WINTER 06

Lens

A New Way of Looking at **Science**

Releasing the power of light

How imaging science is transforming medicine

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Lens –
A New Way of Looking
at **Science**

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Cover: **Scientists ply the glow of the firefly to plumb the mysteries of the body. Illustration by David Cutler.**

El hambre es el primer ojo del cuerpo
(Hunger is the original eye of the body)

–VERÓNICA VOLKOW

Lens **is published three times a year by Vanderbilt University Medical Center in cooperation with the VUMC Office of News and Public Affairs and the Office of Research. *Lens*® is a registered mark of Vanderbilt University.**

Our goal: to explore the frontiers of biomedical research, and the social and ethical dimensions of the revolution that is occurring in our understanding of health and disease. Through our *Lens*, we hope to provide for our readers – scientists and those who watch science alike – different perspectives on the course of discovery, and a greater appreciation of the technological, economic, political and social forces that guide it.

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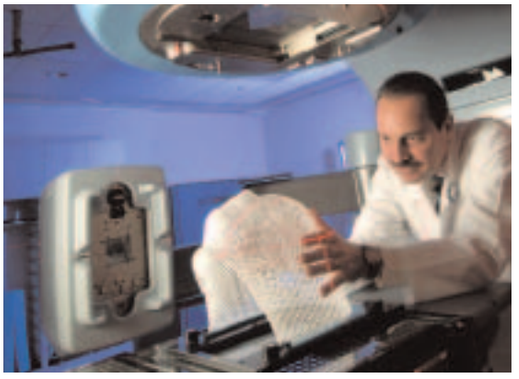

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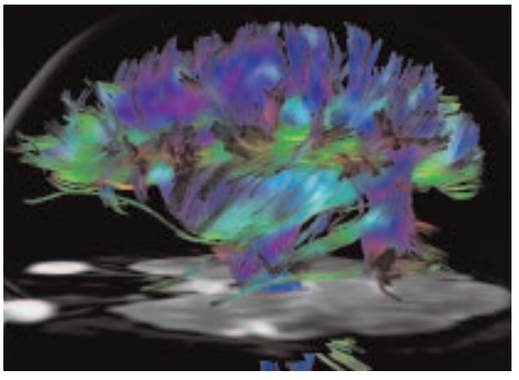

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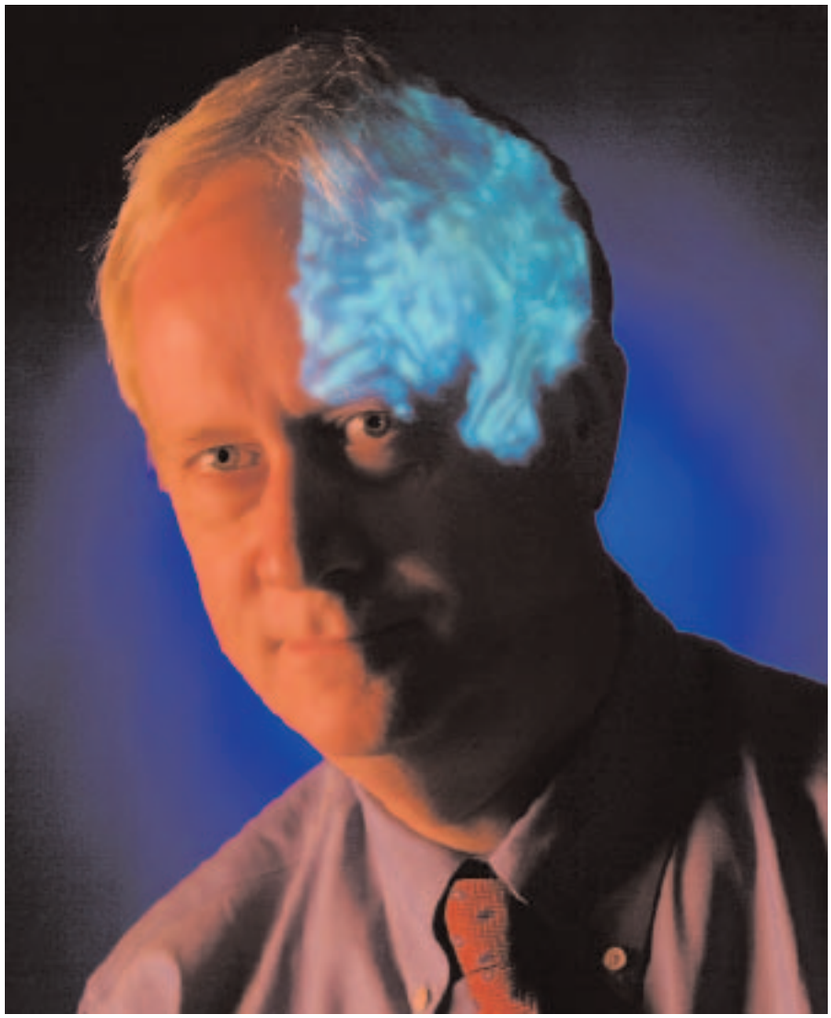

Biology, biomarkers and the workings of the human brain

The extraordinary reach of imaging science

By John C. Gore, Ph.D.

Director, Vanderbilt University Institute of Imaging Science
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Professor of Molecular Biology & Biophysics, and of Physics

After X-rays were first discovered in 1895, their strange and wonderful properties were almost immediately exploited for medical uses. They gave physicians for the first time the ability to "see" inside the human body non-invasively, and a whole new medical specialty, diagnostic radiology, was created. A little over a century later, a similar revolution is occurring with the development of a multitude of advanced technologies capable of providing a broad array of information to biomedical scientists and clinicians.

Imaging science is the new discipline that connects discoveries in the basic sciences and engineering to applications in biology and medicine. The new technologies build on advances in other fields such as molecular biology and proteomics, and have enormous potential to improve clinical care and to make important contributions to medical research.

Over the last few years, a compendium of powerful imaging techniques has been developed, not only for clinical medicine but also for basic research. Imaging today plays a central role in patient management and care. Radiological imaging methods such as X-rays and nuclear imaging, computed tomography (CT), magnetic resonance imaging and spectroscopy (MRI, MRS), positron emission tomography (PET) and ultrasound imaging are essential for the diagnosis of numerous disorders, for providing crucial insights into the pathophysiology of many types of disease, and

Pictured at left: John C. Gore, Ph.D., with three-dimensional rendering of a functional magnetic resonance image (fMRI) of the brain projected onto his forehead.

Pictured below: Brain image obtained by Vanderbilt scientists on a 7 Tesla MRI scanner at Philips Medical Systems reveals small structures such as tiny blood vessels (white dots in the dark gray regions of the image and magnified inset) that are beyond the resolving power of conventional scanners. An identical scanner will be installed at Vanderbilt in the spring of 2006. One Tesla is roughly 20,000 times the strength of the magnetic field of the earth. The 7 Tesla scanner allows scientists and clinicians to study brain structure, function, and neurochemistry at an unprecedented level of detail.

Courtesy of Vanderbilt University Institute of Imaging Science

for obtaining measures of the response of patients to treatments.

In vivo imaging methods also have widespread applications in research, for the elucidation of biological structure and in the study of fundamental biochemical, molecular and physiological processes. Imaging can be used in many different ways: to assess tissue structure and for quantitative morphometry, such as measuring the growth or regression of tumors; to measure intrinsic tissue characteristics and composition, such as tumor cell density or neural myelination; to map various metabolic and physiological properties, such as blood flow or oxygen use; and to detect and quantify fundamental processes at the molecular and cellular levels, such as the expression of specific genes.

Much of imaging research today is aimed at the development of biomarkers in order to derive information on specific biological processes or responses to treatment. For example, in patients with cancer, imaging-based biomarkers of tumor vascular properties may be used to predict early in the course of the disease whether a particular treatment regimen will be successful.

The development of functional brain imaging by MRI and the study of neurochemistry with MRS and PET are two other recent advances that have had a major impact on our understanding of brain architecture and function, allowing us to understand the neural basis for both normal and abnormal behaviors.

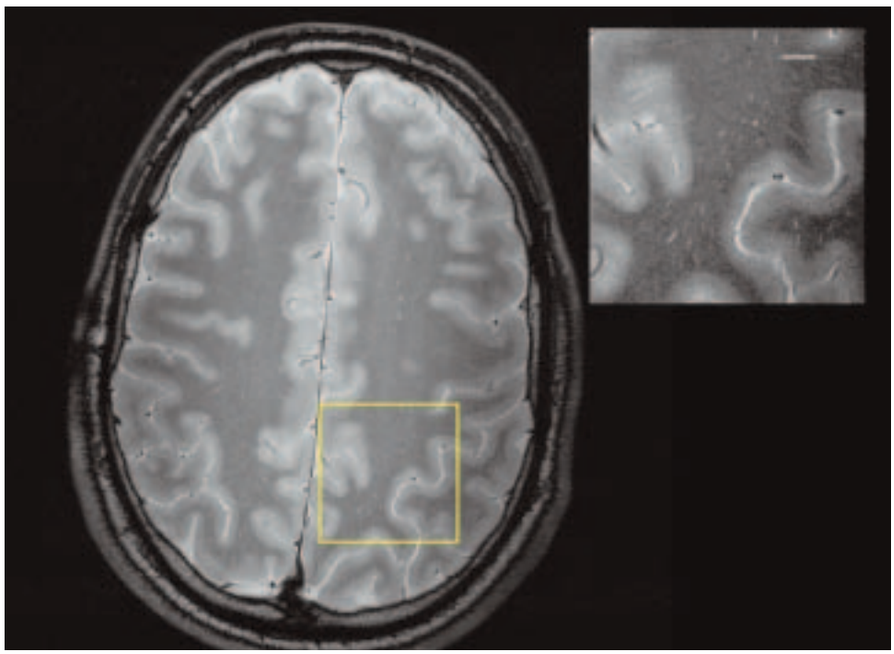
New technological developments and advances in molecular sciences, such as the development of novel agents that can target specific receptors, have expanded the applications of imaging to the molecular level, especially through the use of optical or nuclear detection methods. The result is that imaging applications permeate almost all current areas of medical research.

