

IMAGING: Portraits From Life

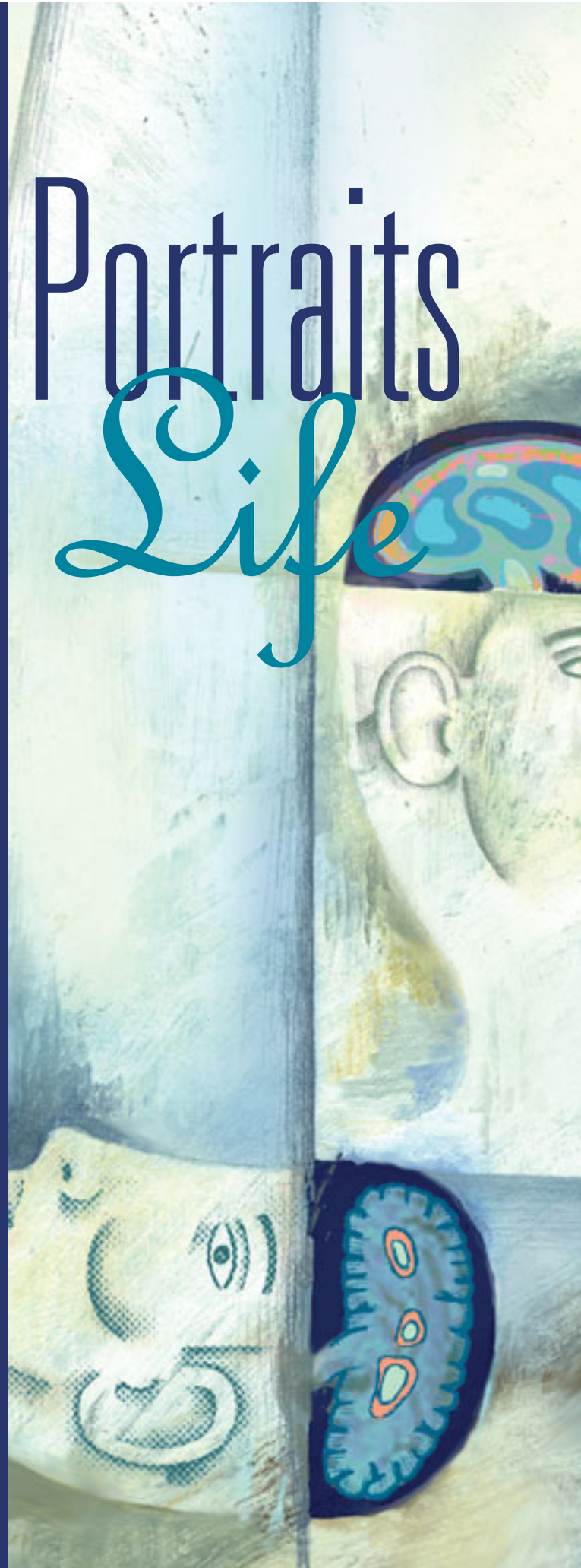
Recent developments in optical imaging let researchers catch cellular processes in the act.

BY NANCY K. MCGUIRE

*C*linical researchers often assess a drug's effectiveness by asking how a patient feels after taking it. If the "patient" is a lab mouse, this method is not particularly effective. The researcher could take a biopsy to determine what the composition of a particular piece of tissue was at the time the biopsy was taken. It's a step in the right direction, but what a researcher really wants is to watch cells in action, preferably without harming the patient in the process. A picture, or better yet, a series of pictures, would be ideal.

In the century-plus since X-rays first captured the human skeleton on film, a wide swath of the electromagnetic spectrum has been pressed into service to illuminate various organs and cellular processes. Photographic film has largely given way to digital data files, making it possible to examine and compare images quantitatively and repeatably. Real-time imaging has replaced static snapshots with movies. The rise of genomics and proteomics has fueled the drive toward imaging on a microscopic scale, and laser technology has made this possible. In deference to the human brain's limitations in comprehending vast amounts of data, images are commonly presented as tomograms, two-dimensional optical "slices" that portray the structures and processes at a given depth below the surface.

