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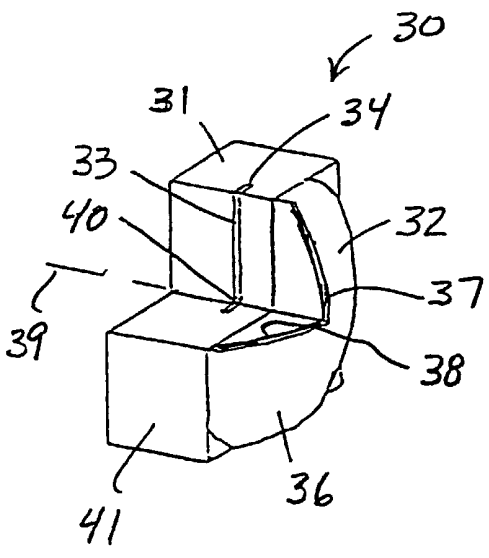
(54) Title: OPTICAL ELEMENT FOR FLOW CYTOMETRY



(57) Abstract: An optical element for use in a flow cytometer is provided that comprises a base member having a portion defining a flow channel, and an aspheric concave reflective surface having an optical axis intersecting the flow channel. Some light scattered or emitted by cells or other particulate material in a sample stream passing through the flow channel is collected and reflected by the aspheric concave reflective surface to form an image at an image plane external to the optical element.

OPTICAL ELEMENT FOR FLOW CYTOMETRY

Field Of The Invention

The present invention relates to an optical element for collecting and projecting light scattered or emitted by the passage of particulate material through a beam of light. More specifically, the present invention relates to an optical element that combines the functions of a fluid flow delivery system, and a high-efficiency light gathering optic into a compact unit for use in a flow cytometer.

Background Of The Invention

Flow cytometers are used in biomedical research for counting, characterizing and sorting cells and other particulate material in a fluid. A typical flow cytometer works by passing individual cells or particles in a flow stream through an excitation site, where they are intersected by a high-intensity light beam or laser beam. If a particle is present, light from the beam is scattered by the particle, collected, and directed to a sensor that measures properties of the light to count and characterize the particles. Additional measurements may be made by staining the cells with fluorescent dyes or by reacting the cells

with fluorescent reagents that are biologically reactive with the cells, using the laser beam to induce fluorescence, and using the sensor to detect properties of the light emitted due to fluorescence to characterize various properties of the cells.

Flow cytometers are widely known and have been used for more than two decades. Numerous improvements and modifications have been developed that enable measurement of a variety of cellular properties, and enhance the accuracy and efficiency of the measurements. Also, many advancements in improving optical efficiency of the light gathering optics of flow cytometers have been achieved.

Some previously known systems improve the efficiency of light gathering by placing the excitation site within an ellipsoidal reflective chamber (typically referred to as a flow cell), or within a reflective chamber formed by a combination of spherical, ellipsoidal and other conic reflectors. These previously known systems typically are constructed so that the excitation site is located at a first focal point of the optical system, and light scattered or emitted by the sample is collected by a sensor positioned at a second focal point of the optical system.

For example, U.S. Patent No. 3,946,239 to Salzman et al., U.S. Patent No. 4,188,543 to Brunsting et al., U.S. Patent No. 4,189,236 to Hogg et al., and U.S. Patent No. 4,871,249 to Watson all describe systems that use enclosed reflective chambers. The chambers include windows for illuminating the sample stream and for light collection, and typically also have openings for introducing and removing the sample stream.

Although the light collection efficiency of the foregoing devices may be high, enclosed reflective chambers collect light scattered in all directions and direct the light to the focal spot by different paths using different numbers of surfaces, and thus do not form a true image of the sample. In addition, such previously known systems typically require additional optics to collect and redirect light in a manner useful for spectral processing and detection.

