OPTICAL TRAPPING

Diploma paper by
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ABSTRACT

In this report we present some theory and experiments on optical trapping of small dielectric particles. We have accelerated dielectric particles with a focused laser beam. A trap consisting of two focused beams has been constructed. With a single-beam trap we have captured different kinds of dielectric particles with diameters in the range from 25 nm to 620 nm. PMMA (polymetylmetaacrylat), polystyrene and silica particles have been used.

The influence of different types of microscope objectives on the performance of the trap was investigated. The stability of the single-beam trap was characterized through measurements of particle "life time" in the trap and the depth of the potential well. We have also performed measurements of the scattered light from the trapped particles.

Some successful attempts to capture biological material *in vivo* have been made using yeast cells. Finally lipid droplets and cubisoms containing medicine, a novel pharmaceutical product, was trapped indicating interesting possibilities for cell-level pharmaceutical studies.

1. INTRODUCTION

In many modern applications there has been a problem in controlling small objects. The need for a nonintrusive pair of tweezers for objects in the nm-μm range is apparent. In biological research such tweezers could be used in order to manipulate bacteria, viruses, cells and large molecules. Cell-level studies of the new pharmaceutical methods using cubisoms or liposoms to deposit medicine with delayed release is another possible field of application for a system that controls small particles.

One method to obtain such a pair of tweezers was mainly developed by A. Ashkin at Bell Laboratories, New Jersey, USA. He started in the late sixties experiments investigating the force on particles due to radiation pressure from visible laser beams. In 1970 he presented the first successful attempt to accelerate and trap small dielectric particles suspended in water.[1] By using transparent, dielectric particles he could minimize the thermal forces. These were, and still are, a great problem in optical trapping. He used a focused laser beam to accelerate a particle only by radiation pressure. In the same report he presented the first application where two oppositely directed beams created a potential well (i.e., the first double-beam trap).

During the eighties Ashkin and co-workers developed the theory behind the double-beam trap. Together with J. P. Gordon he discussed the forces which make the particles move into the center of the beam.[2,3] They showed that the gradient forces are necessary to create an optical trap.

In 1985 Ashkin and J. M. Dziedzic presented radiation trapping by alternating light beams.[4] This method proved to work where the usual cw trapping did not, (i.e., where it was not possible to obtain a gradient force.)

The great break through in the technique came in 1986. Together with J. M. Dziedzic, J. E. Bjorkholm and S. Chu Ashkin presented the first single-beam trap.[5] They showed that a backwards gradient force appeared when a strongly focused laser beam was used on dielectric particles. This force in combination with the oppositely directed force from the radiation pressure resulted in a potential well. They observed trapping in the Mie as well as in the Rayleigh scattering regions. Particles in the range from 25 nm to 10 μm were trapped.

In the following years Ashkin and Dziedzic used the single-beam trap in different applications. In a report from 1987 they present experiments in which they have used the single-beam trap to capture and manipulate viruses and bacteria.[6] The trapped organic material did not seem to have been damaged. In this report they also present some S/N measurements on the scattered light from trapped material.

Further experiments on trapping biological material *in vivo* has been made and in 1990 Ashkin together with some biologists presented a paper in which the trap was used to measure transport properties of mitocondria.[7]

In December 1990 a group of Japanese scientists submitted a report in which they presented optical trapping using semiconductor lasers.[8] They managed to capture particles and yeast cells in a beam from a diode laser at $\lambda = 1.33$ μm. These results are very intresting as the biological damage decreases in the IR-region due to the lower absorption in this wavelength range.

There are a few other scientists who have been working in the same field. In the end of 1990 a group of scientists at University of California published a report in which they present experiments using both argon and Nd:YAG lasers.[9] Besides the trapping of microspheres they have succeeded in trapping mitotic cells and sperm cells.

In Sweden, Lech Wosinski at the Institute of Optical Research in Stockholm has made some experiments using double- and single-beam traps. He has also discussed the theory of double- as well as single-beam trapping.[10]

One cannot discuss the trapping of small dielectric particles without mentioning the similar methods for trapping atoms and ions. Ashkin presented a method to trap atoms in 1978.[11] The method is closely related to the one used in optical trapping of dielectric particles and laid the foundation for today's expanding activity in, e.g., laser cooling and optical molasses.[12,13]

We started our experiments in an attempt to obtain very small controlled light sources. These are to be used in experiments on new methods for high-resolution near-field microscopy on biological objects *in vivo*.[14] The resolution of such a microscope is ultimately limited only by the size of the light source.

2. THEORY

2.1. Introduction

Dielectric particles positioned in a Gaussian laser beam will be accelerated. This phenomena is due to the transfer of momenta from the photons to the particle, when the beam is reflected and refracted at the surface of the particle. The forces are shown in Fig. 2.1.

