Making Light Work of Cell Surgery

Using lasers as both scalpel and tweezers, researchers can perform delicate operations on cells; in the future, such capabilities may be valuable in treating infertility

THE DOCTOR LEANS OVER THE PATIENT, carefully guiding his laser scalpel. The light touches the patient's exterior and begins to carve a tiny hole. Whoops! The laser was set too high and it causes quite a bit more damage than the doctor intended. The patient dies. But not to worry. This patient won't need any last rites, nor will the next of kin be filing any malpractice suits.

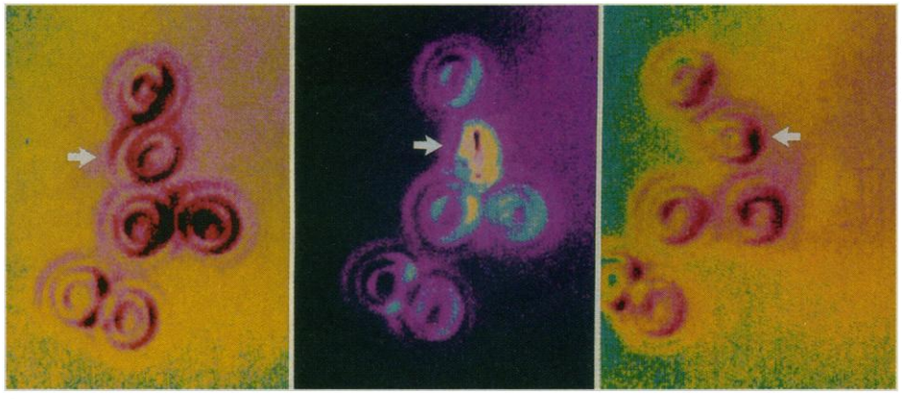
That's because the patient is a carrot cell in a petri dish. The doctor is Michael Berns, a cell biophysicist at the Beckman Laser Institute and the University of California at Irvine, and the "operation" was an attempt to drill a hole through the cell's outer wall and membrane in order to insert new genes into its chromosomes.

Berns is one of a handful of scientists around the world who are doing cell microsurgery with lasers. Over the years researchers have developed a variety of ways to use lasers as scalpels or drills: snipping off bits of DNA from the chromosomes, cutting holes in membranes, and damaging or destroying parts of the cell to see how the cell functions without them. But recently, they have added a valuable new tool to their laser tool kit—a way to grab hold of a cell with laser light and move it around without damage—and this addition promises to open up a great many fresh applications. Already, it has allowed researchers to perform laser operations that had been impossible before, such as fusing two cells.

These laser techniques offer a unique chance to do basic research on individual cells, says Yung Sheng Liu of General Electric Research Center in Schenectady, New York, who chaired a session on lasers in biology and medicine at a recent meeting of the American Physical Society. But in addition to the research applications, laser surgery could prove to have clinical applications, especially in treating infertility. One of Berns' goals, for example, is to use lasers to improve the success rates of in vitro fertilization for men whose sperm have difficulty crossing the protein barrier that surrounds an egg.

The idea of putting lasers to work on cells is almost as old as lasers themselves. The first experiments were done in 1962, just 2 years after the invention of the laser. Marcel Bessis at the Kremlin Bicetre, a hospital in Paris, damaged different parts of cells with a ruby laser and observed how their behavior changed.

Berns himself entered the field in 1966, with an experiment aimed at studying the development of a millipede's legs by destroying a small piece of a millipede embryo. The piece was too inaccessible to target by conventional means, he remembers, and the laser seemed a natural thing to try. Although technical problems torpedoed the original series of trials, Berns became hooked on doing laser microsurgery on cells.



Michael Berns

Optical tweezers. *A laser grabs a single red blood cell (indicated by arrow) and rotates it through an angle of 180° while leaving its neighbors unmoved.*

Some of his early studies involved snipping an end off a chromosome during mitosis, or cell division. The tip, separated from the rest of the chromosome, would "float off into the cytoplasm and be absorbed," Berns says. With care, he could produce living cells with missing genetic material and get clues about what the genes on the damaged part of the chromosome were doing.

