Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7914–7918, October 1989
Cell Biology

Internal cell manipulation using infrared laser traps

(laser cell surgery/optical tweezers/viscoelasticity/mechanical properties/cytoplasmic streaming)

A. ASHKIN AND J. M. DZIEDZIC

Laser Science Research Department, AT&T Bell Laboratories, Holmdel, NJ 07733-1988

Communicated by James P. Gordon, July 5, 1989

ABSTRACT **The ability of infrared laser traps to apply controlled forces inside of living cells is utilized in a study of the mechanical properties of the cytoplasm of plant cells. It was discovered that infrared traps are capable of plucking out long filaments of cytoplasm inside cells. These filaments exhibit the viscoelastic properties of plastic flow, necking, stress relaxation, and set, thus providing a unique way to probe the local rheological properties of essentially unperturbed living cells. A form of internal cell surgery was devised that is capable of making gross changes in location of such relatively large organelles as chloroplasts and nuclei. The utility of this technique for the study of cytoplasmic streaming, internal cell membranes, and organelle attachment was demonstrated.**

Infrared laser traps have recently been developed using the forces of radiation pressure that offer a way of micromanipulating entire living cells and even organelles within cells (1, 2). By using these traps, preliminary observations were made of the elastic behavior of the cytoplasm when small particles were optically displaced inside of living cells (1). Measurements have also recently been made on the compliance of bacterial flagella using laser traps (3). One of the unique features of this manipulative technique, sometimes referred to as the "optical tweezers" technique, is the ability to apply controlled manipulative forces inside of cells while leaving the cell wall intact. We show here that application of highly local internal forces makes it possible to study the mechanical properties of living cytoplasm with minimal damage. There is essentially no precedent for such a capability in the extensive literature on micromanipulation techniques and measurement of mechanical properties of biological materials. Standard micromanipulation techniques based on microgy (4–6), although capable of removing organelles from within cells, are not easily controlled and are fairly destructive of the cell. Weak variable magnetic forces have been applied to magnetic particles ingested into living cells for studying the viscoelastic properties of the more fluid parts of cells (7–9). Extensive measurements exist external to cells on the mechanical response of individual muscle fibers isolated from muscles (10–13). The mechanical properties of organic gels of actin and other organic polymers have been much studied (14–16) using standard rheological instruments (17, 18). External forces applied to living cells using a "cell-poker" have been used to probe gross mechanical properties of living cells (19). A related technique measures the local pressure needed to aspirate cell membrane into a micropipette (20). The principal limitation on the optical tweezers technique for internal cell manipulation is the restricted magnitude of the force consistent with minimal optical absorption damage to the cell.

In this largely qualitative study, we explore areas of internal cell manipulation in plant cells where optical tweezers can make significant changes in internal cell structure. We have discovered the ability of traps to pull out long thin filaments of cytoplasm from different parts of the cell. These so-called artificial filaments are observed to exhibit, in striking fashion, the viscoelastic properties of plastic flow, necking, stress relaxation, and set, in close analogy with the known physical behavior of polymeric fiber materials (21, 22) and the more limited measurements on biological samples. By using filaments, one has the unique capability of probing point to point and temporal variations of the mechanical properties of cytoplasm in essentially unperturbed cells. As pointed out by Allen (23) and Allen (24), the local rheological properties of plant cells and amoeboid cells are not well characterized and are needed for a full understanding of cytoplasmic streaming and amoeboid movement. We also show that an appreciation of viscoelastic properties enables gross changes in the internal structure of the large organelles of the cell. This amounts to a limited form of optical cell surgery. Effects due to internal membranes are observed in experiments on making connections between cytoplasmic filaments and between optically displaced organelles. The displacement and reconnection of large organelles give rise to novel cell structures not normally found in nature. In other experiments, we see and strongly affect the phenomenon of cytoplasmic streaming and particle transport. Streaming motions represent the response of the cytoplasm to its own internally generated forces of motility (23–27). Traps also offer a unique approach to the study of these processes. We believe that the optical methods demonstrated here will enhance the prospects for use of laser traps for micromanipulation in cell biology.