U.S. Patent 3,989,381 to Fulwyler, describes an optical chamber or flow cell having a spherical reflective portion for use in a flow cytometer. Instead of enclosing the excitation site within a reflective chamber to collect light scattered in all directions, however, Fulwyler uses a spherical reflective portion to reflect some of the scattered light, and direct it back through the sample towards a collection lens. Light passing through the collection lens is refracted into parallel beams, and is directed towards a photodetector. Thus, the spherical reflective surface functions as a light gathetering enhancer, rather than as an imaging optical element.

Refractive light collection systems, such as that described in the foregoing Fulwyler patent, may cause optical and chromatic aberrations. Such aberrations may be particularly severe near the edges of the lens, or wherever a significant refractive discontinuity is encountered. Such refractive discontinuities in the optical path also may reduce the light collection efficiency of the system. Systems that use refractive elements typically attempt to reduce such aberrations by filling the flow cell with a fluid having an index of refraction similar to the index of refraction of the material from which the

collection lens is formed, thereby decreasing the number of refractive discontinuities in the optical system. Optical aberrations may also be reduced by passing light through refractive boundaries or discontinuities at normal or near-normal angles.

In view of the foregoing, it would be desirable to provide an optical element for use in a flow cytometer that provides high-efficiency light gathering, and that produces high-quality images with reduced chromatic aberration.

It would further be desirable to provide an optical element for use in a flow cytometer that combines a flow channel for a sample stream, an excitation site, and a reflective light collection and imaging element into a single compact unit.

Summary Of The Invention

It is an object of the present invention to provide an optical element for use in a flow cytometer that provides high-efficiency light gathering, and that produces high-quality images with reduced chromatic aberration.

It is also an object of the present invention to provide an optical element for use in a flow cytometer that combines a flow channel for a sample stream, an excitation site, and a reflective light collection and imaging element into a single compact unit.

These and other objects of the present invention are accomplished by providing an optical element having a base portion defining a flow channel, and a mirror portion defining an aspheric concave reflective surface. The mirror portion preferably comprises a convex aspheric shape coated with a layer

of reflective material to form an aspheric concave reflective surface relative to the flow channel. The aspheric concave reflective surface preferably has a relatively large collection angle to provide high-efficiency light collection and to direct collected light to form an image an a plane external to the optical element.

The optical element is constructed from an optically clear material, and the base and mirror portions of the optical element may be integrally formed. Alternatively the base and mirror portions may be assembled from separate components, for example, a plano-convex element, reflectively coated on the aspheric surface, and a base element.

Where separate components are employed, the components are optically coupled with a coupling material having a refractive index matching the components joined, so as to reduce refractive discontinuities in the optical path. In addition, the sample stream preferably comprises a fluid selected to have an index of refraction that closely matches the index of refraction of the material from which the optical element is constructed.

Methods of using an optical element constructed in accordance with the present invention also are provided.

Brief Description Of The Drawings

The above and other objects and advantages of the present invention will be apparent upon consideration of the following detailed description, taken in conjunction with the accompanying drawings, in which like reference characters refer to like parts throughout, and in which:

FIG. 1 is a block diagram of a previously known flow cytometer;

FIGS. 2A and 2B are, respectively, a cutaway perspective view and a side view of an illustrative optical element constructed in accordance with the principles of the present invention;

FIG. 3 illustrates a ray trace resulting from use of the optical element of FIGS. 2; and

FIG. 4 is an exploded view of an alternative embodiment of an optical element of the present invention.

Detailed Description Of The Invention

Referring to FIG. 1, a block diagram of a previously known flow cytometer is described. Such a system is described, for example, in U.S. Patent No. 3,989,381 to Fulwyler, which is incorporated herein by reference. Flow cytometer 10 generally includes pressurized sheath fluid tank 11 coupled via inlet line 12 to sheath flow tube 13, pressurized sample fluid tank 14 coupled via inlet line 15 to sample tube 16, flow cell 17, light source 18, light collector 19, light detector 20, processing circuit 21, and waste tank 22. Light source 18 may comprise, for example, one or more lasers having different wavelengths, depending upon the intended application of flow cytometer 10.

