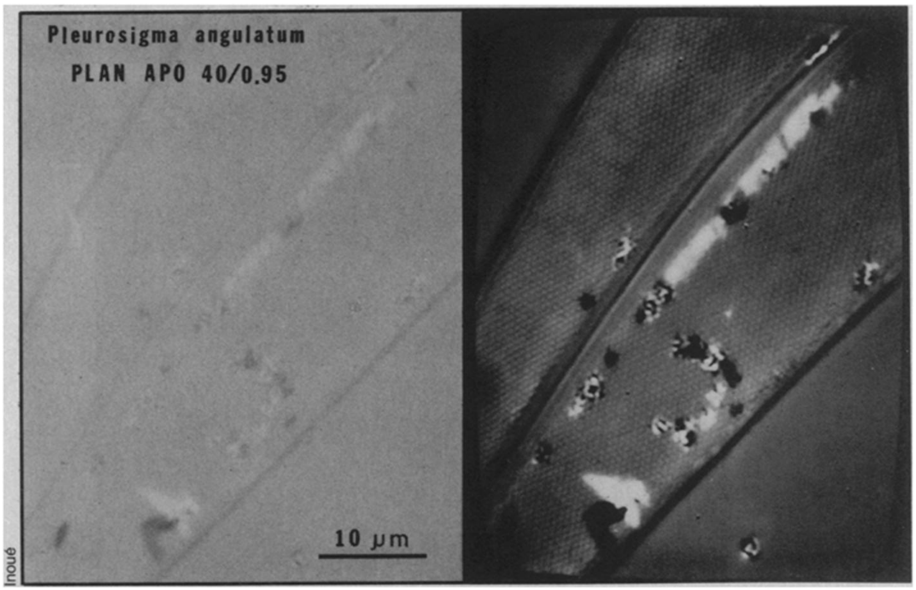
Cell-e-vision

Add standard video equipment to a laboratory microscope and biologists find better contrast, higher sensitivity, thrilling action and peace of mind



Video monitor (right) improves image of diatom viewed in polarized light microscope.

BY JULIE ANN MILLER

More and more biologists are finding themselves glued to a TV screen. And they're not all watching "Dallas" or even "Nova." They are watching protozoa flopping around in a termite's gut or the beating of a bundle of bacterial flagella. In the past two years many biologists have discovered that a television camera aimed through a light microscope can improve the contrast, sensitivity and quality of images, as well as capture movement and produce instant data records.

"We can use the microscope in wholly unconventional ways to produce 'images' invisible to the eye but seen clearly on a television monitor," explains Robert D. Allen of Dartmouth College. "This has opened up a whole new world and has narrowed significantly the gap between the useful ranges of the light and electron microscopes."

"Subtle, hitherto unobserved, dynamic changes in cellular organization and fine structure can now be analyzed with precision and speed in living cells," agrees Shinya Inoué of the Marine Biological Laboratory in Woods Hole, Mass., and the University of Pennsylvania.

At his videomicroscope, Allen views filamentous pseudopodia of a marine protozoan. A chicken intestinal cell with 100-nanometer-diameter microvilli is inset.

At the recent meeting in Cincinnati of the American Society for Cell Biology Allen and Inoué each presented images recently captured by video microscopy. Later at a crowded, hastily scheduled workshop, other scientists eager to begin using the technique quizzed the microscopists on aspects ranging from which knob to turn for specific adjustments to how best to negotiate prices with dealers of the commercially available video equipment.

The new use of video equipment — basically a closed circuit TV camera, a monitor and a videotape recording machine—allows microscopists to see structures one-half to one-fourth the size of the smallest that can be seen with a light microscope. With a video set-up, a structure only 25 nanometers wide is clearly visible, although its image is swollen. Electron microscopy can achieve much higher magnification, but the specimen must be killed in the preparation of the sample. In contrast, the new video microscopy does not damage living cells so it is especially valuable for studying such processes as cell movement and the translocation of material within cells.

"It's fantastic what we can see," Allen says. "Every time we turn the instrument on and look at a cell we know well, we find something new and exciting." For instance, Allen and his wife, Nina Allen, have seen particles hopping along very thin filaments in algal cells. They also have observed in unprecedented detail blood platelets, which are minute cell fragments that play a role in blood clotting.

With a different videomicroscope setup, Inoué has captured in polarized light the rotating flagella of a single bacterium and the beating cilia of another microorgansim called a *Stentor*. He has watched the growth in laboratory culture of spikelike sea urchin skeletal structures and the action of cilia in the balance organ of a snail. Perhaps most exciting are the rapid reactions of a sea cucumber sperm as it encounters an egg (see p. 238).