MATERIALS AND METHODS

The single-beam infrared laser trap consists of a highly convergent 1.06-μm laser beam focused within the viewing plane of a standard optical microscope (1). It can trap and manipulate cells and particles from tens of microns down to submicron sizes (1, 2, 28). For large cells, where the beam focal volume of a few μm^3 is smaller than the cell, the trap acts much as a laser tweezers, which exerts strong forces near the boundaries of the cell, where the cell curvature and light refraction are largest. For small (submicron) organisms and particles, it exerts maximum force when the particle is at the point of maximum intensity gradient within the beam focal region. Traps of this type are capable of trapping a remarkable range of cell shapes and particle types (1, 2).

Manipulation is accomplished by fixing the trap in space and manipulating the stage and sample chamber in x, y, and z directions with micropositioners. For internal cell manipulation, we often use piezoelectric controls on the coordinate micrometers. Samples of *Spirogyra* and water-net (*Hydrodicton*) algae cells were collected from the back pond of the AT&T Bell Laboratories at Holmdel. Scallion (*Allium cepa*) cell samples came from a local supermarket.

In this work, we discovered a further capability of traps—namely, the ability to pull out a cytoplasmic strand or filament from an essentially planar internal interface. This occurs in the absence of any particles in the trap. When we

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

move the trap across a cytoplasmic boundary such as exists, for example, between the higher index of refraction layer of cytoplasm surrounding the nucleus of a scallion cell and the lower index material of the central vacuole, the trap pulls out a long thin strand of high index cytoplasm into the lower index material. We can understand the initial surface forces causing this effect as due to the sum of the forces of the individual rays of the trap converging on the planar index discontinuity. For any ray, irrespective of its incident direction, there are two main contributions to the net surface force pointing out from the high to the low index medium: simple refraction and the change in P/cn, the effective momentum per second of the light within dielectric media (29, 30), where P is the power, n is the refractive index, and c is the velocity of light. There is also a normal force due to surface reflection but this is in general negligible. Surprisingly, this gives a substantial outward normal surface force, irrespective of the orientation of the planar interface even when the plane is vertical and rays are converging on the surface from both sides. Fig. 1 is a photograph of a so-called "artificial" elastic filament pulled out from the cytoplasm surrounding a scallion nucleus into the central vacuole. The trap initially at A creates a normal surface force that pulls the cytoplasm into a hemispherical dome and finally a long filament as the trap moves from A to B.

RESULTS AND DISCUSSION

Artificial Filaments. Artificial filaments or strands provide a way to observe the viscoelastic properties of cell cytoplasm. Filaments of different elastic properties were pulled from different regions of plant cells—for example, from the thin layer of cytoplasm adjacent to the cell wall into the central vacuole, from the edges of organelles such as chloroplasts or nuclei into the nearby cytoplasm, or from the boundaries of the many naturally occurring streaming cytoplasmic channels into surrounding areas. Strands pulled into the central vacuole can often be hundreds of microns long and span almost the entire cell. Filament diameters vary and often are submicron. When a filament is released from the trap, it snaps back to its point of origin with speeds from about 100 μm/sec to a few μm/sec depending on its point of origin and whether it is released quickly or after being held for tens of seconds. After release, a small blob of a few cubic μm at the origin is the only remnant of the filament. If one rapidly pulls out a strand at a lower optical power level, one often loses it after a finite displacement due to increasing tension, and it snaps back. If, however, one stops and waits after shorter displacements, the extension can be essentially arbitrarily large.