ILLUSTRATION: FERRUCCIO SARBELLA





InSPECTor Gadget and his PET CAT

Tomographic medical imaging techniques date back to the early 1960s, when D. E. Kuhl and R. Q. Edwards introduced single-photon emission computed tomography (SPECT), a precursor to today's CT (computed tomography) and PET (positron emission tomography) scans. Before a SPECT scan, the patient is injected with or inhales radionuclide-labeled compounds that emit gamma radiation. An array of photon detectors rotates around the patient to collect data from many angles. The photon intensity of a SPECT image is less than that of a PET scan, limiting the image resolution to about 7 mm. However, SPECT maintains advantages in the availability of suitable radionuclide markers, particularly for the brain and head, and the cost of the instrument, which is roughly one-third that of a PET instrument. In a recent study, SPECT images of the brains of cocaine addicts showed damage patterns similar to those observed in stroke victims (1). This suggests that cocaine addicts could benefit from similar treatments, an observation that was borne out in a subsequent clinical trial.

PET imaging has been used to map the spatial and kinetic aspects of drug distribution in the brain, as well as drug metabolism and blood flow patterns. The patient inhales or is injected with radionuclide-labeled compounds that emit positrons. When positrons collide with electrons, they annihilate each other, emitting two gamma rays with an intensity that is proportional to the marker concentration. As many as 500 detectors encircle the subject, recording approximately 500,000 annihilation events per tomographic slice. The photons follow a predictable path from the site where they are produced, and the detector arrays trace this path back to the source with a precision of 1–2 mm.

Unfortunately, many of the radionuclides used for PET have very short half-lives, making it necessary to synthesize and purify the tracer compounds, administer them to the patient, and obtain the images within a span of a few minutes. This requires the patient to travel to one of a few existing cyclotron facilities where the radionuclides are produced. Some researchers have used the short-lived radionuclides to their advantage, however, administering them to patients several times each day to create time-lapse sequences revealing pharmacokinetic and metabolic effects.

Halogen-substituted marker compounds made an appearance in the late 1990s (2). The positron-emitting ^{18}F and ^{123}I isotopes in these compounds have half-lives of 110 min and 13.3 h, respectively, making it possible for the tracer compounds to be shipped off-site. Thus, patients can undergo diagnostics in regional clinics rather than at cyclotron facilities. Fluorinated naphthyl methoxy piperidines, for example, bind to serotonin receptors in the brain, inhibiting the binding and transport of serotonin to the receptors and enabling researchers to differentiate major depression from other psychiatric disorders such as obsessive-compulsive disorder, Parkinson's disease, Alzheimer's disease, and alcohol abuse.

The tomographic technique most familiar to the general public is the CT scan, sometimes referred to as computed axial tomography, or CAT. In contrast to conventional X-ray images, which superimpose outlines of every organ or bone between a stationary X-ray source and the film or detector, a CT scanner uses a moving X-ray beam to illuminate the desired cross section from many angles. The

scattered X-rays are then captured by a ring of detectors surrounding the patient, and a computer reconstructs the image of a thin “slice”. Commonly used medical CT instruments can obtain only axial sections, because the patient is situated perpendicular to the detector ring (hence, the “axial” in CAT). This limits the usefulness of CT scans in identifying structures such as pituitary tumors, which are more easily visualized using the longitudinal sectioning capabilities of MRI (3). If multiple CT scans are to be used to track the progress of a course of therapy, the cumulative X-ray dosage must be monitored carefully to avoid harming the patient. Despite these limitations, CT scanners have a price advantage (a CT scan is roughly half the price of an MRI scan), and the detector rings are smaller and quieter than MRI tubes, causing less distress to patients. (See “The anatomy of metabolism”, p 37, for more on MRI.)

