Laser Scissors and Tweezers

Researchers are using lasers to grasp single cells and tinier components in vises of light while delicately altering the held structures. These lasers offer new ways to investigate and manipulate cells

by Michael W. Berns

The intense, pure beams of light known as lasers are now standard components of such commonplace objects as compact-disc players and printers. The everyday presence of lasers does not mean, however, that they have been reduced to performing only pedestrian tasks. Imagine focusing a beam specifically onto an organelle, a structure within a living cell. Consider further that the beam can actually grasp that minuscule entity and hold it in place. Now imagine that while this "microbeam" acts as tweezers, a second beam serves as scalpel or scissors to conduct delicate surgery on the organelle.

Even in a world accustomed to lasers, such musings have the ring of science fiction. Nevertheless, much as medical surgeons guide micromachined tweezers and scissors through endoscopes to perform minimally invasive surgery on organs, the cell biologist can now use "laser tweezers" and "laser scissors" to perform minimally invasive manipulations on living cells and their organelles.

Laser scissors came first. Almost three decades ago Donald E. Rounds and I, while at the Pasadena Foundation for Medical Research, suggested lasers might be wielded to probe the structure and function of cells and organelles [see "Cell Surgery by Laser," by Michael W. Berns and Donald E. Rounds; SCIENTIFIC AMERICAN, February 1970]. Our early work focused on defining the parameters of our lasers (such as wavelengths of light and durations of exposure) and on determining which organelles could be successfully manipulated with light beams that could alter intracellular regions as small as 0.25 micron in diameter. (The diameter of an average human hair is about 100 microns.)

During the intervening years, my colleagues and I found that laser scissors could be used to study organelles of the nucleus, such as chromosomes and the mitotic spindle that segregates chromosomes during cell division. Lasers also facilitated studies of cytoplasmic constituents—namely, mitochondria (the energy factories of cells) and such structures as microfilaments, microtubules and centrosomes—involved in maintaining cellular architecture and transporting molecules within cells.

Putting Lasers to Work

Although we do not always know exactly how lasers produce the specific changes they make in cellular components, we can nonetheless generate certain alterations reproducibly and without compromising the target's structure or environment. For example, the traditional biological tools of light and electron microscopy show that laser scissors can produce a particular change in a chromosome, deep within a cell. Early work by our group demonstrated that scissors can inactivate a selected part of a chromosome in dividing cells—specifically, a region containing genes that control construction of a nuclear organelle known as a nucleolus. What is more, the alteration persisted in the cloned progeny of those cells, all of which possessed inactive versions of the genes in that same region.

The alteration to the chromosome—a lesion less than a micron in size—appears as a lightened region when the live cell is viewed under a phase-contrast light microscope. Careful transmission electron microscopy, capable of 10,000 to 100,000 magnification, reveals that same region to be a cleanly defined structural alteration, with the chromosomal material on either side of the lesion, as well as the cytoplasm surrounding the chromosome, apparently unaffected. The "lightening" seen in light microsco-py is actually the result of a change in refractive index rather than a complete physical removal of material—the laser is changing the chemical and physical properties of the chromosome without totally destroying it.

The cell membrane can likewise be studied, via a gentle perturbation of its fluidity. The membrane can even be incised, the laser cutting a micron-size hole that seals within a fraction of a second. Through this technique, called optoporation (pore production through optical means), molecules can be inserted into a cell when the pores are open without permanently damaging the membrane.

Optoporation may be especially suitable for genetic manipulation of plants, which have rigid cell walls that are relatively impenetrable compared with the supple membranes of animal cells. At the University of California at Irvine, my colleague Hong Liang and I have taken advantage of optoporation to insert genes into single rice cells; these genetically modified cells gave rise to whole plants in which every cell carried and expressed the introduced genes. This work, when considered with the inactivation of nucleolus genes, demonstrates that laser scissors can be employed either to insert or to delete genes.

