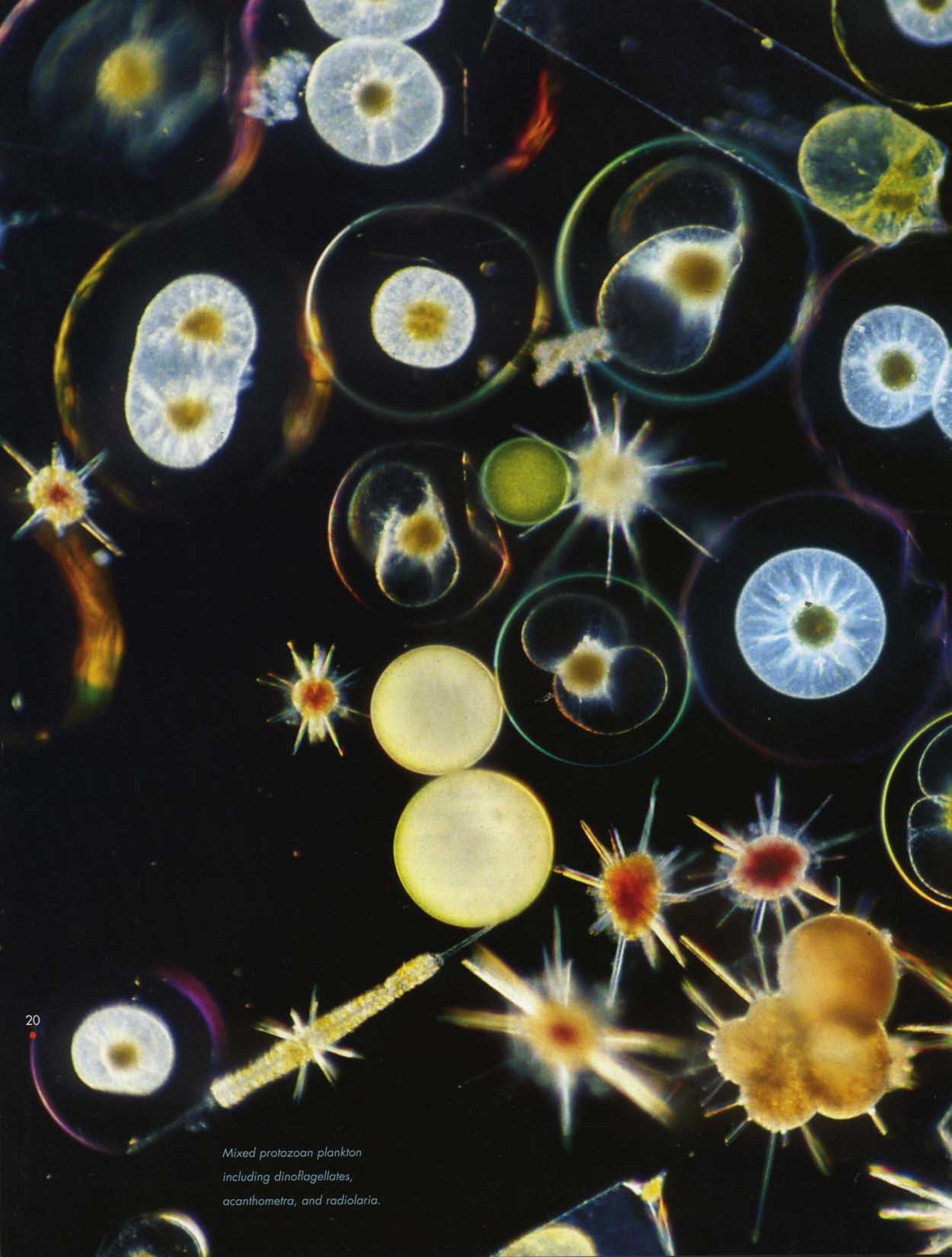
