

tion of optics elements includes an adjustably mounted diverging lens 12 and a high convergence lens 23.

Lens 12 is adjustable in any of three dimensions (x, y, z) by manipulating adjustable mount 13. It is important that lens 12 expand that spot size of light beam 11 to cover a substantial area on the surface of lens 23. As shown in FIG. 1, diverging light beam 14 impinges on a large portion of the facing surface of lens 23 so that relatively high intensity of beam 14 fills the aperture of lens 23. In order to create the forces required for operation of the single-beam gradient force optical trap, it is desirable that lens 23 be capable of focusing to a spot size less than λ approaching $\lambda/2$. In an example from experimental practice, lens 23 is a strong or high convergence water immersion microscope objective lens having a numerical aperture of approximately 1.25 (measured in water), wherein the numerical aperture is defined as the refractive index for the medium multiplied by the sine of the half angle covered by the converging light beam. Element 24 depicts the liquid (water or oil) in which lens 23 is immersed for improved optical coupling into cell 25.

The optical trap is shown within cell 25 with particle 27 captured in the trap. Particle 27 is suspended in a liquid medium such as water, for example, which is enclosed by cell 25. Cell 25 is a transparent enclosure for enclosing the suspended biological particles or a transparent slide from which particle containing droplets can be hung. In one example, cell 25 has dimensions of 1 cm. \times 3 cm. \times 100 μ m.

The position of cell 25 is adjustable in three dimensions (x, y, z) by the use of adjustable mount 26. In practice, mount 26 is useful in locating and manipulating the biological particles.

Viewing of biological particles in the trap is accomplished directly or through the use of a monitor. While other types of viewing such as viewing directly in cell 25 are possible, it is an added feature of the present invention that the viewing is accomplished through the same lens objective which simultaneously creates the optical trap.

Illumination for viewing is provided by visible light source 29 and is projected through converging lens 28 onto the particles in the field of view. High resolution viewing occurs with the aid of lens 23 through which the visible light passes toward either the eyepiece 22 or the monitor 18. For direct viewing, visible light shown as a dashed line is reflected from beam splitter 19 to microscope eyepiece 21. Infrared blocking filter 22 is placed in front of eyepiece 21 to isolate the viewing optics (viewer's eye) from back reflections from cell 25. For monitoring, the visible light passes through beam splitter 19 and is reflected from beam splitter 15 toward infrared blocking filter 17 and finally monitor 18. Infrared blocking filter 17 isolates the monitor from back reflections from cell 25.

In FIG. 2, the apparatus shown in FIG. 1 is augmented by a second infrared laser source and optics to create a second single-beam gradient force optical trap in cell 25. Infrared laser source 30 generates light beam 31 impinging on adjustably mounted diverging lens 32. Lens 32 causes beam 31 to emerge in a diverging pattern as light beam 34. Adjustment of lens 32 is accomplished in three dimensions (x, y, z) via adjustable mount 33. Light beam 34 is reflected by mirror 35 which coincidentally permits transmission of light beam 14. This would occur by judiciously choosing different wavelengths of operation for the separate laser sources. On

the other hand, element 35 can be realized as a beam splitter which would reflect approximately half of the light beam incident thereon and transmit the remaining half. As shown in FIG. 2, light beam 34 is converged by lens 23 to form a second trap in cell 25. Particle 36 is confined in the second trap.

While not shown, it should now be apparent to those skilled in the art that a second trap may be created in the cell by utilizing an additional set of optics including another high convergence microscope. The second trap may be created from light entering the cell on the side opposite the beam for the first trap or, for that matter, at any angle to the beam for the first trap.

Manipulation or orientation of particles is achieved by grabbing each end of a rod-like particle, for example, and moving it at will.

In operation, it is necessary to move the trapped biological particles into the viewing plane. This is carried out by adjusting the position of the diverging lens or lenses. Similarly, translation, separation or isolation of the biological particles is easily affected by adjusting mount 26 by the desired amount.

FIGS. 3 through 5 show several modes of operation for the same optical trap. FIG. 3 shows the conventional mode of operation in which the focus of the beam from lens 23 lies within cell 25 and the trapping action relies on the backward gradient component of the optical force. Depending on the size of the particles, it is possible to trap up to approximately four or five particles within the trap at one time.

Both modes shown in FIGS. 4 and 5 require less intensity than for the trap in FIG. 3. In FIG. 4, the bottom plate of cell 25 provides the backward trapping force and the gradient provides the transverse trapping force. It is possible to trap approximately twelve or more biological particles at one time. In FIG. 5, the scattering force of the focused light beam provides transverse confinement due to its inward direction; backward trapping is supplied by the bottom plate of cell 25. In the latter mode of operation, it is possible to trap significantly greater numbers of particles than for the modes shown in FIGS. 3 and 4.

Various biological particles have been isolated, confined and transported in this type of optical trap. For example, some biological particles successfully trapped are tobacco mosaic viruses (See Ashkin et al., *Science*, Vol. 235, pp. 1517-20 (1987).), yeast, E. coli bacteria, blood cells containing hemoglobin, and complex cells or parts of cells containing chlorophyll structures.

In general, the biological particles investigated do not have the regular shape of the dielectric spheres studied earlier. For example, passive, string-like organisms were trapped wherein the organism was approximately 50 μ m long and approximately 1 μ m in diameter. In the case of tobacco mosaic virus, the particles resemble a cylinder about 200 angstroms in diameter and 3100 angstroms long.

It is a significant attribute of the present invention that particle motility is preserved and reproductivity of the particles is maintained. Reproduction by trapped biological particles has been observed with offspring remaining in the trap. In other words, the optical trap permits non-destructive manipulation of biological particles at optical powers approaching several hundred milliwatts.

It should be noted that the use of infrared light results in a lower intensity trap at the focal spot for the same laser power than for traps using visible light. However,