The pointillist perspective

The confocal microscope was introduced commercially in the 1980s as a noninvasive means of producing high-resolution images of thin optical sections, similar to those obtained using the tomographic techniques. Confocal images are not only better resolved than conventional optical microscope images, they provide quantitative measurements because the data are collected in digital form. Subcellular structure images are easily accessible (Figure 1), and planar images can be stacked to construct 3-D representations. Today’s confocal microscopes use a laser light source to illuminate a small area of the specimen and a pinhole in front of the detector to eliminate the haze and blur produced by light coming from above and below the focal plane of the specimen. The specimen is scanned relative to the source and detector, producing a series of point images that are assembled as a computer bitmap.

Fluorescence-labeling confocal microscopy has proven especially useful for tracking protein–protein interactions at a cellular level. For example, if various proteins are labeled with differently colored fluorescent compounds, the area of the image that contains a combination of the colors indicates where the proteins are closest together—the most likely area for interaction (Figure 2). Conventional micrographs show the true separation between structures in the plane of the image, but they are less able to gauge the distances between structures directly above and below the structure of interest, all of which appear superimposed in the image. In contrast, confocal micrographs represent only the thin plane of the sample that coincides with the focal plane. Out-of-plane structures do not appear in the image, and vertical distance is measured by comparing a series of

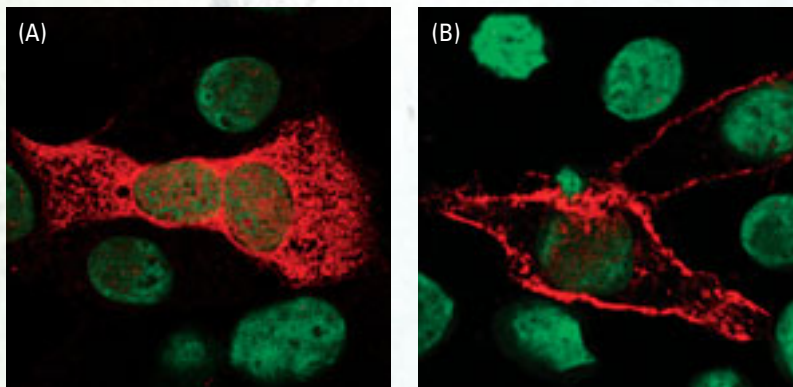


Figure 1. Confocal laser scanning micrograph of fixed HeLa cells 24 h after transfection with Ca²⁺-promoted Ras inactivator (CAPRI). (A) Unstimulated cells. (B) Cells fixed 10 min after ionomycin-induced Ca²⁺ mobilization. Red: CAPRI-expressing cells detected by indirect immunofluorescence using primary CAPRI antiserum and a secondary TRITC-conjugated antibody. Green: DAPI staining of cell nuclei. (Images courtesy of Peter Lockyer, The Babraham Institute, Cambridge, U.K.)

images taken at different depths. Computer software can select the optimum pinhole size automatically to compensate for variations in optical slice thickness caused by differences in the excitation and emission characteristics of specific dyes. This simplifies data analysis for images where more than one dye is used. Multitracking is another compensation technique—the instrument alternates rapidly between excitation lines from each dye, making it possible to monitor live-cell processes and reduce cross-talk between dye emissions.

Light microscopes, including confocal microscopes, can attain a resolution of about 0.2 μm , which is sufficient for general cellular structures. Extending the technique to specific interactions between proteins requires even better resolution. An adaptation of confocal microscopy called fluorescence resonance energy transfer (FRET) takes the resolution down to less than 0.01 μm . FRET imaging measures the nonradiative transfer of photon energy from one excited fluorophore to another. The amount of energy transferred depends on the distance between the two fluorophores, providing a means of measuring the distance.