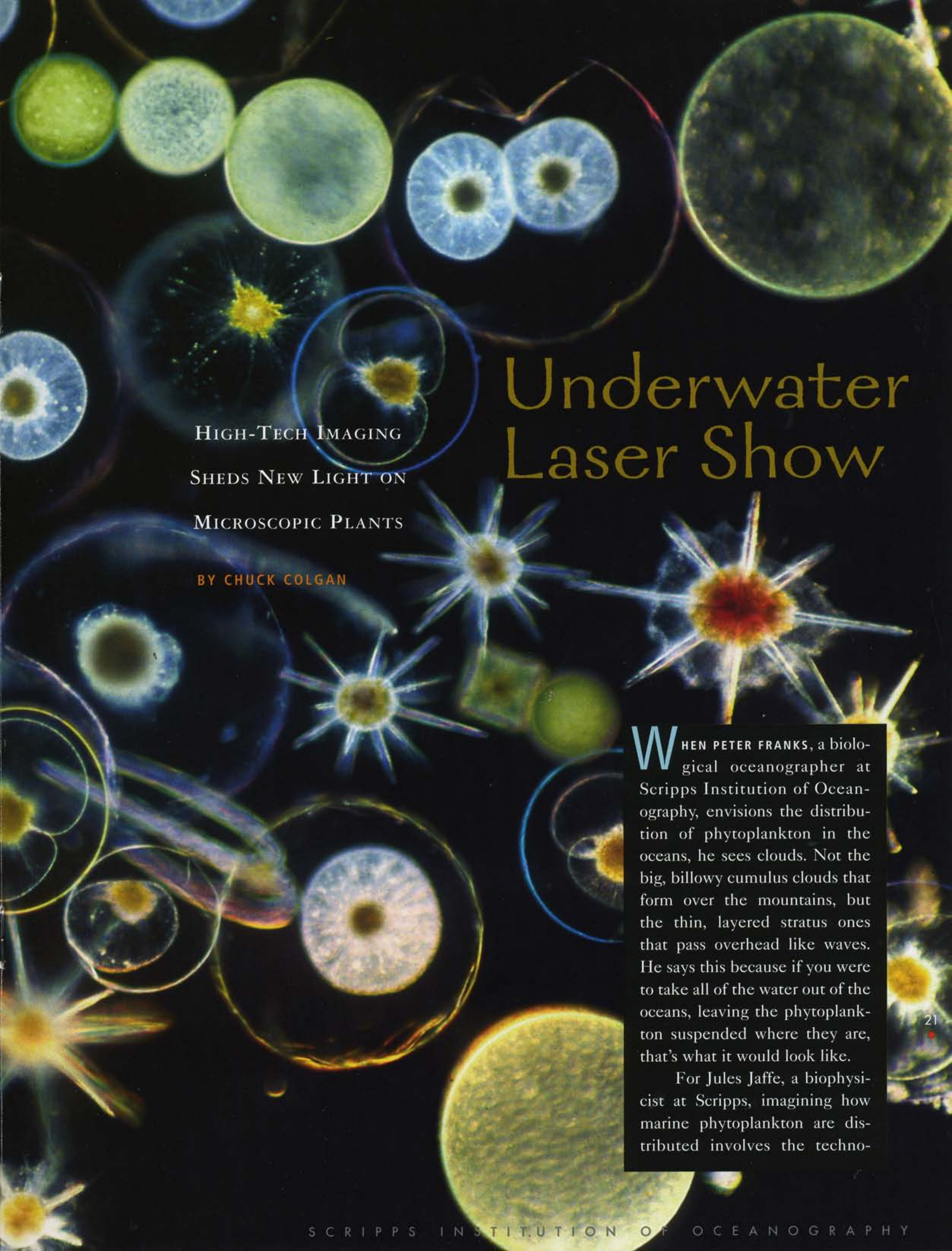