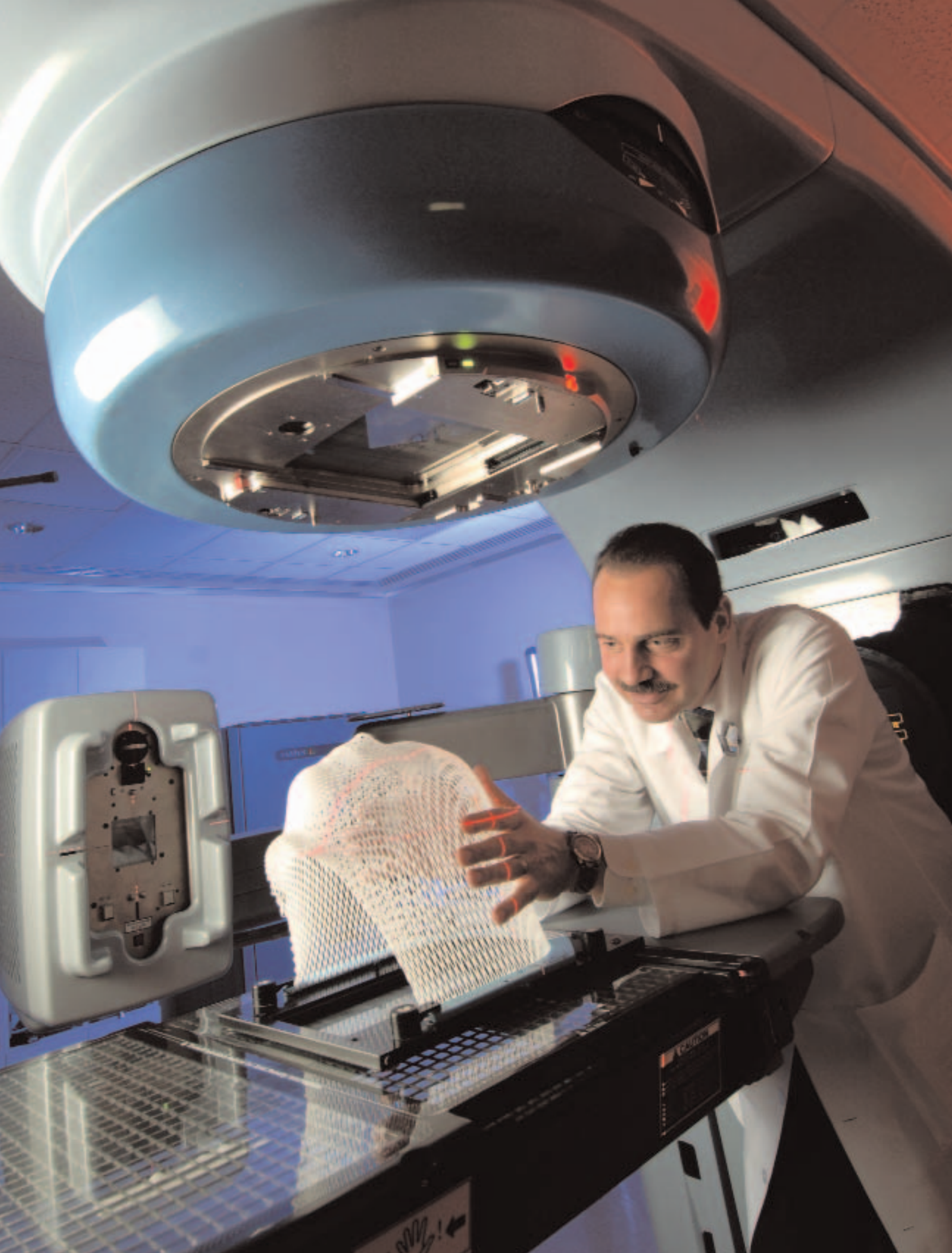
The greatest successes for applications of imaging science in the future will come from environments where the complementary natures of different imaging approaches is realized, and where experts in basic sciences and technical aspects of image formation and analysis work closely with biomedical scientists who ask appropriate questions. Vanderbilt University Medical Center has taken a lead in establishing a new, multidisciplinary Institute of Imaging Science (VUIIS), in recognition of the pervasive importance and intellectual vitality of imaging.

VUIIS provides Vanderbilt researchers with state-of-the-art research imaging of animals and human subjects across a broad range of modalities. It comprises an expert faculty that includes physicists, engineers, computer scientists, chemists, physiologists and clinical scientists, working together to address important problems within imaging science and applications of imaging. The Institute manages an impressive array of imaging resources, including systems dedicated to the study of preclinical models of disease such as microPET, microCT, optical, ultrasound and MR imaging of small animals. It also will shortly house a 7 Tesla human scanner for MRI and MRS, one of fewer than 10 such systems in the world, and the flagship for exciting new research directions.

These facilities will be integrated, along with chemistry labs dedicated to the development of new probes for molecular imaging and computing labs for advanced image analysis, in a new 42,000-square-foot building that will be completed in the fall of 2006. The new Institute will provide Vanderbilt a world-class research facility in all aspects of biomedical imaging, and provide an exemplary training environment for specialists as well as other research scientists in the use of imaging. The faculty and trainees within VUIIS are already engaged in numerous projects applying imaging methods in cancer biology, basic and clinical neurosciences, metabolic disorders and clinical trials.

This issue of *Lens* highlights some of the current areas of emphasis in imaging science at Vanderbilt and elsewhere. **LENS**





Piercing the body with precision

How imaging is aiding the fight against cancer

By Bill Snyder

The scalpel is giving way to the scan – at least in some cases of cancer.

New imaging technologies are raising hopes that doctors soon will be able to locate tumors with pinpoint accuracy, and track their hour-by-hour response to treatment – without the need for surgery.

Coupled with recent advances in genetics and molecular biology, imaging is speeding the discovery and evaluation of safer, more effective treatments that can stop tumors in their tracks.

"In the past 10 years we've made tremendous strides in improving imaging of cancer," says Dennis E. Hallahan, M.D., chairman of Radiation Oncology at Vanderbilt University Medical Center. "In the near future we will be using functional imaging to image pre-cancer."

At Vanderbilt, for example, a sophisticated technique called dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is being tested in women with breast cancer.

The goal: to see whether new "targeted" therapies are shrinking tumors by disrupting their blood supplies. If successful, the technique could avoid the need for repeat biopsies, says Tom Yankeelov, Ph.D., director of Cancer Imaging in the Vanderbilt University Institute of Imaging Science.

"Particularly in breast cancer there's currently no adequate . . . some would say there's no non-invasive method at all, to determine whether or not a tumor is responding to treatment," Yankeelov says.

"It's really a sad state of affairs," he says. "Repeat biopsies are not really an option because you have to go under the knife each time – who wants to do that?"

In addition, "biopsies by definition only sample a portion of the tumor. It is entirely possible that you could sample a section of tissue that is free of active disease and miss the sections that are actively proliferating.

"That is why imaging is so powerful," Yankeelov says. "You can get a more complete description of the tumor status, and you can do it non-invasively."

That's the aim of DCE-MRI, a modified MRI technique in which a contrast agent is injected into the patient to outline the profusion of fragile, leaky blood vessels that spring up to feed growing tumors. *(See next page)*.

Nearly 40 anti-angiogenic drugs, which inhibit the growth of these vessels, are now in clinical trials. Advanced imaging technologies like DCE-MRI, by detecting changes in blood flow and vessel permeability or "leakiness," for example, may help doctors determine whether the tumor is responding – even after the first course of chemotherapy.

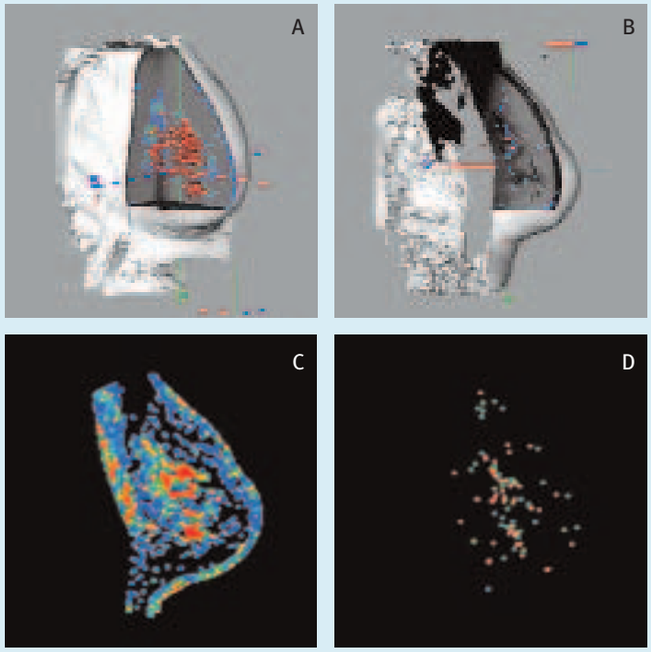
More work needs to be done, however, before DCE-MRI will be ready for the clinic. "I personally think (it) is really just another tool in the toolbox," Yankeelov adds.

