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Optical Trapping and Manipulation of Viruses and Bacteria

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Optical trapping and manipulation of viruses and bacteria by laser radiation pressure were demonstrated with single-beam gradient traps. Individual tobacco mosaic viruses and dense oriented arrays of viruses were trapped in aqueous solution with no apparent damage using ~120 milliwatts of argon laser power. Trapping and manipulation of single live motile bacteria and *Escherichia coli* bacteria were also demonstrated in a high-resolution microscope at powers of a few milliwatts.

WE REPORT THE EXPERIMENTAL demonstration of optical trapping and manipulation of individual viruses and bacteria in aqueous solution by laser light using single-beam gradient force traps. Individual tobacco mosaic viruses (TMV) and oriented arrays of viruses were optically confined within volumes of a few cubic micrometers, without obvious damage, and manipulated in space with the precision of the optical wavelength. The ability of the same basic optical trap to confine and manipulate motile bacteria was also demonstrated. We have used the trap as an "optical tweezers" for moving live single and multiple bacteria while being viewed under a high-resolution optical microscope. These results suggest that the techniques of optical trapping and manipulation, which have been used to advantage with particles in physical systems, are also applicable to biological particles. Optical trapping and manipulation of small dielectric particles and atoms by the forces of radiation pressure have been studied since 1970 (*1–4*). These are forces arising from the momentum of the light itself. Early demonstrations of optical trapping (*1*) and optical levitation (*5*) involved micrometer-size transparent dielectric spheres in the Mie regime (where the dimensions d are large compared to the wavelength λ). More recently optical trapping of submicrometer dielectric particles was demonstrated in the Rayleigh regime (where $d << \lambda$). Single dielectric particles as small as ~260 Å (*6*) and even individual atoms (*7*) were trapped with single-beam gradient traps (*8*).

Single-beam gradient traps are conceptually and practically the simplest. They consist of only a single strongly focused Gaussian laser beam having a Gaussian transverse intensity profile. In such traps the basic scattering forces and gradient force components of radiation pressure (*1, 3, 4, 8*) are configured to give a point of stable equilibrium located close to the beam focus. The scattering force is proportional to the optical intensity and points in the direction of the incident light. The gradient force is proportional to the gradient of intensity and points in the direction of the intensity gradient. Particles in a single-beam gradient trap are confined transverse to the beam axis by the radial component of the gradient force. Stability in the axial direction is achieved by making the beam focusing so strong that the axial gradient force component, pointing toward the beam focus, dominates over the scattering force trying to push the particle out of the trap. Thus one has a stable trap based solely on optical forces, where gravity plays no essential role as was the case for the levitation trap (*5*). It works over a particle size range of 10^5, from ~10 μm down to a few angstroms, which includes both Mie- and Rayleigh-size particles.

The sensitivity of laser trap effectiveness to optical absorption and particle shape is of particular importance for the trapping of biological particles. Absorption can cause an excessive temperature rise or additional thermally generated (radiometric) forces as a result of temperature gradients within a particle (*9*). In general, the smaller the particle size the less the temperature rise and the less the thermal gradients for a given absorption coefficient (*10*). Particle shape plays a larger role in the trapping of Mie particles than Rayleigh particles. For Mie particles both the magnitude and direction of the forces depend on the particle shape (*3*). This restricts trapping to fairly simple overall shapes such as spheres, ellipsoids, or particles whose optical scattering varies slowly with orientation in the beam. In the Rayleigh regime, however, the particle acts as a dipole (*6*) and the direction of the force is independent of particle shape; only the magnitude of the force varies with orientation. A significant conclusion of this work is that important types of biological particles in both the Mie and Rayleigh regimes have optical absorptions and shapes that fall within the scope of single-beam gradient traps.