In Europe, laser-scissors manipulation of gametes (sperm and egg) has been applied recently in the human clinic, as part of a procedure called assisted hatching. The scissors thin or remove a small area of the protective zona pellucida of eggs that have been fertilized in a laboratory dish. The very early embryos are then placed in the womb, where the thinning of the zona appears to abet implantation. Thinning can also be accomplished by more conventional techniques, but the laser method works without toxic chemicals that can damage the embryo. The most extensive human study to
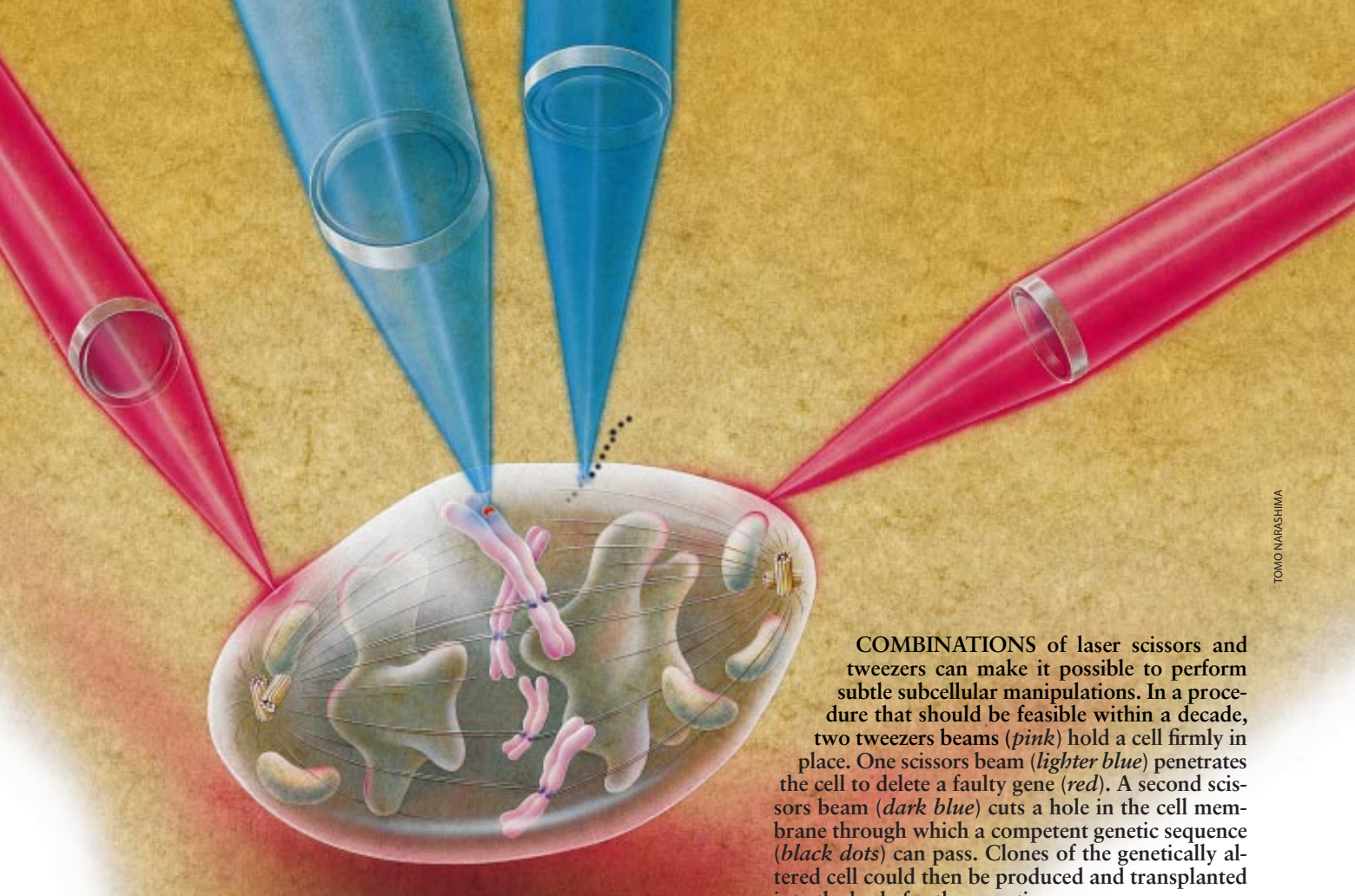
    Laser Scissors and Tweezers