Since then Berns has seen a great improvement in laser microsurgery. Looking back 20 years, he says he feels he was a bit like a modern-day doctor contemplating the prospect of performing surgery with a butcher knife. "In the early days, we had only one type of laser available—the ruby laser," he remembers. "Nowadays we have all sorts of lasers. They offer different wavelengths and some can be fired in very short pulses." The importance of having lasers with a variety of wavelengths is that different parts of the cell absorb light at different wavelengths, and thus each responds better to some lasers than to others. Pulsed lasers deliver a lot of power in a short time, destroying the target quickly and avoiding a buildup of heat that can damage the surrounding tissue.

Two other major improvements make laser surgery a much more precise operation today, Berns says. One is an increasing sophistication in the handling of the lasers—he now uses computer interfaces to help guide the lasers and computer-enhanced images to better see what is going on. The other is a much better "feel" for the tools. "We've really begun to understand better how the photons interact with the cells at the molecular level," he says.

Perhaps because of the interdisciplinary nature of the field, and perhaps because most researchers who are interested in both lasers and biology have concentrated on direct medical applications, few researchers have done extensive work on laser surgery of cells. GE's Liu says the only other group he is familiar with is that of Karl Otto Greulich at the Physical Chemical Institute in Heidelberg, Germany. Nonetheless, the recent advances in the field seem likely to attract much more interest in coming years.

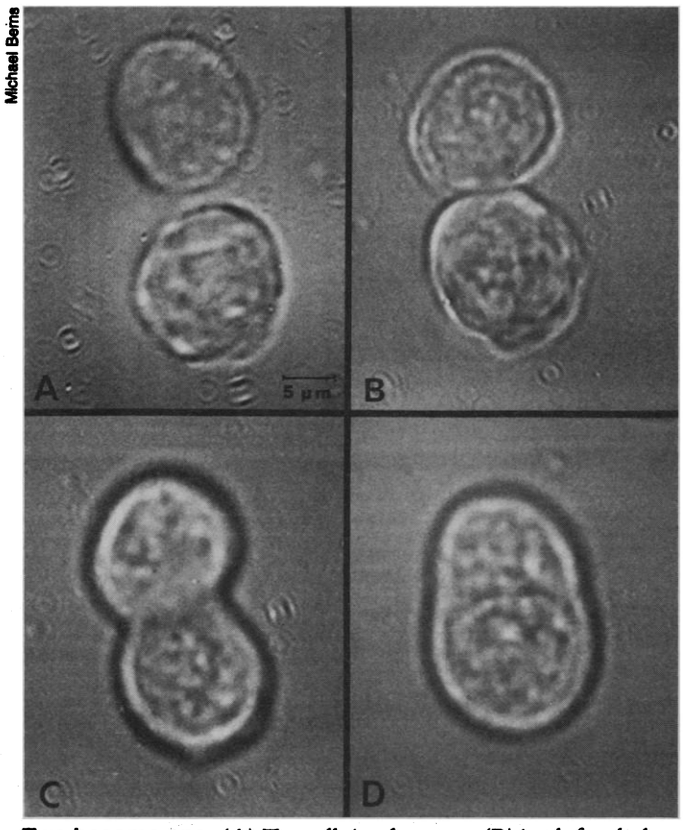
A recent experiment illustrates just how precisely modern-day laser systems can be controlled. Berns' student Wen Tao used a Nd-YAG (neodymium-yttrium-aluminum-garnet) laser to slice through the network of

microtubules—the tiny fibers in a cell that play a role in cell movement, cell shape, and the movement of the chromosomes during mitosis—and watched how they grew back. He found that the microtubules regenerated at varying speeds, contradicting one theory that predicted the whole network would develop as a unit. The work should help cell biologists understand how microtubules function, Berns says.

Although the laser cut wide swathes through the microtubules, it did not damage the remainder of the cells or their membranes and left the cells fully functional. Indeed, one cell underwent division just an hour after the experiment. This is far different from what most people expect—that laser light will destroy everything in its path. The laser is so selective, Berns explains, because the peak intensity of the light is concentrated in a sphere about 0.5 micrometer across. Outside that sphere the power of the laser drops sharply. Being able to reach through the cell membrane and cut something without affecting the membrane or other parts of the cell gives the laser a big advantage over other cell surgery techniques that depend on physically reaching into the cell.

