

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 May 2006 (11.05.2006)

PCT

(10) International Publication Number
WO 2006/050355 A2

(51) International Patent Classification:
G02B 26/08 (2006.01)

(21) International Application Number:
PCT/US2005/039453

(22) International Filing Date:
1 November 2005 (01.11.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/624,096 1 November 2004 (01.11.2004) US

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **JONES, David** [GB/DE]; Zwezscheweg 38, 35037 Marburg (DE). **POHL, Torsten** [DE/DE]; Huteweg 5 Niederweimar, 35096 Marburg (DE). **ECKHARD, Brockmann** [DE/DE]; Gottfried-Keller-Str. 29, 35039 Marburg (DE). **STORCK, Winfried** [DE/DE]; Wilhelmschickstr. 13, 35287 Amöneburg (DE). **KAUFMAN, Jonathan, J.** [US/US]; 112 Willow Street, Apt. 1A, Brooklyn, NY 11201 (US).

(74) Agents: **KOLAKOWSKI, William, F.** et al.; Dykema Gossett PLLC, 39577 Woodward Avenue, Suite 300, Bloomfield Hills, MI 48304 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A COMPACT PORTABLE RUGGED FLUORESCENCE MICROSCOPE SEALED AGAINST DUST AND WATER

(57) Abstract: A microscope whose construction is made from a water tight, dust sealed case where the microscope optical components are embedded in a block of machined material or attached to a shock proof frame. The case may be treated with a scratch free and chemically inert surface that may be decontaminated or sterilized. Based on light emitting diode (LED) illumination the microscope has a folded light path to be compact, thus light and easily portable. By using an especially efficient light condenser system designed for maximum use of the available light and choice of a small but powerful LED the microscope may be battery powered for long periods of time extending into days. Preset controls reduce the amount of manipulation to make operation as simple as possible. The X-Y stage is similarly simple and also dust and water protected.



WO 2006/050355 A2

A COMPACT PORTABLE RUGGED FLUORESCENCE MICROSCOPE SEALED AGAINST DUST AND WATER.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention is related to light microscopy and fluorescence microscopy systems and in particular a method to make the fluorescence microscope relatively insensitive to dust, water and shock simple to use and very portable. This will allow fluorescence diagnosis of malaria, tuberculosis, HIV/AIDS, and other diseases to be performed in the field, to reduce maintenance to a minimum and to allow rapid instruction in its use.

2. Discussion of Related Art

Most microscopes, especially fluorescent microscopes, are sensitive to dust and to environmental conditions, such as spills of salt water, which can enter the case and corrode sensitive optics and electronic parts. They are also sensitive to shocks. Thus they are not easy to use in the field or in conditions where conditions are not optimal. Many light, portable microscopes have been made over the last 100 years, designed for lightness and portability, but not to resist environmental influences or shock. Also many of these field microscopes have reduced optical power so that bacteria, for instance, may not be resolved. There is thus a crucial need for a new fluorescent microscope that is adapted for use in the field, as the impact that such an instrument could have on world health is potentially enormous.

SUMMARY OF THE INVENTION

It is accordingly an object of the present invention to provide a fluorescent microscope that is sealed and protected from water and dust.

A further object is to provide a fluorescent microscope that is protected from mechanical shock.

An additional object is to provide a fluorescent microscope that has minimal controls for optimal use in the field some or all of which may be preset, and a yet still further object is to provide a fluorescent microscope that may be powered by a battery.

One further object is to provide an x-y stage that itself is sealed and protected from water and dust.

A still further object of the present invention is to provide an x-y stage that produces x-y motion of a microscope slide through use of magnetic force.

Another object of the invention is to provide a focusing stage that does not allow for over-focusing and slide breakage.

An object of the present invention is also to provide a low cost, portable battery powered fluorescent microscope that can find extensive use in the developing world for diagnosis and management of disease, such as tuberculosis, malaria, and HIV/AIDS.