A sample to be analyzed or sorted by flow cytometer 10, generally cells or other particulate material, is mixed with saline solution or distilled water in sample tank 14 to form a sample fluid. The sample may be treated with stains, fluorescent reagents or dyes to provide emissions in predetermined wavelength regions when subjected to predetermined

types of illumination. The sample fluid is injected under pressure into sample tube 16.

Sheath fluid, which generally comprises the same fluid used to form the sample fluid, is injected into sheath flow tube 13 and forms an annular flow coaxial with the sample fluid. Because the sheath fluid travels past the exit of sample tube 16 at much greater velocity than the sample fluid, the sheath fluid entrains the slower moving sample fluid. As the sample fluid attains the velocity of the surrounding sheath fluid, the diameter of the sample fluid stream generally is reduced to a diameter on the order of the thickness of a single cell or particle suspended in the sample fluid.

The resulting sample stream, comprising the reduced diameter sample fluid coaxially surrounded by the sheath fluid, passes through flow cell 17, where it is illuminated by beam 24 generated by light source 18. Beam 24 intersects the sample stream at excitation site 23. As described in the above-incorporated Fulwyler patent, flow cell 17 may be filled with quiescent sheath fluid through which the high-velocity sample stream passes with relatively little loss of sample material. The sample stream then passes into waste tank 22.

Flow cell 17 may include one or more spherical reflective elements that direct light from the excitation site to light collector 19. When a cell or other particulate material in the sample stream passes through the beam, the light is scattered. Alternatively, the cells in the sample may have been dyed using a fluorescent dye or a fluorescent reagent, causing them to fluoresce when the cells pass through the beam.

Some of the scattered or emitted light is reflected by the reflective interior of flow cell 17 toward light collector 19, typically a lens or other optical system, which focuses the collected light onto light detector 20. A spectral discriminating device, typically an optical filter or spectrally dispersing device 19a is placed in the optical path before the detector 20 to limit the light incident on the detector to the desired wavelength regions. Light detector 20 produces a signal indicative of properties of the detected light, and sends the signal to processing circuit 21, typically a microcomputer programmed to function as a data recorder. Processing circuit 21 may be used to count or characterize the cells or other particulate material in the sample stream.

Once the sample stream exits flow cell 17, it typically is collected in waste tank 22. Alternatively, with the addition of additional components that are not shown, but well-known in the art, system 10 may redirect some of the sample stream into a sorting chamber (not shown), based on the characterization of a sample obtained when the sample passed through flow chamber 17. Accordingly, flow cytometer may be employed for sorting cells or other particulate material having certain characteristics, as is known in the art.

Referring now to FIGS. 2A and 2B, a perspective view of optical element 30 constructed in accordance with the principles of the present invention is described. In FIG. 2A, an upper-right quadrant has been cut away to more clearly reveal internal structure of the optical element. Optical element 30 constitutes an aspheric reflective imaging viewing orifice and may

be substituted in place of flow cell 17 in flow cytometer system 10 of FIG. 1.

Optical element 30 preferably comprises base portion 31 and mirror portion 32. Base portion 31 comprises an optically clear material having a portion defining flow channel 33 having inlet 34 and outlet 35. Mirror portion 32, also preferably formed from an optically clear material, includes convex aspheric portion 36. Convex aspheric portion 36 is coated with thin layer 37 of a broadband reflecting material, such as aluminum, a broadband dielectric interference stack, or gold, which is deposited using techniques that are per se known, for example, by vapor deposition.

When coated with reflective material, convex aspheric portion 36 forms aspheric concave reflective surface 38 having optical axis 39. Excitation site 40 is located within flow channel 33 along optical axis 39 of aspheric concave reflective surface 38. In operation, a beam of high-intensity light is directed through lateral face 41 of base portion 31 to intersect the sample stream passing through flow channel 33 at excitation site 40.