COMBINATIONS of laser scissors and tweezers can make it possible to perform subtle subcellular manipulations. In a procedure that should be feasible within a decade, two tweezers beams (*pink*) hold a cell firmly in place. One scissors beam (*lighter blue*) penetrates the cell to delete a faulty gene (*red*). A second scissors beam (*dark blue*) cuts a hole in the cell membrane through which a competent genetic sequence (*black dots*) can pass. Clones of the genetically altered cell could then be produced and transplanted into the body for therapeutic use.

date, performed by Severino Antinori's group at the Associated Research Institute for Human Reproduction in Rome, reported a greater than 50 percent increase in pregnancy rates in more than 200 women whose embryos had undergone laser zona thinning compared with women who had not undergone the procedure. The first multicenter trials of laser zona thinning have just gotten under way in the U.S. Similar studies are in progress in Australia and await government approval in Israel.

In collaboration with Nancy L. Allbritton's group at Irvine, my laboratory is using laser scissors in another way as well—to open a single cell so that its chemical components can be analyzed at any given moment. We take advantage of what can sometimes be an unwanted side effect of laser scissors: development of a minuscule cloud of ionized gas called a microplasma that forms when laser light is focused on or above the glass microscope slide on which the cell rests. The expansion and contrac-

tion of this microplasma generates mechanical stresses that, in turn, can rupture the cell. (Physicians exploit a similar effect on a macroscale to pulverize kidney stones and some cataracts via a laser-initiated shock wave; see "Laser Surgery," by Michael W. Berns; SCIENTIFIC AMERICAN, June 1991.)

By positioning a tiny glass capillary tube just above the cell, we are able to collect its contents and analyze them, producing a snapshot of the cell's biochemistry at that moment. This technique has the potential for major applications in single-cell analytical chemistry. One goal is to determine the exact identities and concentrations of proteins important in cancer at the single-cell level.

Expanding Understanding of Scissors

In all applications of laser scissors, investigators need to achieve precision and selectivity. Precision refers to the targeting of the laser beam exactly to the correct point; selectivity pertains to the

controlled alteration of the target while leaving the surroundings unaffected.

Achieving precision is relatively straightforward, thanks to the high level of optical elements in today's microscopes. Objectives, or lenses, are precisely machined and chromatically corrected throughout the visible spectrum so that all wavelengths focus at nearly the identical point in space; researchers using multiple laser beams of different wavelengths thus can confidently focus those beams to the same point.

With a state-of-the-art, oil-immersion microscope objective at 100 magnification, the physics governing the size of a laser focal spot result in that spot's being slightly smaller than the laser wavelength. A neodymium yttrium aluminum garnet (Nd:YAG) laser operating at a wavelength of 532 nanometers can produce a focused spot of 499 nanometers, or 0.499 micron. Precision, however, can be better even than it first appears. Our laser of choice produces a Gaussian distribution of energy—that
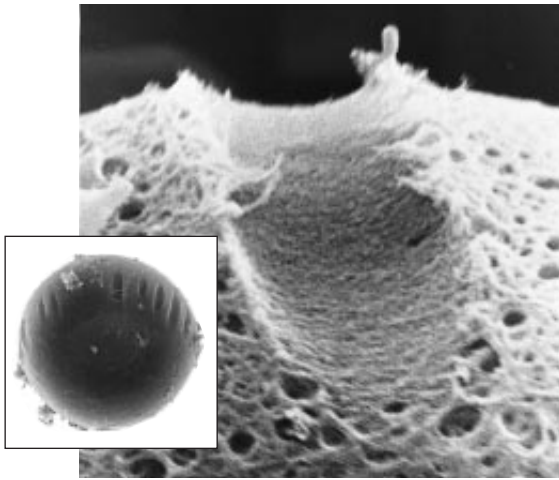
FEATS ACCOMPLISHED by laser scissors include etching of grooves (*left*) in the zona pellucida around a fertilized mouse egg (*inset*) to facilitate implantation. They also include removing a V-shaped wedge from a chromosome (*right*) in a living cell from a kangaroo rat. At its widest, the wedge is about one micron across.

MICHAEL W. BERNS

is, the energy forming the focal spot can be characterized by a bell-shaped curve. Because only the peak of the curve may have sufficient energy to alter a particular target organelle, the effective spot can be significantly smaller than the diameter of the measured focal spot.