Newer imaging techniques can measure glucose metabolism, hypoxia (lack of oxygen) and the diffusion of water molecules in and out of cells – indicators of how big the tumor is, how "healthy" it is, and whether it is surviving attempts to kill it.

By tracking various markers that have been tagged with a radioisotope, PET also can tell whether a tumor is dying – or proliferating. Similarly, mass spectrometry techniques can detect changes in the expression of various proteins by tumors in response to treatment.

Pictured left: Dennis E. Hallahan, M.D., chairman of Radiation Oncology at Vanderbilt University Medical Center, adjusts a plastic frame that holds the head still during treatment using an image-guided radiation therapy system, called Trilogy, made by Varian Medical Systems.

Photo illustration by Dean Dixon



Starving a tumor

Fragile, leaky blood vessels nourishing a breast tumor are revealed with the help of dynamic contrast-enhanced MRI. The red voxels (three-dimensional data points) produce a 3-D volume rendering of blood flow (perfusion) and leakiness (permeability) before treatment (A).

In the same patient after chemotherapy (B), a drastic reduction in perfusion/permeability indicates treatment is successfully "starving" the tumor by disrupting its blood supply.

(C) and (D) are single-slice images taken from the center of the 3-D volume renderings before and after treatment. The hope is that this kind of analysis will enable doctors to determine early on whether the tumor is responding to therapy.

Courtesy of Tom Yankeelov, Ph.D.

Whether these techniques can predict the outcome of therapy and its impact on patient survival remains to be proven clinically.

Another technical challenge: "registering" the different images – mapping coordinates representing the same anatomical point so that the same "voxel," or three-dimensional piece of data, lines up in each of them.

Guiding the scalpel

Registration already is an integral part of stereotactic surgery and radiosurgery, the precise guidance of scalpels and radiation beams to remove abnormalities, including tumors, with minimal damage to surrounding tissue.

New techniques developed by Vanderbilt engineers and computer scientists are extending the reach of the neurosurgeon and radiation oncologist even further. Their contributions are proving to be invaluable, especially for treatment of aggressive, infiltrating glioblastomas of the brain.

"The visual cues we have at surgery are really poor," explains Reid C. Thompson, M.D., director of Neurosurgical Oncology at Vanderbilt. "There isn't often a discrete edge … maybe there's a slight discoloration … maybe the tumor just feels a little different."

As a result, he says, "you either don't take out enough tumor in the brain, and we know that's probably not good in terms of prognosis, or you take out too much, which is an obvious problem."

To further define the margins of the tumor during surgery, Anita Mahadevan-Jansen, Ph.D., and her colleagues in the Department of Biomedical Engineering have developed an optical probe that within 30 seconds can differentiate between normal and abnormal brain tissue based on the spectra of light bounced off of them.

A recent clinical study concluded that the instrument can achieve what amounts to an "optical biopsy" with "near-instantaneous feedback," improving the percentage of tumor that is removed during surgery and reducing operating time and expense.

Michael I. Miga, Ph.D., assistant professor of Biomedical Engineering and director of the Biomedical Modeling Laboratory, has harnessed a widely used commercial technique, laser range scanning, to adjust for changes in the surface of the brain as the surgeon cuts into it.

By repeatedly sweeping a laser beam across the brain surface, the scanner produces "point clouds" or sets of three-dimensional points that – in clinical studies – have accurately predicted the changing locations of the tumor as well as nearby blood vessels and other delicate structures during the operation.

The development of these techniques owes much to the rich, longtime collaboration between Vanderbilt engineers, computer scientists and neurosurgeons.

Leaders in this effort include J. Michael Fitzpatrick, Ph.D., and Benoit M. Dawant, Ph.D., professors in the Department of Electrical Engineering & Computer Science; Robert L. Galloway, Ph.D., professor of Biomedical Engineering; and Neurosurgery Department chair George S. Allen, M.D., Ph.D.

During the past 15 years, Vanderbilt researchers have improved the registration of preoperative images with anatomical information collected by an optical probe during surgery. The combined image, projected onto a computer screen in the operating room, helps the surgeon hold a true course through tissue topography that changes with each touch of the scalpel.

Dawant, who with Fitzpatrick co-directs the Medical Image Processing Laboratory, has developed computational "atlases" of the brain and liver – marked by recognizable anatomical guideposts such as blood vessels – that can be "warped" to fit individual patient cases.

Similarly, Galloway and his colleagues have teamed up with surgeons to create intraoperative guidance systems that use optical tracking or articulated "arms" to track the surgical position in three dimensions. *(See page 7)*

The primary software platform, called ORION, for Operating Room Image-Oriented Navigation, can be modified to support neurosurgical and surgical applications, including ones such as liver surgery, where the target moves with patient breathing.

All this would not have been possible were it not for the astronomical increase in computing power, Miga adds.

"We solve about 80,000 coupled equations in a model, and we do that in about 15 to 18 seconds," he says. "And when I say 80,000 equations, I'm talking about essentially a spatial understanding of how the organ we're looking at is deforming or moving."

Hitting the target

Thompson says the next logical step in cancer imaging is to develop a molecular imaging agent, "some unique marker in the tumor that you could target … that would allow the tumor to fluoresce" as the surgeon peers through the operating microscope.

With Vanderbilt chemistry professor Darryl J. Bornhop, Ph.D., Thompson is investigating another class of fluorescent molecules, the rare-earth lanthanide chelates, which potentially could be used to delineate tumors both in MRI scans and under the operating microscope.

Many cancer cells express a high density of peripheral benzodiazepine receptors (PBRs), named for their ability to bind anti-anxiety drugs like Valium and Xanax. To PK-11195, a compound that binds tightly to PBR, Bornhop attaches fluorescing complexes of lanthanide chelates.

In animal studies, the injected marker shows unique, dual capabilities: Bornhop's hybrid shows up in MRI scans, and its fluorescent tag can be observed through the microscope. If it works in humans, the surgeon could match the fluorescence seen during surgery to the pre-operative MRI.

While PBR targeting also may be useful in the treatment of other tumors, including those of the breast and colon, it alone may not be enough, Bornhop cautions. A "cocktail" of chemicals will probably be needed – especially to monitor how a tumor is responding to therapy.

One possible avenue: cell adhesion molecules, which play important roles in inflammation, cell migration, cell signaling – and cancer.

About a decade ago, Hallahan and his colleagues at the University of Chicago observed that the inner linings of tumor blood vessels sprouted these distinctive glycoproteins (carbohydrate-protein complexes) when zapped by a dose of radiation. He wondered how he could capitalize on this phenomenon.

After moving to Vanderbilt to chair the Department of Radiation Oncology in 1998, Hallahan assembled a diverse team that included Todd D. Giorgio, Ph.D., associate professor of Biomedical Engineering and Chemical Engineering.

The researchers began searching for fragments of proteins – short sequences of amino acids called peptides – that would hone in on tumor blood vessels.

Hallahan hoped the peptides would bind specifically to these radiation-induced markers inside tumor blood vessels. When tagged with radioisotopes, these tiny guided missiles could be used to monitor the effectiveness of drugs designed to shut down the tumor's blood supply. They also could deliver their own toxic payloads.

The researchers found an amino-acid sequence, arginine–glycine–aspartic acid or RGD, that bound specifically to the markers.

In a preliminary feasibility study, they labeled the peptide with a gamma ray-emitting radioisotope, injected it into patients receiving high-dose radiation to treat metastatic brain tumors, and watched the tumors light up in images taken by a gamma camera. "This study shows that it is feasible to guide drugs to human neoplasms by use of radiation-guided peptides," they reported in 2001.

Next, the researchers coated "nanoparticles" (about the size of a virus) with fibrinogen, a blood-clotting protein that contains the RGD sequence, tagged the particles with a radioisotope, injected them into tumor-bearing mice, and blasted the tumors with radiation. Not only did the blood vessels light up, but the fibrinogen coating apparently triggered clots to form inside the vessels, blocking blood flow and causing the tumors to shrink.

Currently the researchers are searching for peptides and antibodies that zero in on tumor blood vessels following low-dose irradiation in combination with Sutent (SU11248).

"That's why it takes so long to get a drug or an antibody to market," explains Raymond L. Mernaugh, Ph.D., director of the Molecular Recognition and Screening Facility in the Vanderbilt Institute of Chemical Biology who is participating in the research. "You go through all these steps to prove that you have something that's very specific, doing exactly what you want."

Sutent is a "targeted" cancer therapy, now in clinical trials, which blocks an enzyme key to the development of tumor blood vessels. Not all tumors or patients respond to targeted therapy, however. Hallahan's goal is to develop a way of determining within hours, rather than weeks, whether the drug is working.

Meanwhile, Giorgio and his colleagues have identified peptides capable of penetrating the nuclei of cells in the breast, and which potentially can differentiate normal cells from tumors. By attaching gold nanoparticles to the peptides, this method could generate an early and extremely precise view of breast cancer.