A related object is to provide a portable compact fluorescent microscope that may be decontaminated and find application in defending against bioterrorism.

The present invention is directed towards a fluorescent microscope which is protected against dust, liquid and shock. The light source is enclosed within the case and the controls are simplified and reduced so that the case has few projections and very few controls. All joints, mechanical parts, controls and optical parts are protected by seals such as gaskets and/or 'O' rings or 'X' rings so that dust and liquid cannot penetrate the case nor allow humidity to affect the optical components which are part of the microscope. The case may be made of a corrosion free metal or plastic or made from a hard metal that is further treated to provide a very hard and chemically inert surface, such as an anodising process or by a plasma coating process that can for example coat stainless steel with titanium nitride. This makes the surface of the microscope chemically inert and resistant to virtually all chemical sterilization procedures.

The present invention is a light, portable, battery powered but optically powerful fluorescent microscope that is (i) water and dust proof; (ii) incorporates a very efficient light emitting diode (LED) condenser which is specifically designed for a 50 μ m square LED which allows maximal usage of the light output coupling this into the objective. Microscopes that are difficult to set up and use are complex and require not only require long training but are also very difficult to use in the field; therefore we have simplified the controls by the use of preset alignments and minimal controls. The operators of microscopes often break glass slides by over-focusing. We have therefore introduced a safety feature to prevent over-focusing and glass slide breakage. The x-y stage of the microscope is a complex mechanical instrument where the controls are linked; thus one control always moves with the other. In contrast, we have designed a new mechanism whereby both x and y controls are fixed and the

mechanism is encased in a waterproof and dustproof box. The specimen slide holder is coupled to the x-y mechanism by magnetic force.

In a presently preferred embodiment of the invention, a single piece of suitable plastic, such as polyoxymethylene (POM), is precision machined to accept the optical components so that no post alignment is necessary. In the magnification path conventional components are used. Microscopes are made to several conventions. We describe here a microscope built for 160mm tube length optics, but other tube lengths, such as infinity-corrected optics may also be built in this way. A description may also be found in the article by D. Jones et al., LED Fluorescence Microscopy et al., Vol. 40/2 Proceedings RMS, June 2005, pp. 91-96, and which is incorporated by reference hereinto.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a drawing of a presently preferred embodiment of the invention.

Figure 2 is a schematic drawing of a presently preferred embodiment of the invention.

Figure 3 is a drawing showing an aspherical LED condenser of the present invention.

Figure 4 is a drawing showing a specimen slide holder.

Figure 5 is a drawing showing an x-y stage of a presently preferred embodiment of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

With reference to both Fig. 1 and Fig. 2, a piece of POM is precision machined using a computer controlled drilling and milling machine (CNC machine). Light enters an objective (9) and is first reflected by a front surface mirror (2) to a second front surface mirror (3) into either an eyepiece or to an imaging device such as a CCD chip or CMOS chip (14). For fluorescence, an excitation semiconductor light source or LED (7) is switched on and light passes into an aspherical LED condenser (8). After passing through the excitation filter (6) the light is further focused onto a specimen through the objective by classical epifluorescence optics. A dichromatic mirror (5) reflects short wavelength light and passes longer wavelength light into the eyepiece through a further emission filter (4).

With additional reference to Figure 3, the LED condenser is specially designed for the specific excitation LED used. The lens was designed to fulfil a large number of demands: (1) only one lens; (2) numerical input aperture as high as possible; (3) all output rays within a given solid angle; (4) fixed diameter (5) compact shape (6) no critical dimensions (e.g., thickness); (7) magnification not much higher than 4 (depends on the diameter of the light source); and (8) even illumination of the sample. Goals (1) and (6) are needed for low cost series production. With (2), the lens is able to collect a large number of photons and passes them into the output cone. Due to the fact that this application is for illumination rather than imaging, it is not necessary--in fact it is an advantage--to have all radiant rays converging in a focal plane. Some of the above constraints are in conflict with one another; for example, (1) together with (2) cannot fulfill (7). In a presently preferred embodiment of the invention, the lens design starts with (3), which is of the highest priority. Next an LED is selected with an throughput close to the solid angle and the area of the target.

