the plants, when they dry up."

But news of the loss of the Jasper Ridge checkerspot has highlighted for many the question of when, if ever, scientists studying a shrinking population should intervene to save it. "That's very controversial," says FSU's Simberloff.

The Fish and Wildlife Service's David Wright, for one, thinks more could have been done to help out the Jasper Ridge checkerspot, given its status as a threatened and much-studied species. "I don't intend this as a criticism of Stanford, but I regard this as a wake-up call... for anyone interested in conservation." He says that "if a population has survived at a location for tens of thousands of years, it likely has the reproductive capability to recover from environmental vagaries"—if given a helping hand. Wright suggests that researchers might have experimented with increasing the amount of habitat available to the butterflies by restoring grazing or controlled burns or weeding part of the reserve.

JASPER RIDGE BIOLOGICAL PRESERVE

Serpentine splendor. Native grasslands on Jasper Ridge during spring bloom.

By most accounts, the case for intervening is strongest when the threatened population is genetically distinct. Murphy says that concern doesn't apply to Jasper Ridge, where "there were no unique alleles." Ehrlich concurs, saying that the vast reserve of Kirby Canyon checkerspots is "very similar" to the Jasper Ridge populations. But Susan Harrison, an associate professor of environmental studies at the University of California, Davis, who did her graduate work with Ehrlich and Murphy on the Kirby Canyon checkerspots, asserts that "nobody really knows the answer to that because the studies weren't done." Comprehensive genetic studies of *Euphydryas* populations along the western United States were done in the late 1960s and early 1970s, but used a method which has since been shown to be unreliable, says Alan Launer, research associate at Stanford's Center for Conservation Biology.

Reed Noss, a population extinction expert and professor at Oregon State University in Corvallis, says that whether to intervene "really depends on the management goals for a particular area." Small populations "have a high chance of going extinct," he says, and "from a metapopulation standpoint, it probably doesn't matter" if the Jasper Ridge butterflies have disappeared because of the reservoir of bugs at Kirby Canyon. But where a whole species is winking out, and efforts to protect habitat haven't been effective, interventions may be warranted. "If a species is really on the brink, and we see that an intervention can be done, we have an obligation to do that, just ethically," he says.

Watching the Jasper Ridge checkerspots disappear was an important research opportunity, Murphy contends. "Watching this population hang on at about a dozen individuals was one of the more enlightening aspect of our study of the species," he says. Indeed, says Ehrlich, "just trying to keep *Euphydryas* going on Jasper Ridge would give us less information" than observing the extinction.

Either way, the question of observation versus intervention is "something the community needs to discuss more," says Wright. He also would like to see *Euphydryas editha* back on Jasper Ridge. "I want to work with them to reintroduce the butterfly to Jasper Ridge. If there are special permits that are needed, I'm more than happy to put that on my list of priorities."

Ehrlich and Murphy share Wright's enthusiasm for a reintroduction. "We will be trying a reintroduction," Ehrlich promises. But Murphy notes a possible snag: "Stanford is very sensitive to the legal implications of putting the butterfly back in the habitat. Stanford now has grasslands that are free of listed species. If they wanted to build on these habitats, they frankly could."

And while Stanford has no plans for construction on Jasper Ridge, says Stanford spokesperson Janet Basu, it isn't planning to reintroduce the checkerspot butterfly, either. "It looks like the reintroduction won't happen, at least [not] in the short term.... There's been no forward action on this," Basu says.

Come mid-March, graduate students will again be taking to Jasper Ridge to search for the checkerspot. If none are seen, then the extinction will be official. "It's the loss of a symbol, and it's another example of population extinction," mourns FSU's Simberloff. "You now have more and more examples of a depressing trend."

–Ellen McGarrahan

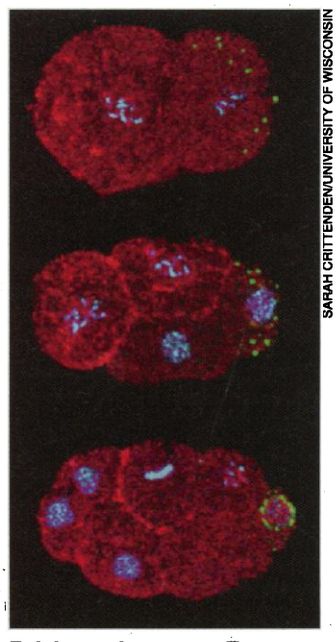
Ellen McGarrahan is a free-lance writer in San Francisco.

BIOCHEMISTRY

Photons Add Up to Better Microscopy

The first view through the light microscope opened up the cellular world for 17th century biologists. Now, a new kind of microscope could do the same thing for the world of biochemistry, letting 20th century biologists follow molecules in real time within living cells.

On page 530, applied physicist Watt Webb and his colleagues at Cornell University in Ithaca, New York, describe how they tapped photon physics to view a key brain chemical called serotonin inside living cells. The significance of the achievement goes beyond serotonin, however. Webb's method, which uses the additive energies of multiple photons to excite fluorescence from molecules that previously couldn't be observed without damaging or killing the cell, should open new vistas for any biologist interested in tracking specific molecules in tissue.



SARAH CRITTENDEN/UNIVERSITY OF WISCONSIN

Rainbow microscopy. Two proteins (red, green) and DNA (blue) fluoresce in a two-, four-, and eight-cell nematode embryo.

The technique will enable researchers to probe deeper into cells and to monitor molecules in living samples much longer than previously possible, Webb says. Joseph Lakowicz, a biochemist at the University of Maryland School of Medicine in Baltimore who is also developing new fluorescence spectroscopy techniques, agrees: "Webb has really changed the paradigm of microscopy."

In current microscopic methods, biologists often visualize cellular components by tagging them with molecules that fluoresce when excited by light of the correct wavelength. But the fluorescence technique is limited because many dyes and cellular components, such as proteins, fluoresce only when excited by short-wavelength, high-energy photons that can overheat the cell or drive toxic chemical reactions. In addition, because the entire sample is illuminated, stray

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 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

D. NORMILE

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

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