By focusing on the membrane of a cell instead of an interior target, investigators can drill holes in the membrane itself, as well as in the tough outer walls that surround plant cells. This could be a boon to genetic engineers who want to introduce new genes into cells. Wen Tao did just this in one experiment. He placed cells in a solution containing foreign DNA that he wanted the cells to take up. When he then used a laser to punch a hole in an individual cell, the membrane would close up within a millisecond or so, but that proved long enough for the genes to slip through every now and then. Later tests showed that the new genes were incorporated into the cells' chromosomes and were active there.

Although researchers already have several techniques of inserting genetic material into cells, the laser method has some advantages, Berns says. It is easier to perform than direct injection with a tiny needle, which takes a steady hand and a great deal of practice. And it may prove to be a good way to get through the tough outer walls of some plant cells, such as pollen, that resist other gene-implanting techniques.



Two become one. *(A) Two cells in a laser trap; (B) just before the laser strikes the intersection of the two cells; (C) after ten pulses, the membranes start to fuse; and (D) after 5 minutes the fusion appears to be a single cell.*

Choosing which laser is right for which job is currently a combination of trial and error and educated guesswork, Berns says. "If you know what your target is and what wavelengths it absorbs, that tells you which lasers to try first." Past that, it's a matter of testing different lasers, power settings, and pulse lengths. If you get it wrong, it's immediately obvious, he says. Too little power, and nothing happens; too much, and the cell dies. It's particularly dramatic when drilling a hole in a cell's membrane, he says. Set the laser power slightly too high and "the whole cell is gone—blown to smithereens."

In just the past year, Berns has found a way to use lasers for something other than cutting and drilling. Arthur Ashkin of AT&T Bell Laboratories was the first to point out that a laser can trap objects as large as viruses, single cells, and bacteria (see *Science*, 26 August 1988, p. 1042). A low-power laser can hold a small cell in its focus for several minutes without damaging it or, in the case of larger cells, the laser can grab onto an internal organelle and move it around the cell's interior.

Berns exploited this discovery early last year to learn a surprising detail about how chromosomes move during mitosis. Scientists do not understand exactly how the two sets of chromosomes are pulled into the two halves of a dividing cell, so Berns decided to pull on the chromosomes with his "optical tweezers" just to see how they would respond. In a series of tests, he focused the laser next to chromosomes that had briefly stopped moving and tried to pull them opposite to their original direction. The result: "All of a sudden the chromosome starts moving away from the direction we're trying to pull it." Not only that, but it races at speeds 10 to 20 times faster than normal during cell division.

Exactly what triggers this vigorous reaction isn't clear, but Berns hypothesizes that something—probably the microtubules that pull the chromosomes into the two halves of the cell—senses that the chromosome is being pulled the wrong way and reacts by pulling even harder in the right direction. The next experiment, he says, will be to cut the connections to the microtubules and then pull on the chromosomes and see what happens.

With both scalpel and tweezers in his laser tool kit, Berns naturally couldn't wait to use them together, and in January he reported success in fusing two cells by holding them side by side in a laser trap and firing a second laser at their intersection. In several trials he, Rosemarie Wiegand Steubing, and student Bill Wright found that the laser disrupted the cells' membranes enough that the two membranes started to grow together. Within 5 minutes, the two cells had completely coalesced inside one membrane.

Steubing, who came to Berns' team from the Heidelberg group, says that so far none of the fused cells have reproduced. In principle, there is no reason they shouldn't, she notes, since other researchers have fused cells chemically and they survived and reproduced. Steubing predicts that it will be only a matter of time before the team discovers the proper combination of laser parameters and cell-handling techniques to achieve equally good results. The potential advantage of the laser technique is that a researcher can exert more control over the process and can watch as it is going on.

Such cell manipulation with lasers may turn out to have important applications in studying and treating infertility problems. Last year, Berns and Yona Tadir, on sabbatical from the University of Tel Aviv in Israel,

used the laser trap to test how much power sperm cells produce as they swim through a solution. By first trapping a sperm and then lowering the laser power until the sperm could escape the trap, the researchers were able to get a measure of the force each sperm exerted. Some infertility problems in men seem to stem from a low motive force in their sperm, and this technique may offer a way to understand what causes that.

