United States Patent [19]

Hogg

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[45] **June 17, 1975**

[54] **PARTICLE STUDY DEVICE**

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[73] Assignee: **Coulter Electronics, Inc.**, Hialeah, Fla.

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[21] Appl. No.: **473,127**

Related U.S. Application Data

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[52] **U.S. Cl.** ..... **324/71 CP**

[51] **Int. Cl.** **G01n 27/00**

[58] **Field of Search** ..... 324/71 CP; 73/432 PS

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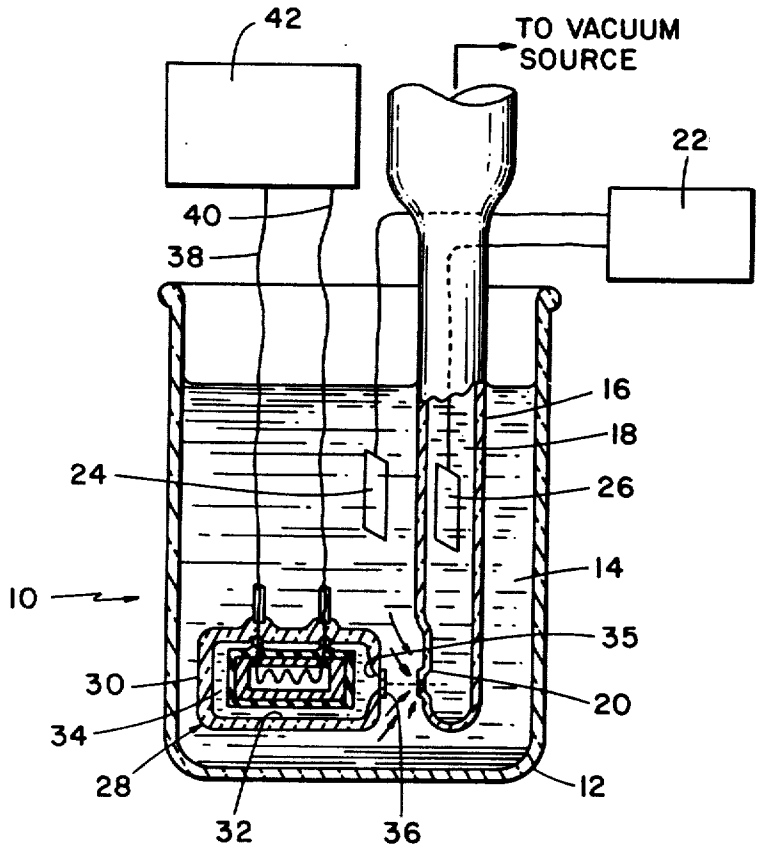
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Primary Examiner—Alfred E. Smith
Assistant Examiner—Rolf Hille
Attorney, Agent, or Firm—Silverman & Cass, Ltd.

[57] **ABSTRACT**

A particle study device wherein a non-diluted specific minute amount of fluid sample containing particles is ejected by an ejecting mechanism into a flow stream leading to a sensing zone in the particle study device. The ejecting mechanism includes a hollow body having a thermal expansion device mounted therein and a power supply circuit which supplies a predetermined amount of electrical energy to the thermal expansion device to raise the temperature of same a predetermined amount and cause the same to expand thereby to eject from the hollow body a specific amount of fluid sample having a volume displacement equal to the volume increase of the thermal expansion device. Preferably the hollow body is part of a syringe including a plunger. The syringe facilitates the picking up of fluid sample of which only a minute specific portion is ejected toward the sensing zone of the particle study device.