Mixed protozoan plankton including dinoflagellates, acanthometra, and radiolaria.



Underwater Laser Show

HIGH-TECH IMAGING SHEDS NEW LIGHT ON MICROSCOPIC PLANTS

BY CHUCK COLGAN

WHEN **PETER FRANKS**, a biological oceanographer at Scripps Institution of Oceanography, envisions the distribution of phytoplankton in the oceans, he sees clouds. Not the big, billowy cumulus clouds that form over the mountains, but the thin, layered stratus ones that pass overhead like waves. He says this because if you were to take all of the water out of the oceans, leaving the phytoplankton suspended where they are, that's what it would look like.

For Jules Jaffe, a biophysicist at Scripps, imagining how marine phytoplankton are distributed involves the techno-

Above, Biologist Peter Franks is surrounded by images of phytoplankton in his Scripps office. **Right**, Biophysicist Jules Jaffe leans on the framework of an underwater instrument at a test pool at Scripps.

logical challenges of making unobtrusive observations of miniscule organisms in a dynamic three-dimensional environment. With one eye on the oceans and one on the electronics marketplace, he develops noninvasive instruments that employ cutting-edge acoustic, laser, an optical devices in ways their manufacturers never intended. The development of these technologies, Jaffe explains, was paid for by medical research, the military, and laser-light show producers—his job is to transition the tools for use in the oceans.

Franks and Jaffe have teamed up over the past 12 years at Scripps to share ideas on how to merge interests in phytoplankton ecology and ocean engineering. They are part of the community of biologists, physical oceanographers, chemists, and other marine scientists who are striving to better understand the finer ecological details of phytoplankton—the free-floating and mostly microscopic plants of the sea that are the base of the marine food web and that contribute about half of the oxygen produced by all plants on Earth.

Even though phytoplankton have been described and cataloged since the discovery of the microscope, very little is known about the structure of their communities. Knowing more about these organisms will help resolve some of the basic questions about rates of productivity and chemical cycling in the oceans, as well as help create a baseline for understanding how changing ocean conditions may affect future food resources and global climate.

AMAZING PHYTOPLANKTON

Phytoplankton are critical to life on Earth.

They are a major source of oxygen, producing about 50 percent of the total supply. Every second breath you take is courtesy of phytoplankton. Phytoplankton are also the primary source of food in the oceans, creating an estimated 200–250 billion tons of high-energy organic material each year. For comparison, humans consume about three billion tons of food annually. Phytoplankton absorb, convert, and repackage nutrients and minerals from seawater that travel through the marine food web, eventually fueling life even at the deepest depths of the oceans. This procedure is also a major factor in the cycling of nutrients, minerals, and carbon dioxide as well as other greenhouse gases in the oceans and atmosphere, with plankton locking up such compounds in their cells. When they are eaten and then excreted, these materials eventually make their way to the deep ocean.

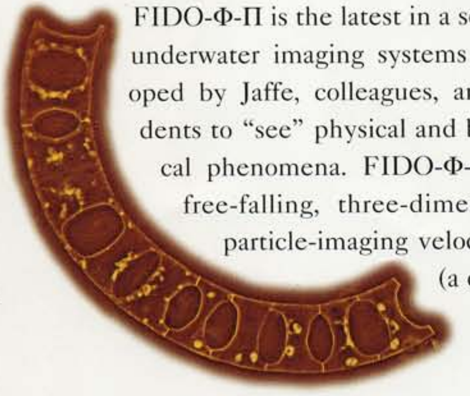
Ranging in size from one micron to one millimeter (0.000039–0.039 inches), phytoplankton (like all plants) use photosynthesis to pro-

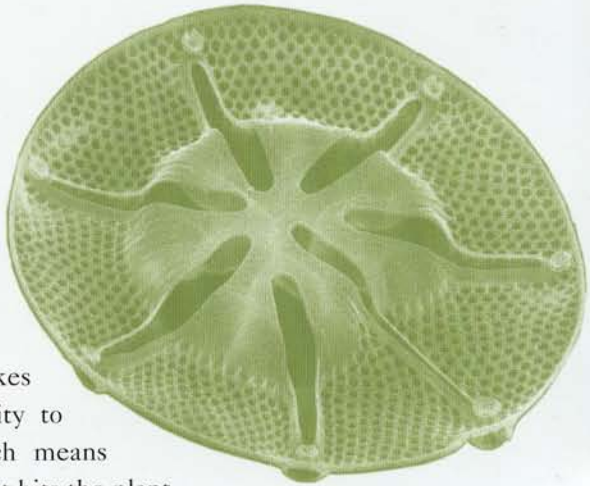
Below, *The underwater imaging system FIDO-Φ-Π prior to launch at sea and at a deep-water test tank at Scripps.*