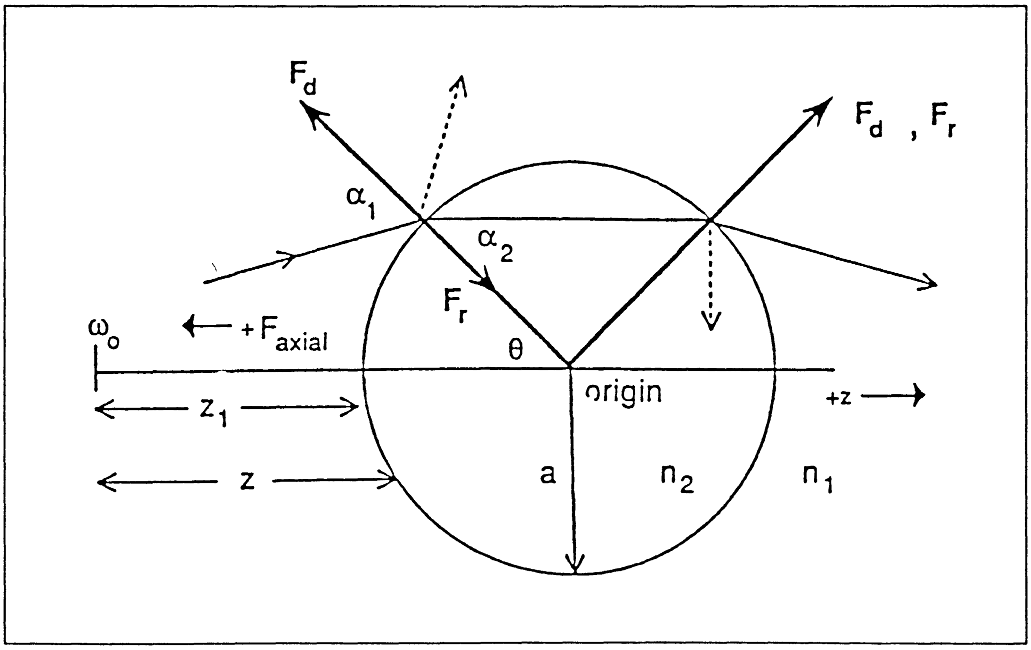


Fig. 2.1. Force components at a spherical surface. The thin solid ray is the incident beam, and continues through the sphere as the transmitted beam. The dashed rays correspond to the reflected beam at the two interfaces.(From Ref. 9.)

The part of the force that is due to reflection of photons is the integration over the sphere of[9]

$$F_r = \frac{2n_1 PR}{c} \cos(\alpha_1) \qquad (2.1)$$

and the part of the force that is due to refraction is an integration over the sphere of

$$F_d = \frac{PT}{c}((n_1^2 + n_2^2 - 2n_1n_2\cos(\alpha_1 - \alpha_2))^{1/2} \qquad (2.2)$$

where R and T are the reflectance and transmittance of the beam at the surface, n_1 and n_2 are the refractive indices of the surrounding medium and the particle, respectively, α_1 and α_2 are the incident and transmitted angles, P is the power on a small area of the sphere and c is the speed of light. The acceleration is in the direction of the beam because of the symmetry of the radiation that is hitting the particle. For very small particles, as discussed in section 2.2.2.1., the force acting on the particle can be divided into two parts. The contribution in the direction of the beam is called the scattering force while the contribution in the direction of the field gradient is called the gradient force, which correspond to the reflection and refractive forces, respectively.

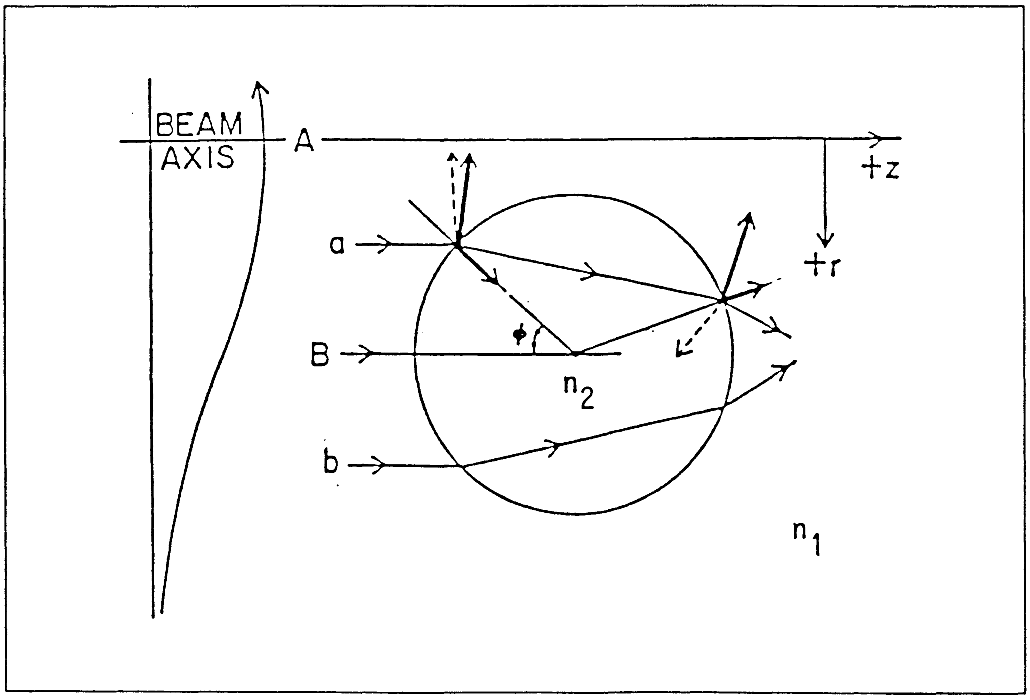


Fig. 2.2. A dielectric sphere situated off axis A of a TEM_{oo}-mode beam and a pair of symmetric rays a and b. The forces due to a are shown for $n_2 > n_1$The sphere moves toward +z and -r.(From Ref. 10.)