28 Claims, 10 Drawing Figures



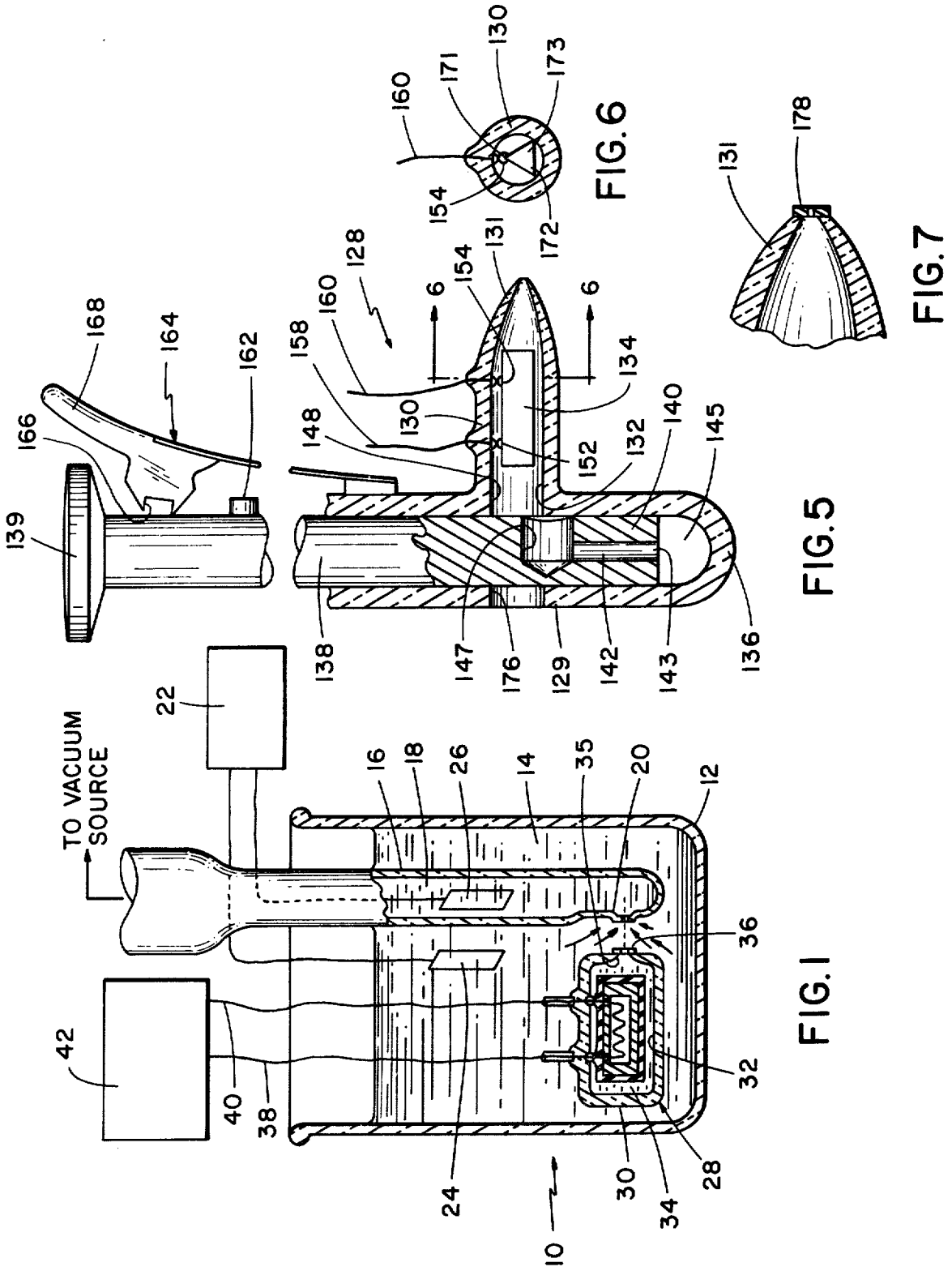


FIG. 1

FIG. 5

FIG. 6

FIG. 7

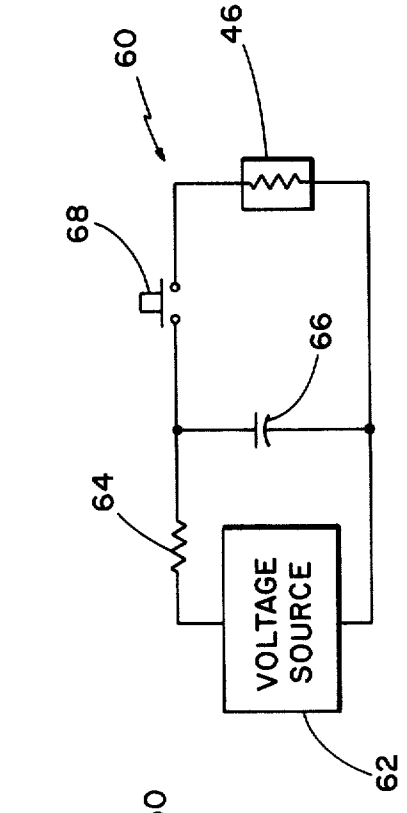


FIG.3

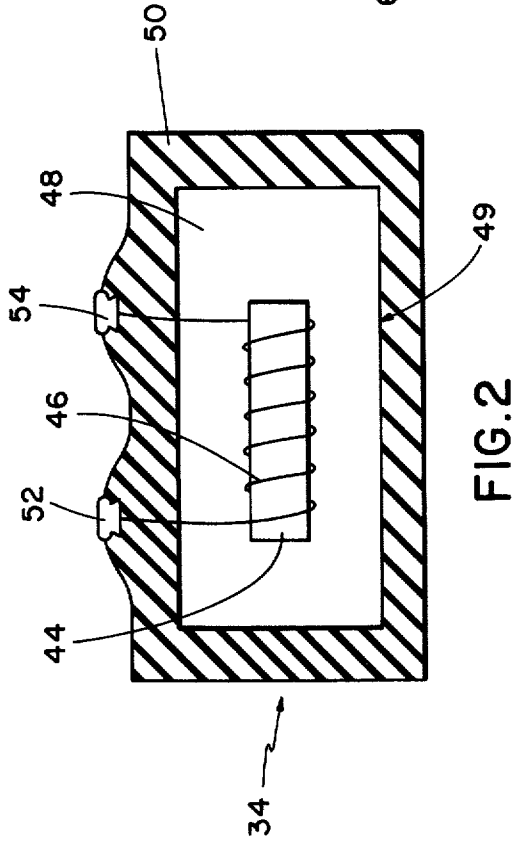


FIG.2

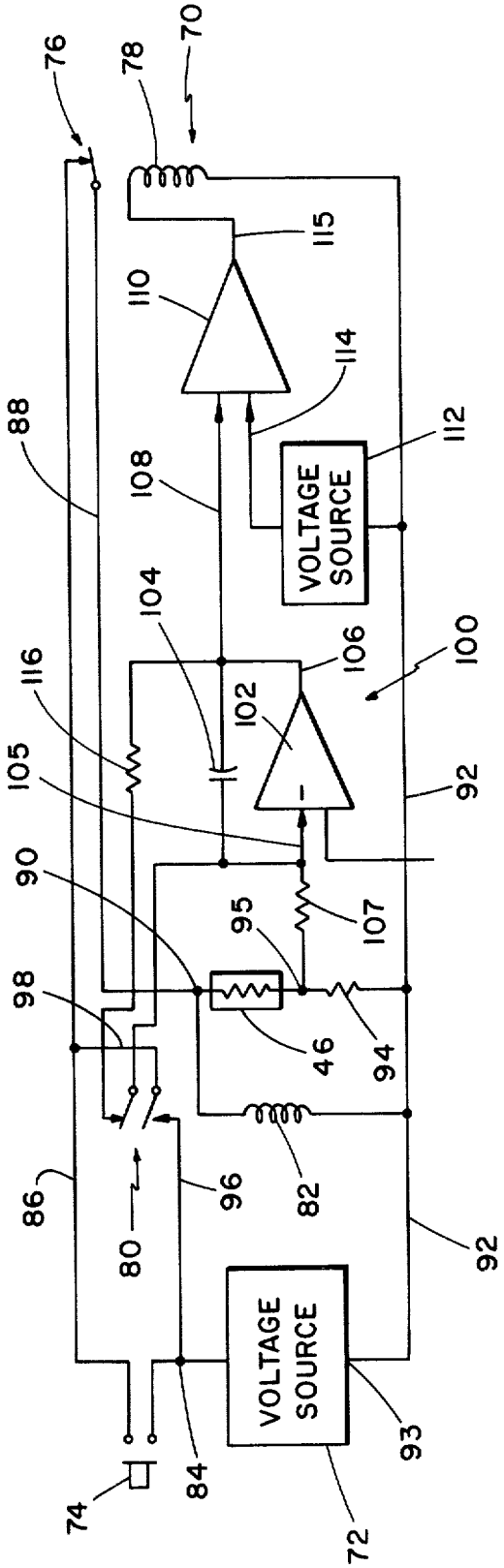


FIG.4

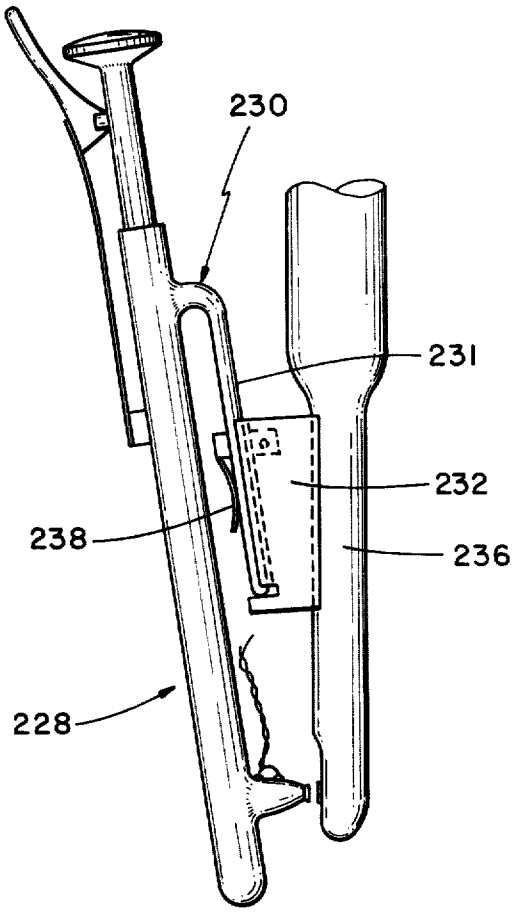


FIG.8

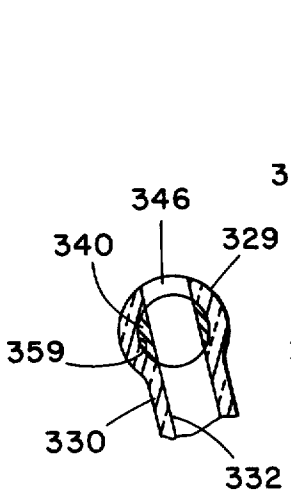


FIG.10

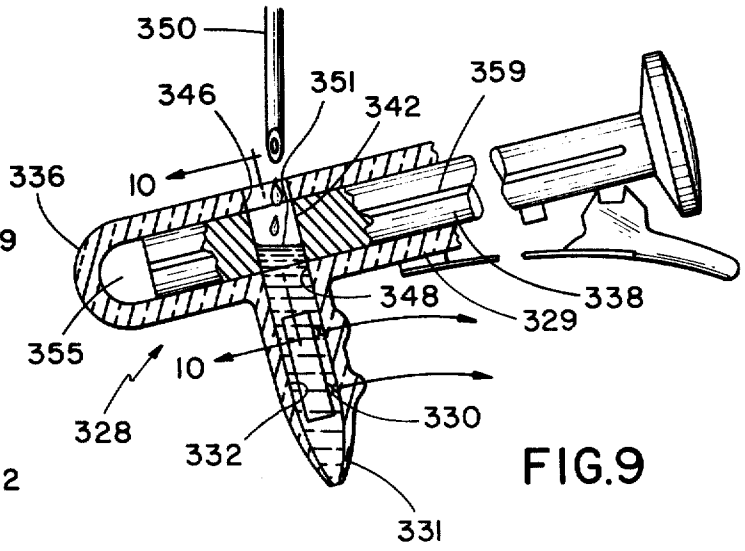


FIG.9

PARTICLE STUDY DEVICE

This is a division of application Ser. No. 279,436 filed August 10, 1972, now U.S. Pat. No. 3,859,012.

BACKGROUND OF THE INVENTION

The invention relates to a non-diluting particle study device and more specifically to a device for ejecting a non-diluted specific minute amount of fluid sample containing particles into a flow stream leading to a sensing zone in the particle study device.

Heretofore, in the field of particle analysis and particle study, such as the study of red and white blood cells in a blood sample, it has been common practice to dilute the blood sample and then to pass a portion of the diluted sample through a sensing zone in a particle study device. The blood is diluted because the normal human blood count is five million cells per cubic millimeter and it is only necessary to study or analyze one hundredth of that amount, namely, a volume of 0.01 cubic millimeters.

In studying a blood sample, the blood cells in a given amount of the sample are counted by passing a portion of the diluted blood sample through a sensing zone in a particle analyzing device, such as a Coulter type particle analyzing device which operates on the Coulter sensing principle disclosed in U.S. Pat. No. 2,656,508 issued Oct. 20, 1953 to Wallace H. Coulter.

According to this principle, when a microscopic particle in suspension in a fluid electrolyte is passed through an electrical field of small dimensions approaching those of the particle, there will be a momentary change in the electric impedance of the electrolyte in the ambit of the field. This change of impedance diverts some of the excitation energy into associated electrical circuitry, giving rise to an electrical signal. Such signal has been accepted as a reasonably accurate indication of the particle volume for most biological and industrial purposes.

One apparatus of the Coulter type includes first and second vessels each containing a body of fluid electrolyte. The second vessel is smaller and is immersed in the electrolyte in the first vessel. An electrode extends into the electrolyte in each vessel and electric current flows between the electrode through an opening in the side wall of the second vessel, the opening consisting in a minute aperture commonly referred to as a Coulter aperture. Flow of liquid between the vessels is caused by applying vacuum to the second vessel. According to the Coulter principle, particles passing through the aperture from one body of electrolyte to the other body of electrolyte will change the impedance of the electrolyte contained within the aperture and this change in impedance is sensed by the electrodes. This change generates an electrical signal in the form of a particle pulse which is then counted by the electrical circuitry of the particle analyzing device.

