

light or fluorescence can lower the contrast of the image. Confocal microscopy partially solves this problem with a pinhole, placed in front of the photodetectors, that blocks out all but the focused light.

Webb got his first glimpse of a way around the wavelength limitation 9 years ago. A colleague had created synthetic molecular "cages" filled with the neurotransmitter acetylcholine that could be inserted in nerve cells to study whether this neurotransmitter's effects depend on the site of its release. He needed Webb to come up with a way to deliver enough energy to a particular part of the cell so the cages there would disintegrate and release the neurotransmitter—without killing the cells.

Webb realized he could do this if he focused a laser beam and delivered the photons in short, intense pulses. The densely packed photons would have a good chance of hitting the cages in pairs, delivering a one-two-photon punch that is equivalent, energetically, to a single hit by a photon with twice as much energy (or half the wavelength). The lower energy of the photons would help minimize the damage to the cells, and the photons would be sufficiently dense to deliver a double dose only along the plane of focus. "You don't burn up the cells above or below the plane of focus," Webb says.

But Webb—and later, others—quickly realized that those two photons could also stimulate fluorescence. "That was the conceptual breakthrough," says John White of the University of Wisconsin, Madison. Indeed, in work reported in 1990, Webb demonstrated that two-photon excitation could be used in fluorescent imaging technology (*Science*, 6 April 1990, p. 73). He showed, for example, that he could follow the moving chromosomes in dividing cells by stimulating fluorescence of a dye attached to the DNA.

In the current work, Webb and his colleagues have now gone a step further. By delivering light in shorter, brighter pulses from a titanium sapphire laser, they raise the odds that three photons will simultaneously strike individual molecules. The energy of all three add together, extending even further the range of fluorescence excitation and making possible the use of photons of even longer wavelengths.

When tested on leukemia cells, which like nerve cells contain serotonin but are easier to work with, these triple hits were enough to make serotonin fluoresce, without the need of any external dye. Based on the amount of fluorescence, Webb was able to measure the amount of serotonin in the tiny granules that store the chemical until it's released. Others have tried to visualize serotonin molecules in the granules, but only in fixed tissue, not living cells, says Webb.

While Webb was working out the logistics of three-photon excitation, others were

hot on the same trail. In 1995, Maryland's Lakowicz began to test the potential of three photons to excite fluorescence from various dyes and biological molecules. At the same time, Victoria Centonze in White's lab made an unexpected observation. A cell that she expected to emit just red fluorescence under her microscope also emitted blue light. She and White didn't realize at first that the blue emission was the result of three-photon hits exciting a second dye that was also present, but their colleague David Wokosin did go on to demonstrate that that was indeed the case.

White's team has now used a single laser to excite fluorescence by both two-photon and three-photon absorption in the same specimen. In work published in the September 1996 issue of *Bioimaging*, the researchers report that this allowed them to follow three different biological molecules, each tagged with a different dye. White estimates that the strategy will make it possible to follow up to five molecules simultaneously in living tissue.

Also, the longer wavelengths of light that can be used in multiphoton excitation don't scatter on their way through tissue, as do

shorter wavelengths, so "you can probe deeper into the cell," White adds. Webb's group has looked 390 nanometers into skin and observed how sun-damaged elastin shatters into tiny pieces. And White says he can peer two to five times deeper into zebrafish embryos than he could with conventional confocal microscopy techniques.

Currently, the only multiphoton instruments are those the researchers put together, but Cornell has granted Bio-Rad Laboratories in Hercules, California, a license to develop multiphoton excitation into a commercial instrument. This will likely cost several hundred thousand dollars, however, until laser technology improves. But White, who has consulted for the company but otherwise doesn't stand to gain from the new product, expects that researchers will quickly come to appreciate what this new microscope has to offer. "It has few disadvantages compared with the confocal microscope and quite a few advantages," he says. "I suspect that it will largely supersede the confocal microscope."