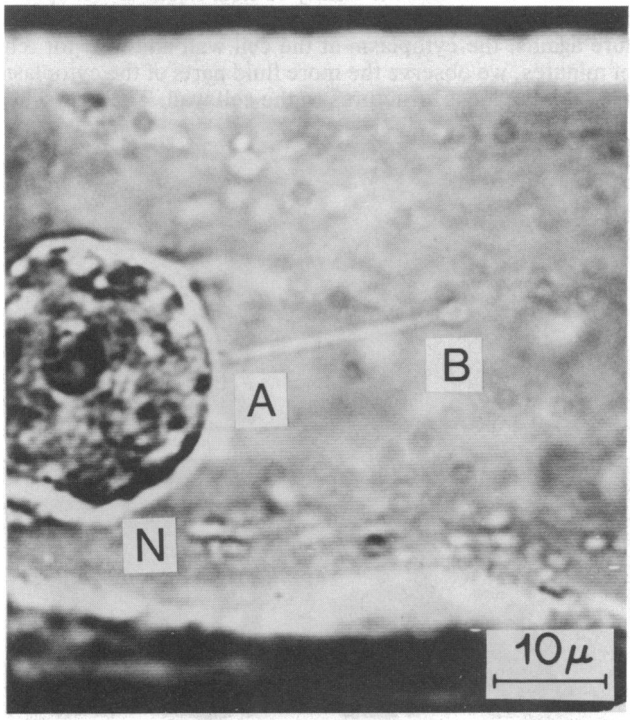

FIG. 1. Artificial cytoplasmic filaments in a scallion cell. The laser trap originally located on the surface of the nucleus (N) at A is moved to B, pulling out the viscoelastic filament AB into the central vacuole.

If a filament is held extended for tens of seconds and one then rapidly moves the trap back toward the origin, one finds that at some point the filament no longer contracts but begins to sag due to loss of tension. After a few seconds, the strand tightens up again as it apparently continues to shrink slowly under relaxed conditions. Insight into how the filament extends itself when pulled can be gained by observing a pattern of fixed particles attached to the filament that act as markers. Although some filament expansion does occur, the principal gain in length comes from the feeding of new material from a short "neck-like" region that protrudes out a few microns from the point of origin.

Another striking property of filaments is their ability to "walk" over cytoplasmic surfaces. If one pulls out a filament normal to a surface and then displaces the trap parallel to the surfaces, the point of origin of the filament simply "walks" over the surface, following the trap. This serves to minimize the length and tension of the filament. "Walking" can occur over the surfaces of complicated shaped objects like the nucleus or its cytoplasmic supporting strands.

Remarkably, the whole range of rather complex mechanical effects observed above in living cytoplasmic filaments is closely analogous to the physical properties of viscoelastic fiber materials as seen in polymer science (21, 22). At their simplest, physical viscoelastic polymeric fibers and solids closely follow a Hooke's law stress–strain relation at low forces or stresses, up to a so-called yield point. Beyond this point, the solid starts to exhibit plastic flow. Large displacements (strain) are now possible with little increase in stress, and the material extends out principally at the expense of a "necked-out" region. If the extension is too rapid for plastic flow to occur, there is a rapid rise of stress and the fiber breaks. At fixed extension (strain) beyond the yield point, the stress relaxes steadily with time as the material continues plastic deformation. This in turn allows further extension. Upon removal of stress, a plastic fiber does not return to its original configuration but ends up with a "permanent set." There is also a transient set component due to relaxation of the deformed plastic material.

The fact that the mechanical properties of living cytoplasm so closely mimic those of viscoelastic polymers is not entirely surprising. Indeed, many polymeric materials and fibers are of organic origin, such as rubber, lattices, keratin, wool, etc. At the cellular level, the viscoelasticity of cytoplasm arises from the polymeric properties of actin and microtubule filaments that make up the basic support matrix and transport system of cells (14, 25, 31). Rapid transitions in cytoplasmic properties (ectoplasm–endoplasm transitions and possible sol–gel transitions) are considered important processes in cell motility (25, 31, 32) and contribute to local variations in mechanical properties. Only limited measurements exist on viscoelastic properties of living cytoplasm in prior work. Evidence of yield, stress relaxation, and shear-rate dependent viscosities was seen in experiments using ingested magnetic particles (7–9). In "poking" experiments, hysteresis was seen in the force needed to indent living mouse fibroblast cells (20). Measurements external to cells on mus-

cle fibers show evidence of stress relaxation, crosslinking, and set (11, 12). Extensive viscoelastic measurements have been made on artificial actin gels that serve as models of cytoplasm of the variation of stress, compliance, and viscosity with shear rate (14–16). Strong non-Newtonian and shear thinning behavior was seen.