Similarly, the recent discovery of neural stem cells could lead to improvements in the early detection and treatment of gliomas. Scientists believe these stem cells, the source of normal brain tissue, under some circumstances can be transformed into tumors.

"Let's say you could image the stem cells," Thompson imagines. "Then you could see that your therapy made (the abnormal cells) go away … I hope we would get to a point where if somebody came in with a tumor we'd be able to simply flip the switch and shut it off and keep it from progressing … without having to do surgery.

"It is absolutely changing the way we think about these kinds of cancers." **LENS**

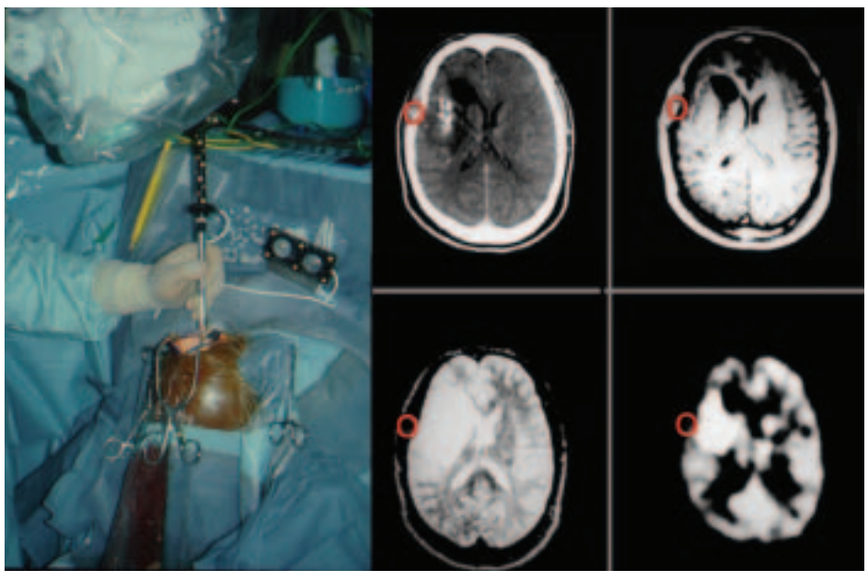


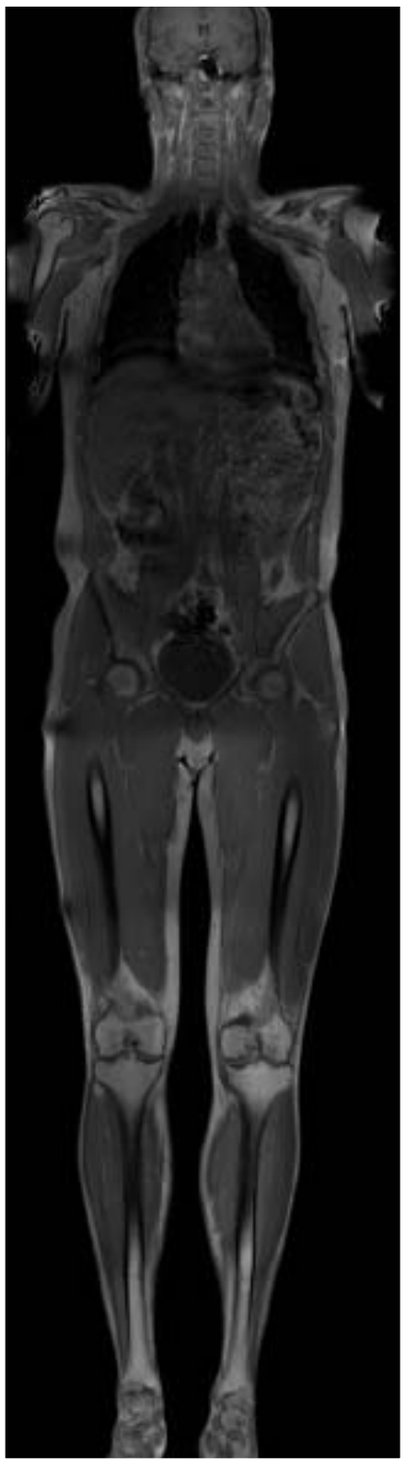
Image-guided brain surgery
A tracked surgical probe collects data from a brain tumor during surgery (left). The information is used to index pre-operative images to the correct slice of the tumor (right) and to display the surgical position on that slice on a monitor in the OR. The CT image (upper left), PET image (lower right) and two MR images (upper right and lower left) update in real time as the probe is moved.

Courtesy of Robert L. Galloway, Ph.D.

Autopsy of the living

A brief history of imaging science

By Melissa Marino



On the evening of Nov. 8, 1895, an accidental discovery ushered in a scientific and medical revolution that would allow us to see inside the living human body for the first time.

While conducting an experiment with cathode rays, Wilhelm Roentgen, Ph.D., noticed a strange glow on a distant cardboard screen. Knowing that cathode rays could not pass through the obstacles between his cathode ray tube and the glow, he proposed the existence of a novel type of penetrating ray, which he called the "X-ray." After spending several solitary weeks analyzing the rays, Roentgen published his findings in late December, along with an eerie X-ray photograph (radiograph) of his wife's hand.

Roentgen's discovery opened up the human body without a single incision. Soon, the medical profession was using X-rays to locate lodged bullets and bone fractures. With the further refinement of the technology and the development of contrast agents, even soft tissues came into focus.

Rock 'n' roll

Despite the improved resolution of contrast-enhanced X-ray images, overlying bones obscured some parts of the body from view. By moving the X-ray tube and film in tandem, bones that stood in the way were blurred out and a single cross-sectional slice through the body was highlighted.

This new technique, called tomography, was first described by the Dutch radiologist Bernard Ziedses des Plantes in 1931. Tomography could produce a series of images that could be stacked to give the physician information about volume. The first commercial tomograph, called the laminagraph, was built at the Mallinckrodt Institute of Radiology at Washington University in St. Louis in 1937.

Although laminagraphs are related to modern computed tomography (CT), such technology had to await the dawn of the computer age. A major player was a London-based electronics firm, Electric and Musical Industries Limited (EMI), perhaps best known as the Beatles' record label.

Supported in part by the sales of Beatles records, the EMI group, led by Godfrey Newbold Hounsfield, developed and brought the first CT scanner to market in 1971. As image resolution improved and scanning speed increased, the CT scan soon became the "standard of care" for suspected brain disorders. It has since become a powerful method for imaging the body as well.

Over the seven decades that passed between the first X-ray devices and the modern version of CT, the basis for the next wave of medical imaging – one that didn't involve harmful radiation – was slowly taking shape.

Resonance

In the late 1930s, physicists discovered that atomic nuclei containing odd numbers of protons (such as hydrogen) would align themselves with a strong magnetic field and revert to their original state, or "relax," when the field was turned off. This change could be detected by the radiofrequency waves given off in the process.

Since bodily tissues differ in their water content (and consequently, hydrogen content), scientists realized that this "nuclear magnetic resonance," or NMR, could be used to distinguish between soft tissues – and possibly to detect disease.

In 1971, Raymond Damadian, M.D., a physician at Downstate Medical School in Brooklyn, N.Y., used NMR to distinguish excised cancerous tissue from normal, healthy tissue.

Two years later, Paul Lauterbur, Ph.D., a chemist at the State University of New York at Stony Brook, introduced rotating magnetic field gradients and computer algorithms to assemble a two-dimensional image from NMR data. Using this technique, he produced the first NMR image of a living subject, a clam.

Damadian and colleagues followed in 1977 with the first NMR image of a human subject.

Peter Mansfield, Ph.D., a physicist at the University of Nottingham in England, developed mathematical calculations that allowed faster acquisition of the NMR image. His work led to the "fast" or "functional" MRI (fMRI), which could acquire images at the rate of 30 to 100 frames per second.

In 1989, Seiji Ogawa, Ph.D., a physicist at AT&T Bell Laboratory in New Jersey, described the phenomenon – called Blood Oxygenation Level Dependent (BOLD) effects – that forms the basis for functional MRI (fMRI). The changes in oxygenation of blood hemoglobin in "activated" brain regions perturb the local magnetic environment, serving as a natural contrast agent.

Since changes in the BOLD signal depend on the changes in blood flow and oxygenation, fMRI provided a measure of brain activity and the unparalleled ability to safely and non-invasively probe the physiological basis of neurological and psychological disorders as well as normal cognitive function.

Medical 'Geiger counters'

Despite the attractiveness of MR as a radiation-free, and presumably safe, imaging technique, nuclear technology has spawned some of the most sensitive and powerful imaging methods to date – single photon emission computed tomography (SPECT) and positron emission tomography (PET).

The discovery of naturally-occurring radioactive elements, like uranium, polonium and radium, in the late 19th Century sparked the "atomic age." However, these naturally radioactive elements are not normally found in the body, so their medical use was limited.

In 1934, the creation of artificial radioisotopes of normally non-radioactive elements common in the body (including carbon, oxygen, nitrogen and fluorine) gave physicians the tools they needed to adapt radioactive compounds for medical purposes. As these radioisotopes decayed, they produced gamma rays, which could be detected with a Geiger counter.

Deriving an image was not the priority at first; the goal was to detect "hot spots" in the body where the radioactive compounds accumulated. But, in 1951, Benedict Cassen, Ph.D., at UCLA built the scintiscanner, a device that scanned the body using pen-sized gamma ray detectors and created a crude print-out of those hot spots. Acquiring a usable image from these radioactive compounds suddenly seemed possible.