—Elizabeth Pennisi

## PLASMA PHYSICS

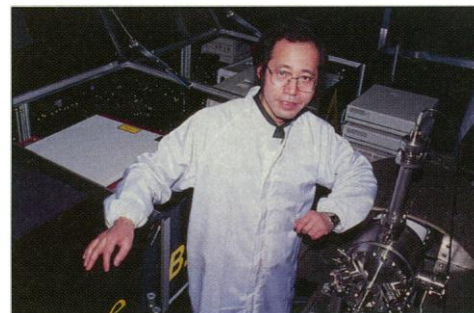
### More Powerful Pulses Please and Puzzle

**OSAKA, JAPAN**—If results presented at an international meeting\* here hold up, researchers will have taken a sizable step toward creating a new generation of compact particle accelerators powered by laser pulses. A team at the Japan Atomic Energy Research Institute (JAERI) led by physicist Kazuhisa Nakajima says it has succeeded in accelerating electrons to energies of from 100 million to more than 300 million electron volts. That's still well short of the energies needed for high-energy physics experiments, but it's more than three times higher than those reached in earlier experiments. The announcement has stirred both excitement and caution, however, because theorists can't explain the achievement.

"The results, if you take them at face value, are extremely impressive," says Chan Joshi, a University of California, Los Angeles (UCLA), electrical engineer and a pioneer in laser acceleration. "But there are aspects of the results that are hard to understand." Among other things, he and his colleagues wonder how the laser pulse could have remained sharply focused for long enough to drive the intense accelerations reported by the group, which is part of JAERI's year-old effort to push the development and use of compact, short-

pulse lasers (*Science*, 5 January 1996, p. 26).

In principle, accelerating electrons with such lasers sounds easy. A laser pulse shot into a gas ionizes it and creates a wake, much



D. NORMILE

**Wake-up call.** Nakajima's results are controversial among short-pulse laser physicists.

like a speedboat zipping across a pond. Electrons riding on the wake can be accelerated to high energies. The advantage over conventional accelerators is that the acceleration occurs over a much shorter distance, which could sharply cut the size, cost, and energy requirements of accelerators. But the effect occurs only if the laser pulse can somehow remain within a narrow channel for several centimeters instead of dispersing within a fraction of a millimeter, as it does normally.

Theoretical studies predict that, at very high laser energies, the interaction of the laser and the plasma, or ionized gas, creates a sort of lens in the plasma that focuses the laser light

\* The 2nd Japan-U.S. Workshop on Interactions of High-Power Waves With Plasmas and Matters, Osaka, Japan, 16–18 December 1996.

and propagates it along the narrow path needed for high acceleration. The JAERI group, however, claims to be seeing these phenomena, called self-focusing and self-channeling, at much lower energy levels. As evidence, they cite the shape of the fluorescence excited by the laser in the plasma, the spectrum of the laser light scattered out of the plasma, and direct measurements of the diameter of the laser beam, which they say remained small for several centimeters. But UCLA's Joshi and others say the evidence is not conclusive. "What is lacking is a measure of laser intensity in the channel," Joshi says.

The experimental observations are bolstered by computer simulations by JAERI physicists Yasuaki Kishimoto and James Koga. When the researchers assumed that the laser pulse itself ionized the gas, which is the approach used in most experiments, they found that self-channeling did not occur even at the theoretically predicted energy levels. However, if the gas were ionized ahead of the pulse, then self-channeling occurred well below the critical power that theory predicts. Koga speculates that this occurs in some experimental setups when a spike of energy from the laser pulse precedes the rest of the pulse and ionizes the gas.

His interpretation could explain why some groups, using laser pulses with different characteristics, have not detected self-channeling. But it leaves open the mechanism through which the self-focusing and self-channeling could be occurring. Says Koga, "We're wary about saying anything too strongly" about self-channeling. But Toshiki Tajima, a physicist at the University of Texas, Austin, who also does theoretical work at JAERI, says there is no other explanation for the results: "We may not understand the mechanism, but there has to be self-channeling."

The unexpected self-channeling laser isn't the only puzzle in the results, however. Wake-field acceleration is supposed to work only at a specific plasma density for any given laser pulse length, yet the JAERI group has seen electron acceleration over a broad range of plasma densities. They are convinced, however, that a wake field is responsible. In one set of experiments, the group varied the timing of the injection of the electrons relative to the laser pulse at different densities. Nakajima says the results—electrons injected too early or too late did not gain energy—support his contention.