duce food, so they must stay within the ocean's upper 100–200 meters (330-660 feet) where there is sufficient sunlight. The traditional way of studying phytoplankton is to collect samples with fine-mesh nets or water bottles. Usually, great efforts are taken to confine the sampling to prescribed depths, which allows biologists to establish phytoplankton identification, habitats, and ecology. But because the phytoplankton are removed from their habitat, it's difficult to determine how they were arranged in the ocean, their relative distances from one another and other species, or where they were minutes or even seconds before capture. To help resolve the spatial and temporal relationships of phytoplankton, Franks and Jaffe have designed a unique research strategy and an automobile-sized instrument with a name that sounds like a college fraternity: FIDO-Φ-Π (pronounced "fido-phi-pi").

MOVING IN ON THE MINUSCULE

FIDO-Φ-Π is the latest in a series of underwater imaging systems developed by Jaffe, colleagues, and students to "see" physical and biological phenomena. FIDO-Φ-Π is a free-falling, three-dimensional particle-imaging velocimeter (a device that measures water flow) that uses a blue-green laser and high-sensitivity cameras to produce incredibly detailed images of plankton community structure and water velocity patterns.





The imaging system takes advantage of a plant's ability to produce fluorescence, which means that when a high-energy light hits the plant, most of the light is absorbed, but some is reemitted as a lower energy light. When you shine a blue-green laser on a plant, about two percent of the light comes back as red. The amount of red light is directly proportional to the amount of chlorophyll-a, the photosynthetic pigment in plants. The only thing that does this in the ocean is phytoplankton.

Predecessors to FIDO-Φ-Π were instruments tethered to a research ship by cables so that large two-kilowatt power sources could run the three-watt underwater lasers. Being tied to a ship meant that the instrument had a tendency to move up and down in the water, which disturbed the animals and plants being observed and, in turn, distorted the data being recorded. Today, lasers used for light shows can produce the equivalent laser output from 100 watts of power, allowing the scientists to package both the laser and a battery in a compact watertight container, thus eliminating the need for a cable. When Jaffe told the laser manufacturer what he had done, it "completely blew their minds."

At sea, the one-ton FIDO-Φ-Π is lifted up off the deck by the ship's winch and carefully maneuvered by hand and ropes into the water. After the imaging system is released, it slowly descends through the water column at a rate of three to five centimeters (one to two inches) per second as a blue-green laser is projected from its bottom that illuminates a 24 x 24 centimeter (9.5 x 9.5 inch) sheet of water at about 75 centimeters (30 inches) below the instrument. Two low-light sensitive digital cameras with special filters to image the fluorescence are angled at the laser sheet. As the light hits the phytoplankton, they fluoresce. Every two seconds a sequence of five images is recorded by each camera. These "mini-movies" can be used to track particle motions, which for plankton, are mostly the motions of the water. The stereo images give different views of the same water, allowing calculation of the three-dimensional velocities in the imaging plane. The images have a resolution of about 100 microns (0.0039 inches), which is sufficient to observe individual cells and distinguish between single dinoflagellates and long diatom chains. Onboard computers store the images and control the instrument's mechanical systems and sensors. When FIDO-Φ-Π reaches a depth of about 50 meters (165 feet), pistons are activated to change the vehicle's buoyancy and it floats to the surface to dump its data via a

PLANKTON PICTURE SHOW

SOMETIMES SCIENTISTS DO the strangest things while pursuing the finer details of the subjects they study.