Thus a particle positioned in a Gaussian beam will be affected by a force in the direction of the field gradient as well as in the direction of the beam. The inward force is due to that the field is not symmetric over the particle as long as it is positioned off-axis in the beam. Thus the larger intensity incident on the part of the particle near to the axis of the beam will result in a net force pulling the particle toward the beam axis as shown in Fig. 2.2.

When focusing the Gaussian beam the refractive forces may be directed opposite to the reflection forces. Thus the particle is affected by a backward force in the direction of the field gradient when it is placed beyond the focal point in a focused beam. This forms the basis of the single-beam traps discussed in section 2.2.2.

2.2. Optical traps

Optical traps divide into two subgroups, traps using one beam and traps using several beams.

2.2.1. Double-beam traps

It has been shown that it is impossible to construct a trap only using the scattering force[3]. Furthermore, a particle in a Gaussian beam will be dragged into the centre of the beam as discussed above. This leads to the double-beam trap where two Gaussian beams are directed toward each other. Fig. 2.3. shows the principle of a double-beam trap. The two beams in such a trap are weakly focused so that the intensity becomes weaker in the direction of propagation of the beam. Because of this there will be an equilibrium point at which the intensity of the two beams is equal thus giving us axial stability. Another reason why the beams should be slightly focused is the fact that the thermal motion of the trapped particles would drive the particles out of the trap using an unfocused beam, unless a very powerful laser is used.

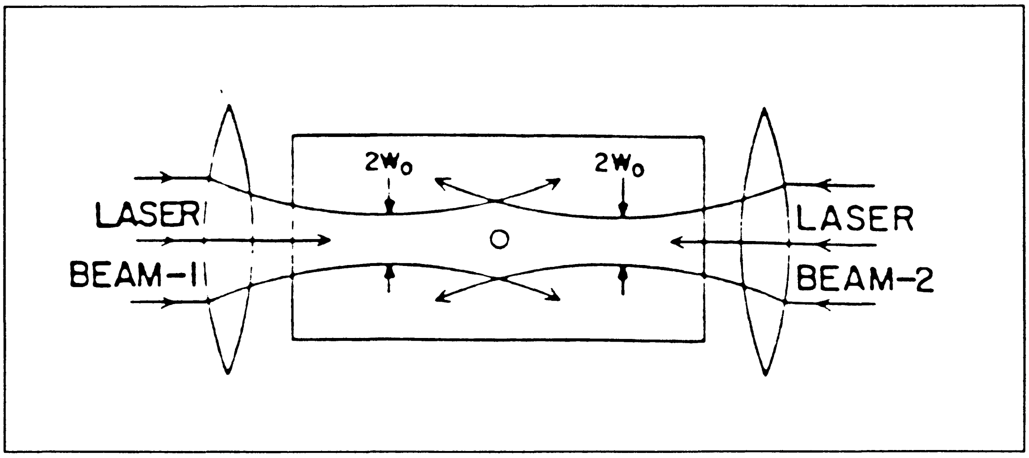


Fig. 2.3. The principle of a double-beam trap. The two beam waists are marked in the figure.(From Ref. 1.)

An alternative to the two cw beams mentioned above is to have two alternating beams, which can give stable trapping of particles under conditions where stable cw trapping is not possible. This configuration is also used to trap atoms.[11]

2.2.2. Single-beam traps

In a single-beam optical trap the axial stability is obtained by letting the scattering force of a strongly focused beam interact with the gradient force or with the gravitational force. The latter configuration has the disadvantage that if gravity is turned off or reversed the particle is driven out of the trap by the scattering force. The first configuration is the most simple trap possible.

The advantages of this trap compared with the double-beam trap is that in the latter the two beams has to be aligned along the same axis with μm precision, which may be difficult. Fig. 2.4. shows the principle for a single-beam trap.