When making a blood analysis a dilution of blood in electrolyte is placed in the first vessel. Then vacuum is applied to the second vessel to cause diluted blood to flow from the first vessel through the aperture into the second vessel for a specific period of time. The second vessel is filled with electrolyte, probably including prior dilutions.

To make a fairly accurate measurement of particle concentration, one must accurately measure or meter the amount of fluid which passes through the sensing zone during a period of time when the electrical circuitry of the device is operative. This can be accomplished by passing fluid through the sensing zone at a given flow rate for a specified period of time. Apparatus utilizing fluid flow metering systems of this type in a fluid analyzing device are disclosed in U.S. Pat. Nos. 3,577,162 and 3,654,439.

In most Coulter type particle analyzing devices, the metering is accomplished with a fluid metering apparatus of the type disclosed in U.S. Pat. Nos. 2,869,078, 3,015,775 and 3,271,672. Such metering apparatus includes a closed fluid system hydraulically connected to the second vessel. The closed fluid system includes a connection to a vacuum source and a mercury manometer. When operating the device, vacuum is applied to the closed fluid system to raise the mercury in the manometer and to draw some fluid sample into the second vessel. The connection to the vacuum source is then closed and the manometer, by reason of the mercury flowing downwardly to its original position, causes liquid to be drawn through the aperture and generates signals indicating the beginning and the end of an analytic run in a period during which an accurately metered volume of fluid is passed through the aperture. The metered volume of fluid is equal to the volume within the manometer between two electrodes.

It will be understood from the foregoing description of a Coulter type particle analyzing device that it is necessary to dilute a quantity of blood, to make an accurate determination of dilution and to accurately meter the fluid flow through the Coulter aperture in order that an accurate count of blood cells can be made. A simpler way of making the particle analysis or study would be to pass a specific minute amount of undiluted blood through the Coulter aperture and thereby eliminate the manometer system. Heretofore this has not been practical since it is difficult to eject a specific minute amount of undiluted blood into the flow stream of electrolyte flowing toward and through the aperture. The present invention overcomes this difficulty by providing a device for ejecting a specific minute amount of particle-containing fluid such as blood into the flow stream leading to a sensing zone in a particle analyzing device. Preferably, the ejecting device is used in a Coulter particle study device for ejecting a specific minute amount of blood toward a Coulter aperture thereby providing a non-diluting Coulter particle study device.

SUMMARY OF THE INVENTION

According to the invention there is provided in a particle study device a mechanism for ejecting a specific minute amount of fluid sample, the ejecting mechanism including structure for receiving and temporarily storing a given amount of sample, a thermal expansion device mounted within the structure, and a source of energy for supplying a predetermined amount of energy to the thermal expansion device to raise the temperature of the device to cause the device to expand thereby to eject a specific minute amount of fluid sample from the structure.