Optical element 30 preferably may be manufactured as a single compact monolithic unit, and preferably comprises a transparent optical material, such as laser grade fused silica, quartz, or an optical grade plastic or appropriate optical glass with low base refractive index or high dispersive power ("ABBE value"). Monolithic construction of optical element 30 facilitates easy assembly and replacement of the optical system in a flow cytometer, and results in an optical system that is significantly more compact than the optical systems used in a previously known flow cytometers.

High optical collection efficiency is achieved by refractive matching of the fluid passing through flow channel 33 with the transparent material from which optical element 30 is constructed. In addition, aspheric concave reflective surface 38 preferably has a relatively large collection angle (i.e. numerical aperture greater than 1.0) and covers an area such that light is collected from a cone with an apex angle of 90° or more relative to excitation site 40.

Accordingly, assuming light scattered or emitted from the cells or other particulate material within the sample stream has an isotropic distribution, a collection angle of 90° will result in approximately 20% of the total light emitted being collected by aspheric concave reflective surface 38, and used to form an image. Because aspheric optics are used to form reflective surface 38, near diffraction-limited performance is provided for on-axis operation, despite the large collection angle.

Optical element 30 may be inserted in the system of FIG. 1 in place of flow cell 17. In operation, the sample stream, either with or without an outer annulus of sheath fluid, enters optical element 30 through inlet 34. To minimize the optical aberrations, the sample stream completely fills flow channel 33. The sample stream exits optical element 30 through outlet 35.

As illustrated in FIG. 3, laser 42 generates focused excitation beam **L**, which is directed through lateral face 41 of base portion 31 orthogonal to flow channel 33. Excitation beam **L** intersects and illuminates the sample stream at excitation site 40.

As a cell or other particulate material passes through excitation site 40, illumination by excitation beam **L** causes scattered emitted light, such as fluorescence, phosphorescence and/or other types of emission to occur, depending upon the dyes, stains or reagents used in the sample stream, and the types of illumination employed.

Light scattered or emitted from the cell or particular material forms light rays **A** that strike aspheric concave reflective surface 38 of mirror portion 32. Aspheric concave reflective surface 38 reflects some of light rays **A** to form reflected rays **A'**. Rays **A'** are focused by aspheric concave reflective surface 38 to generate image **I** at image plane **P**, external to optical element 30. Image **I** may be detected using any of a variety of known photodetection means, and may be used for spectral processing and detection, or other measurements as is known in the field of flow cytometry.

In addition, because unfocused light falling on image plane **P** may represent a source of potentially interfering background noise, an apertured screen may be placed just before the image plane in alignment with optical axis 39 to mask the detector from such unfocused light. Applicant expects that contributions to the light impinging on a photodetector may be reduced, for example, to less than 0.2%, by using such an apertured screen.

Advantageously, the reflective optics used to collect, redirect, and generate the image in the optical element of the present invention are expected to introduce little or no chromatic aberrations. Although the light travels through refractive materials

(i.e. the optical element), and encounters boundaries having significant refractive discontinuity (e.g. when the light exits the optical element), chromatic aberration is expected to be insignificant, because the light crosses such boundaries at near normal angles. Additionally, since the fluid that fills flow channel 33 preferably has its refractive index matched to that of the material of optical element 30, the number of refractive discontinuities encountered is reduced.

Referring now to FIG. 4, an exploded view of an alternative embodiment of an optical element constructed in accordance with the present invention is described. Optical element 50 comprises two separate components: aspheric plano-convex element 52 having thin layer 53 of reflective coating disposed on aspheric convex surface 54 to form aspheric concave reflective surface 55; and base cuvette 56 defining flow channel 56. Aspheric plano-convex element 52 and base cuvette 56 preferably are constructed of the same optically clear material, and preferably are coupled together using layer 57 of a suitable optical adhesive or gel that closely matches the refractive index of the material from which components 52 and 56 are constructed.