Selectivity—controlled alteration—can also be achieved as is evident from applications that have been developed so far. Nevertheless, investigators do not yet know how to guarantee selectivity in many new applications (other than by empirically noting what works) because of incomplete understanding of the interactions between laser light and biological targets. Such a circumstance is not unusual in science: people took aspirin for a century before researchers unraveled how it worked at the molecular level. Still, knowing exactly how a drug or a technology operates cannot help but lead to better

ENERGY DELIVERED to cells (energy dose), which is measured in joules per square centimeter (J/cm^2), depends on the irradiance of the lasers themselves and on how long a cell is exposed to the light. (Diagonal dotted lines represent constant energy doses.) Some of the effects that can be achieved in cells at different combinations of irradiance and time are highlighted. At least some heating (*red*) is common over a wide range of energies. Dark red is where heat is the dominant mechanism.

applications. Therefore, as might be expected, scientists are working intensively to clarify the complex interactions between laser and living cell.

Several factors make defining exact laser effects difficult. Measuring and recording events in submicron regions of a single living cell represents a formidable challenge, as does controlling the energy of the laser beam within such small volumes. Despite these obstacles, we can honestly say we are not completely in the dark. From macroscopic studies, we understand that various physical and chemical processes can potentially come into play when lasers interact with organic tissue. These processes can be trig-

gered by the absorption of single photons or by the nearly simultaneous absorption of multiple photons.

Single photon absorption may simply heat the target; it can also initiate chemical reactions that produce free radicals or other cell-damaging products. And photons of high energy (such as in the ultraviolet) may even break molecular bonds, thus tearing molecules apart, in a process called photoablation. Furthermore, multiple photons can be absorbed so closely in time as to be equivalent to a single, high-energy photon. Such multiphoton absorption can drive chemical reactions or lead to the kind of molecular dissociation seen when single ultraviolet photons are absorbed. Any of these scenarios, or any combination, may operate in a submicron cell target.

In addition to the absorption properties of the target, another key factor that determines the laser's effect is the irradiance of the impinging light (the energy reaching a target's surface in a given time period, measured in watts per square centimeter). With pulsed lasers that expose the target to light in periods ranging from microseconds down to femtoseconds (10^{-15} second), the irradiance can be enormous. Laser effects following absorption in tissue exposed for relatively long periods (hundredths of seconds to minutes) are well known and include heating—which can cause molecular denaturation, coagulation or vaporization—as well as chemical reactions that can create deleterious photoproducts, such as free radicals. We are less

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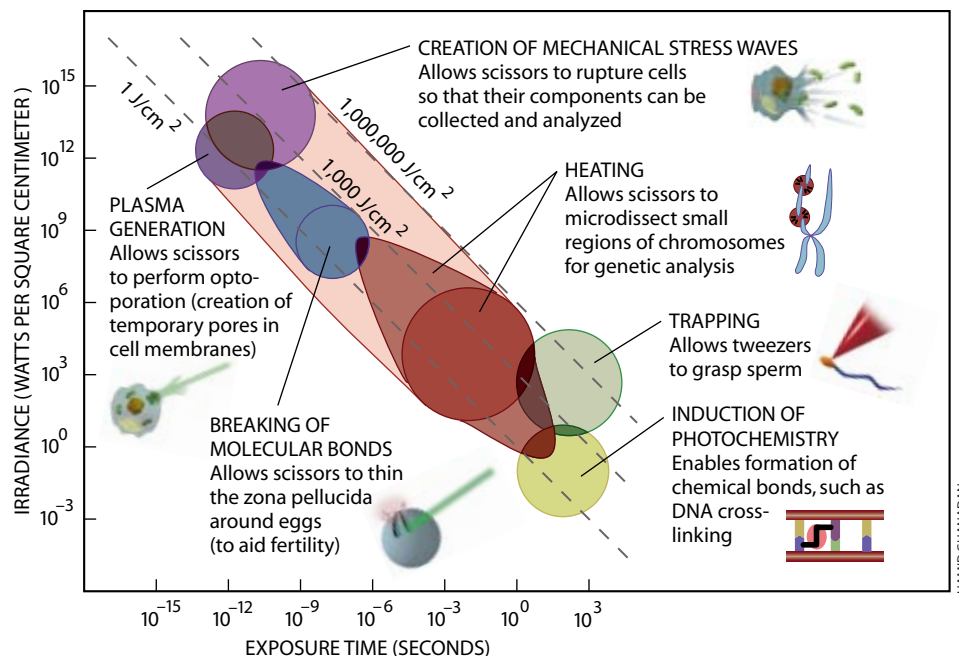
certain, though, of the effects of the high irradiances in the volumes in which we work intracellularly—a sphere one micron in diameter has a volume of less than a femtoliter.