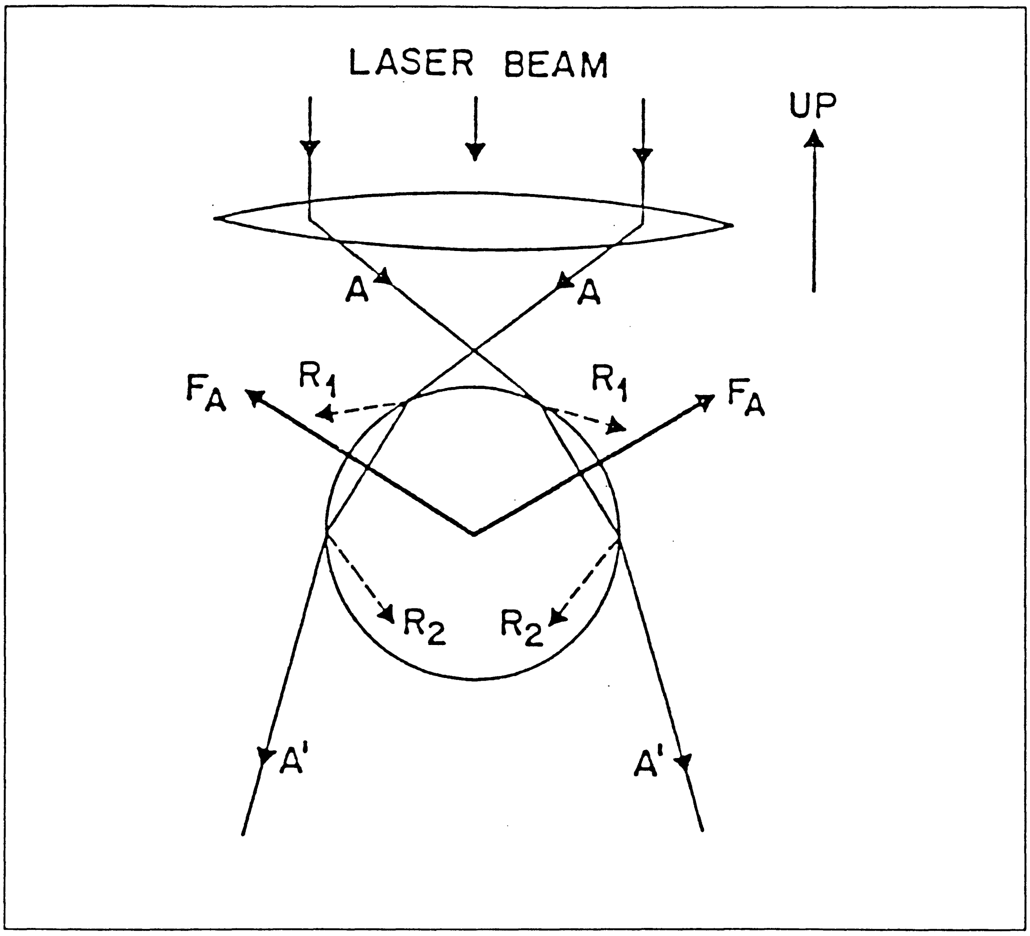


Fig. 2.4. Diagram showing the ray optics of a spherical Mie particle trapped in water by the highly convergent light of a single beam gradient force trap. The reflected beams are denoted R_1 and R_2, F_A is the total force.(From Ref. 10.)

2.2.2.1. Small particles

The theoretical description of the forces acting on a particle in a laser beam is based on either Mie theory or Rayleigh theory, depending on the size of the particle. In some of our experiments we used polystyrene latex particles in a water suspension yielding an effective index $m=(n_{latex}/n_{water})$ of about 1.25. For such particles Rayleigh theory is valid for particle diameters up to 0.2λ[15] with an error of less than 2%. For larger particles Mie theory has to be used.

For Rayleigh particles the gradient force on the particle in an electromagnetic field is the Lorentz force that can be written[16]

$$\mathbf{F}_{grad}=(\mathbf{p}\cdot\nabla)\mathbf{E}+\frac{1}{c}\cdot\frac{d\mathbf{p}}{dt}\times\mathbf{B}, \qquad (2.3)$$

where $\mathbf{p}$ is the dipole moment of the particle. Taking $\mathbf{p}=\alpha\mathbf{E}$ we can rewrite this expression as

$$\mathbf{F}_{grad}=\alpha((\mathbf{E}\ \nabla)\mathrm{E}+\frac{1}{c}\cdot\frac{d\mathbf{E}}{dt}\times\mathbf{B}, \qquad (2.4)$$

Using the identity

$$(\mathbf{E}\ \nabla)\mathbf{E}=\nabla(\frac{1}{2}\ \mathrm{E}^2)\text{-}\mathbf{E}\times\nabla\times\mathbf{E}, \qquad (2.5)$$

and Maxwell's equation

$$\nabla\times\mathbf{E}+\frac{1}{c}\cdot\frac{d\mathbf{B}}{dt}=0, \qquad (2.6)$$

we can rewrite the force as

$$\mathbf{F}_{grad}=\alpha(\nabla\ (\frac{1}{2}\ \mathrm{E}^2)+\frac{1}{c}\cdot\frac{d}{dt}(\mathbf{E}\times\mathbf{B})). \qquad (2.7)$$

Since the contribution from the second term is small, Eq. (2.7) is[16]

$$\mathbf{F}_{grad}=-\frac{n_1}{2}\alpha\nabla\mathrm{E}^2=-\frac{n_1^3a^3}{2}\frac{(m^2-1)}{(m^2+2)}4\pi\varepsilon_o\nabla\mathrm{E}^2, \qquad (2.8)$$

where m is the effective refractive index, a is the radius of the particle and ε the vacuum permeability. This is called the gradient force. It is directed in the negative z-direction.