As a first test for trapping of small biological particles we tried TMV, a much studied virus that can be prepared in monodisperse colloidal suspension in water at high concentrations (*11, 12*). Its basic shape is cylindrical with a diameter of 200 Å and a length of 3200 Å (*13*). Although its volume of about 470 Å^3 is typical of a Rayleigh particle, its length is comparable to the wavelength in the medium and we expect some Mie-like behavior in its light scattering. TMV particles have a negative charge in solution (*14*) and an index of refraction (*15*) of about 1.57. Our virus samples were prepared from the same batches used in experi-

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ments on the self-alignment of TMV in parallel arrays in dense aqueous suspensions (*13, 16*). These samples, suitably diluted, were studied in essentially the same apparatus (*6*) previously used for trapping of silica and polystyrene latex colloidal suspensions and Mie-size dielectric spheres (see Fig. 1).

When one visually observes the 90° scattering from untrapped viruses in the vicinity of the focus, one sees the random-walk motion and intensity fluctuations, or flickering, characteristic of Brownian diffusion in position and orientation. These rotational intensity fluctuations of about an order of magnitude result from the large changes in polarizability of a cylindrical particle with orientation in the light beam (*17*). At laser power levels of about 100 to 300 mW we begin to see trapping. The capture of a virus manifests itself as a sudden increase in the 90° scattering, as shown in Fig. 2. As more viruses are captured in the trap, we see further abrupt changes in scattering. If we block the beam momentarily (at points B) the trap empties and the trapping sequence repeats. Not only does all apparent positional Brownian motion disappear when viruses are captured but the intensity fluctuations characteristic of a freely rotating TMV particle are also greatly reduced (Fig. 2). This strongly indicates the angular alignment of the individual and multiple viruses within the trap. In previous work on trapped silica colloids we deduced that the successive particles entering the trap do not coalesce into a single particle but form a dense fixed array of separated particles (*18*), presumably held apart by electrostatic repulsion. It is likely therefore that charged viruses in the trap also form a dense array of separate particles. We further suspect that the trapped viruses are oriented parallel to one another as in dense oriented arrays of free TMV suspensions (*13, 16*).

The size of an unknown captured particle can be obtained by comparing the magnitude of its 90° scattering with that from a polystyrene latex calibrating sphere of known size, in the same trap. This comparison technique was used in our previous work on colloidal silica (*6*). It gives high accuracy for spherical Rayleigh particles because of the r^6 dependence of Rayleigh scattering. In the Rayleigh limit, all parts of the particle radiate in phase as a single dipole. Since TMV is not strictly a Rayleigh particle, we might expect interference effects due to optical path differences from different parts of the same particle to reduce the scattering below the full Rayleigh value. However, if we compare the 90° scatter of the average of 72 single particles from one sample, measured over a period of several days, with the 90° scatter from the calibrating sphere, using the Rayleigh formula, we find an effective volume of about $(450\ \text{Å})^3$. This volume corresponds to a cylinder 200 Å in diameter and about 3100 Å long, which is quite close to the volume of TMV. We conclude from this that we are looking at single TMV particles, and further that the axis of the TMV particle in the trap is oriented closely perpendicular to the beam axis along the optical electric field, since only then can all parts of the cylindrical virus radiate in phase at 90° as a Rayleigh particle. Looking at the size uniformity of our 72 particles we find, assuming that all differences in scatter are due to changes in the length of the viruses, that 75% of all particles lie in a length range within about 20% of the average. Thus we find that this particular sample is quite monodisperse and the measured length is $L = 3100 \pm 700$ Å.

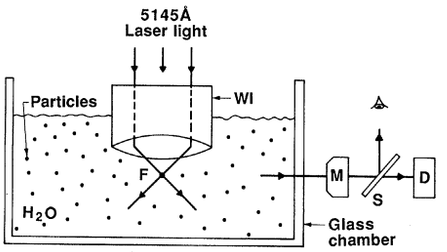

Fig. 1. Apparatus used for optical trapping of TMV particles and mobile bacteria. Spatially filtered argon laser light at 5145 Å is focused to a spot diameter of about 0.6 μm in the water-filled chamber by the high numerical aperture (1.25) water-immersion microscope objective (WI) forming a single-beam gradient trap near the beam focus (F). The 90° scattering from trapped particles can be viewed visually through a beam splitter (S) with a microscope (M) or recorded using a photodetector (D).