A major challenge, therefore, is to determine the boundaries at which increases in irradiance will change the effect on the target—for example, discovering the level at which the laser's effect switches from heating of the target to the generation of microplasma-induced shock waves. Irradiance effects and boundaries are still incompletely defined but are certainly affected by the duration of the laser pulse and by the absorbance properties of both the target and its physical environment.

Laser-irradiance boundary studies represent a dynamic area of research, with our group especially concentrating on the submicron realm. The next few years should see great progress in the characterization of laser-target interactions, which will create the opportunity for ever more delicate applications of lasers in subcellular domains.

Despite the unknowns, extremely accurate laser ablation, in which we destroy or inactivate specific regions of targets, can be performed in virtually any cell component that can be visualized through the light microscope. As eagle-eyed baseball great Ted Williams might have put it, "If I can see it, I can hit it."

In the near future we should be able to surpass even that visual limitation. For example, we might employ light-absorbing molecules (LAMs) capable of finding and binding to a sequence of DNA in a gene we wish to manipulate. This sequence could be too small to see, or its location within a chromosome might be unknown. Bathing the entire cell in light of the correct wavelength would energize the LAMs, which in turn would inactivate the bound gene. (The process could also potentially activate that gene or other genes, by transferring energy to initiate a cascade of chemical reactions or by simply inactivating a suppressor gene.) The rest of the cell merely would be bathed in light briefly and be none the worse after the exposure.

Laser scissors, with their short, intense pulses of light, led the way in our attempts to perform microscopic surgery on cells and molecules. More recently, they have been joined by the equivalent of the forceps—laser tweezers.

To the nonphysicist, the use of light to trap and move an object is counterintuitive. That light can heat or burn,

measure or calibrate makes sense. But the idea of light creating a force that can hold and move an object may seem as fanciful as a *Star Trek* tractor beam. Still, light has momentum that can be imparted to a target. The resultant Lilliputian forces fall far below our sensory awareness when, for example, the sun's light shines on, and imperceptibly pushes against, us. But these forces can be large enough to influence biological processes at the subcellular level, where the masses of objects are infinitesimal.

What Tweezers Can Do

In the mid-1980s Arthur Ashkin of AT&T Bell Laboratories discovered that a continuous-wave, low-power (under one watt) laser beam could "optically trap" individual bacteria and protozoa. He and his co-workers demonstrated—first with a blue-green argon ion laser and then with the infrared Nd:YAG laser (to which cells are more transparent)—that whole cells and their organelles could be grasped and moved about.

Steven Chu, a 1997 Nobelist in physics, and his colleagues at Stanford University later showed that laser tweezers could also grip molecules. They conjugated transparent polystyrene beads to the ends of coiled, naked DNA and used optical trapping forces to pull the beads and stretch the DNA molecule to its full length [see "Laser Trapping of Neutral Particles," by Steven Chu; SCIENTIFIC AMERICAN, February 1992]. Steven M. Block of Princeton University and Michael P. Sheetz of Duke University have employed optical trapping of molecules to study kinesin motors, the proteinaceous cellular structures that drive the whiplike action of a flagellum or a sperm tail and move intracellular particles and organelles.

LASER AS TRACTOR BEAM can hold an object in its grasp because of basic physical principles, such as the conservation of momentum. The refraction of any pair of symmetric laser light rays within the beam produces forces. A typical set (rays A and B) produces forces F_a and F_b. These result from the response by the target to the change in momentum of the light rays. If the focal point lies before the center of the target, the sum of any pair of such forces is a total force (vertical arrow F) pulling the target toward the beam. A focal point after the center of the target would push the target. Focal points to the left or right of the center would move the target left or right.