While manufacturing optical element 50 from separate components may introduce assembly tolerance issues resulting in slight losses in optical efficiency and image quality, optical element 50 may be much easier to manufacture. In addition, aspheric plano-convex elements 52 having different curvatures may be coupled to base cuvette 56, depending upon the intended application of the flow cytometer. It will of course be apparent to one skilled in the art that the optical element of the present invention may be constructed in

other ways. For example, an optical element that is split at the position of the flow channel may provide some additional benefit in ease of manufacture.

While preferred illustrative embodiments of the present invention are described above, it will be evident to one skilled in the art that various changes and modifications may be made without departing from the invention. It is intended in the appended claims to cover all such changes and modifications which fall within the true spirit and scope of the invention.

What Is Claimed Is:

1. An optical element comprising:

a base member including a portion defining a flow channel; and

an aspheric concave reflective surface having an optical axis that intersects the flow channel to define an excitation site, the aspheric concave reflective surface optically coupled to the flow channel to generate an image at an image plane.

2. The optical element of claim 1, wherein the aspheric concave reflective surface comprises:

a mirror portion having an aspheric convex surface; and

a layer of reflective material disposed on the aspheric convex surface.

3. The optical element of claim 1, wherein the flow channel further comprises an inlet disposed on a first lateral face of the base member and an outlet disposed on an opposing lateral face of the base member.

4. The optical element of claim 1, wherein the base member defining the flow channel and the aspheric concave reflective surface are integrally formed.

5. The optical element of claim 2, wherein an optical adhesive is used to optically and mechanically couple the base member to the mirror portion.

6. The optical element of claim 2, wherein an optical gel is used to optically couple the base member to the mirror portion.

7. The optical element of claim 1, wherein the aspheric concave reflective surface has a collection angle of at least 90°.

8. The optical element of claim 1, wherein the optical element has a numerical aperture greater than 1.0.

9. The optical element of claim 1, wherein the optical element is constructed of material selected from a group consisting of laser grade fused silica, quartz, an optical grade plastic and an optical grade glass.

10. An optical element comprising:

a base member having a portion defining a flow channel;

an aspheric plano-convex portion optically coupled to the base member; and

a layer of reflective material disposed on the aspheric plano-convex element to form an aspheric concave reflective surface, the aspheric concave reflective surface having an optical axis that intersects the flow channel to define an excitation site.

11. The optical element of claim 10 wherein the base member and the aspheric plano-convex element are integrally formed.

12. The optical element of claim 10 wherein the base member and the aspheric plano-convex element are optically coupled using a material selected from a group consisting of an optical adhesive and a gel.

13. The optical element of claim 10, wherein the aspheric concave reflective surface has a collection angle of at least 90°.

14. The optical element of claim 10, wherein the optical element has a numerical aperture greater than 1.0.

15. The optical element of claim 10, wherein the aspheric concave reflective surface generates an image at a location external to the optical element.

16. The optical element of claim 10, wherein the optical element is constructed from a material selected from a group consisting of laser grade fused silica, quartz, an optical grade plastic and an optical grade glass.

17. A method of characterizing particles in a sample stream comprising:

providing an optical element comprising a base member having a portion defining a flow channel and an aspheric concave reflective surface optically coupled to the flow channel;

passing the sample stream through the flow channel;

illuminating the sample stream at an excitation site to generate scattered emitted light;

collecting the scattered emitted light using the aspheric concave reflective surface;

redirecting the scattered emitted light collected by the aspheric concave reflective surface to generate an image; and

detecting the image at the image plane to characterize the particles.

18. The method of claim 17 wherein passing the sample stream through the flow channel further comprises providing a sheath fluid having an index of refraction substantially equal to an index of refraction of a material from which the optical element is formed.

19. The method of claim 17, wherein redirecting the scattered light collected by the aspheric concave reflective surface to generate an image further comprises generating the image at an image plane at a location external to the optical element.

20. The method of claim 17 wherein collecting the scattered light using the aspheric concave reflective surface further comprises collecting scattered light from a cone-shaped region having an apex angle of at least 90° relative to the excitation site.