Kwan Je Kim, a physicist at Lawrence Berkeley National Laboratory on sabbatical at Kyoto University, says questions about the results indicate that the whole area of wake-field acceleration is "a bit immature." But Tajima is more optimistic: "A year ago, we never thought the field would make such rapid progress." Theoretical explanations, he adds, will come hand in hand with additional data.

—Dennis Normile

## CANCER RESEARCH

# Designing Therapies That Target Tumor Blood Vessels

The word "cancer" conjures up images of a cohort of rampaging cells, burgeoning into life-threatening tumors that dispatch their metastatic offspring to ravage other parts of the body. Traditional cancer treatments have been based on attacking the rebel cells directly, by removing them surgically or attempting to destroy them with radiation or chemotherapy. But a new wave of potential cancer therapies aims to kill these hostile armies not by direct attack, but by shutting off their supply lines: the blood vessels through which tumors get the oxygen and nutrients they need to live and grow.

Work reported today advances this anticancer strategy further, giving a boost to two different means of cutting tumors' lifelines. The most common one aims to prevent tumors from forming the new blood vessels necessary to nurture their growth. To block the process, called angiogenesis, researchers have identified agents that interfere with the endothelial cells that build the new vessels, by preventing them from responding to growth factors or suppressing their ability to chew their way through surrounding tissues. The second approach seeks to block blood vessels that have already formed.

These antiangiogenic and antivascular measures have already produced encouraging results on animal tumors, and today's reports add to the promise that has already launched a dozen or more candidate drugs toward the ultimate test in the clinic. In work described on page 547 of this issue, Philip Thorpe and his co-workers at the University of Texas Southwestern Medical Center in Dallas show that they can shrink or even eliminate tumors in mice by giving the animals agents that trigger blood clot formation in existing tumor-feeding vessels. And in today's issue of *Cell*, Judah Folkman and his colleagues at Harvard Medical School report their discovery of a factor called endostatin that is the most potent yet in a growing collection of molecules that block new blood vessel formation. Endostatin, Folkman's group reports, can shrink large tumors down to microscopic size in mice.

To see tumors shrink so dramatically un-

der treatment is "outstanding ... better than my best hopes," says Noel Bouck of Northwestern University, who is also working on antiangiogenesis drugs. "If it just works for human tumors, it will be fabulous."

That, of course, is a very big "if," and one that applies to all the new strategies. As cancer researchers know only too well, many approaches that have looked promising in animals have died a quiet death after proving ineffective in humans. Still, antiangiogenic therapy may have unique strengths. For one, these drugs might avoid one of the main handicaps of conventional cancer therapies: the development of drug resistance, which ultimately leads to treatment failure.

"Cancers have a formidable ability to acquire resistance to any therapeutic modality we throw at them ... chemotherapy, radiation therapy, immunotherapy," says tumor biologist Bob Kerbel of the Sunnybrook Health Science Center at the University of Toronto. But the cells of a tumor's blood vessels—which are the target of the new therapies—are normal and thus less prone to mutate than cancer cells. As added bonuses, an effective vessel-targeting therapy

should be useful for many types of cancer because all tumor-feeding blood vessels are essentially the same, and delivering a drug to the vessels should be much easier than getting it into all the cells of a solid tumor.

The idea of attacking a tumor's blood supply took some time to catch on. Back in the 1970s, when Folkman proposed that tumors have to induce new blood-vessel growth to obtain the nourishment they need, other researchers were skeptical. "The view of most scientists was that tumors didn't need blood-vessel growth at all, that they could grow with the supply that was there," Folkman says. Over the next decade, it became increasingly clear that Folkman was right, as his group showed that tumors contain newly formed blood vessels and secrete diffusible factors that cause those vessels to grow. In the early 1980s, Folkman's lab and others isolated several of those factors and showed that they trigger blood-vessel growth. They also identified the first antiangiogenic agents, platelet factor 4,



**Lifelines.** A human eye cancer attracts new blood vessels.

ANTHONY ADAMS