Consider Erdem Karaköylü, a biological oceanography graduate student at Scripps. He recently applied glue to loose strands of his hair to stick to the backs of squiggling, miniscule zooplankton so that he could secure them in place while taking their pictures. Oddly, he wasn't doing this to simply photograph the tiny animals, as his real interest is in what and how much they discharge waste.

Karaköylü is participating in investigations with his thesis advisor Peter Franks and others to understand the behaviors and contributions of copepods, the predominant group of zooplankton. Considered to be the most plentiful multicellular animals on the earth, outnumbering even insects, they constitute the largest source of protein in the oceans.

Typically, 1–2 millimeters (0.04–0.08 inches) long, copepods feed directly on phytoplankton and are food for many fishes and whales. Large-scale copepod grazing, followed by fecal production, repackages organic molecules near the surface into pellets that sink rapidly to the seafloor. These pellets accelerate transportation of carbon dioxide and minerals to the deep sea and serve as food for organisms at these depths.

Traditionally, biological oceanographers have calculated contributions by copepods to the downward movement of organic materials by counting catches in water or net samples, then estimating the total population and extrapolating their fecal output.

When a high-speed, slow-motion camera was loaned to Franks's lab, Karaköylü realized that he could observe copepod fecal production up close. Not only did he get amazing visual images of the animals, but by using colored lights and filters that show fluorescence, he could see the phytoplankton that the copepods were eating. Because copepods are semitransparent, Karaköylü watched the food as it was digested and excreted.

Now he wants to know if something more can be learned by using the technique. This summer he will be recording copepod feeding and fecal pellet production in Dabob Bay in Puget Sound, Washington, as part of a team from Scripps and the University of Washington.

If Karaköylü can help improve estimates of copepod fecal production, he'll know if it was worth literally pulling his hair out.

Graduate student Erdem Karaköylü and his amazing photograph of a copepod suspended on one of his hairs.

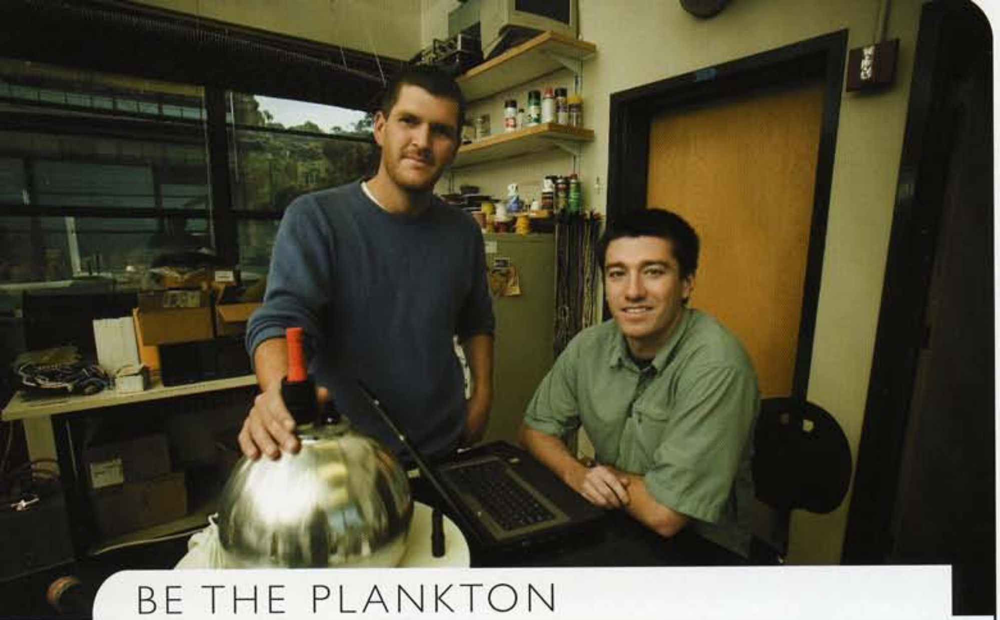
wireless connection, then submerges for another profile.