It should be understood that the most efficient use of the radiant energy of the LED is to match a small area LED which is easier to match to the throughout of the target area (the exit pupil of the objective). It needs to match the boundary of the source as closely as possible to the boundary of the target in the spirit of the edge ray principle, which is discussed more fully in the article by Ries, H. and A. Rabl, "The edge ray principle of nonimaging optics," in J. Opt. Soc. Am. A, 1994. 11(10): p. 2627-2632, which is incorporated by reference hereinto. Since, for practical reasons, rotational optics are preferred, a planar source such as an LED is superior to a volume source such as gas discharge lamps because the skewness distribution is better matched to that of the target, and is described in the article by Ries, H., et al., "Performance Limitations of Rotationally Symmetric Nonimaging Devices," J. Opt. Soc. Am. A, 1997. 14: p. 2855-2862, which is incorporated by reference hereinto. We tailored an aspheric single lens aplanatic condenser following the principles outlined in the publication by G.D. Wasserman and E. Wolf, "On the theory of aplanatic aspheric systems," Proceedings of the Physical Society B, 1949. 62(2): p. 2-8, and which is incorporated by reference hereinto. The condenser images the LED into the exit pupil of the microscope objective and thereby leads to a confocal Köhler-type illumination. This has the additional advantage of homogenizing any visible structure of the LED such as the electrodes in the field of view.

It should be appreciated that the design of the present invention leads to an optically powerful microscope, one which has a high numerical aperture (N.A.). In the

presently preferred embodiments of the invention, N.A. = 0.65 at x40 magnification and N.A. = 0.8 at x60 magnification. It is understood that high N.A. leads to enhanced resolution (R) through the well known expression, $R = 0.63 \lambda / \text{N.A.}$, where λ is the wavelength. In the present embodiments of the invention, it is understood that at x40 magnification, a N.A. greater than 0.6 is to be utilized, and at x60 magnification, a N.A. greater than or equal to 0.8 is utilized.

The power emitted by the LED is another unique aspect of the design of the present invention. The power specification permits the imaging of a wide variety of target specimens, and depends on the magnification. For x10 magnification, a minimum power of 1 mW is required, for x20, a minimum power of about 5 mW is required, for x40 a minimum power of about 15 mW is required, for x60 a minimum power of 30 mW is required, for x80 a minimum power of 60 mW, and for x100 a minimum power of about 100 mW is required. In general, the expression $P = M^2 / 100$ may be used to compute the approximate minimum power required (P), where xM is the magnification.

Thus, it should be appreciated that the apparatus as disclosed herein excites a fluorescent or phosphorescent molecule of a specimen within a field of view, the molecule having a known excitation wavelength, comprising:

- (a) an LED light source capable of emitting an output light within a preselected wavelength band correlated with the excitation wavelength at a preselected power;
- (b) an electronic controller coupled to the light source for controlling the intensity of the output light;
- (c) an optical system for converting the output light into an excitation beam having pre-selected distribution of light flux suitable for simultaneously illuminating the field of view, wherein said optical system has a high numerical aperture and at least one magnification factor; and
- (d) a waterproof and dustproof case which incorporates said LED, said electronic controller and said optical system.

In the presently preferred embodiment of the invention, the focusing system has a limited travel to prevent over-travel and breakage of the glass slide, that is to protect the glass slide from breakage. To accommodate slides of different thicknesses a slide holder is used that keeps the upper surface of the specimen slide at a fixed distance from the objective, thus allowing the focus mechanism to be reduced in travel and also to be more exact. In the present embodiment of the invention, the holder

accepts standard slides of thicknesses of around 1mm (standard) up to 6mm (blood counting chambers) and slides of a standard length of 75mm and a width of 25mm.