Even more important, laser surgery may offer a way to promote in vitro fertilization for men with weak sperm cells. Tadir, now back in the Department of Obstetrics and Gynecology at Beilinson Medical Center in Tel Aviv, estimates that perhaps as many as 1% of all couples have infertility problems because the man's sperm cannot penetrate the zona pellucida, which is the glycoprotein coating surrounding the egg cell.

Berns and his collaborator, Ines Rojas of the Department of Obstetrics and Gynecology at the University of California at Irvine, hope to be able to poke holes in the zona pellucida to help sperm get in that otherwise could not. After initial experiments on hamster eggs, Rojas says, "We've been able to make the holes and the eggs look fine. Now we want to see if we can get sperm to go through this little hole."

Medical researchers have tried other techniques to help weak sperm into the egg, Tadir says, but generally without success. For instance, some clinicians have used tiny needles to physically inject a single sperm through the zona pellucida, but there have been no more than a handful of pregnancies across the world using this method, Tadir adds. The few successes with microinjection indicate, however, that if the sperm can be transported across the zona pellucida without damage to either sperm or egg, the resulting embryo should be normal.

Several technical problems remain, such as the difficulty of handling a very large cell (the egg) and a very small cell (the sperm) simultaneously in a laser trap. But if laser surgery lives up to its promise for in vitro fertilization, it could be just what the doctor ordered. ■ ROBERT POOL

Marking the Ice Ages in Coral Instead of Mud

Understanding the cause of the ice ages hangs in the balance as researchers dispute land and marine climate records

FOR OCEANOGRAPHERS, the mystery of the procession of the ice ages was solved more than a decade ago: the natural orbital wobbles, wriggles, and wiggles of planet Earth cause the global climate to swing from mildness to frigidity and back again, time after time.

But late in 1988 a group of terrestrial researchers looking for climatic records beneath dry land instead of deep beneath the sea renewed a battle the oceanographers thought they had won. Fifty meters down in a water-filled Nevada crevasse called Devil's Hole, the terrestrial workers found a well-preserved record of climatic variations that they claimed flatly contradicted the deep-sea record linking Earth's orbital variations with the ice ages. That gave heart to a whole cadre of paleoclimatologists who had never been impressed by the marine evidence.

As the renewed clash enters its second year, the latest evidence seems to be shifting in favor of the oceanographers again. It comes not from the deep sea or a crack in the Nevada desert, but a sort of no man's land where both sides have been seeking the upper hand: those strips where land and sea meet, the offshore coral reefs. The decisive weapon there would be a new way to date climate records.

Marine scientists originally became convinced that orbital variations pace the ice ages by dating the climate record preserved in the carbonate sediments at the bottom of the sea (also see box on p. 32). For example, marine scientists found that the last warm interglacial period (about 127,000 years ago) roughly coincided with an orbitally induced maximum in the amount of sunlight falling on the Northern Hemisphere's arctic lands. That seemed just the thing to melt glacial ice and usher in a 10,000-year break after the ice age cold, the researchers reasoned. Similar coincidences through the previous five 100,000-year ice age cycles only strengthened their confidence in the orbital variation hypothesis.

Many terrestrial climate researchers were never completely convinced that the oceanographers were right, but in the absence of terrestrial records as dependable as those from the deep sea, the skeptics had to accede to a truce-like quiet. Then hydrogeologist Isaac Winograd of the U.S. Geological Survey in Reston, Virginia, and USGS colleagues in Reston and Denver rolled out their big gun: a 260,000-year climate record in the carbonate deposits of Devil's Hole, 120 kilometers northwest of Las Vegas. That record looked as good as any marine record, maybe better, its discoverers claimed, because they had been able to date it more directly than any deep-sea sediment ever had been. And in this record the ice ages had not been in step with orbital variations. In particular, the previous interglacial period appeared to have ended at least 147,000 years ago—20,000 years before the oceanographers say it did.

That stung marine scientists. "There's something wrong with [Winograd's age for the previous interglacial]," asserts oceanographer William Ruddiman of Columbia University's Lamont-Doherty Geological Observatory. "There is a virtually unanimous consensus in the marine community," he told *Science*, that an age in the range of 120,000 to 126,000 years for the previous interglacial is a good one. The reasons seem

R. J. Hoffman

Deep dive. *This carbonate deposited in a flooded Nevada crevasse challenges the idea that variations in Earth's orbit pace the ice ages.*