Each phytoplankton cell in the blue-green laser sheet is a source of red light—its fluorescence. The resulting images of phytoplankton cells floating in the ocean look much like photographs of stars floating in space, with hundreds to thousands of tiny points of scattered white light. While the recorded light is red, Franks plots the intensity of the red fluorescence in black and white, which makes it easier to visualize.

EXPECT THE UNEXPECTED

The team's findings confirm that the amounts of phytoplankton and fluorescence increase slowly and smoothly with depth as they approach the bottom of the light zone and then decrease into deeper and darker depths. What Franks and Jaffe found that they didn't expect is that the types of plankton change significantly and regularly over vertical distances as small as 60 centimeters (two feet). In general, the layers of phytoplankton communities seem to change about every two meters of depth, which has huge implications for how zooplankton find their food and how nutrients are distributed in the oceans.

"What we are seeing is that at the small scale, from a half millimeter to 12 centimeters [0.02 to five inches], the physical dynamics of the water are mixing them," Franks said. "But on a slightly larger scale, at 20



Above left, *Fernando Simonet (left), associate development engineer, and graduate student Paul Roberts are part of the research team designing autonomous underwater explorers.*

BE THE PLANKTON

ONE OF THE DAUNTING QUESTIONS in marine biology is what happens to larvae after they have hatched and spend a brief period of their lives as plankton, before eventually maturing into fish, lobsters, or abalones.

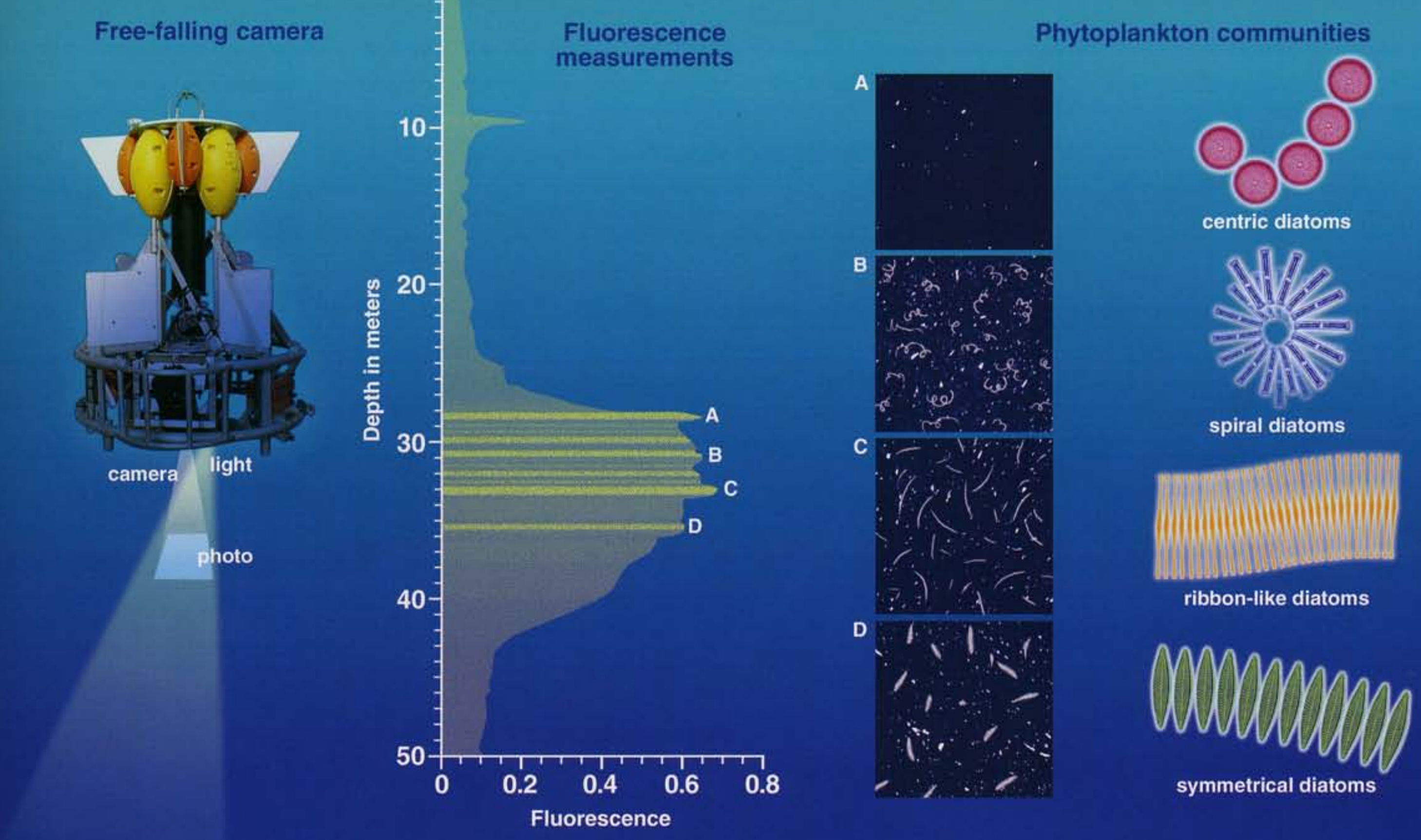
Knowing the fate of the more than 70 percent of marine species that have planktonic life stages is critical to ocean ecology. Such knowledge is increasingly central to the development of coastal marine reserves as a method for safeguarding fish and other commercially important marine stocks. How do you determine the boundaries of a reserve when what you are trying to protect is free-floating—and to who knows where?