In 1968, the first nuclear imaging machine, single photon emission computed tomography (SPECT) was built. However, the seminal advance in nuclear imaging came in 1975, when Michael Phelps, Ph.D., and Edward Hoffman, Ph.D., at Washington University in St. Louis reported their development of PETT (positron emission transaxial tomography), later shortened to "PET."

When a positron emitted by a decaying radioisotope collides with a nearby electron, two gamma rays traveling in opposite directions are produced. Using a hexagonal array of gamma detectors and computational methods similar to those that generated CT images, the Washington University scientists built a device that could construct three-dimensional "maps" of positron emission deep within the body.

PET was primarily used for research purposes until 1979, when another milestone – the development of radioactive FDG (fluorodeoxyglucose), a glucose analog – further propelled the technology into the medical field. With FDG, physicians can track the metabolic activity of cells, aiding in the diagnosis of cancer and other diseases.

Smiling in the womb

In 1877, the discovery of piezoelectricity laid the foundation for one of the safest and most economical methods of seeing into the body – ultrasound.

Piezoelectric crystals generate a voltage in response to applied mechanical stress, including sound waves. During World War I, they were used in the first sonar devices to detect sound waves bounced off enemy submarines.

In 1937, sound waves were first transmitted through a patient's head to derive a crude image of the brain. Ultrasound ultimately found its niche in obstetrics and gynecology in the 1950s following reports of the damaging effects of X-rays on the fetus.

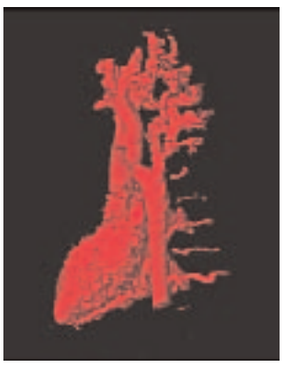
Since then, ultrasound has morphed from flat black-and-white, two-dimensional images intelligible only to trained professionals to the sharp and startling 4-D "movies" of the fetus kicking, yawning and smiling in the womb.

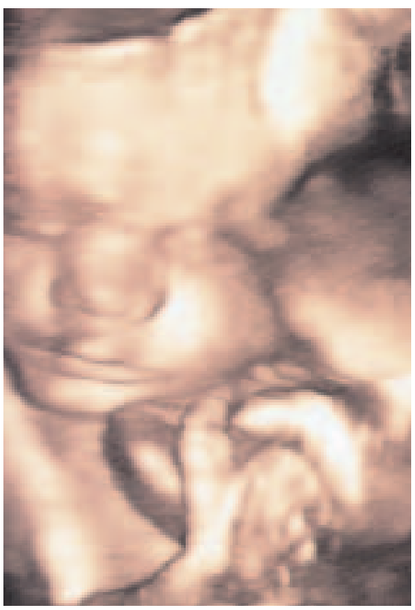
Because ultrasound also can capture the movements of heart muscle and valves, an application called echocardiography is now used to examine the heart – before and after birth. **LENS**

Pictured at left: Whole body scans, like this magnetic resonance image, can help physicians determine the extent of cancer spread throughout the body. They also could be used routinely to screen healthy people for a gamut of diseases, although this use is controversial. Courtesy of the Vanderbilt University Institute of Imaging Science.

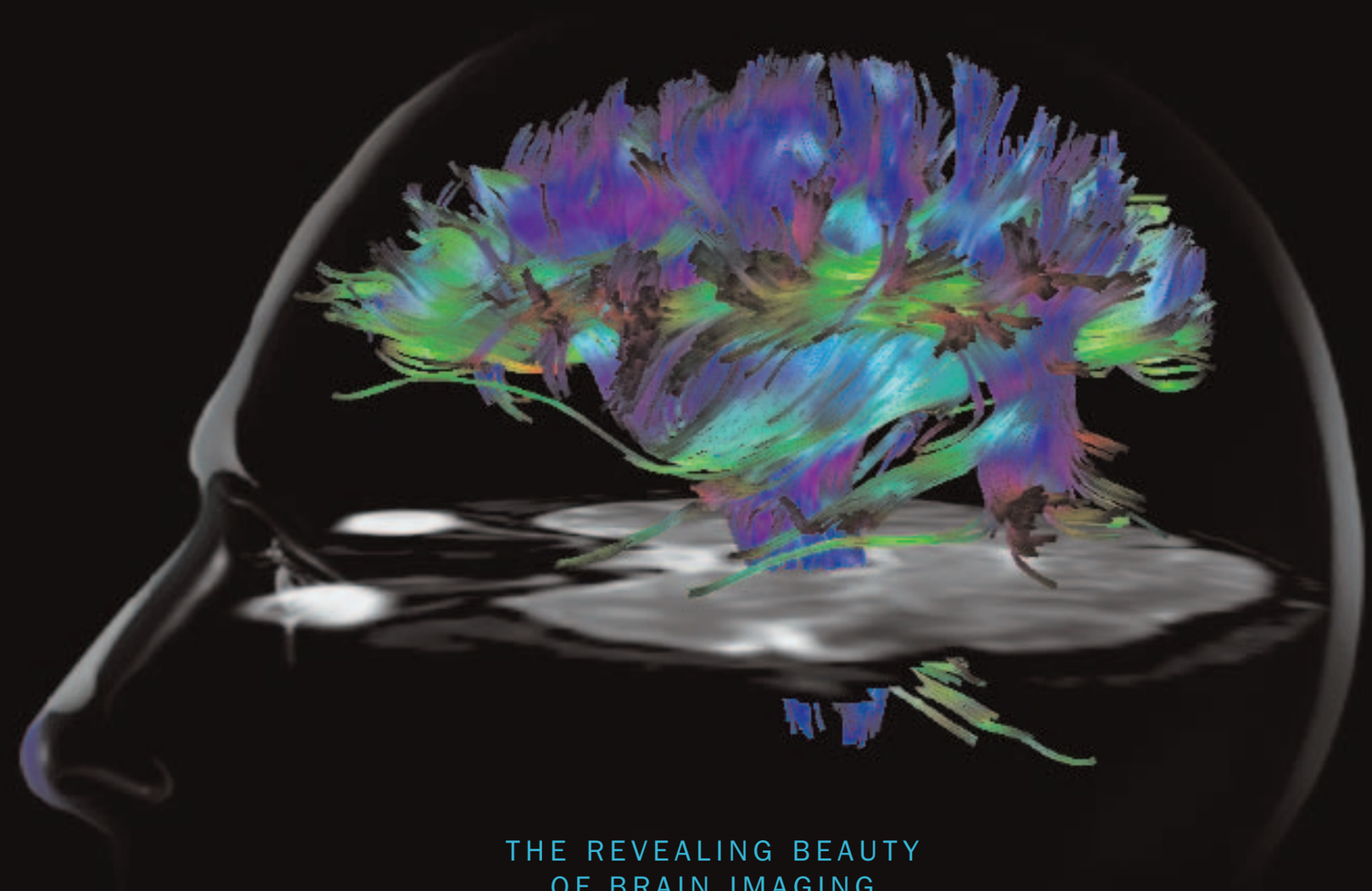
Pictured below: (Top) 3D reconstruction of an MRI of the heart and mediastinal vessels in a child with a congenital narrowing of the aorta. (Bottom) 3D ultrasound image of the fetal face.

Courtesy of Marta Hernanz-Schulman, M.D., and David A. Parra, M.D. (top), and Arthur C. Fleischer, M.D. (bottom).





Picturing the MiND *at* work



THE REVEALING BEAUTY OF BRAIN IMAGING

By Melissa Marino

Since the discovery of the X-ray, scientists have tried to take pictures of the mind at work. One hundred ten years later, they have never been closer. Soon it may be possible to predict – and avert – the development of drug addiction, to individualize therapy for schizophrenia and other disorders, and to preserve and even augment brain function.

"Traditionally, imaging meant radiology – you went to the X-ray department. Imaging is now much more broadly based," says John C. Gore, Ph.D., director of the Vanderbilt University Institute of Imaging Science.

Now, techniques like functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) provide much more than a static snapshot of the brain's form. They can illustrate the symphony of activity that underlies memory, addiction and love. They can also resolve dysfunction related to psychiatric and neurological disease and watch how drugs and educational interventions "normalize" brain activity.

"Can we measure the effect of a behavioral therapy? Can you tell whether someone is going to recover from aphasia? Those are questions that radiologists have not previously thought much about," Gore says. "They now have the tools to do it."

The explosion of fMRI studies in psychiatry and psychology has revealed volumes of information about individual brain regions and their principal functions.

While this may convey the rather simplistic notion that the brain is highly compartmentalized, another imaging technique based on MRI, diffusion tensor imaging (DTI), may help weave these isolated islands of brain activity back together again into a functional network.

"With fMRI, you can look at brain function – where in the brain certain information is processed," says Adam W. Anderson, Ph.D., associate professor of Biomedical Engineering and of Radiology and Radiological Sciences at Vanderbilt.

Pictured at left: Diffusion tensor image illuminates "white matter," bundles of long fibers (axons) that transmit signals between different parts of the brain. Colors indicate the direction in which the bundles are running (green = back to front, red = side to side, blue = top to bottom). The gray "base" – an MRI slice through the brain showing the eyes – and the outline of the head are included to help orient the image in space. The bundles dangling beneath the base are going to the brainstem and temporal lobe. Such images could be used to help surgeons excise brain tumors without damaging fiber bundles.