With additional reference to Figure 3, the slide holder is constructed as follows. Two side pieces are grooved of a sufficient depth that together with the springs (5) which are held onto the side pieces by a screw (4) the slide is pushed up and located firmly onto the top of the groove. A rod (2) keeps the side pieces apart and stabilizes the construction, secured by a grub screw (3). Small magnets on the underneath of the side pieces couple the slide holder to the x-y stage mechanism described below.

With additional reference to Figure 5, an x-y stage is designed to keep all moving parts inside a sealed box for dust and water protection. As may be seen, the L shaped magnet holder is connected to both the x-mechanism (from left to right) and the y-mechanism (from front to back). The magnet holder slides along rods and along the bottom plate and top plate (not shown), which act to stabilize the mechanism, using a low friction material, which in the present embodiment is Teflon. In the presently preferred embodiment of the invention, two (2) mm diameter magnets are sufficiently strong (e.g., niobium ferro boron) to couple through the top plate to corresponding magnets on the specimen slide holder. Both top and bottom plates have a clear window for the transmission light source. Two controls turn threaded rods which are also sealed as they pass through the casing by 'O' rings. The threads of the mechanism are of a standard microscope type where a rotation of 360 degrees produces a linear motion of 3 mm.

Referring again to Figures 1 and 2, the drawings illustrate a fluorescent microscope with an epifluorescence illumination. A high power LED (8) (for instance a 472nm LED from Nichia) of more than 1mW output power) is coupled to the optical system through a custom made polymethylmethacrylate (PMMA) aspherical lens (7). An interference filter (6) for instance from Omega Optical USA, removes light higher than 505nm. A dichromatic mirror (obtainable from, e.g., Omega Optical) reflects the excitation light down towards the objective (9) (for instance a x 40 achromat NA 0.65 from Edmund Optical part number M36-133), which focuses the excitation light onto the specimen which rests on the stage (12). The specimen is focused by turning the focus wheel (11) which is also sealed by an 'O' ring (13). The fluorescence light passes back through the objective, through an emission edge filter (4) (510-690nm from Omega Optical) and is focused onto the eyepiece (1) (ES M36-130). To make the microscope more compact, the light path can be folded by mirrors (2,3). For transmitted light a second green LED (5, Nichia) is placed either in line with the optical

axis or through a classical high numerical aperture condenser, for instance from Lomo (St. Petersburg, Russia) and placed at the periphery so that the light illuminates the sample at an oblique angle. It should be understood that this configuration increases the contrast of the sample in non-fluorescence mode. Oblique lighting is also a classical fluorescence technique existing for over 60 years and may also be employed here.

To achieve a good shock-proof and shock-resistant design the microscope is constructed around a monolithic block of a metal such as aluminum or a plastic (such as polyoxymethylene, or PVC, PPS, ABS, PEEK, etc.) which is precision machined or cast with high precision in aluminum or plastic. The block contains electronics that regulate the light sources and also regulate the battery charging. An imaging chip (14), for instance the Texas Instruments (Japan) TC253SPD-30 may also be included that can be swung into the light path to record the image (14). Alternatively the mirror (2) may be swung out and a C mount adapter used for camera attachment through a port in the top case. In one alternative embodiment, a camera image from the microscope is transmitted wirelessly for analysis by experts at a remote (to the microscope) location.

To achieve dust and water tightness all the joints (as shown in Figure 2) are sealed with 'O' rings of neoprene or Teflon. The objective and the eyepiece are similarly sealed. Where the stage support (12) and focus (11) mechanism pass through the case, these are also sealed in the same way.

The aluminum outer case may be treated for instance by an anodizing process to reduce wear. In the case of a microscope where a tougher outer surface than aluminum is used, such as stainless steel, which is abrasion resistant and chemically inert, we use a stainless steel outer case which is treated in a plasma chamber to coat the surface with zirconium nitride, which is chemically resistant and is very hard. In this case Teflon O rings are used instead of neoprene for chemical resistance. It should be further understood that the entire microscope is sealed in order that it may be decontaminated, and thus can find application in defending against biochemical and biological agents.