A small enough object that is relatively transparent to laser light of a particular frequency refracts incident laser beams, bending the light. As a result of this refraction, momentum is transferred from the light to the target. When the geometry of the arrangement of light beams and target is correct, the momentum imparted to the target pulls the target in the direction of the incident laser beam, and the beam can thus hold the target in place [see illustration below]. By moving the beam, the laser operator can pull the target from place to place.

The beams of scissors and tweezers differ significantly in duration and intensity. Whereas the scissors employ short pulses of high irradiance, tweezers make use of continuous, low-irradiance beams. The target must be transparent to tweezers so that the beam will pass through the object without significant energy being absorbed and converted to destructive heat or even generating damaging photochemistry.

A single beam can grasp a cell or organelle, but that object may still be able to wag; a second beam can lock the target in place, in a vise of light. Laser-tweezers beams typically use light at wavelengths between 0.7 and 1.06 microns and of 25 to 500 milliwatts of power in a focal spot between 0.5 and 1.0 microns in diameter. Such a beam generates forces in the piconewton range, more than sufficient for trapping cells and moving organelles inside and outside of cells.

Laser tweezers make new kinds of experiments possible. My Irvine colleagues Michael Cahalan, Bruce J. Tromberg,
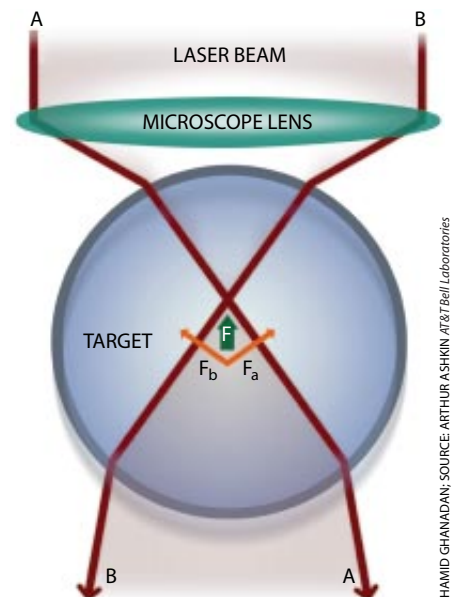
LASER BEAM

MICROSCOPE LENS

TARGET

A   B

F_b F_a

F

B   A
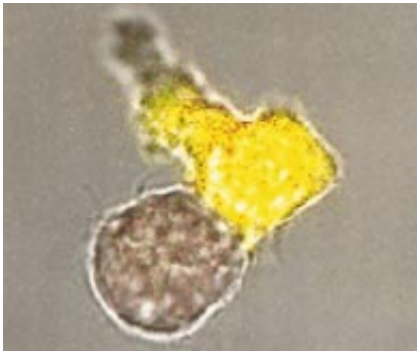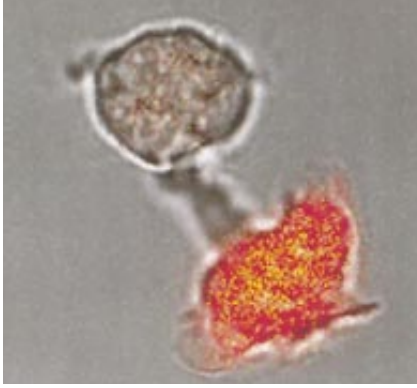
POLARITY OF *T* CELLS is borne out in studies made possible by laser tweezers. *B* cells, which provoke calcium release by *T* cells, were carefully positioned alongside *T* cells using tweezers. Positioning of the *B* cell at one end of a quiescent *T* cell elicited no change; a fluorescent red stain in the *T* cell remained red (*top*). But when the *B* cell touched the other end of the *T* cell, calcium was released, signaled by yellow fluorescence (*bottom*).

Xunbin Wei, Tatiana Krasieva and Paul Negulescu recently employed them to analyze the relation of form to the function of the immune system's *T* cells. Presentation of foreign molecules, or antigens, by the immune system's *B* cells starts a cascade of reactions that includes an increase in calcium ion concentration in the *T* cells. This rise in turn leads to the *T* cell specialization and proliferation so vital in immunity.