A significant factor in our ability to use traps for probing viscoelastic properties of living cells is the relatively low values of yield point of much of the cell's cytoplasm. For plant cells, we find yield points from as low as $\approx 10^{-8}$ dynes/μm^2 for the more fluid streaming parts of the cytoplasm up to values of 10^{-6}–10^{-4} dynes/μm^2 for the stiffer cytoplasm of artificial filaments. The maximum surface stress available using light traps is about 2×10^{-4} dynes/μm^2 (corresponding to ≈ 0.8 W applied at a focal spot of ≈ 0.5 μm in diameter). This greatly exceeds the stresses of approximately 10^{-8}–10^{-7} dynes/μm^2 used in magnetic particle experiments (9). It also exceeds the measured yield stress values of pure actin gels (32) of about 0.5–3×10^{-8} dynes/μm^2 and the stiffer shear-enhanced values of stress of about 10^{-5} dynes/μm^2 for gels of actin plus α-actinin crosslinking protein (15). The maximum stress using traps is also closely equal to the value of pressure needed to indent cells in the poking experiments. The pressure to distort the surface of red blood cells (erythrocytes) was measured to be about 50 times less ($\approx 4 \times 10^{-6}$ dynes/μm^2) using the micropipette technique. In unpublished results using optical tweezers, we distorted the surface of erythrocytes with tens of milliwatts of power, which corresponds to $\approx 2 \times 10^{-6}$ dynes/μm^2. Another factor in our filament experiments is the apparently low surface tension of internal interfaces. We attribute this to the presence of internal membranes at these cytoplasmic interfaces. If we ignore the viscoelastic stress, then the surface force required to pull out a filament of radius r into the initial shape of a hemispheric dome is at least $2\pi rS$, where S is the surface tensions (8). The fact that we can pull out filaments from a focal spot about 0.5 μm in diameter using powers of 0.1 W or less implies a surface tension of a cytoplasmic interface about 10^3 times less than an air–water interface.

The advantages of this technique for studying viscoelastic properties are its simplicity and ability to probe much of cell's cytoplasm on a point to point basis and as a function of time and treatment with minimal disturbance. By inducing an alternating motion in the microscope stage, one can hope to make dynamic stress and viscosity measurements using artificial fibers and small trapped organelles in analogy with rheological techniques used external to the cell.

Manipulation of Large Organelles. An understanding of the viscoelastic properties of cytoplasm also makes possible the micromanipulation of the larger organelles of the cell. Initial attempts to move larger cell structures such as chloroplasts or nuclei, which are held in place by thick cytoplasmic connections, only resulted in plucking out of filaments from the surrounding cytoplasm. If, however, we apply force to a large organelle slowly, and stop after observing a slight displacement, we can then wait for plastic flow and force relaxation to occur. Assuming we are beyond the yield point, it then becomes possible to advance slowly with increasing displacements, until large changes are made in the position of the organelle. During this process, the thick cytoplasm holding the organelle gradually pulls out, "necks down," and ends up as, at most, a few weak strands, leaving the organelle essentially free. If one stops the process at an intermediate displacement where the cytoplasm is not too severely "necked down," the organelles creep back close to their original locations. Fig. 2 shows some of the drastic changes that can be made in the position of the spiral band of chloroplast of a *Spirogyra* cell. Fig. 2 *a* and *b* are views of the undisturbed cell close to the surface and the mid-plane. In Fig. 2*c*, the left end of the chloroplast spiral was pulled free from the outer cytoplasmic skin into the central vacuole close to the nucleus. In Fig. 2*d*, the loosened chloroplast is lifted close to the upper surface of the cell. Similarly, we have pulled off the central section of the chloroplast spiral and the fairly rigid nuclear support structure from their original location at points P and Q of Fig. 3*a* and moved them into the central vacuole to points R and S of Fig. 3*b*. This frees the nucleus from the cell wall and rotates it by about 45°. We also find that the cell has the ability to heal itself. If we "park" detached chloroplasts or the detached nuclear support structure against the cytoplasm at the cell wall and wait for tens of minutes, we observe the more fluid parts of the cytoplasm reattach the loose structures to the cell wall. The reattached

