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Micro scalpel offers unprecedented precision

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CUTTING things up to see how they work has a long, if gruesome, tradition in medicine. In the UK, early anatomists sliced open the corpses of executed criminals and grave-robbled cadavers to get a look at what was inside. Others performed surgery on living animals - slitting here, removing an organ there - to figure out which parts were vital, which merely useful, and what they all did. Thanks to these knife-wielding researchers, we know volumes about how the body works - but only down to a certain level.

At the microscopic scale, it's a different story. The cooperative workings of nerve cells, say, or the capillaries in the brain, are largely mysterious. Our knives are too crude to work on such tiny things. What if we had a scalpel small enough to dissect this micro world?

Wish granted. A new tool for performing delicate surgery on individual cells is opening up a new biological world. It can precisely cut or remove parts of a cell without killing the cell itself. With its help, we can measure the tension and elasticity of a cell's own framework - features that can cause disease when they go wrong. We can unravel mysteries about the intricate and dynamic network of molecules inside cells that governs their behaviour and often breaks down in illnesses such as cancer. What's more, this micro scalpel is already giving new insights into the mechanics of the motors that allow bacteria to swim and the ways that nerve cells can repair themselves. "Now we can begin to look at the functions of individual molecules in the physical context of where they operate in the cell," says Donald Ingber, a cell biologist at Harvard Medical School and Children's Hospital Boston.



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Cellular surgery

"For the first time, people had operated on a living cell without damaging or killing it"

This minuscule scalpel takes the form of a femtosecond laser, a device that can vaporise cell structures with a precision of a couple of hundred nanometres or less. Of course, lasers are nothing new in surgery: they have been used for several decades to cut biological tissue, and are well known in eye surgery. But the femtosecond laser is in a different class. First, the beam can be tightly focused on an area about a tenth that zapped by other lasers. Moreover, rather than burning off surface tissue, it can target a point inside a cell or under the skin - leaving the outer layer and surrounding areas undamaged. **"The beauty of the femtosecond laser is that it only affects the focal area, and it gets below the surface,"** says David Kleinfeld, a biophysicist at the University of California, San Diego.

Laser sharp

The key feature of the femtosecond laser is this: it shoots extremely short pulses of light and it does so thousands of times a second. Each shot lasts only 10 to 100 femtoseconds - 10^{-14} to 10^{-13} seconds - but

produces a spot as hot as the sun in its target material. Between pulses much of that heat dissipates, so it doesn't build up and damage surrounding tissue. In the end, the target material turns into a plasma of electrons that boil off, but leave a tiny amount of dust. "It's a very efficient process. Almost all the energy goes to ablate the material," says Adela Ben-Yakar, an engineering physicist at the University of Texas at Austin.

In the early 1990s, Harvard University physicist Eric Mazur began using a femtosecond laser to trigger tiny explosions inside glass, with the aim of creating cavities that could be used for storing data. But by the end of the decade, he had realised that the device might also be used to blitz structures inside cells and tissues. In 2000 Mazur's physics group teamed up with Ingber's cell biology group and began shooting the femtosecond laser at targets in cells. And sure enough, they found they could wipe out a single mitochondrion - the organs within a cell that generate its energy. No one had ever done this before, but the achievement was more impressive still: the team also managed to knock out a mitochondrion without bursting the cell's outer membrane, damaging other mitochondria in the same cell or killing the cell itself. For the first time, people had operated on a single living cell.

That demonstration opened up the possibility of manipulating biological systems at a scale that had been mostly off-limits before. At present, most scientists' finest tools - the needles used in microsurgery - are too bulky for working with any but the largest of cells, such as egg cells. Even then, a high proportion of cells don't survive the operation. Other ways of knocking out cell structures or functions, such as chemicals or gene mutations, can often only target whole cells, or are not very controllable or precise in their effects. The femtosecond laser, on the other hand, can zero in on a single structure just 100 nanometres to 10 micrometres across - the realm of organelles, single cells, bacteria and simple tissues.