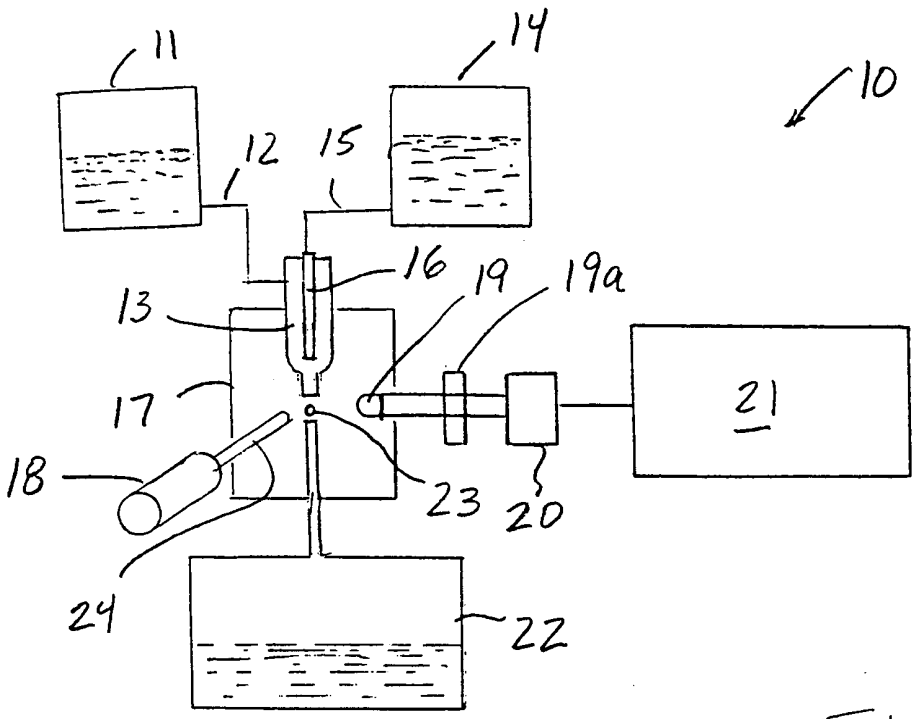


FIG. 1
(PRIOR ART)