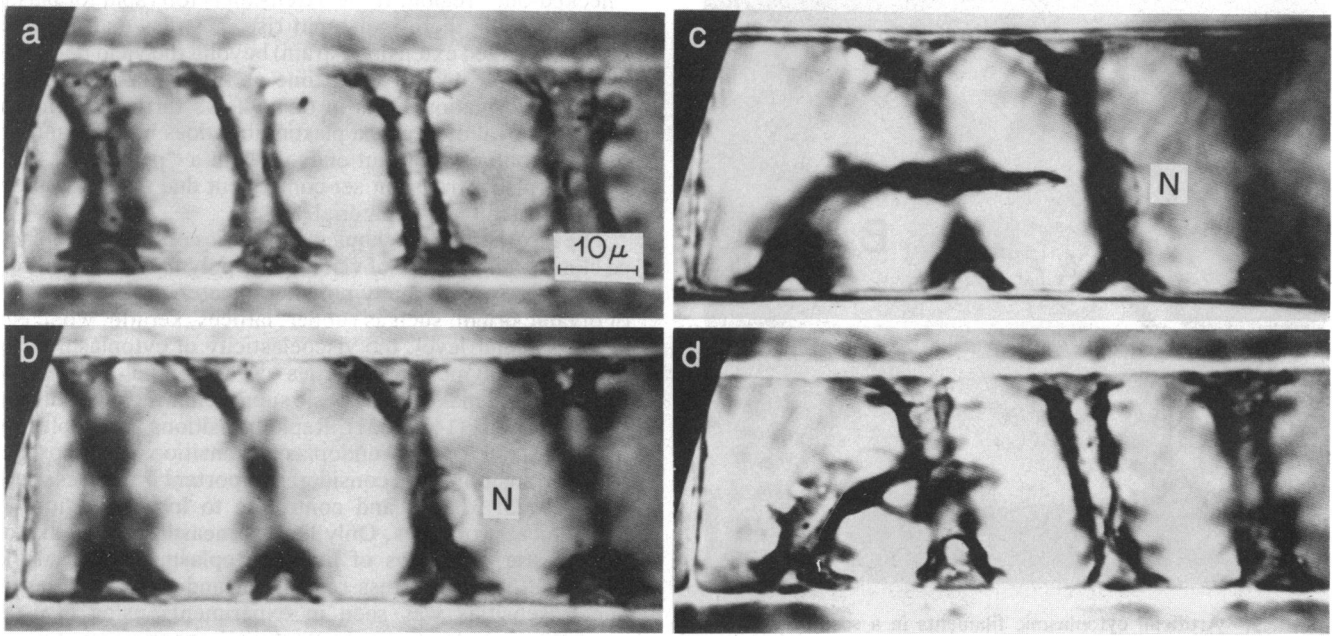

FIG. 2. Manipulation of spiral chloroplast of *Spirogyra* from its undisturbed position (*a* and *b*) to grossly new locations (*c* and *d*). In *c*, the lefthand piece of chloroplast was moved into the vacuole near the nucleus (N). In *d*, it is raised to the cell surface.