Not much size information can be deduced from the observed changes in 90° scatter as additional virus particles enter a trap and form an aligned array, since the combined scattering field in such cases is the result of interference of the fields from each particle of the array. Not only are the positions and phases of the fields of the various particles unknown, but they probably change as additional particles enter the trap. Examples of destructive interference and a decrease in total 90° scattering as additional particles enter a trap are seen at times of 7.4 and 10.8 minutes in Fig. 2. In a related experiment (*18*) on the angular distribution of scattered light from trapped colloidal silica particles we found that when a similar decrease in 90° scattering occurred on entry of an additional particle, it was possible to find another direction where the phases added constructively and actually gave an increase in scattering.

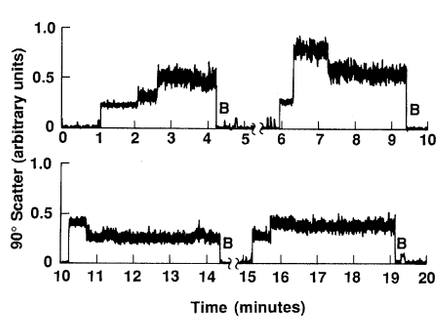

Fig. 2. Scattered light observed at 90° as successive TMV viruses enter the optical trap. At times labeled "B" the trapping beam is momentarily blocked, releasing the viruses. The trap subsequently refills with new virus particles.

In the above discussion we deduced the optical alignment of TMV along the optical field E on purely experimental grounds. This also makes sense energetically since this optical alignment results in the maximum polarizability α of the cylindrical virus. Indeed, the TMV, when aligned, not only feels the deepest trapping potential $\alpha E^2/2$ but also experiences strong realigning torques when rotated, due to the angular dependence of $\alpha E^2/2$. More detailed information on the angular orientation of TMV within the trap could be obtained from scattering experiments with an additional low-power probe laser beam of different wavelength and varying directions of polarization relative to the virus axis.

Another conclusion that can be drawn from the data of Fig. 2 is that it is possible to trap viral material without any gross optical damage. Indeed, single viruses have been trapped for tens of minutes with no apparent changes in size as indicated by the constant magnitude of the scattering. The viability of the virus after trapping was not examined. In previous experiments optical damage was a serious problem, which limited the trapping of small Rayleigh-sized polystyrene latex particles and even to some extent silica particles. For silica this damage was subsequently eliminated (*18*) by *p*H changes or additions of potassium silicate to the solution, which points to a surface photochemical reaction as the damage mechanism. For virus particles the fact that the strong optical absorptions are in the ultraviolet probably contributes to their optical stability in the visible light range. As with silica colloids (*6*) we were able to manipulate captured TMV particles within their environment by moving either the light beam or the entire chamber. This implies the ability to separate trapped viruses by means of a simple flushing technique, for example.

The major problem encountered in the experiment was one of reproducibility. Thus far we have had two batches of dense virus (10 and 50% by weight) and had successful

trapping runs with samples diluted from each batch which lasted several days. However, with other samples from the same batches we trapped many fewer TMV-sized particles. Instead we trapped mainly larger-sized clumps and smaller-sized single particles. These larger trapped clumps usually decrease in size (damage) in just a few minutes. Large clumps, which also decayed in time, were previously trapped in our silica experiments (*6*). The smaller-sized trapped particles are stable in size and are probably just smaller pieces of virus. On this assumption, the lengths of some of these smaller viral pieces that we observed at trapping powers of ~1.5 W were ~270 Å. If these small particles are typical of the index of refraction of other proteins, then this observation implies an ability to trap proteins with molecular weight $M \geq 3 \times 10^6$ at a power of 1.5 W.