The recently developed Nipkow disk system uses two rotating disks, one with 20,000 microlenses and one with 20,000 pinholes in the same pattern, rotating at 1800 rpm. Instead of scanning the sample, the disk illuminates many points in the sample simultaneously, creating an image in real time. Another refinement, adaptive optics technology, was first used for the large telescope mirrors in astronomical observatories. Computer-controlled actuators adjust the angles of each section of a deformable mirror to correct for ripples in a light wave as it passes through air, the specimen, the cover slip, or other image-distorting interfaces.

That light in your eyes

Optical coherence tomography (OCT) relies on an IR light backscattering effect similar to the acoustic backscattering used to produce ultrasound images. Because the frequencies and bandwidths of IR light are much higher than those of medical ultrasound signals, image resolution is 4–20 μm for OCT, compared with 110 μm for high-

frequency ultrasound. (Resolutions down to 1–2 μm have been achieved using developmental instruments and high-end lasers, but just under 10 μm is more typical for clinical instruments.) An optical fiber that can be run through a catheter or endoscope delivers light directly to the imaging site, producing images in real time and eliminating the need for tissue biopsies. Fluorophores are not needed to produce OCT images,

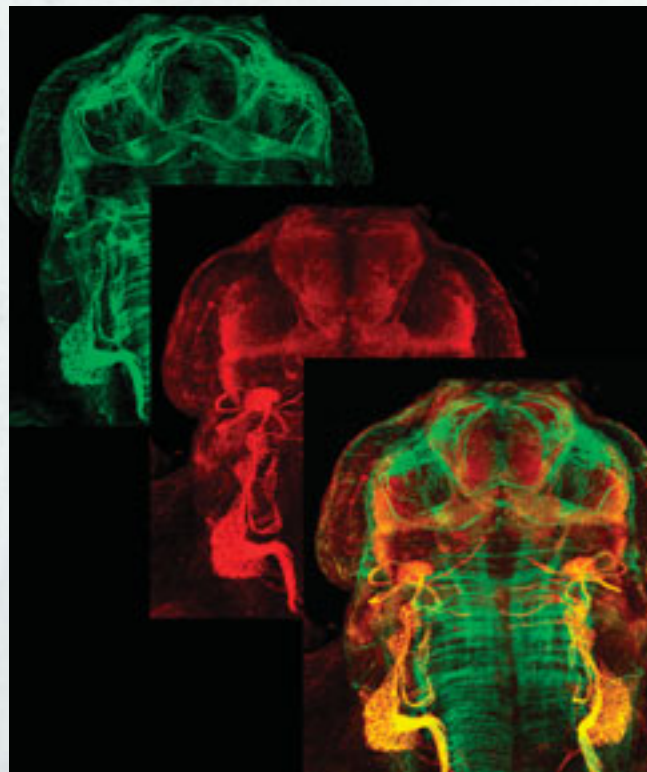


Figure 2. Confocal micrograph shows a zebrafish embryo (dorsal view) that has been labeled with two types of antibodies against cell adhesion molecules. Different subpopulations of axons in the nervous system fluoresce red or green. Superimposing the images shows areas where red and green fluorescently labeled proteins coincide (yellow). (Image courtesy of Martin Bastmeyer, Friedrich-Schiller-Universität Institut für Allgemeine Zoologie, Jena, Germany.)

another advantage of the technique.

Michelson interferometers process the optical signals, producing a 3-D transverse or longitudinal image. Spectroscopic information is extracted from the backscattered light using computer deconvolution analysis similar to that used for FTIR spectroscopy. The depth of the image slices can range from 20 to 200 μm , thin enough to track processes at a cellular level.

