choosing the focal depth of a photographic lens

MICROCOPY RESOLUTION TEST CHART,
NATIONAL BUREAU OF STANDARDS
STANDARD REFERENCE MATERIAL 1010a
(ANSI and ISO TEST CHART No. 2)
We, INTERNATIONAL REMOTE IMAGING SYSTEMS, INC., of 9825 De Soto Avenue, Chatsworth, California 91311, United States of America hereby apply for the grant of a standard patent for an invention entitled:

"A METHOD OF OPERATING A MICROSCOPIC INSTRUMENT"

which is described in the accompanying complete specification.

DETAILS OF BASIC APPLICATION

- Number of Basic Application: 676,190
- Name of Convention Country in which Basic Application was filed: United States of America
- Date of Basic application: 29 November, 1984

Our address for service is:

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DATED this TWENTY-FOURTH day of OCTOBER 1985

INTERNATIONAL REMOTE IMAGING SYSTEMS, INC.

By: 

Registered Patent Attorney

TO: THE COMMISSIONER OF PATENTS
AUSTRALIA

SBR: ep 99T

FORM 10
DEPARTMENT OF JURISPRUDENCE

COMMONWEALTH OF AUSTRALIA

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made for a patent for an invention entitled:

"A METHOD OF OPERATING A MICROSCOPIC INSTRUMENT"

I/We, Fred H. Deindoerfer

of 9221 Encino Avenue
Northridge, California 91325
United States of America

do solemnly and sincerely declare as follows:

1. I am/we are the applicant(s) for the patent
   (or, in the case of an application by a body corporate)
   I am/we are authorized by INTERNATIONAL REMOTE IMAGING SYSTEMS, INC.

   the applicant(s) for the patent to make this declaration on
   its/their behalf.

2. The basic application(s) as defined by Section 141 of the
   Act was/were made

   in United States of America

3. I am/we are the actual inventor(s) of the invention referred
   to in the basic application(s),
   (or where a person other than the inventor is the applicant)

   Fred H. Deindoerfer

   of 9221 Encino Avenue
   Northridge, California 91325
   United States of America

   is/are the actual inventor(s) of the invention and the facts upon
   which the applicant(s) is/are entitled to make the application are
   as follows:

   The said applicant is the assignee of the actual inventor.

4. The basic application(s) referred to in paragraph 2 of this
   Declaration was/were the first application(s) made in a Convention
   country in respect of the invention(s) the subject of the application.

   Declared at Chatsworth, this 8 day of Jan., 1986.

   INTERNATIONAL REMOTE IMAGING SYSTEMS, INC.

   Signature of Declares(s) 11/81

   Fred H. Deindoerfer, President
A method of operating a microscopic instrument having a microscopic means analyzing particles in a fluid sample flowing with a sheath fluid in a flow chamber, said chamber having an inlet and an outlet and a passageway extending from the inlet to the outlet with the passageway having an imaging area which said microscopic means is directed, and a means to distribute the fluid sample and the sheath fluid into substantially a planar flow from the inlet to the imaging area, said planar flow characterized by a width and a thickness, said sheath fluid and said fluid sample are conveyed from said inlet to the outlet, said microscopic means having an optical lens for focusing on said imaging area; said method comprising

- selecting the flow rate of the sheath fluid to produce planar imaging flow at the imaging area;
- directing said microscopic means at said imaging area in a direction parallel to said thickness;
- choosing the working distance of said optical lens to be greater than the distance from the image plane to the outside of said chamber at said imaging area;

.../2
choosing the focal depth of said optical lens to be much less than the thickness of said chamber at said imaging area; and maintaining the flow rate of said fluid sample and said sheath fluid such that the particles of said fluid sample flow within the focal depth of said optical lens, at said imaging area.
Name of Applicant: INTERNATIONAL REMOTE IMAGING SYSTEMS, INC.
Address of Applicant: 9825 De Soto Avenue, Chatsworth, California 91311, United States of America
Actual Inventor: FRED H. DEINDOERFER
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Complete Specification for the invention entitled:
"A METHOD OF OPERATING A MICROSCOPIC INSTRUMENT"

The following statement is a full description of this invention, including the best method of performing it known to us.
Abstract

