wherein the particles pass through a restricted opening in a direction parallel to a beam of light passing therethrough whereby the intensity of light emitted through said opening is diminished by the particle passage.

It is still another object of this invention to provide an apparatus for counting particles suspended in a transparent fluid wherein the particles pass through a small opening in an opaque film through which a beam of light is directed, and means is provided for inspecting said opening during operation of the apparatus.

It is yet another object of this invention to provide an apparatus for counting particles suspended in a transpar-

ent fluid which is forced through a restricted opening from one chamber to another chamber, said apparatus comprising means for easily removing excess fluid from said other chamber in preparation for further measurements.

According to the present invention, a beam of light from a light source is directed in a direction perpendicular to the surface of an opaque element and axially with respect to a restricted opening therein. The fluid containing the particles to be counted passes through this restricted opening. The device of this invention operates with a light stream of diminishing intensity, and microscope imaging, which led to difficulties in prior devices, is not necessary. The construction of the device is therefore simplified, and operation thereof is facilitated. Since errors inherent in prior devices are eliminated, the apparatus of this invention permits very accurate measurements to be made and accurate distribution curves to be drawn.

## Brief description of the drawings

Other novel features and advantages of this invention will be apparent from the following description and the drawings in which the features of this invention are shown by way of example.

FIGURE 1 is a schematic drawing of the apparatus of this invention.

FIGURE 2 is an enlarged, vertical section of the counting tube.

FIGURE 3 is a schematic drawing of an alternative embodiment of this invention which includes means for inspecting the restricted opening.

FIGURE 4 is a schematic drawing of still another alternative embodiment of this invention wherein additional optical elements and a miniature photoelectric cell are employed.

## Description of the preferred embodiments

In the present invention an opaque film with a hole in it is employed. For the examination of blood, the hole can have a diameter of about 0.02 mm., for example. Fluid suspensions to be examined are passed through this hole. With this system, a particle suspension, aerosol, or blood which has been diluted in a predetermined proportion with a stabilizing solution to an almost colorless, slightly turbid solution, is examined. The hole is traversed by a strong beam of light axially aligned therewith.

On the other side of the hole a photocell is positioned to be impinged by the light which has passed through the hole. A photoelectric current is thereby generated by impinging light. Every time a particle passes through the hole, the light which impinges upon the photocell will be slightly weakened. The resulting current impulses are amplified and can, in a known manner, be delivered either to a converter and a mechanical counter or to an analog computer.

The magnitude of the current impulses depends on the sizes of the particles, other conditions being the same, with a large particle producing a stronger impulse than a smaller particle. The amplifier can be provided with a discriminator which would exclude impulses below a predetermined magnitude so that only those impulses would be counted which represent particles having a certain minimum size. The process therefore makes it possible to ascertain the size distribution of the particles.

If the red corpuscles are dissolved with a diluent solution, the white blood corpuscles which occur in much smaller numbers can be counted.

The simplest form of the apparatus of this invention for the examination of suspensions is shown schematically in FIG. 1. A tube **1** of clear transparent glass which is closed at its lower end and which serves as a counting tube has its lower portion immersed in an optical tank **5**. The tube **1** has near its lower end a side opening which is covered by a film **2** which is sealed tightly to the margin



of the opening, as by cementation. The film **2** consists of opaque material, e.g., of a platinum foil 0.01 mm .thick. The foil **2** is provided with a tiny hole or restricted opening **3**, in line with the optic axis. The hole **3** serves as a counting hole and when used for blood examination should have a diameter of about 0.02 mm. The upwardly extending portion of the counting tube **1** has its upper end attached to a vacuum pump P for maintaining a partial vacuum in the tube down to a few mm. of mercury.

In front of the tank **5** a source of light **6** is positioned, the light being focussed by a lens **7** on the hole **3** of the counting tube. The light which traverses the hole **3** falls upon a photoelectric cell **8**, which can for example be an alkali cell whose terminals are connected to a counter C of known construction.

For the examination of a suspension **4**, for example, of blood, a known volume is diluted in a definite ratio and is poured into the tank **5**. After application of the vacuum the suspension in the tank will flow through the hole **3** into the counting tube **1** and will accumulate in the latter. The dilution is preferably such that only one blood corpuscle of the suspension **4** passes through the hole **3** at one time so as to cause a momentary weakening of the light from the source **6** which will in turn be converted into a momentary weakening of the electric current from the photocell.