The force in the positive direction of z is the scattering force. For an incident wave of unit intensity, the intensity of the scattered wave at a distance r from the particle is given by[16]

$$I= \frac{16\pi^4 a^6}{r^2\lambda^4}\cdot\frac{(\varepsilon_2-\varepsilon_1)^2}{(\varepsilon_2+2\varepsilon_1)^2}\cdot\sin^2\psi, \tag{2.9}$$

where ψ is the angle between the dipole and the scattering direction, a is the radius of the sphere and λ is the wavelength and ε_2 and ε_1 is the dielectric constant in the sphere and the surrounding medium, respectively. Integrating this expression over the surface of a sphere results in the total scattered power

$$P_{sca}= \frac{128\pi^5 a^6}{3\lambda^4}\frac{(m^2-1)^2}{(m^2+2)^2}I_o \tag{2.10}$$

where $m^2=\varepsilon_2/\varepsilon_1$. For Rayleigh particles in a medium of index n_1 the scattering force in the direction of the incident power is $F_{sca}=n_1{*}P_{sca}/c$. In terms of the intensity I_0 and effective index m the force is

$$F_{sca}= \frac{128 I_0\pi^5 a^6}{3\lambda^4(m^2+2)^2 c}(m^2-1)^2 n_1. \tag{2.11}$$

A small spherical particle in a focused Gaussian beam will be affected by the gradient force and the scattering force simultaneously. When the gradient force is dominating the particle will be dragged backwards and vice versa. At some spatial position beyond the focal point there is an equilibrium point where the total force acting on the particle is zero. This is the point at which the particle will be trapped if it enters the beam not too far away from the equilibrium point.

By calculating the ratio R between F_{grad} and F_{scat} as a function of distance along the z-axis we obtain the theoretical potential well. Assuming a Gaussian beam R is

$$R = \frac{F_{grad}}{F_{scat}} = \frac{3n_1^2 \lambda^6 z(m^2 + 2)}{16\pi^6 a^3 \omega^4 (m^2 - 1)\left[1 + \frac{\lambda^2 z^2}{\pi^2 \omega^4}\right]} \tag{2.12}$$

where ω is the $1/e^2$ beamwaist.

Fig 2.5 shows such a calculation for a sphere with an effective index m=1.65/1.33 and a radius of a=50 nm. The wavelength is λ=514 nm, the $1/e^2$ focal spot is ω=500 nm, z is the distance from the focus and c is the speed of light. The index m=1.65/1.33=1.24 is the effective index for polystyrene latex spheres in water. As mentioned above we can use Rayleigh theory up to a particle diameter of 0.185λ, with an error less than 2%.[17] This means that if we use m=1.24 and λ=514 nm we can treat particles smaller than 95 nm. If we can tolerate an error of 5% we can treat spheres as big as 0.267λ (i.e., a=137 nm using λ=514 nm).

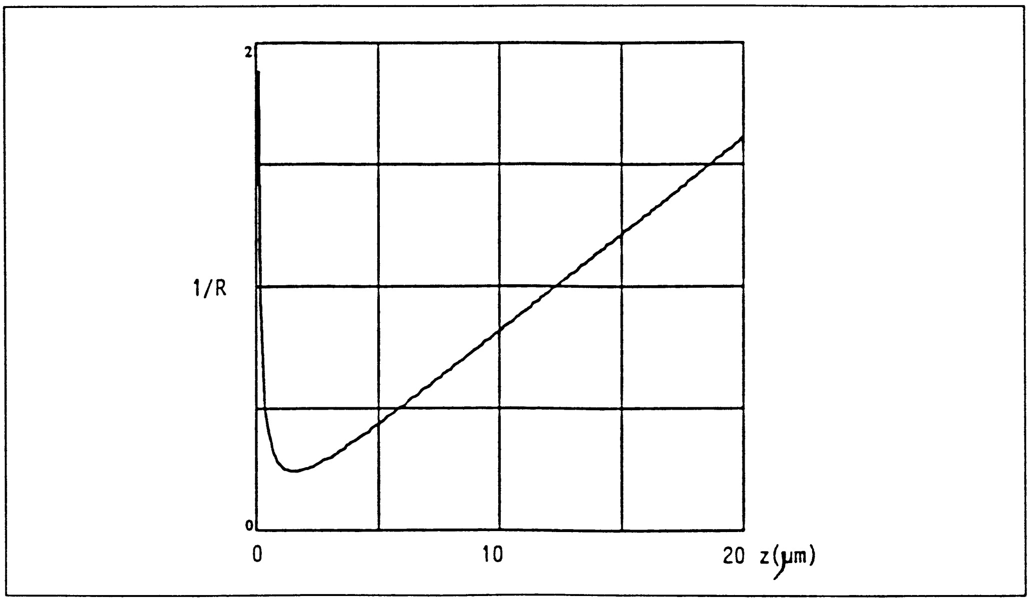


Fig. 2.5. R *as a function of the distance from the beamwaist.*

As one can see the ratio has a maximum when the distance of the particle from the focus is about 1.5 μm. The particle will be trapped at a distance from the focus, where the ratio R is equal to one. In the diagram there are two points where the ratio is one but it is only the point nearest the beam waist that is stable, because a small perturbation on the particle would drive it out of the trap if it was trapped in the other position. If we make the beam waist smaller the particle will be trapped closer to the focus and we will get a larger ratio R, and vice versa. With experimental data as above we will get a critical ratio <1 for all positions on the z-axis for beam waists > 1 μm. Thus particles may not be trapped for larger beam waists.