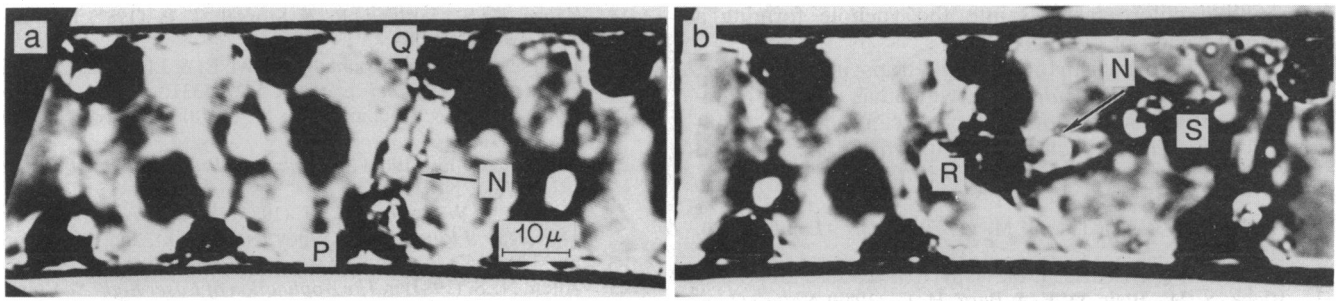

FIG. 3. Gross relocation of chloroplast and nucleus of *Spirogyra*. Material originally near the cell wall at P and Q (*a*), was manipulated into the central vacuole at R and S (*b*), thereby detaching a section of chloroplast and rotating the nucleus (N).

organelles appear to continue to function. With *Spirogyra*, we cannot pull the nucleus free of its support structure, though we can distort it, and move the nucleolus and small particles within the nucleus. With scallion cells it is occasionally possible, when the cytoplasm is not too viscous, to slowly drag the entire nucleus about within the cell along with its network of feeding cytoplasmic channels.

The power level used for manipulation of the larger organelles was often raised to 800 mW at the sample, which is 5–10 times higher than previously used (1). At this power, we still see no obvious thermal damage to plant cells but destruction of some protozoa can occur. In plants, use of high power accelerates the gross manipulation of large organelles. Manipulation occurs more slowly at powers of 100–200 mW. This technique for manipulation of large organelles using the plastic properties of the cytoplasm represents a limited form of cell surgery with which one can effect fairly gross changes in cell structure with minimum disruption to the cell. We believe this will prove useful for the study of cell function. This tweezers technique differs from the previous microsurgical techniques (33, 34) using pulsed high-powered lasers for microdissection and microirradiation.

Particle Motion and Cytoplasmic Streaming. Laser traps are able to affect internal particle motion and cytoplasmic streaming. In scallion cells we can trap and store up collections of the small particles usually seen moving in the well-defined channels of the cell interior or the more diffuse channels near the cell wall. When particles are trapped within a channel, a clump of cytoplasm often collects around them, which locally disturbs the flow. When released, the particles and clump disperse and move on along the channel. If the disturbance to the channels is large, some of the advancing particles will turn and go backward. Artificial side channels or filaments, pulled out from an existing channel, can grow or shrink as cytoplasm and particles flow in or out of them. When the flowing cytoplasm is very fluid, one can capture and transfer particles, with no attached cytoplasmic strands, from one stream to another. The powers required to manipulate streaming particles vary from a few tenths of a watt to a few milliwatts depending on the velocity and viscosity of the cytoplasm.

It is possible using artificial filaments to observe and possibly measure the velocity of cytoplasmic streaming independently of any particle motion within the stream. We find under circumstances of very rapid streaming that filaments, which under low-flow conditions walk over surfaces and come to rest perpendicular to the surface, are now strongly pulled downstream by the current and come to rest at angles as high as 45°.