Inoué observes the sea cucumber sperm as he artificially activates it. The sperm sends out a very fine process that extends 90 microns in less than 10 seconds. As the sperm process is extended, the head of the sperm expresses its contents. Inoué can see the acrosome, the front of the sperm, swell and divide into two compartments as the contents are ejected. "These events that take place inside the acrosome of an activated sperm have never been observed before. Nor has it been possible, so far, to capture these dynamic events and structures with the electron microscope," Inoué remarks.

The basic processes of the video technique are enhancement of contrast and subtraction of background illumination. Allen compares the problem of examining microscopic specimens under dim light to the frustration of trying to detect a pedestrian in a gray raincoat through a dusty car windshield. A TV camera, but not the human eye, can reject the stray light from the dust and make a clear image of the pedestrian. In a manner similar to turning the contrast knob on a television set, biologists using a TV camera can enhance an image in the microscope.

Video techniques for improving faint

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Blood Vessels on TV

The quest for informative images from weak signals extends beyond biology into medicine. There physicians have long used image intensification and television screens to view blood vessels made visible by the X-ray absorption of such opaque materials as iodine injected into a patient's arteries. At the recent meeting in Toronto of the American Association for the Advancement of Science, Andrew B. Crummy described a powerful variation using computer techniques to electronically manipulate the signal. He and colleagues at the University of Wisconsin Center for Health Sciences find the method to be a safe, less expensive, out-patient alternative in many instances to standard arteriography and angiocardiography.

In many different clinical situations a physician needs to see the pattern of blood flow through a patient's arteries. Such information shows where the arteries are obstructed in vascular diseases, including heart disease. Blood flow indicators are also necessary to evaluate artery bypass grafts. The conventional method of viewing the arteries involves inserting a cannula into an artery in the area of interest and injecting a material that is opaque to X-rays. This procedure is costly, because it requires hospitalization, and the patient risks a variety of complications.

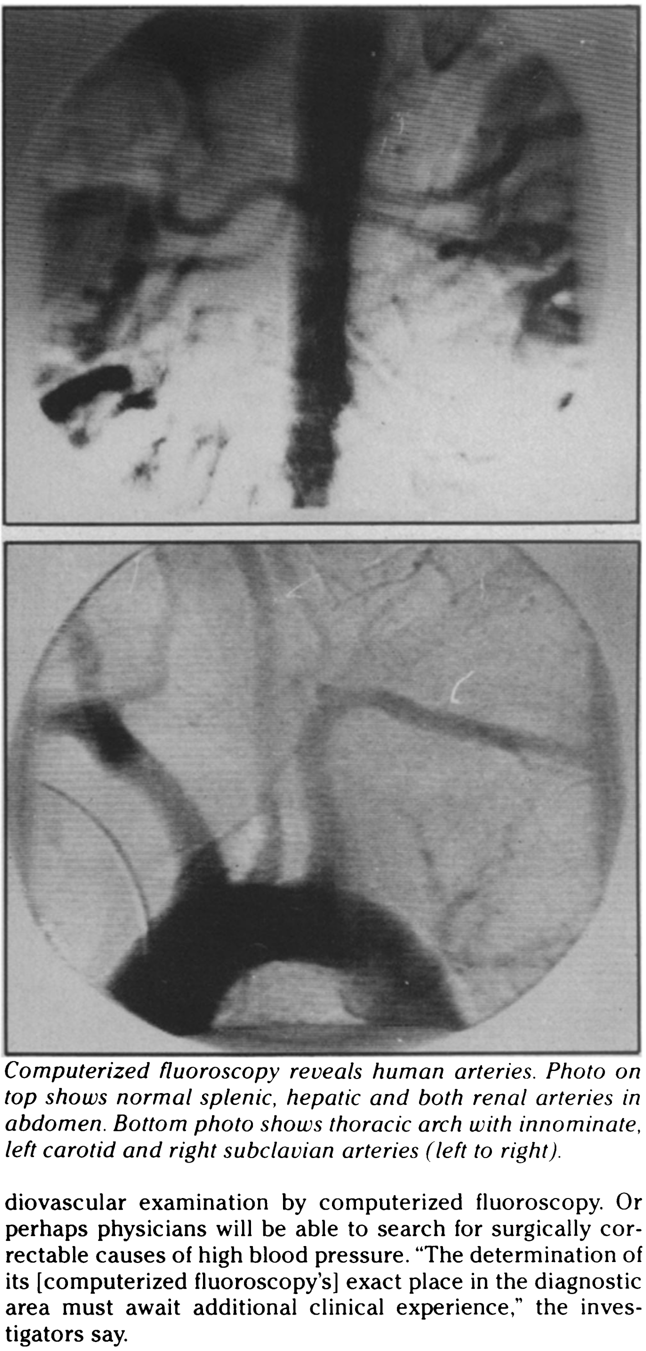
The computer-aided technique, which has now been tested on more than 500 patients, can sense far lower amounts of the opaque material in the blood. Therefore, the contrast dye can be injected into a peripheral vein — a far simpler procedure than injecting into an artery — and enough material goes throughout the circulatory system to be detected in arteries by the "computerized fluoroscopy."