Image courtesy of the Vanderbilt University Institute of Imaging Science; illustration by Dominic Doyle

"With DTI, you can look at the connection between areas of the brain that are processing that information."

From the energy emitted by the nuclei of hydrogen atoms in the presence of a magnetic field, MRI can generate exquisitely detailed images of soft tissues, including the grayish parts of the brain that contain the neurons (called "gray matter").

DTI, on the other hand, illuminates "white matter," bundles of long fibers (axons) that transmit signals between different parts of the brain. In particular, the technique measures "anisotropy," the degree to which water movement is greater along fibers than in other directions.

Anisotropy may help explain why some patients with schizophrenia experience auditory hallucinations. Last year, Swiss researchers reported finding increased anisotropy in the brains of patients who frequently heard voices, particularly in the white-matter tracts that connect hearing and language centers.

Increased anisotropy is a measure of greater connectivity in the axon bundles. Neurons that are too strongly coupled to each other may fire off signals too readily, resulting in over-activation of parts of the brain that process external sounds and language, the researchers reported last year.

For patients with schizophrenia, this may explain the inability to distinguish their own, self-generated thoughts from external speech.

The Swiss report, entitled "Pathways That Make Voices," reinforces the value of DTI in exploring other neurological and psychiatric diseases, says Anderson.

In collaboration with other Vanderbilt researchers, Anderson is using the technique to investigate known white-matter diseases such as multiple sclerosis, and to determine the effects of prenatal cocaine exposure on brain connectivity.

DTI and other MRI techniques also are proving useful to understanding how the developing brain is wired, and how conditions such as premature birth can affect cognitive development. The goal is to find ways to prevent "anatomic disturbances" from impairing "functional capacities," Anderson, Gore and their colleagues at Yale pointed out in a recent paper.

How to Make an Atomic Drug

Ronald Baldwin, Ph.D., is on the front lines of a major effort at Vanderbilt to develop new radiotracers, not only to improve understanding of brain diseases but to speed drug development.

It's an ambitious task.

At the heart of radiopharmaceutical preparation is the cyclotron – a hulking machine entombed by thick concrete shielding with a control panel resembling the cockpit of a jet airliner.

The cyclotron accelerates charged particles, usually protons, in dizzying circles to near the speed of light, until they are sent smashing into a sample of nonradioactive material (carbon, fluorine, oxygen or nitrogen). This collision forces an extra proton into the target atom's nucleus, resulting in a radioisotope that can then be inserted into the pharmaceutical compound – again, not always an easy feat.

To improve safety, ever decreasing amounts of radiation are incorporated into radiopharmaceuticals, making simple chemical reactions tricky.

"There's a lot of art in radiochemistry … a lot of reactions that are 'bread and butter' bench reactions just don't work," Baldwin says, because of the low concentrations of radioisotope used.

With the initiative to develop several new radiopharmaceuticals for both brain and cancer imaging, he has his work cut out for him.

Baldwin doesn't seem to mind, though. He just wants folks to remember where the brilliant images on the screen originated.

"Chemists are often in the background," he says with a smile. "People sometimes forget where (the images) came from."

– MELISSA MARINO

ANNE RAYNER

Ronald Baldwin, Ph.D., prepares radiopharmaceuticals in a shielded cabinet with the help of robotic arms.

"Neuropsychologists have spent a lot of time looking at the brain as it falls apart from injury," adds imaging pioneer Marcus E. Raichle, M.D., professor of Radiology, Neurology and Anatomy & Neurobiology at Washington University School of Medicine in St. Louis. "But it would be equally important to watch it being put together during development."

Right drug, right dose

Schizophrenia remains one of the most challenging brain disorders. Characterized by delusions and hallucinations, the illness disrupts cognition and emotion, profoundly affecting a person's ability to think clearly and interact with others.

Imaging techniques, including PET and fMRI, are aiding understanding of the disease and the development of drugs to treat it.

The classic antipsychotic drugs, such as Thorazine, block receptors for the neurotransmitter dopamine, which also is centrally involved in Parkinson's disease, addiction and other brain conditions. But while these drugs squelch the delusions and hallucinations, in high doses they also cause Parkinson's-like symptoms, including rigidity and loss of muscle control, and they don't improve cognitive function.

In the early 1990s, Herbert Y. Meltzer, M.D., and colleagues at Case Western Reserve University helped establish that clozapine and other second-generation "atypical" antipsychotics could effectively treat psychosis and improve cognition without causing Parkinsonism. This was great news, but how did the two classes of drugs produce such different effects?

That's where imaging came in.

Robert M. Kessler, M.D., and his colleagues at Vanderbilt had developed a number of radiolabeled compounds visible on PET scans that bound to a particular dopamine receptor, called D2, if it was not already occupied by an antipsychotic drug. In this way, the researchers could generate pictures of where in the brains the drugs were working.

Working with Meltzer, who currently directs the Division of Psychopharmacology at Vanderbilt, the researchers found that while the older drugs block D2 receptors throughout the brain, the atypical class selectively binds to receptors in the cortex, the center of higher brain function, but not in areas involved in motor function.

"It's a surprise nobody expected," says Kessler, Roentgen Professor of Radiology and Radiological Sciences and director of the Center for Molecular

DEAN DIXON

"We took a class of drugs which we knew had special clinical effects, but the mechanism was unclear. We found specific patterns of receptor blockade … that we think are responsible, at least in part, for their superior therapeutic properties."

ROBERT M. KESSLER, M.D., DIRECTOR,
CENTER FOR MOLECULAR IMAGING AT VANDERBILT

ANNE RAYNER

Doug Fuchs, Ph.D., and Lynn S. Fuchs, Ph.D., are using fMRI to assess treatment of math disabilities at the Vanderbilt Kennedy Center for Research on Human Development.

Imaging. "It is the same receptor, the same protein" in both places.

The Vanderbilt researchers also have used fMRI to study the effect of drug treatment on specific cognitive functions, such as short-term working memory. By illuminating changes in blood flow and oxygenation, fMRI can create "pictures" of how the brain works under various treatment conditions.

Because individual patients respond differently to different medications, molecular imaging may one day help identify the best drug for a particular patient.

"We may find that schizophrenia is not one disease, but several different types," Kessler says. " … We may be able to determine what factors predispose one to being treated by one class of drugs as opposed to another.

"We may be able to individualize therapy, provide better dosing so that the side effects are spared and the therapeutic benefits are enhanced."

Finding the right dose of the right drug is critical, says Ronald Baldwin, Ph.D., research associate professor of Radiology at Vanderbilt.

"A lot of people are actually overdosed," says Baldwin, who develops novel radiotracers to probe the actions of drugs in the brain. "They are getting more drug than they need to get an effect. With radiotracer imaging, you can look at the receptor that's binding the drug to see how much drug is occupying it."

Mysteries of music and math

Lyric soprano Gloria Lenhoff has sung in opera houses and performance halls all over the country. Her repertoire spans more than 2,000 pieces in 30 languages, yet she can't read music.

Lenhoff has Williams syndrome, a rare neurodevelopmental condition characterized by mild-to-moderate mental retardation, blood vessel disease and – among other surprising traits – an affinity for music.

The genetics of the disorder are well known, but an aberration that slices out a specific a region of chromosome 7 cannot explain Lenhoff's unique gifts.

Brain imaging may help.

Researchers at Stanford University, for example, have used fMRI to study the brains of people with Williams syndrome while they listened to snippets of Bach, Beethoven and Mozart. In 2003, they reported finding "strikingly different" patterns in the way the brains of people with Williams syndrome processed music, compared to normal controls.

When the control group listened to music, the hearing center in the brain's temporal lobe lit up. Among people with Williams syndrome, however, brain activation was more widespread, and included the amygdala, an almond-shaped structure near the base of the brain that plays a key role in emotions.

The finding may provide clues to the comforting power of music, says Elisabeth M. Dykens, Ph.D., associate director of the Vanderbilt Kennedy Center for Research on Human Development.

Dykens, a professor of Psychology, has studied the personality characteristics of people with Williams syndrome and other developmental disorders for several years.

People with Williams syndrome are prone to heightened anxiety and fear of loud noises. At the same time, they can be exceedingly social, highly verbal and friendly.

A recent study led by Dykens found that those who were more musically involved tended to be less anxious and fearful. The goal now is to try to link this observation to specific areas of the brain. "We hope to see how this connection plays out in the imaging studies," she says.

Functional MRI also is proving to be a powerful educational tool.

Gore was among the first to apply fMRI to evaluate reading disabilities while at Yale University, where he directed the Nuclear Magnetic Resonance Research Center.

Children with dyslexia have difficulty recognizing and comprehending written words. Rhyming games and other drills that break down and rearrange sounds to produce different words can help them improve their reading skills.

The brain at rest

The human brain is an energy glutton. Comprising only about 2 percent of body weight, it consumes nearly 20 percent of the body's oxygen intake. Why does the brain need so much energy, even when it is at rest?

Marcus E. Raichle, M.D., a member of the Washington University team that developed PET in the 1970s, believes he may have an answer.

During experimental tasks, some areas of the brain become more active while other areas become less active as measured by changes in metabolism and blood flow. When subjects are studied at rest with their eyes closed, however, activity in these "task-negative" areas goes up, Raichle and his colleagues reported in 2001.