T cells have a polarized appearance, defined by shape and the direction in which they crawl. Titanium sapphire laser tweezers trapped *B* cells and placed them at different locations along the *T* cell surface. With the *B* cell placed at the back end of the *T* cell, no response occurred, and the *B* cell detached from the *T* cell within two minutes. But placement of the *B* cell at the leading edge of the same *T* cell quickly elicited a rise in intracellular calcium ion concentration, meaning that the response cascade was in progress [*see illustration above*]. This finding fits nicely with the notion that *T* cells and other white blood cells migrate in specific directions, in part by responding to signals received by receptors on their front ends.

Laser tweezers can trap even highly motile cells. As my Irvine co-workers Yona Tadir, Gregory J. Sonek and William H. Wright (now at SRI International in Menlo Park, Calif.) first demonstrated, tweezers can grasp individual human sperm cells and manipulate them at will. We took advantage of being able to study the swimming forces of sperm, gradually reducing the trapping power to determine the level at which the sperm could escape. Investigation of this "relative escape force" has provided a method to examine the relation between swimming force, velocity and swimming pattern. One exciting discovery—sperm that swim in a zigzag pattern swim with greater force than straight swimmers—may explain clinical observations suggesting that men with a higher proportion of tacking sperm are more fertile than their counterparts who produce straight swimmers.

Another finding from laser-tweezers studies shows that sperm surgically removed directly from the epididymus (where they mature and are stored prior to ejaculation) of men incapable of ejaculation swim with only one third the force of normally functioning sperm. (Full swimming force appears to require the complete maturation that occurs during passage through the epididymus.) This result helps to explain why fertilization is optimized when these patients' sperm are physically injected into eggs, as opposed to simply having them attempt to fertilize eggs in a petri dish, where success may be a consequence of swimming force. Evaluation of relative escape force should therefore be a valuable screening tool to determine if low fertility is caused by poor motility and could also test treatments aimed at improvement of motility. Thus, laser tweezers should find a role both in clinical management of infertility and in sperm motility research.

Application of tweezers in those roles must be achieved delicately, because exposure to tweezers may hamper motility, probably because of unavoidable thermal or photochemical effects. Heat is actually more of an issue with tweezers than with scissors because of the continuous beam.

Measurements of tweezers-generated heat have been conducted by Tromberg, Sonek and Yagang Liu (now at Beckman Instruments in Fullerton, Calif.), who were able to record temperature rises in irradiated regions of cells. They bound a heat-sensitive molecule, Laurdan, to either artificial cell membranes (liposomes) or Chinese hamster ovary cells in culture and measured changes in fluorescence intensity and in emission wavelengths.

This cellular "microthermometer" detected a rise of 1.15 to 1.45 degrees Celsius per 100 milliwatts of laser power in the focal spot. Tweezers may produce 10 times that power; if heat dissipation is inefficient, deleterious changes in the rates of biochemical reactions, inactivation of heat-sensitive proteins (including enzymes) and even cell death become serious concerns. These Laurdan-dependent measurements represent an important contribution to the growing database of laser-target interactions.

Putting It All Together

Laser scissors and tweezers are each powerful tools in their own right, but techniques making use of both, either sequentially or in combination, allow for even more sophisticated and creative manipulation and alteration of cells. The first application of both laser scissors and tweezers was conducted by my former postdoctoral fellow Romy Wiegand-Steubing. She used an infrared Nd:YAG laser tweezers to move together two human myeloma cells and a pulsed ultraviolet nitrogen laser scissors to cut the adjoining cell membranes; the two cells merged into a single hybrid cell containing the genomes of both. Fused cells can combine valuable attributes within one entity, such as one cell's ability to produce a useful product with another's ability to divide indefinitely.

Furthering the sperm-trapping work, a European group led by Karin Schütze of the Harlaching Center for Laser Applications in Munich has used infrared tweezers to grasp and guide individual bovine sperm into eggs by way of a hole in the zona that was produced with scissors. This technique resulted in fertilization in a small percentage of cases, as did a comparable effort with mouse sperm and eggs.