If a constant partial vacuum is maintained in the counting tube **1**, the diluted liquid or the aerosol will flow at a constant velocity through the hole. In that manner a statistically constant number of darkenings or current pulsations will be produced per second. By the use of known systems of electronic switches, these current pulsations resulting from the darkenings will be registered each second by an ammeter in the counter C which can be calibrated to indicate the number of particles per $mm.^3$ when the suspension flows with a certain velocity.

For automatic indication of the size distribution of the corpuscles, the counter C can be provided with a discriminator for excluding impulses below a certain magnitude from the counter and which is controlled according to a certain program. Instead of the ammeter another known circuit is then connected in which is formed the differential quotient of the current relative to the time. The differentiated current is then delivered to a recorder which records the size distribution curve directly.

For indication by a counter, a measured volume of the suspension or of the blood in a known state of dilution is used. The counter then gives the number of particles contained in such volume, and from such number it is possible to calculate the number of corpuscles per $mm.^3$ of blood by taking into consideration the degree of dilution. By a suitable degree of dilution and of the volume used by the counter, it will be possible for this number to be registered directly by the counter, correct to the tenth power.

A measurement of fluid volume can be effected by an additional device which works on the principle of having the counting tube with the liquid in it serve as a cylindrical lens for the incident light so as to collect parallel rays into a focal line. If in the arrangement shown in FIG. 1 the light which approaches from the right travels along horizontal lines, a focal line will slowly grow in height, together with the height of the liquid in the counting tube **1**. This phenomenon can be used for automatically measuring the counting volume. Above the light source **6** a plane mirror **11** is positioned for reflecting light rays from the source **6** to the counting tube **1**. Two photoelectric cells **9** and **10** are positioned behind the counting tube **1** in line with the plane mirror and spaced vertically from each other so that they will be reached successively by the focal line into which the light from the mirror **11** has been focussed. The photocells **9** and **10** are connected to the amplifier for the mechanical counter in a type of coincidence circuit in such a manner that the amplifier operates only when the lower cell alone

is struck by the light, but not while both cells are in the dark or both are in the light.

After a counting sequence, the counting tube **1** is filled with liquid to above the upper cell **10** and has therefore become inoperative for further counting. To render it operative again, the liquid level in it must be brought down to below the lower cell **9**. This is preferably done by means of the device shown in FIG. 2. In the upper portion of the counting tube, an axially movable piston **12** with an axial bore **20** is slidably mounted. The piston is urged upwardly by a spring **13** interposed between the upper end of the tube **1** and a shoulder at the upper end of the piston **12** until the radial screw **14** reaches the upper end of the longitudinal slot **55** in the tube **1**. At its upper end the piston **12** is provided with a hose coupling **16** for connecting it to a flexible hose leading to the vacuum pump P for maintaining the required degree of vacuum in the tube **1**.

The piston **12** is provided with a second axially parallel boring **18** extending from the lower end of the piston up to a radial boring **18***a*. The outer wall of tube **1** has an opening **19** at a higher level than the upper photocell **10** and in such a position that it will come into registration with the radial boring **18***a* when the piston **12** is depressed.

After a counting has been finished the piston **12** is pressed down into the counting tube **1**, e.g., by hand, until the radial screw **14** reaches the lower end **17** of the slot **15**. In this position the lower end of the longitudinally bored piston will dip into the liquid to below the level of the lower photocell **9**. At the same time the interior of the counting tube **1** is put into communication with the outside atmosphere through longitudinal passageway **18**, and radial passageway **18***a* in the piston and opening **19** in the tube **1**. The air which rushes in through this path drives the liquid through the boring **20** in the piston and through the hose that is connected to the coupling, the liquid being finally discharged into a receptacle (not shown). The piston is then released and is pushed upwardly in the tube **1** by the spring **13**, whereupon a new counting can be commenced. With this system subsequent countings are not invalidated by the presence in the counting tube of residual portions of liquid from previous countings because only those particles will be counted which pass from the optical tank **5** and through the tiny opening **3** into the counting tube **1**.

It is advisable to provide the device with a system for inspecting the hole **3** to see if it is free from contamination or obstruction by large particles. For this purpose the arrangement in FIG. 3 includes a microscope whereby the opening **3** can be observed while the counting is in progress. The microscope comprises an objective **21**, a semi-transparent glass plate **22** positioned at a 45 degree angle to the optic axis and a lens **23**. The plate **22** reflects a small but sufficient portion of the light transversely into the lens **23** through which the hole **3** can be inspected and obstructions recognized.

