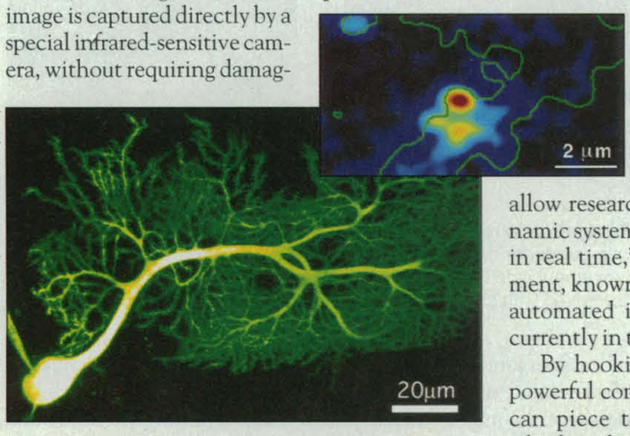
years to micrometers," Zieglgänsberger says.

Their instrument illuminates the sample with obliquely angled beams of infrared light whose wavelengths are out of phase. The image is captured directly by a special infrared-sensitive camera, without requiring damaging dyes or fluorescent markers. Dodt and Zieglgänsberger have used the technique to visualize living brain structures down to the resolution of single spines, which allows them to perform a variety of observations with far greater precision. "It's like fishing in a pond blindly and then having the lake clear so that I can see all the fish," Zieglgänsberger says.



DENK AND SVOBODA, *NEURON* 18, 351 (1997)

Nervous action. Multiphoton image of living Purkinje cell filled with fluorescent dye; calcium ions released by dendritic spine *(inset)* of stimulated cell.

While infrared video microscopy has combined several existing techniques into one device, Taylor and his colleagues at Carnegie Mellon, along with others, are working on bringing together several existing microscopes into one instrument. "The goal is to allow researchers to interact with a dynamic system, like a developing embryo, in real time," says Taylor, whose instrument, known by the acronym AIM—for automated interactive microscope—is currently in the late development stages.

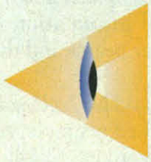
By hooking up the instrument to a powerful computing system, researchers can piece together the 3D images—which otherwise take hours to sort through—very rapidly, Taylor says. That way, researchers could watch a fertilized egg divide, say with multiphoton imaging, then add a drug or reagent and switch to interference microscopy to see how the drug takes effect. "The whole purpose of the AIM is that the data are collected, processed, and displayed during the time of a biological event," Taylor says. "That way a researcher can change the course of an experiment all in the time frame of a biological process such as cell division or locomotion."

While interference and multimodal imaging devices are still in the developmental stages, multiphoton technology is now commercially available. But it comes at a high price. The Cornell Research Foundation has patented the technology and licensed it exclusively to BioRad Microscience Ltd., in Hemel Hempstead, U.K., which is selling the complete instruments for anywhere from $300,000 to $450,000. About $100,000 of that stems from the cost of the laser, while the rest of the major costs are based on meeting European codes for the instrument, according to Webb. Even with that price tag and BioRad's decision not to sublicense, many researchers are optimistic that multiphoton imaging will become widespread. "Its advantages are so clear," says biochemist Steve Potter, who has set up a multiphoton instrument in Scott Frasier's lab at Caltech. "Once the lasers become mass-produced, I predict every confocal [microscope] will become multiphoton."

–Trisha Gura

Trisha Gura is a science writer in Cleveland.

MEDICINE



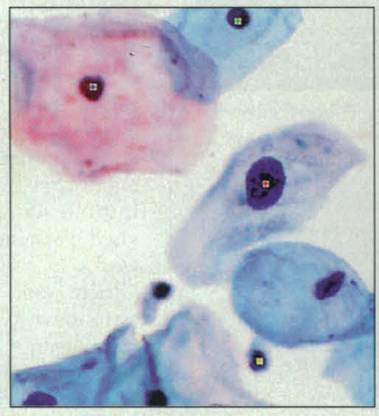
Spectral Technique Paints Cells in Vivid New Colors

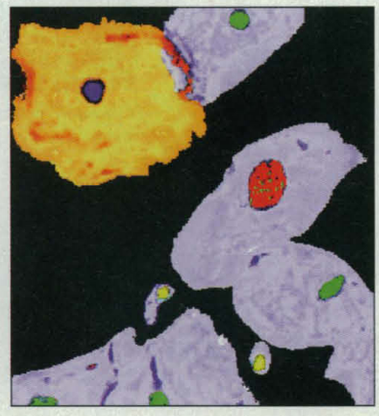
You can think of image enhancement as the art of helping the eye do what it does naturally. Take the two images below. On the left is a conventional micrograph of cells from a pap smear. The cells have been stained to bring out the contrast between different types: Mature epithelial cells are pink-orange, while younger cells stain blue-green, as does the precancerous dysplastic cell in the middle. A pathologist would identify it by its abnormally large nucleus, but it wouldn't be hard to miss.

On the right is the same image, spectrally classified. Richard Levenson and Daniel Farkas of Carnegie Mellon University in Pittsburgh created individual spectra for each pixel in the image with the help of a microscope called the SpectraCube. The microscope divides light from each pixel into beams that travel along paths of varying lengths, then are recombined and allowed to interfere. Mathematical analysis of the resulting interference patterns yields a spectrum.

By comparing each pixel's spectrum to those of reference pixels (boxes on original micrograph), Levenson and Farkas's system identifies groups of pixels with similar spectra and assigns them distinctive colors, making them much easier to tell apart than they are in the original stained micrograph. The nucleus of the dysplastic cell, only subtly different in color from that of a normal cell in the traditional micrograph, is here colored a unique and fiery red, befitting its threatening nature.

The technique had already been applied to cytogenetics by Thomas Ried and Evelin Schröck of the National Center for Human Genome Research in Bethesda, Maryland. They color-coded and differentiated the 24 pairs of human chromosomes after labeling them with tracers that endowed each one with a slightly different spectrum (*Science*, 26 July 1996, p. 494). Having extended the technique to pathology, Levenson and Farkas say it could be used throughout biology to increase the differentiation power of stains, dyes, and fluorescent molecules. "It divides the spectrum into a whole slew of new colors that otherwise couldn't be appreciated by the eye," says Levenson. "It's our belief that important information resides in those colors, and spectral classification can bring it out."





R. M. LEVENSON

–Gary Taubes