Further according to the invention there is provided a method for studying particles in a fluid suspension including the steps of: ejecting a specific minute amount of a fluid sample containing particles into a flow stream leading to a sensing zone; sensing particles in said sensing zone; and producing a signal in response to each particle sensed.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagrammatic view of a particle study device including a vertical sectional view of a sensing zone in the device and a portion of an ejecting mechanism of the present invention positioned adjacent the sensing zone.

FIG. 2 is an enlarged view, partially in section, of the thermal expansion device of the ejecting mechanism shown in FIG. 1.

FIG. 3 is a schematic diagram of one embodiment of the power supply circuit of the ejecting mechanism.

FIG. 4 is a schematic diagram of another embodiment of the power supply circuit of the ejecting mechanism.

FIG. 5 is a fragmentary side elevational view partially in section of another embodiment of the ejecting mechanism of the invention incorporated into and forming part of a syringe.

FIG. 6 is a vertical sectional view taken along line 6—6 of FIG. 5 and in the direction indicated.

FIG. 7 is an enlarged fragmentary sectional view of a modified tip of the syringe shown in FIG. 5.

FIG. 8 is an elevational view of a modified syringe, similar to the syringe shown in FIG. 5, including structure for mounting the syringe in a predetermined juxtaposed position relative to a vessel in a particle study device.

FIG. 9 is a fragmentary side elevational view partially in section of a modified syringe, similar to the syringe shown in FIG. 5, including a piston having a laterally extending passage which facilitates filling of the ejecting mechanism.

FIG. 10 is a horizontal sectional view taken along line 10—10 of FIG. 5 and in the direction indicated.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A particle study device generally of the Coulter type is identified by the reference numeral 10 in FIG. 1. The device 10 includes a first vessel 12 having a body of fluid 14 therein. A second and smaller vessel 16 is situated within the vessel 12 with the lower portion of the vessel 16 immersed in the fluid 14. Preferably, and as shown, the vessel 16 is in the form of a tube which contains a body of fluid 18. Such a tube is known as an aperture tube and has an aperture in the side wall thereof, the aperture being formed in a wafer 20 secured to the side wall of the aperture tube 16 over an opening in the aperture tube 16. One type of wafer construction and mounting is disclosed in U.S. Pat. No. 2,985,830

As indicated, the upper end of the aperture tube 16 is connected to a conventional vacuum source (not shown) which includes a control mechanism operable to connect the tube 16 to the vacuum source for a predetermined period of time. The control mechanism also controls the operation of the circuitry of the particle study device 10, which circuitry is generally indicated by reference numeral 22. The circuitry 22 includes lead connections to two electrodes 24 and 26 which are situated, respectively, in the bodies of fluid 14 and 18. An electric current flows between the electrodes through the aperture in the wafer 20. When vacuum is applied to the aperture tube 16 a portion of the fluid 14 is drawn through the aperture into the tube 16.

In a conventional Coulter particle study device the body of fluid 14 comprises a dilution of blood in an electrolyte such that diluted blood is drawn through the aperture. Particles such as blood cells in the blood passing through the aperture will change the impedance of the electrolyte in the aperture. This change in impedance is sensed by the circuitry 22 which generates an electrical signal, commonly referred to as a particle pulse, each time a particle passes through the aperture. The particle pulses produced in this manner are studied, analyzed, and/or counted by the circuitry 22.

In accordance with the teachings of the present invention the body of fluid 14 is pure electrolyte and does not contain particles, such as would be the case in a conventional apparatus. Instead of placing a diluted sample in vessel 12 to comprise the body of fluid 14, the particle study device 10 of the present invention includes an ejecting mechanism 28 for ejecting fluid sample into a pure body of electrolyte. As will be explained in detail hereinafter the ejecting mechanism 28 is operable to eject a specific minute amount of undiluted fluid sample suspension toward the aperture in the wafer 20. In this way the particle study device 10 is a non-diluting particle study device.

The ejecting mechanism 28 includes a hollow body 30 having a cavity 32 therein. A thermal expansion device 34 is situated in the cavity 32. As shown, one side wall of the body 30 has an opening 35 therein communicating with the cavity 32. The opening 35 is covered by a wafer 36 similar to the wafer 20. The wafer has an aperture therein forming an outlet orifice for the ejecting mechanism 28. This outlet orifice is preferably very small so that surface tension of the fluid in the orifice will prevent any leakage into or out of the cavity 32 after the cavity 32 has been filled with a quantity of the fluid sample to be analyzed. The actual size of the aperture forming the outlet orifice is not critical and it is contemplated that wafers with apertures not meeting established standards for a Coulter type particle analyzing device can be used in the ejecting mechanism of the invention. As a result, rejected Coulter wafers can be salvaged and utilized in the ejecting mechanism of the present invention. A typical wafer will be one having an aperture of the order of 50μ to 100μ.

The thermal expansion device 34 is connected via leads 38 and 40 to a power supply circuit 42 of the ejecting mechanism 28. The power supply circuit 42 includes a calibratable source of electric energy and circuitry for controlling the delivery of energy to the thermal expansion device 34 so that only a predetermined amount of electrical energy is supplied to the device 34 to produce a predetermined increase in temperature of the device 34 and thereby cause a predetermined incremental increase in the volume of the device 34. It will be understood that when the device 34 is heated, it expands - increases in volume. The expansion causes a minute amount of the fluid sample to be ejected through the outlet orifice in the water 36.

As best shown in FIG. 2 the thermal expansion device 34 includes a core member 44 around which is wrapped a resistance element 46, namely a wire conductor, which forms the heating element of the device 34. The core 44 and the body 48 form an expandable element generally identified by the reference numeral 49. Surrounding the body 48 is an insulating jacket 50. As shown two electrical contacts or terminals 52 and 54 are fixed in the jacket 50 and connected to the ends of the wire conductor 46. It will be understood that the leads 40 and 38 extend to the body 30 and are con-

nected to terminals or contacts fixed in the inner wall of the body **30** in position to engage the contacts **52** and **54.**

The electrical circuit for the power supply **42** can take various forms. One such circuit for the power supply **42** is shown schematically in FIG. **3** and is generally identified by the reference numeral **60.** The circuit **60** includes a voltage source **62** which provides a very accurate, precise and constant voltage. The voltage source **62** is connected in series with a current limiting resistor **64** and a capacitor **66.** The resistor **64** controls or limits the charging current to the capacitor **66.** Preferably, the resistor **64** has a relatively high resistance so that the charging current is very low and so that, when the capacitor **66** is connected across the resistance element **46,** the amount of charging current which flows through the resistor **64** compared to the current discharging from the capacitor **66** is very small. As shown the resistance element **46** is connected in series with a switch **68** and this series combination is connected in parallel with the capacitor **66.** When it is desired to operate the ejecting mechanism **28,** the switch **68** is closed allowing the capacitor **66** to discharge through the resistance-heating element **46** and thereby transfer a predetermined amount of energy stored in the capacitor **66** to the heating element **46.** The amount of energy supplied to the heating element **46** is dependent upon the size of the thermal expansion device **34,** the coefficient(s) of expansion of the expandable element **49** and the temperature difference needed to obtain a desired increase in volume of the expandable element **49.**