The microscope objective **21** can also serve to permit the use of a small photoelectric cell, e.g., a photo-transistor, without difficult adjustments. For this purpose, as shown in FIG. 4, a second lens **24** which functions as an ocular, is positioned in line with the objective **21** to strongly converge the light to a focus upon the miniature photocell **25**.

Obviously, many modifications and variations of the invention as hereinabove set forth may be made without departing from the essence and scope thereof, and only such limitations should be applied as are indicated in the claims.

The invention claimed is:

**1.** An apparatus for counting particles suspended in a transparent fluid comprising means defining a restricted opening for passage of the fluid containing suspended particles, a light source means for passing light through said restricted opening in a direction substantially parallel to the axis of said restricted opening, a photoelectric light sensing means positioned in the path of light from said light source which has passed through said restricted opening, whereby the luminosity of light from said light source impinging upon said light sensing means is diminished by the passage of particles through said restricted opening.

**2.** The apparatus of claim **1** wherein the restricted opening is a hole through an opaque element.

**3.** The apparatus of claim **2** wherein the opaque element is a film of opaque material fastened over an opening in the wall of a vessel formed of transparent material.

**4.** The apparatus of claim **3** wherein the film is a metal foil.

**5.** The apparatus of claim **1** wherein a lens means is positioned in the path of the light passing from said light source through said restricted opening for focusing said light on said light sensing means.

**6.** The apparatus of claim **1** wherein a semi-transparent mirror is positioned in the path of the light passing from said light source through said restricted opening, the plane of the reflecting surface of the mirror being positioned at an angle to the path of the light, whereby optical inspection of the restricted opening is facilitated.

**7.** The apparatus of claim **6** wherein a lens means is positioned in the path of light passing from said light source through said restricted opening before it impinges upon the semi-transparent mirror for focusing said light on said light sensing means and another lens means is positioned in the path of light reflected by the reflecting surface of the mirror, said other lens means comprising an ocular of a microscope.

**8.** An apparatus for counting particles suspended in a transparent fluid comprising a vessel formed of transparent material having an opening in one wall thereof, an opaque element fastened over said opening and defining a restricted opening, said vessel having an upright portion, two photoelectric light sensing means positioned at one side of said upright portion and spaced vertically from each other, a light directing means positioned at the side of said upright portion opposite said light sensing means whereby light from said light directing means passing through said upright portion impinges upon said two light sensing means and said light is influenced by the fluid level in the upright portion, a light source means for passing light through said restricted opening in a direction substantially parallel to the axis of said restricted opening, a third photoelectric light sensing means positioned in the path of light from said light source means which has passed through said restricted opening, whereby the luminosity of light from said light source impinging upon said third light sensing means is diminished by the passage of particles through said restricted opening.

**9.** The apparatus of claim **8** wherein the opaque element is an upright film of opaque material.

**10.** The apparatus of claim **8** wherein the upright portion of the vessel is a tube.

**11.** An apparatus for counting particles suspended in a transparent fluid comprising a vessel formed of transparent material having an opening in one wall thereof, an opaque element fastened over said opening and defining a restricted opening, said vessel having an upright tube portion, a piston in said upright tube portion, said piston being shiftable in the axial direction from an upper position to a lower position with respect to the tube portion, said piston having a radial passageway, and a longitudinal passageway extending from the lower end of said piston and communicating with said radial passageway, said tube portion having an opening in the wall thereof in communication with said radial passageway when the piston is in the lower position, said piston having another longitudinal passageway extending from the lower end to the upper end thereof, light source means for directing light through said restricted opening in a direction substantially parallel to the axis of said restricted opening, and a first photoelectric light sensing means positioned in the

path of light from said restricted opening, whereby the luminosity of light from said light source impinging upon said first light sensing means is diminished by the passage of particles through said restricted opening.

**12.** The apparatus of claim **11** wherein a second and a third photoelectric light sensing means are positioned at one side of said upright tube portion and spaced vertically from each other, the upper of said second and third light sensing means being positioned below said opening in the wall of said upright tube portion, a light directing means positioned at the side of said upright portion opposite said second and third light sensing means, whereby light from said light directing means passing through said upright portion impinges upon said two light sensing means and said light is influenced by the fluid level in the upright tube portion.




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