Instantaneous subtraction is the key to the computer application. Crummy describes it as an "on-line digital time and energy subtraction algorithm designed to isolate and enhance the iodine signal from image intensified fluoroscopy." In the simplest procedure, called mask mode, an X-ray image of the area being examined is taken before the arrival of the contrast material, and this background pattern is stored in the computer's memory. As the injected iodine passes through the arteries of the area, the monitor displays a pattern that is the difference, point for point, between the new and the background image. In a typical case, described by Crummy, the iodine signal was made eight times stronger by the subtraction, and the arteries became far more conspicuous on the screen.

So far, efforts have been directed primarily at examination of the principal arteries that supply blood to the head and neck. The investigators have observed constrictions, occlusions, ulcers and bulgings of these carotid arteries. They have also used the technique to study vascular disease in the head, chest, abdomen and extremities. For example, they can use it to determine whether blood vessels beyond an arterial blockage are in good enough condition to justify bypass surgery instead of amputation of the limb.

Crummy does not expect the computerized fluoroscopy to replace the standard techniques completely. Although in some cases it allows physicians to see vessels invisible in the more traditional arteriograph, in other cases the standard method gives more detail. But because the computerized technique is so simple, safe and inexpensive, Crummy believes it can make feasible examination of more patients. Patients with ambiguous neurological symptoms, for instance, are not now given arteriography, but they might benefit from a detailed cardiovascular examination by computerized fluoroscopy. Or perhaps physicians will be able to search for surgically correctable causes of high blood pressure. "The determination of its [computerized fluoroscopy's] exact place in the diagnostic area must await additional clinical experience," the investigators say.

Patient acceptance of the technique opens up exciting possibilities for clinical research. It may be possible to examine arteries in prospective studies, where subjects who have not yet developed symptoms of vascular disease could be observed for many years. Also, the technique might provide information about how blood is supplied to tumors, Crummy suggests. Computerized fluoroscopy thus is expected to expand basic clinical knowledge, as well as provide a simpler and safer important diagnostic procedure.



Crummy

Computerized fluoroscopy reveals human arteries. Photo on top shows normal splenic, hepatic and both renal arteries in abdomen. Bottom photo shows thoracic arch with innominate, left carotid and right subclavian arteries (left to right).

images have been around for many years, but they have been used predominately in military surveillance and the space program. Some microscopes were equipped with television cameras almost a decade ago, but their use has been limited. Allen serendipitously made the discovery leading to the recent dramatic improvements (a discovery made independently by Inoué and colleagues, Inoué says). Allen accidentally misadjusted a camera-microscope set-up; he turned one of the knobs beyond the point of maximal clarity for a person looking into the microscope. As the contrast to the eye worsened, the image displayed on the video monitor got better and better. "It's silly that this wasn't discovered years and years ago," Allen says. "You really have to fiddle with things in order to understand them."

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Verne weighed — including crew, ballast, gondola and balloon — 10,500 to 11,500 lbs. at liftoff and was planned to drift at a maximum altitude of 30,000 feet. In duration and distance records, sports balloonists also lag behind their scientific counterparts. The records to beat are an estimated 600 miles for a hot air balloon, set March 6 by Kris Anderson, Maxie Anderson's son, and 3,314 miles for a helium balloon, set by the two Andersons in May 1980. The *Double Eagle II* set the manned balloon flight duration record of 137 hours.

Anderson and Ida hoped to break both duration and distance records in the *Jules Verne*. At 390,000 cubic feet, it was significantly larger than the 160,000-cubic-foot *Double Eagle II*. Like the *Kitty Hawk* in which the Andersons made their 1980 transcontinental crossing, the *Jules Verne* carried a liquid ballast of antifreeze and water. (Sand, the traditional ballast, freezes at the cold upper atmosphere.) Unlike the *Double Eagle II*, which was made of coated nylon, the envelope of the *Jules Verne* was translucent polyethylene, which absorbed less solar heat and diminished the roller-coaster effect. Even with all these improvements, says Lally, the aeronauts were still defeated before they started because of the daily "balance penalty."

Other global hopefuls are attacking the problem differently. A British team, led by balloon manufacturing company owner Donald Cameron, plans an April launch in a "hot-helium" balloon. In this scheme, the helium in the balloon will be heated by liquid fuel whenever necessary to maintain altitudes. While this is a promising system, says Lally, with whom Cameron consulted last fall, it is also limited by fuel supplies to a 12-day flight time. About two weeks, he said, will be necessary for a round-the-world journey at the altitude Cameron plans to fly.