Measurements of the motive force of cytoplasmic streaming made on the giant alga *Nitella* using centrifugation, external squeezing, and perfusion through opened cells gave values of 1.0–2.0 $\times$ 10^{-8} dynes/μm^2 (25). Since we can generate forces about 3 orders of magnitude larger, we can easily affect the streaming process. However, as pointed out by Allen and Kamiya (24, 25), the inability to measure local viscosity results in an inability to know if changes in streaming velocity are due to changes in viscosity or changes in driving force. Relative viscosity measurements are now possible with tweezers using a small organelle as a moveable test particle. Absolute measurements require a knowledge of the relative index of the particle. Measurements on particles and cytoplasmic drops vented into the surrounding liquid could be made. Effects of temperature, pH, and reagents on streaming have been reported (25, 35). Measurement of local viscosity and forces in such systems with tweezers would be useful.

Cell Membranes and Fusing of Cytoplasm. Another aspect of cytoplasm that affects the ability to manipulate it is the presence of membranes surrounding the outer surfaces of artificial filaments, natural filaments, and organelles. Attempts to fuse or join one filament with another or to organelles or cell surfaces are generally unsuccessful if the cytoplasmic surfaces are held in contact for times up to tens of seconds. Cytoplasmic joints are however possible if one waits considerably longer, presumably allowing the intervening membranes to break down. One unusual structure thus formed (see Fig. 2) consisted of a pair of chloroplasts (C1 and C2) stretched across the central vacuole of a water-net algae and held in place by elastic strands of cytoplasm. The chloroplasts, each with a residual connecting strand, were pulled out from opposite cell walls and held in contact within the same trap long enough for a connecting strand of cytoplasm to form. The positions of C1 and C2 shift as the tension of the connecting filaments varies. In Fig. 4*b*, the trap is also

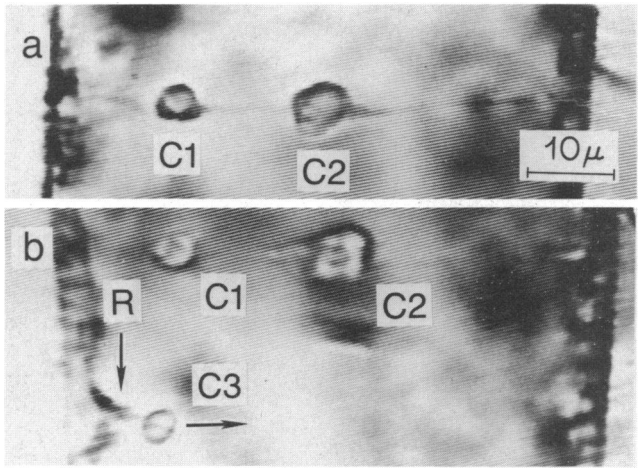

FIG. 4. Unique cell structure made in water-net algae: Chloroplasts C1 and C2 stretched across the vacuole by thin cytoplasmic filaments. The chloroplasts move as the tension of the filaments varies. In *b*, the trap is also pulling chloroplast C3 into the vacuole forming a necked-out region of cytoplasm (R).

seen pulling chloroplast C3 into the vacuole forming a necked-out region of cytoplasm.

An interesting organism to study with traps, in this context, is the giant amoeba *Reticulomyxa*, which has unusual membranes that fuse instantly on contact. It can therefore spontaneously reassemble itself from a suspension of separated pieces (36).