If you want to track plankton, you need an instrument that behaves like plankton, says research oceanographer Jules Jaffe. That's why he and his colleagues are developing devices resembling miniature satellites that follow the currents, temperature regimes, light patterns, and other ocean parameters associated with plankton.

This instrument, an autonomous underwater explorer (AUE), is a 24-centimeter (9.5-inch) aluminum sphere packed with a mechanical buoyancy controller, an acoustic transmitter and receiver, computer equipment, data sensors, batteries, and other electronics. The buoyancy controller allows the AUE to actively ballast itself to maintain a certain depth, follow a prescribed temperature or light level, or be programmed to migrate up and down at set intervals. The acoustic devices provide a communication network between AUEs and a surface buoy that records their locations and then transmits the information via satellite to a ship or to land.

The concept is to submerge 10 or more AUEs in a coastal area to observe and track a species of plankton as they spawn and then disperse over the following days or weeks. The result will be a three-dimensional view of larval dispersal patterns, which is vital for determining the appropriate size of marine reserves and the optimal distances between them.

"We envision a fleet of AUEs that can be deployed easily and rapidly from small boats almost anywhere along the coastline that will permit detailed observation of subsurface circulation patterns for tracking everything from larval dispersion to oil spills and sewage outfalls," Jaffe said.



The Scripps-developed, free-falling camera system records phytoplankton virtually undisturbed by using a laser light and high-sensitivity cameras to photograph fluorescence produced by the tiny plants. As the instrument submerges through the top 50 meters (164 feet) meters of the ocean, it encounters discrete layers of phytoplankton communities whose structure were previously unknown.

centimeters [eight inches], you see gradients and nonrandom distributions of phytoplankton that are being driven by biological and physical dynamics. If you are a zooplankton that eats phytoplankton, this means you have to move at least 12 centimeters vertically to see a significant change in food concentration. There really are no other instruments that will allow us to get this level of detail about spatial relationships."

The images can also be used to study the zooplankton that feed upon the phytoplankton, because the semitransparent animals reveal their gut contents by fluorescence when lit by the laser. This will provide a noninvasive method for measuring the microscale movements of zooplankton in relation to their food and the physical and chemical gradients in their environment. (*See "Plankton Picture Show" on page 25.*)

"This is an entirely new type of instrument that takes advantage of the extensive research conducted in the field of underwater imaging and recent advances made in the area of lasers for such uses as medical diagnosis," Jaffe said. "This system will allow us to see things in the oceans that we have never seen before and to understand ecological relationships. That will be exciting."