Lally contends that there are only two ways to successfully circle the globe using current technology, and both are the offspring of recent developments in long duration scientific ballooning. The first is to fly a zero-pressure balloon of about 10-million-cubic-feet capacity in the tropical stratosphere. Like the troposphere — that part of the atmosphere from the ground to about 50,000 feet — the stratosphere becomes warmer with increasing altitudes. To take advantage of this inverted temperature structure, a balloon could be inflated to fly at 130,000 feet during the day where it is warm, and at night it would only fall to about 70,000 feet, where it would be buoyed by the colder air and continue to fly. The aeronauts would need a pressurized capsule but would not need to ballast. Sailing on the stratospheric winds during the summer months, such a balloon could circle the earth in about 14 days, Lally says.

The second strategy Lally suggests uses a super-pressure balloon. Scientists that use super-pressure balloons can carry only lightweight instruments because heavier loads require bigger balloons and stronger and more expensive materials to withstand the added stresses. With a pressurized gondola and a 500,000-cubic-foot envelope of extremely strong material such as Kevlar — the tradename for a fiber used in some bulletproof vests — and a metalized outer surface to reflect solar radiation, this balloon could fly at about 40,000 feet and circle the earth in 10 to 14 days, says Lally.

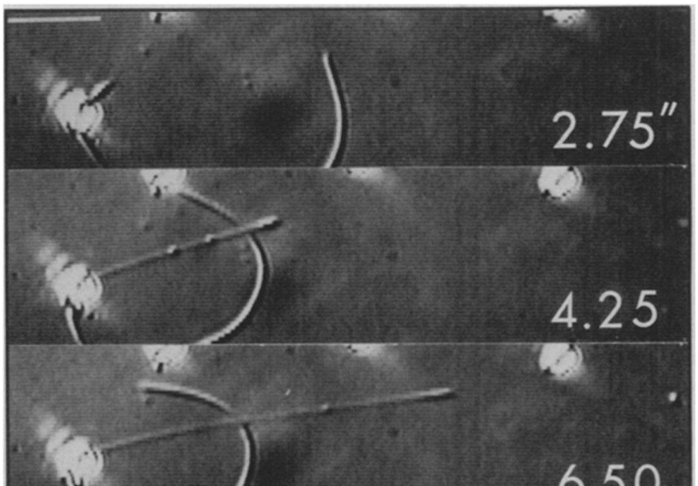
Both of these schemes are currently being used by scientific balloonists. In January, for example, using the first type of system, which is called a radiation-controlled balloon, or RACOON, Lally and coworkers launched a balloon on a successful 14-day global journey from Brazil. Such balloons can be used to carry telescopes for cosmic studies or heavy analytical chemistry equipment. One project proposed for December by Glen Frye of Case Western Reserve University in Cleveland, Ohio, would send a telescope via balloon to study neutrons on the sun.

While Lally's interest in ballooning remains scientific — "people on balloons just get in the way" — he sees no problem applying the same principles to manned flights. With a little more science, the flight of the *Jules Verne* might be more than science fiction. ☐

. . . Microscope

The video equipment can make any microscope give better images, without very fancy (or expensive) systems of lenses, Allen says. "I have a pile of letters on my desk from people who want to do the technique with the microscopes they already have in their labs," he reports. "Everyone who has a decent microscope can now get first-rate images."

The video system is not only impressive when attached to a standard research microscope, but also when it is combined with the "Cadillac" of microscopes, as Allen terms Inoué's system. Inoué first addressed the problem of background and contrast years ago by inventing optical devices, called rectifiers, to produce better images at high magnification. But even his luxury microscope is improved with the addition of an inexpensive ($1,700) television camera. The "off-the-shelf" camera, monitor and tape recorder altogether cost $10,000, only a few percent of the value of the microscope, and Inoué says with newer equipment the cost could be as low as $5,000.



Inoué

Video captures a process shooting out of sea cucumber sperm after an artificial stimulus mimicking contact with an egg. At its most slender portion, the process is 50 nanometers in diameter.

In addition to the clearer images and ability to view smaller structures, the biologists are enthusiastic about other aspects of the technique. Allen estimates it is possible to record images 1,000 times faster and 100 times more cheaply than with photographic methods now in wide use. Because there is no wait for film to be developed, scientists can be certain their data are sufficient and successfully recorded during the experiment. Allen says, "Immediate replay and analysis ensures peace of mind." Another advantage of video is that pictures can be slowed down or sped up for convenient study, or several can be combined to bring out more detail. The system can also feed into a computer for more elaborate data analysis.

In their eagerness, biologists can hardly decide where to turn their new videovision. According to Allen, "We can now expect a number of important breakthroughs to follow in many different kinds of processes in living cells." ☐