A Method Of Operating A Microscopic Instrument

A method is disclosed for operating a microscopic instrument having a microscopic means directed to analyze particles flowing in a fluid sample flowing in a flow chamber. The chamber has an inlet and an outlet and a passageway having a cross-sectional area which decreases substantially as the passageway extends from the inlet to a constriction. Thereafter, the cross-sectional area increases from the constriction to the imaging area. The thickness of the flow chamber, a direction which is parallel to the direction which the microscope is directed, also decreases substantially from the inlet to the constriction. From the constriction, however, the thickness remains a constant. The fluid sample is introduced into the flow chamber at a distance sufficiently far away from the imaging area such that at the imaging area, the fluid sample is flowing in a laminar stream having a constant velocity profile. The microscope is then directed at the imaging area in a direction parallel to the thickness. The working distance of the optical lens in the microscopic means is chosen to be greater than one-half the outer thickness of the flow chamber at the imaging area. The focal depth of the optical lens is chosen to be much less than the thickness of the flow chamber at the imaging area. The flow rate of the fluid sample and the sheath fluid is maintained such that the fluid sample will have a thickness at the imaging area which is less than or comparable to the focal depth of the optical lens.
A Method of Operating a Microscopic Instrument

Technical Field

The present invention relates to a method of operating a microscopic instrument and, more particularly, to a microscopic instrument which has a flow chamber for analyzing particles in a fluid sample flowing in the chamber.

Background of the Invention

Microscopic instruments for analyzing particles, such as biological particles, are well known in the art. Typically, the microscopic instruments are focused on the particles which are on a slide or are suspended in a fluid sample flowing in a flow chamber. The latter is well known in the art. U.S. Patent No. 3,893,766 and RE 29,141 and U.S. Patent No. 4,338,024 disclose a type of flow chamber which can be used with a microscopic instrument for analysis of the particles flowing therein. In both of these references, the flow chamber is characterized by an inlet and an outlet with a passageway extending from the inlet to the outlet. The passageway has an imaging area where the microscopic instrument is directed. The flow chamber has a thickness which decreases substantially as the passageway moves from the inlet to the imaging area. In U.S. Patent No. 4,338,024, a sheath fluid is also introduced into the flow chamber to guide the fluid sample from the inlet to the outlet. In U.S. Patent No. 3,893,766, the sheath fluid is conveyed by sheath flow means which comprises a plurality of tubes extending in the direction of the fluid flow and surrounding the sample tube.

None of the references, however, teaches or suggests the necessary parameters for operating the
Summary Of The Invention

In the present invention, a method of operating a microscopic instrument is disclosed. The microscopic instrument has a microscopic means for analyzing particles that are flowing in a flow chamber. The chamber has an inlet and an outlet and a passageway extending from the inlet to the outlet. The microscopic means is directed at an imaging area between the inlet and the outlet of the passageway. The passageway is characterized by a thickness which decreases initially as the passageway moves from the inlet to the imaging area, reaching a constant thickness, and then remaining constant at the imaging area. A sheath fluid and the fluid sample are conveyed from the inlet to the outlet. The microscopic means further has an optical lens for focusing on the imaging area. The method of the present invention comprises selecting the flow rate of the sheath fluid to produce a planar laminar flow at the imaging area. The microscopic means is directed at the imaging area in the direction parallel to the thickness. The working distance of the optical lens is chosen to be greater than one-half the thickness of the chamber at the imaging area. The focal depth of the optical lens is chosen to be much less than the thickness of the chamber at the imaging area. The flow rate of the fluid sample is maintained such that the thickness of the fluid sample at the imaging area is less than the focal depth of the optical lens.

Description Of The Drawings

Fig. 1 is a cross-sectional view of an apparatus used in the method of the present invention.
Fig. 2 is a greatly enlarged cross-sectional view of a portion of the apparatus shown in Fig. 1.
Fig. 3 is a plan view of an apparatus used in the method of the present invention.
Fig. 4 is a cross-sectional view of the apparatus of Fig. 3.

Detailed Description Of The Drawings
Refering to Fig. 1, there is shown a cross-sectional view of an apparatus 10 useful in the method of the present invention. The apparatus 10 comprises a flow chamber 12, having an imaging area 14 to which a microscopic means 16 is directed. The microscopic means 16 is to one side of the chamber 12. A light source 18 provides the illumination for the microscopic means 16 and is to the other side of the chamber 12. The flow chamber 12 has an inlet 20, an outlet 22, and a passageway 24 from the inlet 26 to the outlet 22. The passageway 24 passes by the imaging area 14. Fluid sample with particles of interest such as blood or urine is conveyed through the flow chamber 12 by entering the inlet 20, and is then conveyed through the passageway 24 to the outlet 22. Sheath fluids are also supplied to the flow chamber 12 through the fluid inlets 26 and 28. The fluid inlets 26 and 28 are to one side and the other side, respectively, of the inlet 20 and upstream of it. The distance from the inlet 20 where the fluid sample enters into the passageway 24 to the imaging area 14 is designated as L. The passageway 24 is characterized by a thickness and a width which decrease substantially as the passageway 24 moves from the inlet 20 to a constriction area 21. From the constriction 21 to the outlet 22, the thickness of the flow chamber remains at a constant, while the width increases. The cross-sectional area of the flow chamber decreases from inlet 20 to the constriction 21. Thereafter, the cross-
sectional area increases. The microscopic means has an optical lens 30 which is shown in Fig. 2.