Now a handful of research groups are trying out the device. Ingber, for example, is training the femtosecond laser on the cytoskeleton - the intricate protein scaffold that holds up every cell. But it is more than a simple support: tugs on one part of the cytoskeleton not only change the cell's shape but can also trigger signals that affect its physiology. Defects in this system, known as mechanotransduction, play a role in diseases such as hypertension, arthritis and emphysema.

Ingber's team uses a traction force microscope to measure the tension on a cell's cytoskeleton fibres and the femtosecond laser to snip individual fibres. So far, Ingber says, he's been surprised to find that a change in the tension of a single fibre can cause one kind of molecule to alter its chemical reactivity while another type of molecule nearby remains unchanged. What's more, snipping a single fibre - one of many thousands in the cell - can alter a cell's entire shape.

Biophysicist Howard Berg of Harvard University is hoping for similar success in probing the mechanics of the molecular motor that powers the well-known gut bacterium *Escherichia coli*. Although *E. coli* is probably the best understood free-living creature on the planet, biologists still don't know how its tiny rotary motors manage to drive the four or five whip-like tails or flagella that spin clockwise and then anticlockwise, propelling the bacterium in search of food. They know a lot about the chemical receptor system that triggers *E. coli* to move in a certain

direction - toward higher concentrations of amino acids and sugars - but not much about the motor itself, such as how it revs up. "We'd like to understand every nut and bolt," says Berg. Nanotechnologists, naturally, will be following his results closely, since they hope to one day create similar machines on this scale.

["We'll know a lot more about the subtleties of networks in cells when we go to design therapies"](#)

Until now, that has been a tall order. The motor is just 45 nanometres wide, and is constructed from about 20 different kinds of parts, embedded in the cell membrane and facing inward. For years, Berg has been trying to gain access to the inner surface, but inevitably any hole he can poke in the membrane using chemicals quickly reseals - either that, or the treatment blows up the whole bacterium. Using the femtosecond laser, however, Berg has found he can delicately cut a 100-nanometre hole in the membrane that stays open. The bacterium dies, but the mechanical parts of the motor and its ability to run remain intact.

Berg is now building a system that will allow him to play around with this flagellar motor and measure its properties. By holding a specially bred extra-long *E. coli* bacterium across two compartments in a lab dish, and cutting holes in the cell membrane for access, he'll control the voltage, ions and signalling molecules on one side of the dish, while measuring the effects on the flagella on the other side. "We're not sure which components are even necessary for the motor," he says. "We might try getting rid of everything and then adding specific parts back."

Understanding cells from an engineering perspective could help us design therapies to manipulate them when they go wrong. That's why last year the US National Institutes of Health (NIH) launched a nanomedicine initiative, which will invest \$6 million this year and again next year in order to establish half-a-dozen nanomedicine development centres. The goal is to encourage cooperation between biological and physical scientists to get better measurements of what goes on in cells, says Jeffery Schloss, co-chair of the NIH nanomedicine implementation group. "If we can fill in this information, we will understand biological systems in a fundamentally new way; we'll know a lot more about the subtleties of pathways and networks in cells when we go to design a medical intervention," he says.

Medical intervention is also on the minds of biologists using femtosecond lasers to probe the nervous system. They hope to uncover basic principles about the brain, behaviour and how nerves repair damage. To do this, they have turned to the 1-millimetre-long roundworm *Caenorhabditis elegans*, because they already know a lot about its simple nerve network: it has just 302 nerve cells and 5000 synapses, and its genes and pattern of development have all been mapped out. Now two teams have used femtosecond lasers to snip individual *C. elegans* nerves - which are less than 1 micrometre thick - and then watch what happens.

One plan is to use surgery on *C. elegans* to investigate neural circuits that control patterns of behaviour. That's the goal of Aravinthan Samuel, a biophysicist at Harvard University. His lab focuses on three forms of behaviour: the worm's movement responses to temperature, pressure and electric fields. He plans to map the neural signalling that occurs, to find the typical routes through the worm's nervous system.