The switch **68** can be a spring biased, push button type switch or can be a relay operated switch. In either case the switch **68** is designed not only to discharge the charge in the capacitor **66** through the resistance element **46** but also to operate the control mechanism (not shown) for connecting the aperture tube **16** to the source of vacuum. If the switch **68** is of the push button type, the operator, after depressing the switch **68** will monitor a conventional visual display device connected to and forming part of the circuitry **22** and observe the signals produced by particles in the specific minute amount of fluid sample which pass through the aperture in the wafer **20.** When the repetition rate of the signals has diminished to a certain point, he will release the switch **68** which will deactivate the counting circuitry-release of the switch could operate a one-shot which applies a signal to the counting circuitry to stop operation of the same. Alternatively, if a relay controlled switch is utilized, a circuit supplying electric current to the coil of the relay will be operated by suitable circuitry, such as a flip flop circuit, when the repetition rate of the particle-caused signals diminish below some predetermined traction of the maximum rate, indicating that all ejected particles have been counted. In this way the ejecting mechanism **28** and the circuit **60** replace and eliminate the prior metering apparatus such as disclosed in U.S. Pat. Nos. 2,869,078, 3,015,775 and 3,271,672.

Another electrical circuit for the power supply **42** is shown in FIG. **4** and is generally identified by the reference numeral **70.** The circuit **70** is operable to supply a given quantity of energy at substantially constant voltage and current levels to the resistance element **46.** This is accomplished by supplying the current from a well regulated voltage source which maintains a constant, precise, accurate voltage independent of current drain and by integrating the current flowing through the resistance element **46.** For this purpose the circuit **70** includes a first voltage source **72** which is a well regulated d.c. power source and supplied an accurate, precise, constant voltage. The circuit **70** also includes a switching mechanism comprised of a spring biased start switch **74,** a normally closed single pole, single throw switch **76** which is operated by a relay coil **78** and a double pole, single throw switch **80** operated by a relay coil **82.** An output terminal **84** from the voltage source **72** is connected to one side of the contacts of the switch **74,** the other side of the switch **74** being connected to a lead **86** connected to the switch **76.** The other side of the switch **76** is connected by a lead **88** to a junction **90.** The relay coil **82** is connected between the junction **90** and a common conductor **92** connected to output terminal **93** of the power source **72.** The resistance element **46** is also connected between the junction **90** and the common terminal **92** but in series with a resistor **94** which functions as a current sensing resistor. The junction between resistance element **46** and resistor **94** is identified by the reference numeral **95.** When the switch **74** is closed a circuit path is established from the output terminal **84** through the lead **86,** the switch contact **76,** the lead **88** and the junction **90** to supply electric current to the resistance element **46** and to the relay coil **82.**

Energization of the relay coil **82** causes operation of switch **80** to establish a connection between the output terminal **84** and the lead **86** through a lead **96,** switch **80** and a lead **98.** As a result, switch **74** can be released and a circuit connection to the resistance element **46** is maintained via the lead **96,** the switch **80,** the lead **98,** the lead **86,** switch **76** and the lead **88.**

For the purpose of monitoring or measuring the current flow through the resistance element **46** over a period of time, an integrating circuit **100** is connected across the current sensing resistor **94** and senses the voltage thereacross, this voltage being indicative of the current through the resistor **94** and hence **46.** The integrating circuit **100** includes an amplifier **102,** a capacitor **104** connected between an inverting input **105** of the amplifier **102** and an output **106** of the amplifier **102,** and a voltage integrating resistor **107** connected between junction **95** and the input **105** of the amplifier **102.** In effect, the resistor **107** converts the voltage across resistor **94** to a current which is integrated by the integrating circuit **100.** The resistor **107** is much larger than resistor **94** so that only a very small current is integrated. The output from the integrating circuit **100** is applied to one input **108** of a comparator **100** and an accurate, precise, constant voltage is supplied from a second voltage source **112** to a second input **114** of the comparator **110.** The output **115** of the comparator **110** is connected to one side of the coil **78,** the other side of the coil **78** being connected to the common conductor **92.**

After a circuit connection has been completed between the output terminal **84** of the voltage source **72** and the junction **90,** current begins to flow through the resistance element **46** and a very small portion of this current is passed through the resistor **107** to the integrating circuit **100** which accumulates a charge on the capacitor **104.** This charging of the capacitor **104** results in the development of a voltage at the output terminal **106** of the circuit **100** and this developed voltage is applied to the input **108** of the comparator **110** and

compared with a predetermined, precise, accurate voltage supplied by voltage source **112** to the input **114** of the comparator **110**. When the developed voltage equals the supplied voltage, the comparator **110** is operated to energize the coil **78**. Energization of the coil **78** causes opening of the switch **76** thereby terminating the current flow through the resistance element **46**. In this way a precise amount of energy, W, is supplied to the resistance element **46**. The energy $W = v \quad idt$. The opening of the circuit connection to the junction **90** also causes de-energization of the coil **82** which in turn causes the contacts of switch **80** to return to the position shown in FIG. **4**. In this position, the switch **80** connects a resistor **116** in shunt relationship across the capacitor **104** for bleeding any charge on the capacitor **104** through the resistor **116** thereby to reset the circuit **70**, namely the integrating circuit **100**, for a subsequent operation thereof.

Again, although not shown in FIG. **4**, it is to be understood that the switch **74** can be of the push button type or of a relay type and is connected to the circuitry **22** in such a manner that operation of the switch **74** also initiates operation of the particle analyzing device. Thus, once the switch **74** is closed, the counting circuits of the circuitry **22** are made operative and the aperture tube **16** is connected to the source of vacuum for initiating flow of the electrolyte and the ejected specific amount of fluid sample through the aperture in the wafer **20**. Also, the circuit **70** together with the ejecting mechanism **28** eliminates the need for a metering apparatus.

Although two specific circuits, circuits **60** and **70**, for the power supply **42** have been illustrated in FIGS. **3** and **4** respectively, it is to be understood that other types of circuits could be utilized. In this respect, instead of utilizing a well regulated power source it is possible for one to monitor the voltage from a non-regulated voltage source from which energy is supplied to the resistance element **46** and at the same time to monitor the current through the resistance element **46**. This could be done by connecting a resistor across the voltage source and measuring the current therethrough. The current through this resistor will vary of course as the magnitude of the voltage varies. Then by means of conventional multiplying circuits the current passing through this resistor and the current charging the integrating capacitor **104** can be multiplied and integrated with respect to time. In this way the energy input per unit time is integrated and when the energy input reaches a predetermined value, the circuit connections to the resistance element **46** can be opened.