If we let the wavelength increase but keep the beam waist constant we will get a much larger ratio, but as the beam waist depends on the wavelength this is not possible for an arbitrary beam waist. If we instead keep the ratio between λ and ω constant, which is possible, we will still get a larger ratio with increasing wavelength, because R depends on λ and ω as λ^6/ω^4.

The stability also depends on thermal motion of the particle in the trap. If the function $B=\exp(-U/kT) \ll 1$ we have thermal stability[8], where $U=n_1\alpha E^2/2$ is the potential of the gradient force. This is equal to requiring that the time to pull a particle into the trap be much less than the time for the particle to diffuse out of the trap by Brownian motion. Calculations made show that an increase of the power from the laser gives better stability while an increase of the wavelength gives less stability. Using a 60 nm silica particle in water with an effective index of $m=1.10$ one obtains a power of 120 mW required to get $B < 1/100$ when $\lambda=514$ nm, T=300 K and the beam waist is 500 nm.

2.2.2.2. Large particles

For large particles with radius bigger than 20% of the wavelength Rayleigh theory is no longer applicable. Mie theory has to be used which result in considerably more complicated calculations. In Fig. 2.6. the results from such calculations are reviewed.[9] The magnitude of the forces due to reflection and refraction on a small spherical particle in a focused linearly polarized beam is given by the integration of Eq. (2.1) and Eq. (2.2) over the particle. By numerical integration one obtains the results shown in Fig. 2.6. where z is the distance from the beam waist to the particle surface. The axial force corresponds to the projection of F_d and F_r along the beam axis as shown in Fig. 2.1.

From Fig. 2.6a. one can see that when the focal spot gets smaller it results in a stronger gradient and thus a more powerful backward force. One can also see from Fig. 2.6b. that the distance from the trapped particle to the focal point becomes greater for greater particles, which means that the angles for the outmost rays still hitting the particle stays almost constant for growing particle diameters. Fig. 2.6c. shows that the trap becomes deeper for higher refractive index of the sphere and Fig. 2.6d. that the effective trapping range becomes greater for higher laser powers. These relations are in accordance with results for Rayleigh particles.