These areas, he says, are nodes of a "default" brain network that functions intrinsically and in a correlated manner during the resting or baseline state. Its activity decreases when an attention-demanding task raises the oxygen requirement in other areas.

Raichle sees purpose and evolutionary significance in this phenomenon. The default system serves as a "sentinel," constantly monitoring the horizons of the external environment (as well as the world within). It also is "forecasting" and preparing for future events based on prior experience.

"The brain is basically in the prediction business," he explains. "We use both our genetically endowed experience plus what we learn in our own experience, and use that to predict what's going to happen next. And we spend most of our brain's (energy) budget on doing that."

Is this activity some aspect of the thing we call "consciousness?"

Perhaps, says Raichle.

If so, could fMRI determine whether a person in a persistent vegetative state really has some flicker of consciousness left?

Time, and further research, may tell.

"Whatever (the default system) is doing is a reflection of what the brain is mainly doing," Raichle says. "We have to back away from the notion that the brain is mainly reacting to the world in which we live, and take the perspective that it is … creating, in the context of ourselves, a model of the world in which we live and expect to live."

– MELISSA MARINO

With fMRI, "you can actually see that in the brain, in the circuits involved in reading," Gore says. Sequential brain scans indicate that brain activity in these children actually "normalizes," or begins to resemble activity seen in children without dyslexia.

Gore, who came to Vanderbilt in 2002, is now working with investigators at the Kennedy Center to extend that work to children with math learning disabilities.

Researchers believe math disability – difficulty in solving math problems – may be a different syndrome or have a different cause than reading disabilities like dyslexia, says Lynn S. Fuchs, Ph.D., who, with her husband, Doug Fuchs, Ph.D., has helped pioneer the diagnosis and treatment of learning disorders.

The Fuchses, who share the Nicholas Hobbs Chair in Special Education at Vanderbilt, and Donald L. Compton, Ph.D., assistant professor of Special Education, are using fMRI to assess how brain function changes in response to an intervention aimed at improving math skills.

"The reason to do the scanning is … to understand how the brain is changing as math improves," Gore explains. "Is it changing in a way that makes it look more like a normal brain, or is it changing in a way that compensates for some kind of structural problem that really can't be changed with intervention?

"If you can actually train parts of the brain to do the job the most efficient way," he adds, "then that is the best thing to do. Imaging can identify optimal strategies."

Predicting relapse and recovery

Addiction is another challenging medical problem that is slowly revealing its secrets to brain imaging.

Through the use of PET and, more recently, fMRI, scientists are beginning to define – anatomically and biochemically – what drugs of abuse do to the brain.

"We've known for probably 30 years or more that using drugs like alcohol for long periods of time causes injury to the brain," says Peter Martin, M.D., professor of Psychiatry and Pharmacology and director of the Division of Addiction Medicine in the Department of Psychiatry at Vanderbilt.

In computed tomography (CT) scans, for example, "the brains of alcoholics seem to have shrunk compared to normal," he says. Yet images of the brain's structure don't correlate well with impairments detected in neuropsychological tests. So, in the early 1990s, "we started looking at what was happening chemically in those regions of the brain that shrink."

Martin and his colleagues used a magnetic resonance technique called MR spectroscopy to measure several brain chemicals including N-acetylaspartate (NAA), which is found primarily inside neurons (nerve cells). The researchers detected lower levels of NAA in the cerebellum in alcoholics, compared to normal controls, suggesting the presence of damaged or dying neurons.

The cerebellum, located at the base of the skull, controls muscle tone, balance and fine movement coordination. It also transmits signals to the prefrontal cortex behind the eyes, which plays key roles in working memory and judgment, as well as emotion, arousal and attention.

Levels of NAA were lowest – indicating the greatest amount of neuron damage – in the cerebella of patients who had started drinking heavily at an earlier age and who had a family history of alcoholism. These patients also relapsed earlier after a period of abstinence than did alcoholics with higher NAA levels, the researchers reported in 2002.

"It's almost as if their brains were more sensitive to developing brain injury," concludes Martin. "In the future, we may be able to do spectroscopy and predict which patients will do well and which ones will not."

Imaging technologies such as fMRI not only can record damage done by drugs; they are shedding light on the processes in the brain – including reward, motivation, memory and craving – that can lead to and maintain addictive behavior.

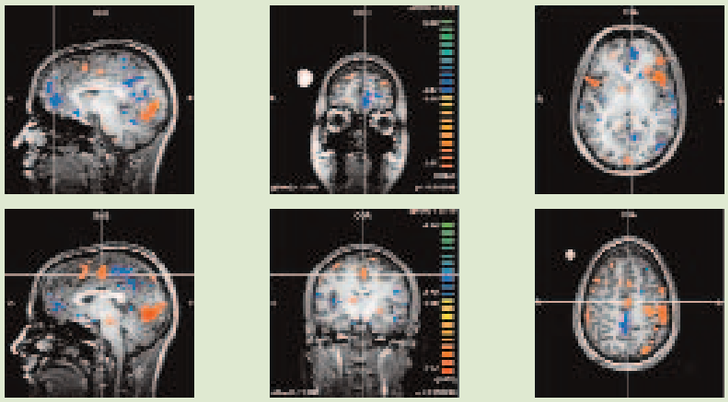
Functional MRI measures the magnetic properties of hemoglobin, the iron-bearing oxygen transporter in red blood cells. Activation of part of the brain increases the demand for oxygen, and thus the intensity of the magnetic signal.

Ronald L. Cowan, M.D., Ph.D., an assistant professor of Psychiatry, and of Radiology and Radiological Sciences at Vanderbilt, is using fMRI to study how brain activation changes over time in response to amphetamine, a powerful stimulant.

By taking a series of images, he hopes to "picture" different stages of drug-taking behavior – from the anticipation of getting the drug, through the experience of euphoria, or the "high," and as the effect of the drug wears off.

"We have little idea what … causes a person to decide to engage in a rewarding activity, be it eating, sex, gambling or drugs," Cowan explains. "We don't know what parts of the brain are involved in the initiation of that activity.

"We're hoping to find a part of the brain early on that gets activated before the experience of euphoria, that actually predicts that it is going to happen," he says. "That might give us a target for therapy, or at least for further study." **LENS**



Picturing the brain in a good mood

Functional magnetic resonance images show the brain of a study participant responding to an oral dose of amphetamine. The drug can produce the experience of a "high" or euphoria when taken in large doses. The images, of three different "sections" or views of the brain, detected changes in two different areas (top and bottom rows) over the course of about 90 minutes when the participant reported "positive" emotions on a mood scale. The blue color indicates deactivation of one brain region, while the orange spot indicates activation of another. While more detailed analysis is required to determine which specific regions respond to amphetamine, imaging studies like this are providing clues to the roles that different areas of the brain play in drug-taking behavior.

Courtesy of Ronald L. Cowan, M.D., Ph.D., and his colleagues at Vanderbilt and the McLean Hospital Brain Imaging Center in Belmont, Mass.

Two paths to the future

Nora Volkow's revolutionary approach to addiction

By Bill Snyder

RHODA BAER

Cocaine was considered to be a "safe" party drug in the high-flying '80s when a young psychiatrist decided to see what it did to the brain.

Using an imaging technique called PET, Nora Volkow, M.D., and her colleagues at the University of Texas in Houston documented areas of "deranged" cerebral blood flow resembling tiny strokes in people who took copious amounts of the drug.

Cocaine was known to constrict blood vessels. Heavy use of the drug had been linked to fatal heart attacks and strokes. Yet their findings at first were greeted with skepticism.

Then in 1986, University of Maryland basketball star Len Bias collapsed and died of a cocaine overdose, and the tide began to turn. "When you go against the current, it takes time to change its course," says Volkow, now director of the National Institute on Drug Abuse (NIDA).

Just as she once helped dislodge widely held notions about cocaine, today this strong-minded scientist is determined to transform the way addicts are treated – or, more often, not treated – by the medical profession and the criminal justice system.

While she sees this a part of her duty as a physician, to serve the most vulnerable members of society, Volkow also acknowledges the world-changing legacy of her great-grandfather, exiled Russian revolutionary Leon Trotsky.

"It's not religious; it's just a sense of humanity, a sense of being part of humankind," she says, her words wrapped in the melodic tones of her native Mexico. "You are alive, you have a certain talent, and you have a responsibility to use it to help others."

"This is the smartest person I know," says Joanna Fowler, Ph.D., senior chemist at Brookhaven National Laboratory in Upton, N.Y., who has worked with Volkow since the mid-1980s. "People just glom onto her. She's like pouring out ideas all day … She can take a problem and very easily see through it; see relationships, simplify things."

At the same time, says Fowler, "she's a very compassionate person ... very much involved in the social impacts of drug abuse.

"Drug addiction impacts enormously even on things that you wouldn't normally think of, like cancer in cigarette smoking, like heart disease, like violence with alcoholics, and accidents and AIDS," she says. "I think we're very fortunate to have a person like Nora."

"I see her as a warrior fighting against a universal enemy," adds her younger sister, Natalia Volkow, Ph.D. "It's such a horrible enemy and so difficult to beat. And that's why I think she chose this subject of study. Nora has never taken the easy way in life, never."

Volkow, 49, displays an intriguing amalgam of traits: athletic drive and stamina (the former competitive swimmer runs six miles every morning before work); an exuberant sense of wonder about the world; and a knack for looking at science through the eyes of an artist (she paints, and her older sister is Mexican poet Verónica Volkow).