CLAIMS**We claim:**

1. Apparatus for exciting a fluorescent or phosphorescent molecule of a specimen within a microscope having a field of view, the molecule having a known excitation wavelength, comprising:

(a) an LED light source capable of emitting an output light within a preselected wavelength band correlated with the excitation wavelength at a preselected power;

(b) an electronic controller coupled to the light source for controlling the intensity of the output light;

(c) an optical system for converting the output light into an excitation beam having pre-selected distribution of light flux suitable for simultaneously illuminating the field of view, wherein said optical system has a high numerical aperture and a magnification factor; and

(d) a waterproof and dustproof case which incorporates said LED, said electronic controller and said optical system.

2. The apparatus of claim 1 wherein said optical system includes an aspherical condenser.

3. The apparatus of claim 1 which includes a fully-enclosed x-y stage.

4. The apparatus of claim 2 which includes a fully-enclosed x-y stage.

5. The apparatus of claim 3 wherein said x-y stage is coupled to a specimen slide through magnetic force.

6. The apparatus of claim 4 wherein said x-y stage is coupled to a specimen slide through magnetic force.

7. The apparatus of claim 1 wherein said semiconductor light source is powered by a battery.

8. The apparatus of claim 1 wherein said x-y stage maintains a fixed distance between an upper surface of a specimen slide and from an objective, whereby said slide is protected from breakage.

9. The apparatus of claim 1 wherein said case is made of a corrosion free metal or plastic.

10. The apparatus of claim 9 wherein said metal is further treated to provide a very hard and chemically inert surface.

11. The apparatus of claim 10 wherein said further treated is an anodizing process.

12. The apparatus of claim 11 wherein said further treated is a plasma coating process.

13. The apparatus of claim 1 wherein said microscope may be sterilized.

14. The apparatus of claim 1 wherein said microscope may be decontaminated.

15. The apparatus of claim 1 wherein said optical system includes means for recording an image of said specimen.

16. The apparatus of claim 15 wherein said means for recording further includes means for wirelessly transmitting said image.

17. The apparatus of claim 1 wherein said case is formed from a monolithic block, whereby said microscope is shock-proof.

18. The apparatus of claim 1 wherein said magnification factor is x10 and said preselected power is about 1 mW.

19. The apparatus of claim 1 wherein said magnification factor is x20 and said preselected power is about 5 mW.

20. The apparatus of claim 1 wherein said magnification factor is x40 and said preselected power is 15 mW.

21. The apparatus of claim 1 wherein said magnification factor is x60 and said preselected power is 30 mW.

22. The apparatus of claim 1 wherein said magnification factor is x80 and said preselected power is 60 mW.

23. The apparatus of claim 1 wherein said magnification factor is x100 and said preselected power is 100 mW.

24. The apparatus of claim 1 wherein said magnification factor is xM and said preselected power is about $M^2/100$ mW.

25. The apparatus of claim 1 wherein said numerical aperture is greater than or equal to 0.65.

26. The apparatus of claim 1 wherein said magnification factor is x40 and said numerical aperture is greater than or equal to 0.80.