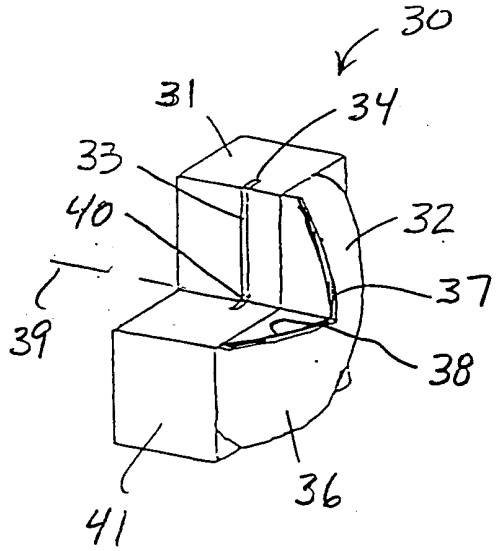


FIG. 2A

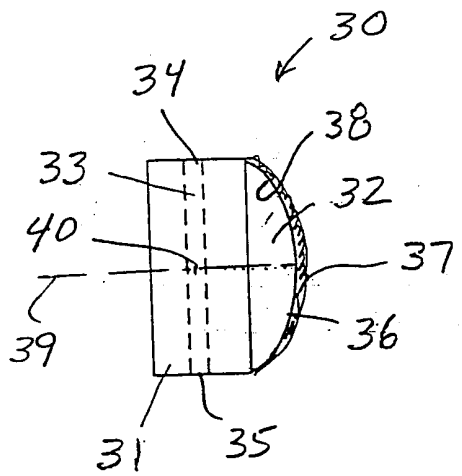


FIG. 2B

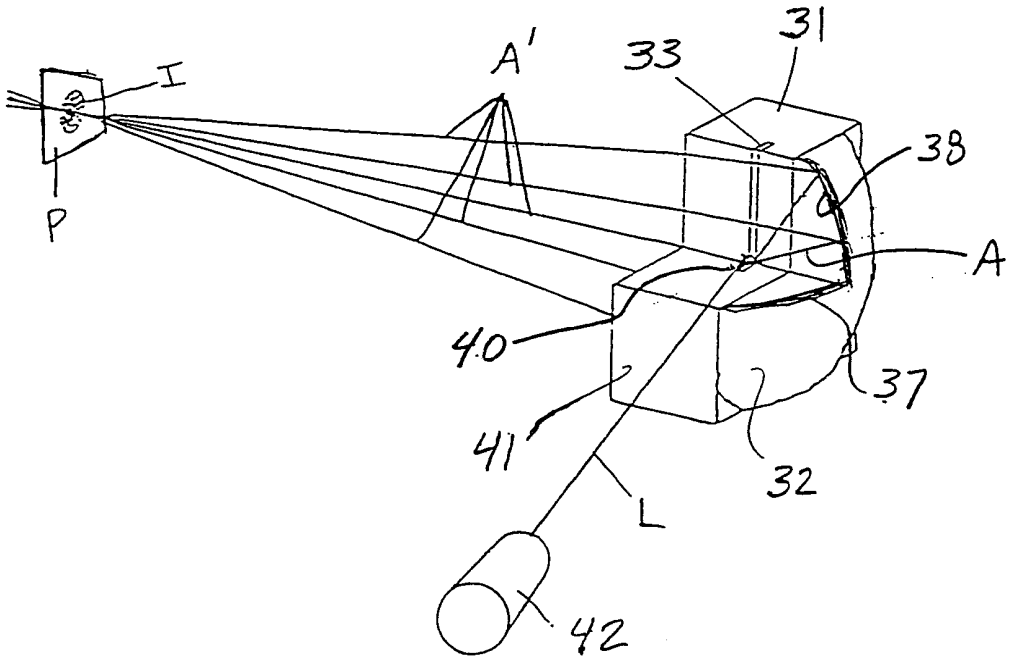


FIG. 3

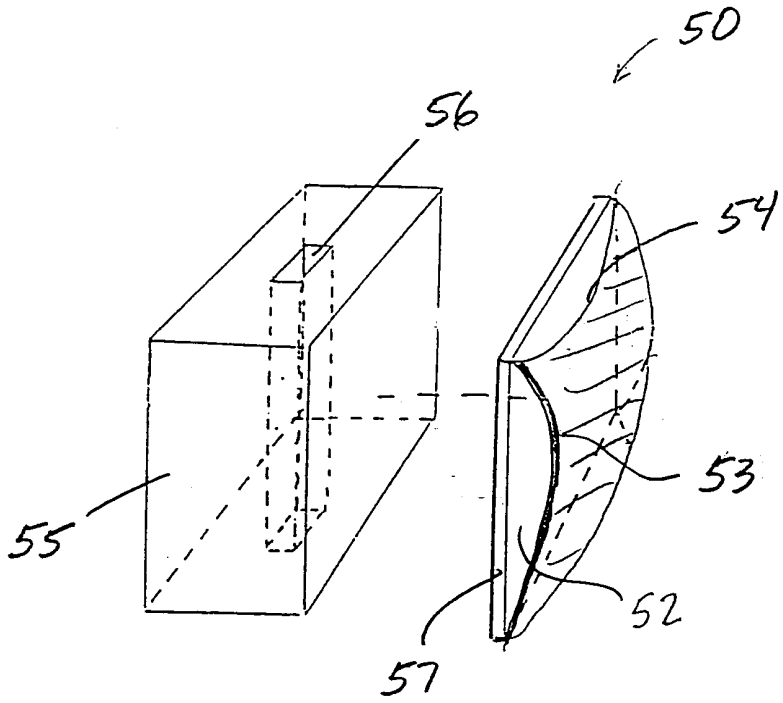


FIG. 4