Referring back to FIG. **1**, it will be noted that the ejecting mechanism **28** is shown schematically and it will be understood that some means must be provided for introducing the liquid sample into the cavity **32** within the body **30**. For this purpose one preferred embodiment of the ejecting mechanism is in the form of a syringe generally identified by the reference numeral **128** in FIG. **5**. The syringe **128** includes an elongate body **129** and a laterally extending projection **130**. The projection **130** tapers to a tip **131**. The projection **30** has a cavity **132** therein in which a thermal expansion device **134**, similar to the device **34**, is situated. An opening in the tip **131** is in communication with the cavity **132**. The elongate body **129** is closed at one end **136**. A plunger **138** is slidably received within the body **129** and extends from an open end of the body **129** opposite the closed end **136**. The outer end of the plunger **138** has a finger manipulatable head **139**. The inner end of the plunger **138** includes a piston **140** having an L-shaped passageway **142** therein. The passageway **142** has one end **143**, which opens into space **145** in the hollow body **129** opposite the closed end **136** thereof, and a second end passageway **147** which opens onto a side of the plunger **138** and is adapted to register and communicate with an inner end **148** of the cavity **132** during at least a portion of the movement of the plunger **138** within the body **129**.

The thermal expansion device **134** has the same general construction as the device **34** and has two contacts **152** and **154** thereon for connecting the heating element therein with the power supply **42**. The device **134** can have any desired cross section, such as a circular cross section, although the preferred cross section is triangular as will be further explained in connection with the description of FIG. **6**.

When the plunger **138** is raised, the volume of the space **145** is increased. This increase in volume creates a suction for drawing fluid through the opening in the tip **131** of the projection **130** so long as the end **147** of the passageway **142** is in communication with the cavity **132**. The filling of the cavity **132** with fluid sample will continue until the opening **147** is completely out of registry with the end **148** of the cavity **132** opening into the interior of the body **129**. At this point the cavity **132** will be closed except for the opening at the tip **131**. When this has been accomplished the tip **131** can be placed closely adjacent the wafer **20** and preferably with the axis of the opening in the tip **131** coaxial with the axis of the aperture in the wafer **20**. The syringe **128** is now ready to eject a specific minute amount of fluid sample toward the aperture in the wafer **20**. This is accomplished by supplying a predetermined amount of energy from the power supply **42** via two leads **158** and **160** to the thermal expansion device **134**. The leads **158** and **160** are, of course, connected to contacts which are mounted within the cavity **132** and which engage the contacts **152** and **154**.

To ensure that the plunger **138** is pulled out sufficiently to close the inner end of the cavity **132**, a locating projection **162** is mounted on and extends outwardly from the plunger **138**. A spring biased latching device **164** having a notch **166** which fits over the projection **162** is mounted on the syringe body **129**. When the plunger **138** is raised the projection **162** will engage and force the latching device **164** outwardly until the projection **162** is in registry with the notch **166** whereupon the latching device will snap inwardly over the projection **162** and prevent further upward movement or downward movement of the plunger **138** due to suction developed in the space **145**. As shown, the latching device **164** has a finger **168** which can be easily manipulated for unlatching the latching device **164** from the projection **162**.

As best shown in FIG. **6**, the cavity **132** within the projection **130** has a cylindrical shape and the thermal expansion device **134** has a generally triangular cross section such that the device **134** has three longitudinal edges **171**, **172** and **173**. The contacts **152** and **154** are disposed on the upper edge **171** and the lower edges **172** and **173** engage the cylindrical inner wall surface of the cavity **132**. Preferably the thermal expansion device **134** has an interference fit in the cavity **132** with the contacts **152** and **154** resiliently engaging the mat-

ing contacts on the inner wall of the cavity **132**. In order to permit an interference fit of the thermal expansion device **134** in the cavity **132**, the insulating jacket thereon is formed from a material having sufficient elasticity whereby the edges **172** and **173** can be deformed slightly when the device **134** is inserted in cavity **132**. Preferably, the insulating jacket material is resilient whereby the contacts **152** and **154** are urged against the contacts in the cavity **132** to make a good electrical connection therebetween. The material, however, must be incompressable so that deformation thereof does not affect the thermal expansion of the thermal expansion device **134**.

To facilitate easy insertion of the thermal expansion device **134** into the cavity **132**, the cavity **132** is formed in the projection **130** by drilling a hole transversely through the syringe body **129** and into the projection **130**. This results in a hole **176** on the side of the body **129** opposite the projection **130**. When the syringe **128** is disassembled for cleaning, the thermal expansion device **134** is then easily removed through the hole **176** and, after being cleaned, is reinserted into the cavity **132** through the hole **176**.

Also, the L-shaped passageway **142** is easily formed by drilling one hole axially into the piston **140** and a second hole radially inwardly from the side of the piston **140**, the two holes intersecting inside the piston **140**.

In order to maintain a very small outlet orifice at the tip **131**, the opening in the tip **131** is preferably covered by a wafer **178** having a very small aperture therethrough, as shown in FIG. **7**.

When the projection **130** is inserted into a fluid sample for filling the cavity **132**, some of the fluid sample is picked up by the outer surface of the tip **131**. To minimize contamination of the electrolyte, the tip **131** is preferably wiped off before the syringe **128** is inserted into the body of electrolyte. However, during the cleaning of the tip **131** with a cloth it is likely that fluid in the aperture will be absorbed by the fabric of the cloth. By having a very small aperture volume which is much smaller than the specific minute amount of fluid sample to be ejected from the syringe **128**, this loss of blood from the aperture in the wafer **178** is insignificant and will not adversely affect the analysis of the fluid sample. Cleaning of the tip **131**, and the possible absorption of sample from the aperture by the cleaning wiper, can be avoided by inserting sample into the cavity **132** through the inner end **148** thereof, as will be more fully explained in connection with the description of FIGS. **9** and **10**.

When utilizing the syringe **128** it is desirable to accurately locate and fix the position of the tip **131** relative to an aperture in a wafer in a particle analyzing or studying device. For this purpose, the syringe **28** preferably has structure associated therewith which cooperates with mating structure on the aperture tube for mounting and holding the syringe **128** in place. A modified syringe **228** having such mounting structure is shown in FIG. **8**. In this respect the syringe **228** has an L-shaped bar **230** which extends from the syringe with one leg **231** of the bar extending parallel to the longitudinal axis of the syringe. The leg **231** of the bar **230** is received in a V mounting block **232** which is fixed to an aperture tube **236**. Except for the mounting block **232** the aperture tube **236** is substantially identical to the aperture tube **16** shown in FIG. **1**. Also except for the bar **230** the syringe **228** is substantially identical to the syringe **128** shown in FIG. **5**. The leg **231** of the bar **230** is held firmly in place by a spring **238** fixed to the mounting block **232**.

In order to ensure proper location of the outlet orifice of the syringe **228**, namely, to ensure that it will be coaxial with the aperture in a wafer in an aperture tube, the syringe is preferably fabricated while mounted in a fixture having a similar shape as the aperture tube **236**. A special microscope is positioned behind an aperture of the fixture, the fixture aperture simulating the aperture in the wafer, and the outlet orifice in the syringe **228** is then aligned with the fixture aperture. When the outlet orifice is defined by the aperture in wafer **178**, the locating of the aperture in the wafer **178** is accomplished by softening the tip **131**, which typically is made of glass, and then positioning the wafer in proper location so that the aperture therein is properly aligned with the aperture of the fixture. Then the glass is allowed to cool and solidify with the wafer **178** properly in place.