The OCT 1, the first commercial instrument, was introduced by the Humphrey Instruments division of Carl Zeiss in 1996 for diagnosing degeneration of the macula, the central region of the retina. With each successive generation of instruments, the price has decreased while the scan speed has increased, so it takes less time to collect the data to produce a complete image (for which the patients are thankful). Components borrowed from the telecommunications industry have been incorporated to improve resolution and achieve real-time image acquisition (4). OCT has proven to be an ideal technique for the early detection of retinal changes, blood vessel patterns, and lesions, critical factors in diagnosing glaucoma and diabetic retinopathy.

In 2000, Asahi Optical Co., Ltd., and LightLab Imaging announced a product development alliance for an endoscopic imaging system for use by gastroenterologists. The following year, Lantis Laser licensed LightLab's IP portfolio to develop OCT for detecting periodontal disease and dental caries and for performing dental restorations. Other clinical trials are under way to assess OCT's

capabilities to detect coronary plaque formation, carcinomas of the esophagus, melanin concentrations, and tissue oxygenation levels. Images of blood vessels with micrometer-level resolution are being used to study arterial abnormalities and verify the position of stents.

Recent adaptations of OCT include polarization imaging, a phase-contrast technique that has been used to assess the depth of burn injuries to skin tissues (5). Doppler OCT produces false-color images indexed to the rate of motion of the objects in the image (blood flow, for example).

Any way you slice it

Visible, IR, or UV light; gamma rays or X-rays. Transmission, backscattering, or fluorescence. With or without stains, tags, fluorophores, or chromophores. Tomographic imaging techniques continue to home in on increasingly smaller structures, reaching toward their goal of catching biomolecular processes “in the act”.

References

- (1) Renshaw, P. F.; et al. *Psychopharmacology* **1999**, *142*, 132–138. See also www.the-scientist.com/yr1999/sept/research_990927.html.
- (2) Goodman, M. M.; Bahjat, F. U.S. Patent 5,919,797, July 6, 1999.
- (3) The Pituitary Foundation. MRI & CT Scans; www.pituitary.org.uk/conference/2001/scans.shtml.
- (4) Reiss, S. M. *Biophotonics Int.* **2002**, *9* (3), 42–48.
- (5) Park, B. H.; et al. *Proc. SPIE* **2000**, *3915*, 243–248.

Further reading

SPECT

Single-Photon Computed Tomography; <http://imasun.lbl.gov/~budinger/medTechdocs/SPECT.html>.

SPECT Image Acquisition and Processing; www.bae.ncsu.edu/bae/courses/bae590f/1995/scarfone/basic.html.

PET

Lok, C. *Nature* **2001**, *412*, 372–374.

McKenna, E. *Modern Drug Discov.* **2001**, *4* (7), 59–62.

CT

Nanocrystal Imaging Corp.; www.nanocrystals.com/NIC.html.

1979 Nobel Prize in Physiology or Medicine; www.nobel.se/medicine/laureates/1979/press.html.

Confocal microscopy

George, J. S.; et al. *Virtual Pinhole Confocal Microscope*; Physics Division Progress Report 1999–2000, Los Alamos National Laboratory: Los Alamos, NM; www.lanl.gov/p/pdfs/papp_pinhole.pdf.

Paddock, S. *Science* **2002**, *295*, 1319–1321.

Two-photon scanning microscopy

Ott, D. *Biophotonics Int.* **2002**, *9* (1), 46–48.

OCT

Boppart, S. A.; et al. Ultrahigh Resolution and Spectroscopic OCT Imaging of Cellular Morphology and Function. In *Proceedings of the Inter-Institute Workshop on In Vivo Optical Imaging at the National Institutes of Health*, Bethesda, MD, Sept 16–17, 1999; Gandjbakhche, A. H., Ed.; U.S. Government Printing Office: Washington, DC, pp 56–61; <http://nb.beckman.uiuc.edu/biophotonics/bopPub.htm>.

Nancy K. McGuire is an associate editor of *Modern Drug Discovery*. Send your comments or questions about this article to mdd@acs.org or to the Editorial Office address on page 3. ■



KEY TERMS: cell biology, clinical, imaging