Referring to Fig. 2, there is shown a greatly enlarged cross-sectional view of a portion of the flow chamber 12 shown in Fig. 1. The portion of the flow chamber 12 shown in Fig. 2 is that portion near the imaging area 14. Optical lens 30 is focussed on the imaging area 14. The optical lens 30 is characterized by having a working distance \( W_d \). In addition, the optical lens 30 has a focal depth \( F_d \). The thickness of the flow chamber 12 at the imaging area 14 is \( D \). Finally, the fluid sample has a thickness at the imaging area 14 of \( t \).

In the method of the present invention, the fluid sample is admitted into the flow stream of the sheath fluid. The fluid (comprising of the fluid sample and the sheath fluid) is passed through the restrictor 21 which has a rectangularly shaped cross section, whose width is many times its thickness. In the imaging area 14, the fluid is maintained in a planar flow. Typically, the width is .813 mm and the thickness is .050 mm. The flow rate of the fluid is chosen such that laminar flow results. As stated in U.S. Patent No. 3,893,766, laminar flow can be maintained by the sheath flow means which comprises a plurality of tubes extending through the center of the conduit in the direction of the flow and surrounding the sample tube. The tubes function to prevent turbulence so that the fluid entering the flow chamber is "collimated" and is non-turbulent. As the fluid sample enters in the flow chamber, the fluid takes the form of a laminar fluid flow. Laminar flow can be achieved without the use of tubes if a slow velocity fluid is introduced into a substantially long conduit prior to entering the flow chamber 12. Typically, the velocity of the sheath fluid in the flow chamber in the imaging area 14 is in the
range of \(0.7 \times 10^3\) mm/sec to \(2.7 \times 10^3\) mm/sec. Once laminar flow is established, the flow of the sheath fluid will have a velocity profile. Preferably, the microscopic means 16 is located at or after the sheath fluid has achieved a substantially constant velocity profile, which is in the nature of the shape of a parabola. Thus, the distance \(L\) is typically 12.7 mm.

Once the distance from the inlet to the imaging area 14 is determined, the outer thickness \(D\) at the imaging area 14 of the flow chamber 12 is determined. The working distance \(W_d\) of the lens 30 must then be chosen to be greater than the distance from the image plane to the outside of the flow chamber 12 at the imaging area 14.

With the working distance \(W_d\) of the lens 30 defined, and with the outer distance \(D\) at the imaging area 14 defined, the focal depth \(F_d\) of the lens 30 is chosen to be much smaller than the outer thickness \(D\).

The thickness \(T\) of the fluid sample at the imaging area 14 must be comparable to or less than the focal depth \(F_d\) of the lens 30. The thickness \(T\) of the fluid sample at the imaging area 14 is determined by the flow rates of the fluid sample and of the sheath fluid. The flow rates of the fluid sample and the sheath fluid can be adjusted thereby varying the thickness \(T\) of the fluid sample at the imaging area 14. Of course as previously noted, the flow rate of the sheath fluid is constrained, in order to produce laminar flow.

In the event, the thickness \(T\) of the fluid sample at the imaging area 14 is greater than the focal depth \(F_d\) of the lens 30, the method of the present invention can still be practiced by varying the flow rates of the fluid sample and the sheath fluid such that the particles of interest in the fluid sample flow in the center of the fluid sample stream, where they are within the focal depth of the optical lens 30.
Finally, to view the particles in the fluid sample, the light source 18 is chosen to be a strobe light. The duration of the strobe of the light source 18 must be sufficiently short to "freeze" the image of the fluid sample. Of course, the duration of the strobe of the light source to "freeze" the image is determined by the rate of flow of the fluid sample. However, once the flow rate of the fluid sample is set, as set forth hereinabove, the minimum strobe duration is then also determined.