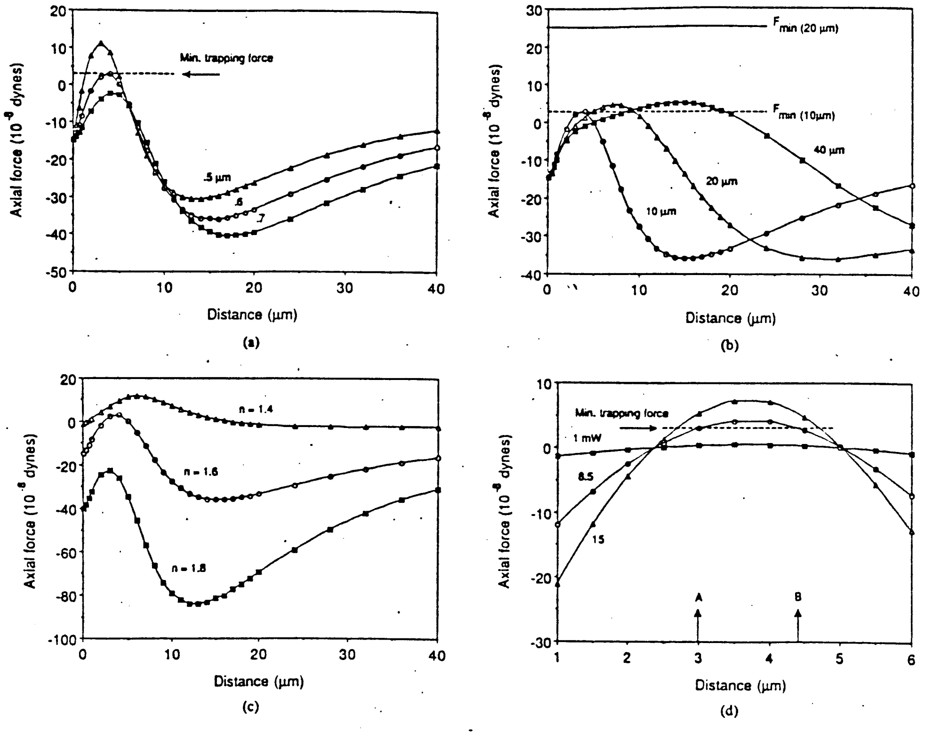


Fig. 2.6.(a) Calculation of the axial force on a microsphere for three different laser beam spot sizes as a function of the distance z. The sphere diameter is 10 *μm* $(n_2 = 1.6)$, *suspended in water* $(n_1 = 1.33)$. *The incident laserpower is* 6 *mW. (b) Calculation of the axial force on a microsphere for three different sphere diameters as a function of the distance z. The laser spot size is* 0.6 *μm. The incident laser power is* 6 *mW. The dotted line denoted by* $F_{min10um}$ *and* $F_{min20um}$ *indicate the minimum trapping force for the* 10 *and* 20 *μm spheres, respectively. (c) Calculation of the axial force on a* 10 *μm diameter microsphere for three different sphere refractive indices as a function of the distance z. The laser spot size is* 0.6 *μm. Laser beam power is* 6 *mW. (d) Axial force on a* 10 *μm diameter microsphere* $(n_2 = 1.6)$ *for three different laser power levels as a function of the distance z. The distance between A and B is defined as the effective trapping range. The spot size is* 0.6 *μm.(From Ref. 9.)*

3.3.6. Light scattering measurements

We have already mentioned that the scattered light from a trapped particle is very intense. Since the particles are to be used as small light sources we thought it would be interesting to measure the signal from a trapped particle. The arrangement is shown in Fig 3.7. The scattered light was directed into a photomultiplier with an 514 nm interference filter. The signal was monitored on a plotter.

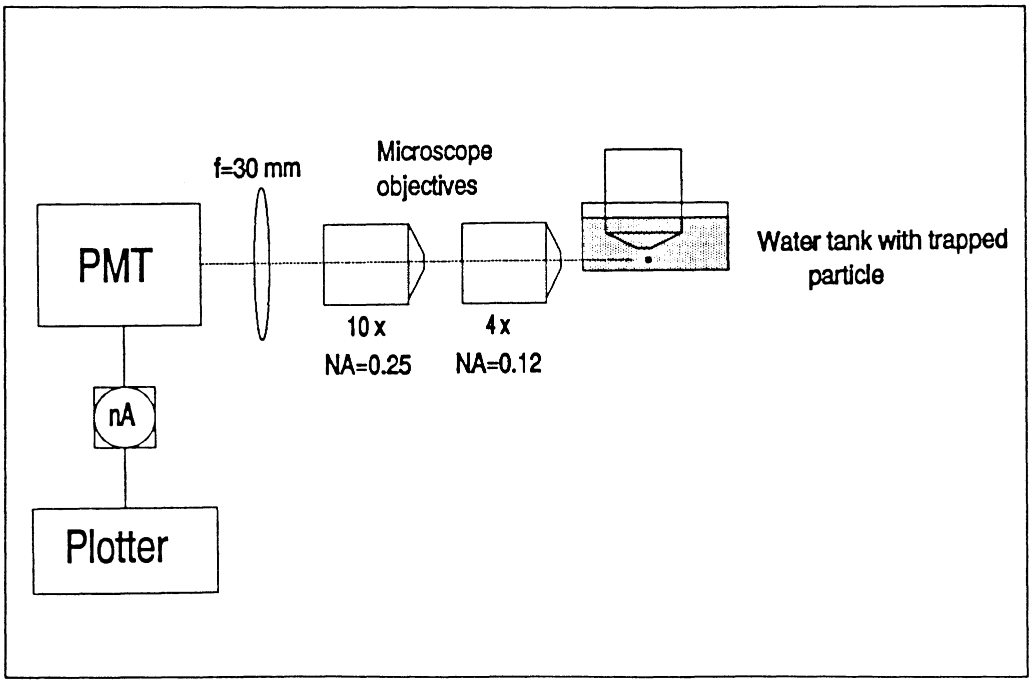
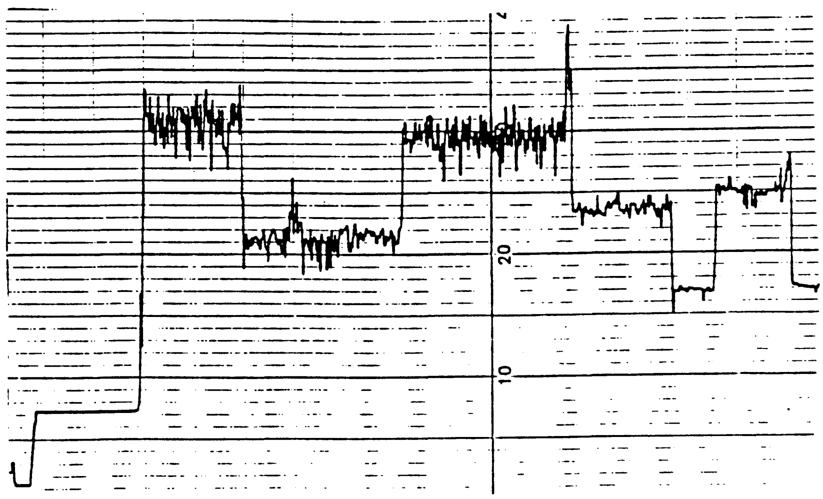
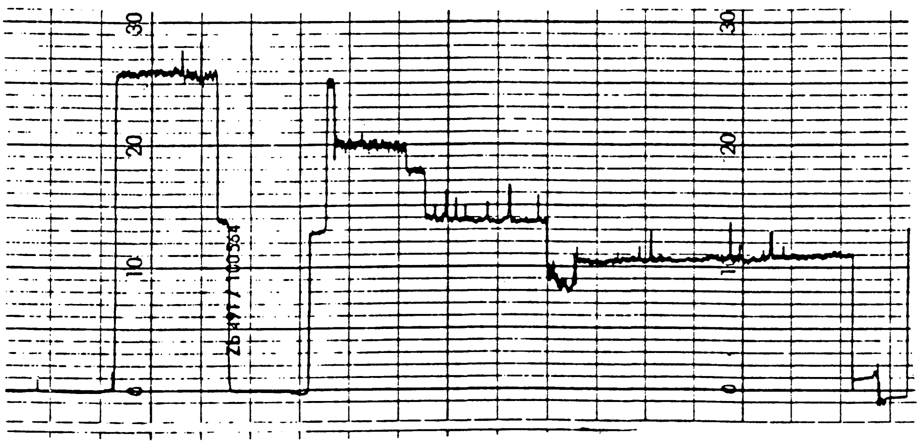