The lack of consistency in TMV trapping could be the result of either the dilution process or the trap geometry. With TMV samples, proper *p*H and low ionic content are needed to avoid polydispersity. At *p*H > 9 the TMV falls apart and at *p*H < 6 it aggregates. We attempted to maintain the *p*H of our diluted samples close to 7. To do this we used deionized or distilled water with small volumes of buffer solution added to adjust the *p*H, with no improvement in the consistency of our results. Regarding trap geometry, we often found laser beam wander to be a problem and usually checked our overall geometry by looking for good trapping of 600-Å silica test particles. It is possible, however, that the trap is much more tolerant of aberrations for the spherical 600-Å Rayleigh particles than for 3200-Å TMV. If the TMV sits transverse to the beam axis, the virus would extend to the edges of the beam to regions not felt by a silica particle. Although it requires more power, a trap with a larger diameter focal spot might be more favorable for a particle of this shape. Another obvious experiment is to study the trapping of more spherical viruses such as tomato bushy-stunt virus (*19*) with a diameter of ~450 Å.

In most of our experiments with silica colloids or TMV in water, we noticed the appearance of some strange new particles in diluted samples that had been kept around for several days. They were quite large compared to Rayleigh particles, on the basis of their scattering of light, and were apparently self-propelled. They were clearly observed moving through the distribution of smaller slowly diffusing Rayleigh-sized colloidal particles at speeds as high as hundreds of micrometers per second. They could stop, start up again, and frequently reversed their direction of motion at the boundaries of the trapping beam, when they encountered a dark region, indicating some sort of attraction toward the light. Their numbers increased rapidly as time went by. When examined under 800× magnification in an optical microscope, they were clearly identifiable as rod-like motile bacteria, propelled by rotating tails. There were at least two types of bacteria, about 0.5 and 1.5 μm in length. Optically they resembled small, transparent Mie particles with an index of refraction close to unity.

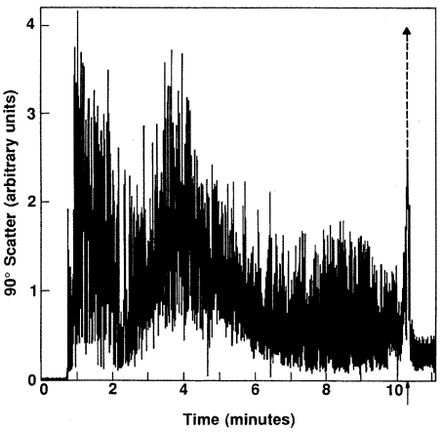

Fig. 3. Scattered light at 90° from a live bacterium trapped by ~5 mW of laser power. At about 10.3 minutes (indicated by arrow) the power was increased to 100 mW. The bacterium was killed and apparently loses much of its cell contents.

When one of these bacteria wandered into or was possibly attracted into the trap, it was captured. It was observable through microscope M either by eye or on the photo detector, as a fluctuating signal, as it struggled unsuccessfully to escape from the trap. The far-field forward scatter from the bacteria could also be seen on a screen placed outside the cell. To help capture the bacteria we initially set the laser power at ~50 mW. Once the bacterium had been captured, we quickly lowered the power to ~5 mW to reduce the possibility of optically damaging the bacterium. Figure 3 shows the recorded 90° scatter as a function of time, taken at 5 mW, as a bacterium propels itself about in the trap. After about 10 minutes we raised and maintained the power at ~100 mW. This, as is seen, was sufficient to kill the trapped bacterium. The light scattering stopped fluctuating and decreased to quite a low value as the bacterium apparently vented some of it contents into the surroundings. The remains of the cell could be held in the trap with laser power as low as ~0.5 mW. It was reported that similar venting of a cell's contents occurred in experiments on the puncture of blood cells by pulsed laser beams (*20*).