To obviate the need to clean off the tip **131**, the syringe **128** can be constructed in a manner whereby fluid sample is inserted into the cavity **132** through the inner end **148** thereof. Such a modified construction of the syringe is shown in FIGS. **9** and **10**, the modified syringe being identified by the reference numeral **328**. The syringe **328** is similar to the syringe **128** and includes an elongate body **329**, a projection **330** with a tip **331**, a cavity **332**, a thermal expansion device **334** mounted in the cavity **332**, a closed end **336** and a plunger **338** slidably received in the body **329**.

The plunger **338** has a piston **340** at the inner end thereof received in the body **329**. Instead of an L-shaped passageway, such as passageway **142** shown in FIG. **5**, the piston **340** has a lateral passageway **342** extending through the piston **340**. The passageway **342** and cavity **332** are formed in one operation by drilling a hole through the body **329**, the piston **340** and the projection **330**. In this way a hole **346**, similar to the hole **176** shown in FIG. **5**, is formed in the body **329** opposite the projection **330**.

To fill the cavity **332** with fluid sample, the piston **340** is manipulated to bring the passageway **342** into registry with inner end **348** of the cavity **332** as shown in FIG. **9**. Then fluid sample, which has been drawn into a hypodermic needle **350** is dispensed into the cavity **332** by holding the needle **350** above the hole **346** and manipulating the needle **350** to drop sample, e.g., blood, into the syringe **328** until the level thereof reaches the position indicated by reference numeral **351**. Air trapped below the fluid sample easily can escape out of the outlet orifice in the tip **331**. The surface tension of the fluid sample, however, prevents fluid flow of the fluid sample through the outlet orifice. After the fluid has reached the level **351**, the piston **340** is withdrawn or pushed in until the passageway **342** is completely out of registry with the inner end **348** of the cavity **332**. The amount of sample in the passageway **342** will, of course be wasted. However, by shearing the body of sample in the cavity **332** and passageway **342**, there is no change in volume of the fluid sample in the cavity **332**. As a result the cavity **332** will be completely filled with fluid sample and is ready for the ejection therefrom of the desired specific minute amount of sample.

To prevent contamination of electrolyte the syringe body **329** has its bottom end **336** closed. The closed space, space **355**, between the inner end of piston **340** and the bottom end **336** of the body **329** is vented to atmosphere by means of at least one air channel **359** formed in the outer surface of the plunger **338** as best shown in FIGS. **9** and **10**. The venting of the space **355** prevents a higher or lower pressure from developing in the space **355**, and thereby prevents a pressure differential from acting on the plunger **338**. Such a pressure differential makes movement of the plunger **338** unnecessarily difficult and could cause uncontrolled movement of the plunger **338**.

In all other respects the syringe **328** is the same as the syringe **128**. Since the syringe **328** does not require wiping off of the tip **331**, and since it can receive blood directly from a hypodermic needle, the syringe **328** may be preferred in blood analyzing devices over the syringe **128**.

It will be apparent that the syringes **128, 228** and **328** are simple in construction and can be easily assembled and disassembled for cleaning purposes.

In practicing the teachings of the present invention it is contemplated that the core member **44** of the thermal expansion device **34** or **134** will be formed of either silver or beryllium oxide. Also the material of the body **48** within which the core **44** is embedded is preferably made from beryllium oxide. The coefficient of thermal expansion of silver is 10.9 (10^{-6}) and the coefficient of thermal expansion for beryllium oxide is 5.3 (10^{-6}). As stated above it has been found that a sufficient quantity of blood for analysis and study of the cells in the blood is 0.01 cubic millimeters of blood. Assuming a temperature rise of 20°F for the sake of convenience and assuming that the expandable element **49** formed by the core member **44** in the body **48** is cylindrical having a length equal to twice its diameter mathematical calculations show that a silver expandable element would have to be roughly 0.085 inches in diameter and have a length of roughly 0.170 inches to obtain an increase in volume of roughly 0.01 cubic millimeters for a 20°F increase in temperature of the expandable element. This is a very convenient size in that it does not require a large syringe. A small syringe can be utilized and a very small amount of blood sample is needed. Also the syringe and the thermal expansion device **34** or **134** are large enough so that they can be readily made utilizing the techniques used in manufacturing miniature resistors.

If a solid beryllium oxide (BeO) cylindrical expandable element is used instead of silver, the volume of the expandable element **49** should be roughly twice the volume of a silver expandable element. In this case, the diameter of the expandable element would be roughly 0.103 inches and the length of the element would be roughly 0.217 inches.

Rough calculations show that an expandable element made from BeO would require 1.09 calories of heat or 4.56 joules of energy in order to obtain an increase of 0.01 cubic millimeters in the volume of the element, if a temperature rise time of 20 second is assumed, and that power would have to be expended in the expandable element at the average rate of 0.228 watts.

One of the advantages obtained with a non-diluting particle counter utilizing the ejecting mechanism **28** or the syringe **128** heretofore described is the fact that much more electrolyte is sucked through the aperture in the wafer **20** than blood. The net effect is exactly the same as if the blood had been diluted. However, dilution does not matter since all the cells in the 0.01 cubic millimeter of blood ejected into the body of electrolyte **14** are to be counted.

The electrolyte being drawn through the aperture in the wafer **20** also serves as a sheath for the blood cells which flow through the aperture. By ejecting the specific minute amount of blood into the fluid vortex formed adjacent the wafer **20** by the electrolyte being sucked through the aperture, essentially all of the blood cells are ensheathed by electrolyte and drawn through the aperture. As a result practically none of the blood cells will be lost in the electrolyte.

The duration of sensing and counting of blood cells is not critical in a non-diluting particle study device of the type heretofore described, since the circuitry **22** can be modified to include an electrical circuit which will measure the interval between pulses generated by particles passing through the aperture. When the interval becomes very large indicating that the expandable element **49** has stopped expanding and blood is no longer being ejected toward the aperture in the wafer **20**, the electric circuit **60** or **70** can be operated to terminate the count and terminate operation of the particle study device. In this way, practically all of the blood cells in the 0.01 cubic millimeter are counted as desired.

Modifications and variations to the ejecting mechanism or the syringe heretofore described will be apparent to those skilled in the art.

What it is desired to be secured and claimed by Letters Patent of the United States is:

1. In a particle study device comprising a sensing zone, means for passing a fluid suspension of particles through said sensing zone and means for sensing the presence of the particles within the influence of said sensing zone and for producing a signal in response to each particle sensed, the improvement comprising means for ejecting a specific amount of the fluid sample containing a plurality of particles into a flow stream leading to said sensing zone, said ejecting means including means for receiving and temporarily storing a given amount of sample, a thermal expansion device mounted within said receiving and storing means, and means for supplying a predetermined amount of energy to said thermal expansion device to raise the temperature of said device to cause said device to expand thereby to eject a specific minute amount of fluid sample from said receiving and storing means.

2. The ejecting means according to claim **1** wherein said receiving and storing means is a syringe.

3. The ejecting means according to claim **1** wherein said receiving and storing means includes a body having a fluid receiving cavity therein and an opening in a wall of the body communicating with said cavity, said thermal expansion device being situated within said cavity.

4. The ejecting means according to claim **1** wherein said energy supply means includes a voltage source having good regulation to provide a substantially constant voltage independent of current drain.