Fig. 3.7. The arrangement for light scattering measurements.

In order to lower the noise signal we used a capacitor of 47 μF which gave us a time constant of RC=47 ms. Some of the results are shown in Fig. 3.8. Observe that we could not trap the 25 nm particles stably. The 60 nm particles were trapped with the water immersion objective (NA=1.20) and the 500 nm particles were trapped with the oil immersion objective.



(a)



(b)

Fig. 3.8. The scattered signal from the trapped particles. (a) 60 nm particles. (b) 500 nm particles.

We noticed in our experiments that it is difficult to keep only one particle in the trap. It was not unusual that there were three particles or more. One solution to this problem is to keep the concentration of particles low but this will mean that the pro bability for trapping decreases since the particles move into the trap by Brownian motion. For the near-field microscopy discussed above, the S/N of the measured scattered light will be important.

The S/N values were rather encouraging. For the 60 nm particles we received a S/N value of 20:1 and for the 500 nm particles it was 100:1. When we made our measurements on the 60 nm SiO_2 particles we realized that the fluctuation in size were rather big. Since the intensity of the scattered light depends on the particle diameter to the 6^{th} power (Eq. (2.9)) even small changes in diameter result in large differences in scattered intensity.

3.3.7. Trapping of lipid droplets and cubisioms

When we began to reach the end of our experiments we thought it would be interesting to trap other things than the small dielectric test spheres used above. From the Department of Food Technology we received lipid droplets and cubisoms containing a medical substance. This types of substances are used for the administration of drugs with a slow release effect. The lipid droplets had a diameter which varied between 2 and 4 μm. The cubisoms tended to differ in size due to coagulation but we think that the diameter was about 1 to 10 μm.

The lipid droplets were the easier to control. With a power of about 160 mW we kept them trapped for about 7 minutes. The cubisoms needed only about 100 mW but they did not stay trapped very long. We noticed that the scattered light fluctuated very much. This may depend on the fact that the trapped material rotated.

Our encouraging results may be useful for studies of drug effect on single cells by actively positioning lipid droplets or cubisoms on cells with the optical tweezer.

3.3.8. Trapping of yeast cells

Our last experiment was an effort to trap biological material *in vivo*. We found that yeast cells were suitable for these tests. We solved ordinary baking yeast in water and tried to catch the cells which had a diameter of about 6 μm. At the beginning we used too much power resulting in that the cells were burned directly. When we used less power we succeeded in trapping cells without any problems. With a power of about 60 mW we managed to hold the cells for 2 minutes. This power corresponds to 30 MW/cm^2 assuming a focal spot diameter of 500 nm. When we increased the power to about 120 mW the cells exploded. Thus we have determined an approximate biological damage threshold. The scattered light from the trapped yeast cells fluctuated very much. In a microscope we could see that they were oval. Thus the intensity fluctuation we noticed probably are due to rotation of the cells in the trap.

4. CONCLUSIONS AND FUTURE WORK

We have constructed a single-beam optical trap and performed some experiments using it. We have investigated some factors which affect the stability of the trap. Experiments have been made in order to test the theory for the forces wich keep a particle trapped. Our measurements of the S/N of the scattered light from a trapped particle are encouraging. The trapped particle may be useful as small controlled light sources for near-field microscopes.

We have also shown that there is no problem in capturing lipid droplets containing medical substances. This may be useful for cell-level pharmaceutical studies. Finally we have been trapping biological material *in vivo* (yeast cells) and determined an approximate damage threshold for the cells.

Several improvements have already been made during the time we have been working with this report. Among other things experiments have been performed using alcohol instead of deionized water in the tank. Alcohol has a refractive index of 1.35 which is a little bit higher than for water (1.33). This small difference is enough to reduce the spherical aberration and the beam focus becomes less diffuse. Using alcohol we have succeeded to trap the 60 nm SiO_2 particles with a laser power of 30 mW. This value can be compared to the one in sect. 3.3.5.2. Using water the minimum trapping power was 120 mW. Even though we have not tested the other particles it is not impossible that we could reduce their minimum trapping power as well.

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