In other experiments we illuminated the entire trapping region with a wide low-power auxiliary red laser beam directed transverse to the trap axis. We then viewed the scene through a red-pass filter with microscope M, either visually or with a video camera and recorder. We could observe the capture of free-swimming bacteria and their subsequent release as the trapping beam was blocked. Several bacteria were occasionally trapped simultaneously. We were also able to demonstrate optical micromanipulation of single trapped bacteria, within the liquid, by moving either the trapping beam or the entire chamber and its liquid.

In the above experiment the low resolution of the side-viewing microscope M and the presence of red laser interference rings made it difficult to resolve details of the trapped bacteria. An obvious extension of this viewing technique is to introduce the trapping laser beam directly into a high-resolution microscope through a beam splitter. By using a water-immersion microscope objective with a high numerical aperture for both laser trapping and viewing through a filter, we were able to simultaneously trap, manipulate, and observe bacteria or other particles with high resolution. For convenience we placed our water samples containing bacteria under a cover slip and used a water-immersion objective designed for operation through the cover slip. An additional lens mounted outside the microscope on an *xyz* mechanical micromanipulator was used to focus the laser trapping beam within the field of view of the microscope and move it about transversely without any need to touch the microscope. We observed the scene either by eye or with a video camera. At power levels as low as 3 to 6 mW we were able to move the beam about and capture a free-swimming bacterium anywhere in the field of view. Once the bacterium had been captured, we could rapidly move it transversely and continue to catch more bacteria until we had a half dozen or more within the trap. Rapid transverse motion without loss of bacteria is possible because of the strong transverse gradient trapping forces. The trapping forces in the axial or *z* direction are stronger in the forward direction of the light rather than in the backward direction (*6, 7*). Thus any rapid upward motion of the focus can result in escape of particles.

At lower power levels, from 1 to 3 mW, and probably less, we discovered another trapping mode in which bacteria were trapped against the bottom surface of the slide. In this low-power surface mode of operation the mechanical surface provides the backward trapping force needed to prevent the escape of the particle out the bottom of the trap in the direction of the weakest trapping force. It is still possible to

move particles about transversely over the surface in this mode because the transverse forces remain quite strong even at the lower power. Bacteria captured by either mode of trapping with powers in the range from 1 to 6 mW have survived for hours in the laser light with no apparent damage.

We performed subsequent experiments using the high-resolution microscope with *Escherichia coli* bacteria. These bacteria are much less motile and could be captured and manipulated rapidly at surfaces and in the bulk fluid with powers as low as a fraction of a milliwatt with no apparent change in behavior or appearance. At powers of 100 mW or more it was possible to observe a shrinkage in the size of the *E. coli* as they become optically damaged in a time of about a minute. With yet another sample of highly motile bacteria we observed a gradual loss in motility of trapped bacteria in about a half-minute with powers as low as 10 to 20 mW. In all cases where optical damage was observed with the 5145-Å green argon laser line it might be advantageous to use other laser wavelengths.

One advantage offered by the high-resolution microscope was the ability to study the trapping forces on bacteria in some detail. For example a bacterium, while being manipulated close to the surface of the slide, would occasionally manage to attach itself to the surface with its tail and remain tethered. Under these conditions it was still possible to optically manipulate the particle in a circle around its tether and observe the action of the optical forces. Although we do not have a complete description of the trapping forces for complex shaped particles like bacteria, it is clear from these experiments that the same qualitative features, based on simple ray-optics and refraction, that account for trapping of Mie-sized spheres (*3, 4, 6*) and spheroids (*21*) still apply here. For example when the beam center is moved toward the edges of a bacterium where refraction is large and asymmetric, we generate large transverse gradient forces which in effect drag the particle with the beam in the direction which recenters the particle on the beam. This is the same basic effect that accounts for the centering of a small Mie particle at the position of maximum light intensity of a large Gaussian beam. However, for the case of a large particle and a small beam, as we have here, it is clearly the local shape of particle that dominates the net force.

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