Samuel's first task was to establish what the worm's normal behaviour is. "Because the worm is so simple, we can reduce its behaviour to robust relationships between stimulus and response," he says. Next, Samuel will use the femtosecond laser scalpel to do brain surgery on anaesthetised worms. "By snipping individual 'wires', we can block information transmission at specific points," he says. By comparing the behaviour of tinkered-with worms with that of normal worms, Samuel expects to be able to link specific nerves, and the neurons they contain, to their roles in these patterns of behaviour.

The new scalpel could lead to remarkable insights into how the nervous system repairs itself, too. Yishi Jin, a neurobiologist at the University of California at Santa Cruz and her team, joined up with Adela Ben-Yakar's physics group, then at Stanford, and used the femtosecond laser to cut nerves in *C. elegans* and then study their regeneration - which might shed light on how to repair nerve injury or treat degenerative nerve diseases.

The Santa Cruz group got excited about trying out Stanford's new scalpel because they had an interesting idea to test. They believed they knew exactly which of the worm's nerves controlled an easily visible form of behaviour: the ability to move backwards. When the genes for certain motor neurons are mutated or knocked out, the worm cannot contract the muscles that push it in reverse gear. So biologists Hulusi Cinar and his wife Hediye Nese Cinar, and applied physicist Mehmet Fatih Yanik, pointed the femtolaser at those same nerves in a normal worm and cut their long axons - the parts of the nerve cell that transmit impulses to other nerve cells. When the worms had recovered from the anaesthetic the team gently tapped them to see if the operation would render them as disabled as the genetic mutants. And it did - at first.

The surprise came when the worms started to recover. The team noticed one worm that, 24 hours after its nerve surgery, regained the ability to move backwards. The scientists say they jumped for joy". It was a very exciting moment - like a miracle," Yanik recalls. More operations on more worms revealed that in about half the cases, the severed axons reconnected and became functional again.

Although many invertebrates can regrow damaged nerves, this experiment showed it for the first time in *C. elegans*, meaning the worm can now be used to study the basic biology of the process. Oliver Hobert, a neuroscientist at Columbia University Medical Center in New York, says, "It's pretty amazing that the two ends can fuse and work again as a nerve fibre. This experiment is a beautiful entry point into further studies on nerve regeneration."

Regeneration game

Now the Santa Cruz group, with the help of Stanford's Yanik, plans to test whether certain factors that are involved in normal nervous system development might spur repair. They need only repeat the experiment on mutant worms that are lacking a particular factor - and many such engineered creatures are available - and then see if its regeneration is impaired or enhanced. Indeed, Hulusi Cinar is already studying one such factor, driven by the hope of discovering new drugs. Meanwhile, Yishi Jin hopes to explore other questions, such as why half of the operated-on worms remain unable to reverse, whether different types of nerves have different regenerative powers, and whether young worms have a higher rate of recovery than elderly worms.

Another self-repairing biological system could also be investigated using the femtosecond scalpel: the network of blood vessels. Kleinfeld's group at San Diego finds the tool is the only way they can selectively cut the tiniest capillaries - 5 micrometres wide - in rats. And it's particularly useful for targeting blood vessels in the brain cortex, especially those lying just beneath the skull. This makes it ideal for modelling strokes, which are caused by damaged or leaking blood vessels in the brain. Ultimately, the team aims to understand the physical "plumbing" of the circulatory system - for example, the fluid dynamics of capillaries and their typical responses to damage or defects. "Parts of the system look pretty robust and other parts don't. At the moment, there's no easy way to predict which is which," says Kleinfeld. "Hopefully, that's what we're going to figure out."

So far, only a limited number of biologists have access to the new scalpel. As Mazur sees it, "Right now there aren't enough experimental groups to investigate the biological applications; the field is in its infancy." Kleinfeld, Mazur and Ben-Yakar are all working on developing the femtosecond laser into a more user-friendly device that serves as an imaging and surgical tool at once.

Until then, physicists and biologists interested in cellular surgery will be hunting for perfect collaborations. That has got to be better than hunting for fresh corpses.

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