One specific embodiment will now be described. A flow chamber 12 has the dimensions of .4 centimeters (width) by .005 centimeters (depth, inner dimension) at the imaging area. The length is 3.81 centimeters. The distance L from the inlet 20 to the imaging area 14 is chosen to be greater than five times the inner thickness (d) of the chamber at the imaging area 14. Preferably, the distance L is 1.27 centimeters. At the imaging area 14, the outer thickness D of the flow chamber 12 is .7 centimeters or less. Preferably, the thickness D is on the order of .157 centimeters. The microscopic instrument is directed in a direction parallel to the thickness D at the imaging area 14. The working distance W of the optical lens 30 is chosen to be .137 centimeters which is greater than one-half the thickness D at the imaging area 14. The focal depth of the optical lens Fd is chosen to be between .6 and 4.5 micrometers, which is much less than the thickness D of the chamber 12 at the imaging area 14. Typically, such an optical lens 30 is one manufactured by American Optical Manufacturing Corporation and has a focal depth of +1.1 micrometers.

Finally, the flow rates of the sheath fluid and the fluid sample are adjusted such that the thickness T of the fluid sample at the imaging area 14 is less than or comparable to the focal depth Fd of the optical lens 30.
The typical flow rates of the fluid sample and of the sheath fluid is in the range of 1 to 2 to 1 to 50. Preferably, the flow rates are .0036 ml/sec and .050 ml/sec, respectively for a total fluid flow rate of .054 ml/sec. The strobe of the light source 18 is at 2 microsecond duration, 60 times per second.

In the present invention, a method is disclosed for determining the optimal operational parameters for the microscopic means which is focused on the imaging area of a flow chamber having fluid sample flowing therethrough.
The claims defining the invention are as follows:

1. A method of operating a microscopic instrument having a microscopic means analyzing particles in a fluid sample flowing with a sheath fluid in a flow chamber, said chamber having an inlet and an outlet and a passageway extending from the inlet to the outlet with the passageway having an imaging area which said microscopic means is directed, and a means to distribute the fluid sample and the sheath fluid into substantially a planar flow from the inlet to the imaging area, said planar flow characterized by a width and a thickness, said sheath fluid and said fluid sample are conveyed from said inlet to the outlet, said microscopic means having an optical lens for focusing on said imaging area; said method comprising selecting the flow rate of the sheath fluid to produce planar laminar flow at the imaging area; directing said microscopic means at said imaging area in a direction parallel to said thickness;

choosing the working distance of said optical lens to be greater than the distance from the image plane to the outside of said chamber at said imaging area;

choosing the focal depth of said optical lens to be much less than the thickness of said chamber at said imaging area; and

maintaining the flow rate of said fluid sample and said sheath fluid such that the particles of said fluid sample flow within the focal depth of said optical lens, at said imaging area.
2. The method of Claim 1 further comprising the steps of:
   admitting said fluid sample into the flow stream of said sheath fluid; and
   passing said sheath fluid with said fluid sample through a restrictor having a rectangular cross-sectional shape with the width substantially greater than the thickness.

3. The method of Claim 1 wherein said microscopic means is positioned at a distance at or after the sheath fluid achieves a substantially constant velocity profile.

4. The method of Claim 3 wherein said profile is in the shape of a parabola.

5. The method of Claim 1, wherein the thickness of said fluid sample at said imaging area is comparable to or less than the focal depth of said optical lens.

6. The method of Claim 1, wherein the outer thickness of said chamber at said imaging area is .7 centimeters or less.

7. The method of Claim 1, wherein said optical lens has a focal depth of .6 and 4.5 µm.

8. The method of Claim 3, wherein the distance from the imaging area to said inlet is greater than five times the inner thickness of said chamber.

9. The method of Claim 1, wherein the ratio of the flow rate of said fluid sample to said sheath fluid is in the range of 1 to 2 to 1 to 90.
10. The method of Claim 1 further comprising the step of strobing the fluid sample at the imaging area at a duration to freeze the image of the fluid sample.

11. The method of Claim 9, wherein the flow rate of said fluid sample is approximately .0036 ml/sec.

12. The method of Claim 10, wherein said strobe operates at approximately 60 times per second of two microsecond flash duration.

DATED this TWENTY FOURTH day of OCTOBER 1985

INTERNATIONAL REMOTE IMAGING SYSTEMS, INC.

Atorneys for the Applicant
SPRUSON & FERGUSON
"A METHOD OF OPERATING A MICROSCOPIC INSTRUMENT"

The following statement is a full description of this invention, including the best method of performing it known to us.

FIG. 1

FIG. 2

FIG. 3

FIG. 4
END