A similar approach, if shown to be safe and effective, could provide an alternative to existing gamete-manipulation procedures in humans and almost certainly will find a role in animal husbandry. Although caution—as well as strict adherence to federal governmental policies—must clearly guide new and experimental procedures, researchers in

MICHAEL W. BERNS

MICROBEAM SYSTEM for laser scissors incorporates a pulsed laser and a phase contrast microscope. The pulses, lasting 10 nanoseconds, are visible as the discrete light bursts along the line of the laser path. The system shown is the immediate predecessor to the confocal ablation trapping system, which has enclosed beams that are not visible.

assisted reproduction have shown considerable interest in laser scissors and tweezers; two companies have already begun to market multipurpose laser workstations for eventual use in fertility clinics. Micromanipulations of sperm and eggs using laser systems should be faster and more efficient than traditional techniques.

The combination of laser scissors and tweezers makes possible unprecedented manipulations of organelles as well as whole cells. In collaboration with Edward D. Salmon of the University of North Carolina at Chapel Hill and Conly L. Rieder of the Wadsworth Center for Laboratories and Research in Albany, N.Y., we initiated studies at the organelle level that used laser scissors to cut chromosomes, which were in the midst of mitosis (cell division). Then, with laser tweezers, we moved the fragments within the cell, our goal being the study of forces exerted by the mitotic spindle, the cellular machinery that pulls replicated chromosomes to opposite ends of a cell as it divides.

Unexpectedly, we were able to freely move fragments that were outside the spindle but were unable to move fragments from the cytoplasm into the spindle. These studies confirmed the existence of a spindle "cage" that blocks foreign material, such as the newly produced chromosomal fragments, from entering the spindle. Because mitosis carries a cell's genetic material to the next generation, it makes sense that evolution developed a system that apparently blocks undesirable material from the spindle area.

These tools are being more fully exploited by Rieder and his colleagues, who have since been able to demonstrate that laser tweezers can inhibit the movement of laser-scissored chromosome fragments already within the spindle. These techniques finally make possible noninvasive studies of forces within the mitotic spindles of single cells. Because the spindle is a key player in cell division, unraveling its workings can lead to more detailed understanding of diseases related to cell division, such as cancer and birth defects.

As part of the National Institutes of Health Laser Microbeam and Medical Program Biotechnology Resource, we have now built a microscope workstation that incorporates two laser-tweezers beams and one laser-scissors beam into a confocal laser fluorescence microscope. The lasers are tunable—that is, they can be set to any desired wavelength. Having all these capabilities in a single, all-purpose workstation meets multiple needs of the cell biologist. A researcher can observe fluorescing cells or organelles through the confocal microscope during and after the period when tweezers and scissors are in operation. This joystick-controlled confocal ablation trapping system (CATS) is available for a wide range of cellular and subcellular studies, including ones that improve procedures necessary for DNA sequencing—deciphering of the order of base pairs, or codes, carried by a stretch of DNA.

My Irvine colleagues Barbara Hamkalo and Al Jasinskas are using this technology to cut out the chromosome's centromere, where the spindle microtubules attach. Investigators disagree over whether this region is likely to be genetically active, but the centromere has been extremely difficult to isolate and analyze for unique active gene sequences. Laser scissors and tweezers should help settle this controversy.

More than 80 years have passed since Albert Einstein laid the theoretical foundation for the existence of lasers. By the early 1960s, thanks to the work of American and Russian physicists, laser light became a reality. Now optical tools incorporating lasers enable biologists to become cell surgeons, probing and manipulating cells and organelles. The application of these technologies has far-reaching implications for medicine, developmental biology, the study of cell structure and function, and the unraveling and manipulation of the human genome. The future of laser-based biological research and applications should be very bright indeed. **SA**

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MICHAEL W. BERNS is the Arnold and Mabel Beckman Professor at the University of California, Irvine. He is also president and co-founder (with his mentor, industrialist Arnold O. Beckman) of the university's Beckman Laser Institute and Medical Clinic. His research interests include genetics, cell motility, laser-tissue interactions and clinical applications of lasers in cancer, ophthalmology and reproductive medicine. He dedicates this article to Beckman, who is 98 years old this month.

Further Reading

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