5. The ejecting means according to claim **1** wherein said thermal expansion device includes an electric heating element, an expandable element which surrounds said heating element, which is made from a material having a given thermal coefficient of expansion

and which has a given volume, and an insulating jacket surrounding said expandable element and said heating element.

6. The ejecting means according to claim 5 wherein said expandable element is made of silver.

7. The ejecting means according to claim 5 wherein said expandable element is made of beryllium oxide.

8. The ejecting means according to claim 5 wherein said expandable element is made of silver and beryllium oxide.

9. The ejecting means according to claim 5 wherein said heating element is a wire conductor and said expandable element includes an elongated core on which said wire conductor is wound, said heating element and said core being embedded in a body which forms part of the expandable element.

10. The ejecting means according to claim 9 wherein said core and said body are made of the same material.

11. The ejecting means according to claim 1 wherein said thermal expansion device includes an electric heating element and said energy supply means includes a voltage source, an energy storing device for storing a predetermined amount of energy and switch means for connecting said storage means to said heating element to discharge said predetermined amount of energy from said storage device into said electric heating element.

12. The ejecting means according to claim 1 wherein said thermal expansion device includes an electric heating element and said energy supply means includes a voltage source, switch means for connecting said voltage source to said heating element and circuit means connected to said switch means and said heating element for monitoring the energy input to said heating element and for opening the connection between said voltage source and said heating element after a predetermined amount of energy has been supplied to said heating element.

13. The ejecting means according to claim 12 wherein said circuit means includes means for intergrating the current blowing through said heating element, a second voltage source, a relay including an operating coil and contacts in the connection between said first voltage source and said heating element, and a comparator having inputs connected to said integrating means and said second voltage source and an output connected to said operating coil, said comparator being operable to compare a voltage developed in said integrating means with a constant voltage supplied from said second voltage source and to energize said coil for causing opening of said contacts when said developed voltage equals said constant voltage.

14. The ejecting means according to claim 1 wherein said receiving and storing means includes an elongate hollow body, a projection which extends from said body, which is integral with said body, which has a cavity therein communicating with the hollow interior of said body, and which has a tip at the distal end thereof, said tip having an opening therein communicating with said cavity, and a plunger in said body, said plunger having a passageway therein, said passageway having a first end which opens into said hollow body opposite a closed end of said hollow body and a second end which opens onto a side of said plunger and which is adapted to register and communicate with said cavity during at least a portion of the movement of said plunger within said body, and said thermal expansion device being situated in said cavity.

15. The ejecting means according to claim 14 wherein said body is open at one end opposite said closed end and said plunger has a portion thereof which extends outwardly from said open end of said body, and wherein said receiving and storage means includes means for limiting outward movement of said plunger to a position where said second end of said passageway is completely out of registry with said cavity.

16. The ejecting means according to claim 14 including means for mounting said body in said particle study device with said tip in a predetermined position relative to said sensing zone in said particle study device.

17. The ejecting means according to claim 1 wherein said receiving and storing means includes an elongate hollow body and a projection which extends from said body, which is integral with said body, which has a cavity therein communicating with the hollow interior of said body, and which has a tip at the distal end thereof, said tip having an opening therein communicating with said cavity, and said body having a hole therein opposite said projection and wherein a plunger is received in said body through an open end thereof and has a lateral passageway therethrough which is adapted to be brought into registry with the inner end of said cavity for enabling filling of said cavity with fluid sample through said hole and said passageway.

18. The ejecting means according to claim 17 wherein the end of said body, opposite said open end thereof is closed, and said plunger has at least one channel therein communicating the space betwen said closed end of said body and the inner end of said plunger to atmosphere.

19. The ejecting means according to claim 1 including means for mounting said ejecting means in position for ejecting the specific minute amount of fluid sample toward said sensing zone in said particle study device.

20. In a particle study device of the type wherein a fluid sample containing particles is passed from one body of electrolyte through an aperture to another body of electrolyte, wherein the impedance through the aperture is changed during the passage of a particle through the aperture and wherein the change of impedance is sensed and used to generate an electrical signal representing a particle passing through the aperture, the improvement comprising means for ejecting a specific minute amount of fluid sample containing a plurality of particles into the first body of electrolyte and toward said aperture, said ejecting means including means for receiving and temporarily storing a given amount of fluid sample, a thermal expansion device mounted within said receiving and storing means, and means for supplying a predetermined amount of energy to said thermal expansion device to raise the temperature of said device and to cause said device to expand thereby to eject a specific minute amount of fluid sample, having a volume equal to the volume increase of the expanded thermal expansion device, from said receiving and storing means.

21. A particle study device according to claim 1 wherein said ejecting means includes means for mounting said ejecting means in a predetermined position relative to said aperture such that an outlet orifice of said ejecting means from which fluid sample is ejected is located closely adjacent said aperture and such that said outlet orifice is coaxial with said aperture.

22. A particle study apparatus comprising a sensing zone, means for passing a fluid suspension of particles through said sensing zone and means for sensing the presence of the particles within the influence of said sensing zone and for producing a signal in response to each particle sensed, said means for passing the fluid suspension through said sensing zone including means for ejecting a specific minute amount of fluid sample containing a plurality of particles into a flow stream leading to said sensing zone, and said ejecting means including means for receiving and temporarily storing a given amount of sample, a thermal expansion device mounted within said receiving and storing means, and means for supplying a predetermined amount of energy to said thermal expansion device to raise the temperature of said device to cause said device to expand and increase in volume thereby to eject a specific minute amount of fluid sample from said receiving and storing means, the volume of said specific minute amount of sample being equal to the volume increase of said thermal expansion device.

23. A method for studying particles in a fluid suspension including the steps of: ejecting a specific minute amount of a fluid sample containing particles into a flow stream leading to a sensing zone; sensing particles in said sensing zone; and producing a signal in response to each particle sensed, said step of ejecting sample being accomplished by heating a thermal expansion device within a sample containing chamber, communicating with said flow stream via a small orifice, thereby to raise the temperature of said device to cause said device to expand and increase in volume to eject from said chamber a specific minute amount of fluid equal to the volume increase of said thermal expansion device.

24. The method according to claim 23 including the steps of supplying electric energy to said thermal expansion device; monitoring the energy being supplied; and terminating the supplying of electric energy after a predetermined amount of electric energy has been supplied to said thermal expansion device.

25. The method according to claim 24 wherein the step of monitoring the electric energy supplied includes the steps of: integrating the current supplied to the thermal expansion device from a constant voltage source; and comparing the voltage developed by said integrating with a given voltage, said termination of the supplying of electric energy occurring when said developed voltage equals said given voltage.

26. The method according to claim 23 including the initial step of filling said chamber.

27. The method according to claim 26 wherein said step of filling said chamber consists essentially in sucking a quantity of a fluid sample containing particles into said chamber through said small orifice.

28. The method according to claim 26 wherein said chamber is formed in a body having an opening larger than said small orifice communicating with said chamber, and said step of filling said chamber includes the steps of introducing fluid sample containing particles through said opening into said chamber until said chamber is filled and then closing said opening.

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