1. Ashkin, A. & Dziedzic, J. M. (1987) *Nature (London)* **330,** 769–771.
2. Ashkin, A. & Dziedzic, J. M. (1987) *Science* **235,** 1517–1520.
3. Block, S. M., Blair, D. F. & Berg, H. C. (1989) *Nature (London)* **338,** 514–518.
4. Crossway, A., Hauptli, H., Houck, C. M., Irvine, J. M., Oakes, J. V. & Perani, L. A. (1986) *BioTechniques* **4,** 320–333.
5. Miller, S. D. (1980) *Am. Biotechnol. Lab.* **71** (1), 30–34.
6. El-Badry, H. M. (1964) *Micromanipulators and Micromanipulation* (Academic, New York).
7. Crick, F. H. C. & Hughes, A. F. W. (1950) *Exp. Cell Res.* **1,** 37–80.
8. Yagi, K. (1961) *Comp. Biochem. Physiol.* **3,** 73–91.
9. Sato, M., Wong, T. Z. & Allen, R. D. (1983) *J. Cell Biol.* **97,** 1089–1097.
10. Huxley, H. E. (1969) *Science* **164,** 1356–1366.
11. Huxley, A. F. & Simmons, R. M. (1972) *Cold Spring Harbor Symp. Quant. Biol.* **37,** 669–680.
12. Cecchi, G., Griffiths, P. J. & Taylor, S. (1986) *Biophys. J.* **49,** 437–451.
13. Haskell, R. C. & Carlson, F. D. (1981) *Biophys. J.* **33,** 39–62.
14. Stossel, T. P. (1978) *Annu. Rev. Med.* **29,** 427–457.
15. Sato, M., Schwarz, W. H. & Pollard, T. D. (1987) *Nature (London)* **325,** 828–830.
16. Zaner, K. S. & Hartwig, J. H. (1988) *J. Biol. Chem.* **263,** 4532–4536.
17. Ferry, J. (1980) *Viscoelastic Properties of Polymers* (Wiley, New York).
18. Zaner, K. S., Fotland, R. & Stossel, T. P. (1981) *Rev. Sci. Instrum.* **52,** 85–87.
19. Petersen, N. O., McConnaughey, W. B. & Elson, E. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5327–5331.
20. Evans, E. A. & Hochmuth, R. M. (1978) *Curr. Top. Membr. Transp.* **10,** 1–64.
21. Nielsen, L. E. (1962) *Mechanical Properties of Polymers* (Rheinhold, New York).
22. Hadley, D. W. & Ward, I. M. (1986) *Encyclopedia of Polymer Science and Engineering* (Wiley, New York), 2nd Ed., Vol. 9, pp. 379–466.
23. Allen, N. S. (1983) in *The Application of Laser Light Scattering to the Study of Biological Motion*, eds. Earnshaw, J. C. & Steer, M. W. (Plenum, New York), pp. 529–543.
24. Allen, R. D. (1983) in *The Application of Laser Light Scattering to the Study of Biological Motion*, eds. Earnshaw, J. C. & Steer, M. W. (Plenum, New York), pp. 519–527.
25. Kamiya, N. (1981) *Annu. Rev. Plant Physiol.* **32,** 205–236.
26. Allen, R. D. & Allen, N. S. (1978) *Annu. Rev. Biophys. Bioeng.* **7,** 497–526.
27. Schliwa, M. (1984) in *Cell and Muscle Motility*, ed. Shay, J. (Plenum, New York), Vol. 5, pp. 1–85.
28. Ashkin, A., Dziedzic, J. M., Bjorkholm, J. E. & Chu, S. (1986) *Opt. Lett.* **11,** 288–290.
29. Ashkin, A. & Dziedzic, J. M. (1973) *Phys. Rev. Lett.* **30,** 139–142.
30. Gordon, J. P. (1973) *Phys. Rev. A* **8,** 14–21.
31. Taylor, D. L. & Condeelis, J. S. (1979) *Int. Rev. Cytol.* **56,** 57–144.
32. Brotschi, E. A., Hartwig, J. H. & Stossel, T. P. (1978) *J. Biol. Chem.* **253,** 8988–8993.
33. Berns, M. W. (1972) *Nature (London)* **240,** 483–485.
34. Berns, M. W. (1974) *Science* **186,** 700–705.
35. Shimmen, T. & Tazawa, M. (1985) *Protoplasma* **127,** 93–100.
36. Koonce, M. P., Eutenauer, V., McDonald, K. L., Menzel, D. & Schliwa, M. (1986) *Cell Motil. Cytoskeleton* **6,** 521–533.