27. The apparatus of claim 1 wherein said waterproof and dustproof case is protected by seals.

28. The apparatus of claim 27 wherein said seals include gaskets.

29. The apparatus of claim 27 wherein said seals include O-rings.

30. The apparatus of claim 27 wherein said seals include X-rings.

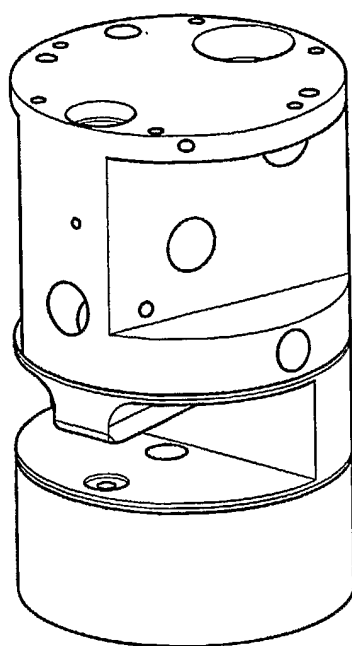


Figure 1

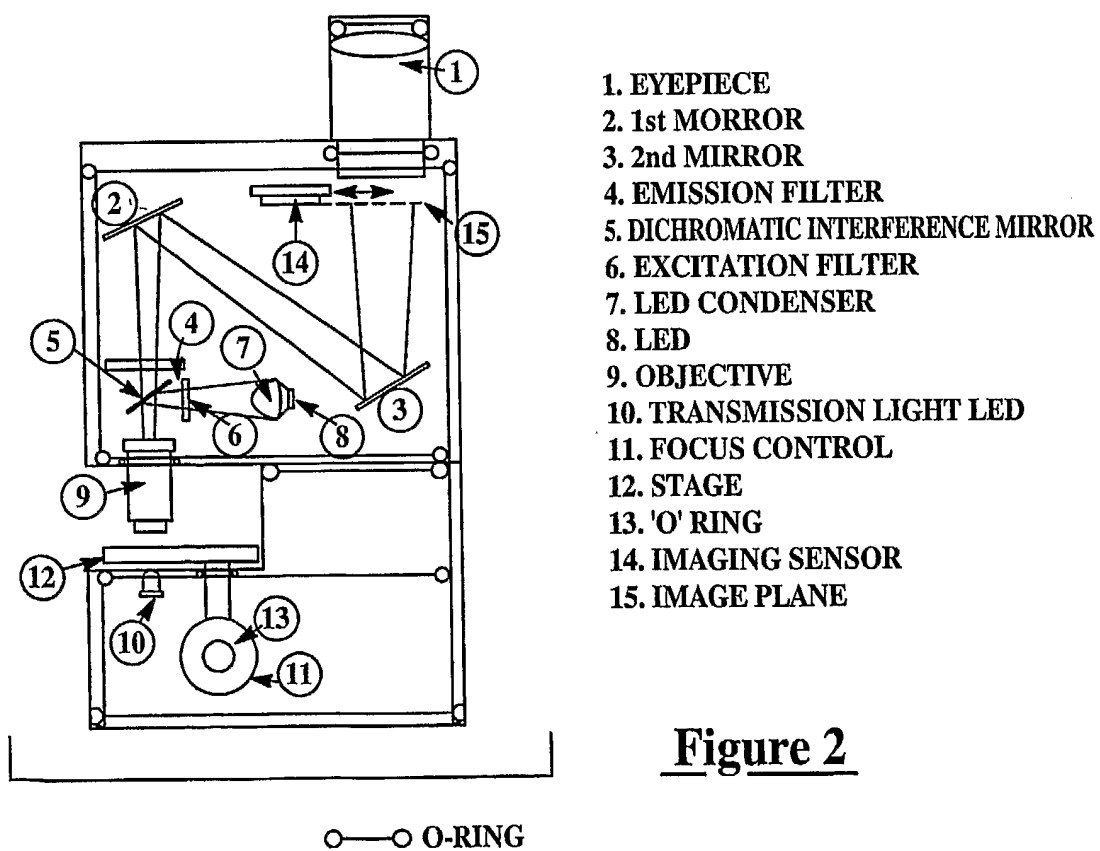


Figure 2

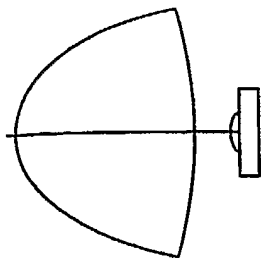


Figure 3

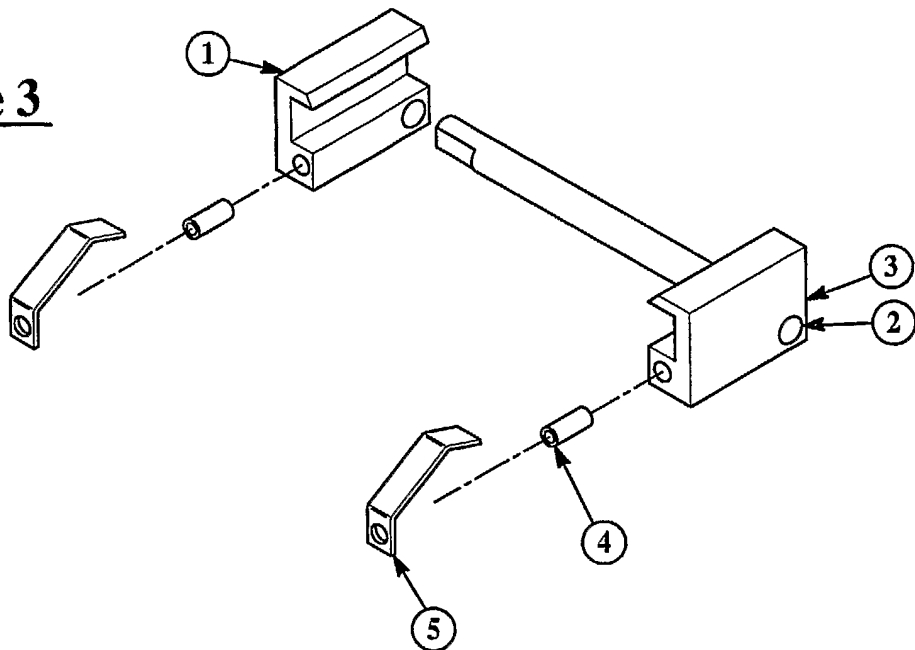


Figure 4

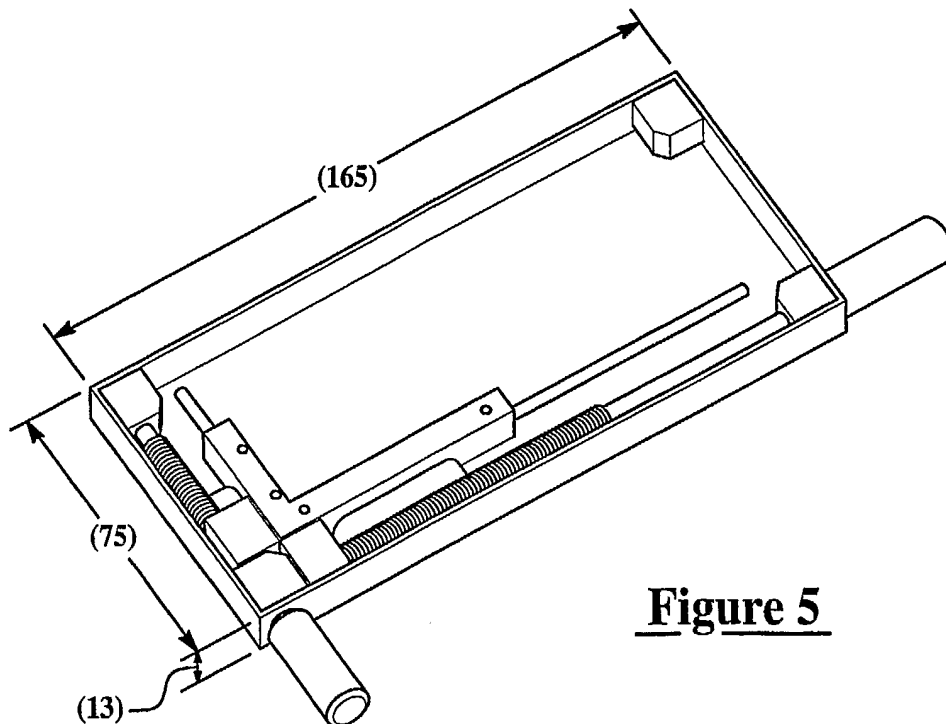


Figure 5