Most importantly, she says, "I'm a scientist. I've always loved science. That's how I see myself."

So far in her career, Volkow has authored or contributed to more than 300 scientific articles. Through groundbreaking imaging studies of the brain's frontal cortex and its dopamine-driven circuitry, she has helped reveal the neurobiological underpinnings of addiction, and how drug-induced changes in brain chemistry contribute to its hallmark craving, compulsion and loss of control.

Two paths

Addiction is not the only area that has come under Volkow's sharp-eyed scrutiny.

She and her Brookhaven colleagues also have linked long-term use of anti-psychotic drugs to the withdrawal and blunting of emotions seen in individuals with schizophrenia; showed how therapeutic doses of Ritalin and other stimulants can improve attention; and found evidence that dopamine, a chemical messenger important in drug addiction, may also play a role in overeating and obesity.

"She really is one of those people who think very much into the future," says former NIDA director Alan I. Leshner, Ph.D., CEO of the American Association for the Advancement of Science. "She's always, in her own research, been leading the cutting edge … She has brought that to NIDA as well. That's exactly where it ought to be."

While she firmly believes in the disease model, Volkow is quick to point out that addiction is not simply the result of particular genes and brain chemicals. "Predisposition is not predetermination," she told a congressional subcommittee last April. "Environment and other biological factors, including family, culture and community, are of

Pictured here, from top: Nora as a teenager; with her mother in 1980; standing (right) with her sister Natalia on the peak of Popocatépetl (the highest active volcano in the Northern Hemisphere), southeast of Mexico City; and with her father. Esteban Volkov says he and his wife, who died in 1997, tried to nurture in their daughters an appreciation of the artistic and intellectual life - and freedom of expression. "I established an absolute true democracy in the family," he says. "There was no imposition. Each one could choose the career that she would like." Natalia earned a doctorate in information systems from the London School of Economics, and is a deputy director general of Mexico's National Institute of Statistics, Geography and Informatics. Her identical twin, Patricia, is a doctor and expert on AIDS.

Photos courtesy of Nora and Natalia Volkow

great importance to the development of addiction and are essential to its prevention."

Drugs aren't the only way to treat addiction. Imaging studies can validate the effectiveness of cognitive and behavioral therapies, as well. Potentially they may help identify social interventions that protect young people from abusing drugs in the first place.

"I always take two paths," Volkow explains, "one path that is going to lead us to the science and the knowledge that will really revolutionize the way that we treat drug addiction, so that five or 10 years from now we will be treating drug addiction completely differently.

"But at the same time, developing strategies that can benefit those that are afflicted by addiction, or interventions that can prevent the use of drugs. We need to get science that looks into the future … but we also need to address the needs right now."

Volkow's path started in the house where her great-grandfather was murdered.

Born Lev Davidovich Bronstein, Trotsky was a brilliant political theorist and proponent of permanent worldwide revolution by the working class. Founder of the Red Army, he was second only to Vladimir Lenin during the early years of Bolshevik rule. After Lenin's death in 1924, Trotsky was expelled from the Soviet Union when Joseph Stalin took control of the government and launched a purge of his rivals.

Trotsky lived for a few years in Turkey, then in France and Norway before eventually finding refuge in Mexico City, where he continued to write books critical of the Stalin regime. Family members who remained in the Soviet Union were imprisoned or shot. In 1940 – shortly after his grandson Esteban moved from Turkey to join him – Trotsky was assassinated by one of Stalin's agents.

Esteban Volkov Bronstein became a chemist and married Palmira Fernández, a fashion designer from Madrid who had fled the Spanish Civil War. They had four daughters, and continued to live in Trotsky's carefully preserved house (it is now a museum).

Volkov's daughters, who end their last name with a "W" instead of the Russian "V," gradually heard details of their great-grandfather's story – but not from their father. "It was a very, very painful period, so he couldn't really speak about it," Nora explains.

They learned from the constant stream of visitors who knocked on Trotsky's door.

"As little girls, whenever somebody rang the bell and asked us to guide them through the house, we did so, and that was a privilege," recalls Natalia. "We usually took a long time talking to them – listening to them."

On one occasion, a group of visitors from South America took the tour, and afterwards Nora got into a lengthy conversation about *One Hundred Years of Solitude*, which she was reading. Later she learned that one of the men with whom she had been talking all afternoon was the book's author, Gabriel García Márquez.

The living brain

"She was a great reader of all kinds of books," recalls her father, now 79. At the same time, "Nora always was a very warm and sweet person. She always showed a great love and passion for animals."

Volkov is not in the least surprised at the meteoric rise of his middle daughter's career. "Nora has a very basic principle," he says. "She always had a very, very great respect for the truth."

Fluent in four languages (including French and German), Volkow received her undergraduate and medical school training at the National Autonomous University of Mexico in Mexico City, where she was recognized as the best student of her undergraduate and medical school classes.

In 1981, after receiving her medical degree, she read an article in *Scientific American* about a new imaging technology called positron emission tomography (PET). She was mesmerized by the splotchy, brilliantly colored images of the living brain. In an instant, the direction of her life changed.

Instead of applying for postgraduate study at the Massachusetts Institute of Technology, Volkow opted for residency training in psychiatry at New York University and the chance to work – in a collaborative research program –with the PET pioneers at Brookhaven, a Department of Energy-operated laboratory near the far end of Long Island.

Five years earlier, a Brookhaven team led by Fowler and Alfred P. Wolf, Ph.D., had produced a radiotracer for glucose, the brain's primary fuel. Colleagues at the University of Pennsylvania used it to create the first images of the living human brain. The intensity of the color on the PET scan reflected the concentration of 18F-fluorodeoxyglucose (FDG), and thus where the brain was active.

By the early 1980s, the Brookhaven team – bolstered by an energetic psychiatry resident – was using PET to study the brains of people with schizophrenia. Their images revealed decreased brain activity in the frontal cortex of patients who had been taking anti-psychotic drugs like Thorazine for long periods of time. "The greater the decrease in brain activity, the greater the 'poverty of thinking,'" Volkow says.

Pictured here: Volkow administers a contrast agent prior to a PET study in 1990. In the background are research nurse Noel Netusil, R.N., (left) and technician Renee Modell, now a nuclear medicine physician.

Courtesy of Brookhaven National Laboratory

"We're doing a disfavor to the well-being ... of patients ... by not addressing the problem of addiction."

Anti-psychotic drugs like Thorazine were known to block the receptors for dopamine, a neurotransmitter that conveys signals between the frontal cortex and other parts of the brain. The frontal cortex, in turn, is involved in a host of cognitive and "executive" functions, from language and memory to impulse control and the ability to solve problems.

While the researchers were unable to determine how much of the withdrawal and blunting of emotions observed in the patients was due to the drugs, and how much to their disease, the study was one of the first to open up the frontal cortex through the window of PET.

After completing her residency in 1984, Volkow moved to the University of Texas in Houston to continue her research at a PET research center founded by cardiologist K. Lance Gould, M.D. Here her path turned again.

The university hospital had no patients with schizophrenia to study, Volkow recalls, but there were plenty of cocaine addicts. So, with the help of addiction specialist Kenneth Krajewski, M.D., and physicists Nizar Mullani, Ph.D., and Stephen Adler, Ph.D., Volkow conducted the first PET studies of the brains of cocaine addicts.

At first, she says, no one believed the images of deranged blood flow, suggestive of stroke. It would be 1988 – three years later – before their findings were published by the *British Journal of Psychiatry*.

By then, Volkow and Adler had married and had accepted positions at Brookhaven.

Volkow, who also joined the faculty in psychiatry at the State University of New York at Stony Brook, says she was tempted back by Wolf's offer to label cocaine for her. "That was extraordinary," she says, "because it actually opened up the first study to be able to look at the dynamics of these drugs in the brain."

Making connections

Soon Volkow and her colleagues, who frequently included Wolf and Fowler, were labeling all sorts of things: D2, a receptor through which dopamine sends its signals; monoamine oxidase, an enzyme that breaks down dopamine; and methylphenidate (Ritalin), which was labeled by Yu-Shin Ding, Ph.D.

In 1993, the Brookhaven group reported that cocaine abusers had lower levels of the dopamine D2 receptor compared to normal controls. Reductions in receptor levels were associated with decreased metabolism, as measured by glucose consumption, particularly in the orbitofrontal cortex and cingulate gyrus.

The cingulate gyrus, a ridge of tissue deep in the brain, is part of the limbic system, associated with mood and emotions. What was surprising was the connection to the orbitofrontal cortex, located just above the eyes, the same area that functions abnormally in patients with obsessive-compulsive disorder and that is believed to underlie their compulsive behaviors.

"We always thought of drug addiction as a disease of the primitive parts of our brain, the limbic parts … the pleasure centers," Volkow said in a 2002 lecture. "And here, the frontal cortex, which epitomizes the higher levels of our 'reasoning' human brain, appears to be involved."

During the next few years, the Brookhaven scientists documented reduced levels of dopamine D2 receptors in the brains of alcoholics, heroin abusers and methamphetamine addicts. In addition, they found methamphetamine caused inflammatory changes in the brain that were associated with loss of memory, attention and motor skills.

In 2001, the Brookhaven group, led by Gene-Jack Wang, M.D., reported that obese people – like those addicted to alcohol, cocaine or methamphetamine – have lower-than-normal levels of dopamine D2 receptors.

"Individuals with low numbers of D2 receptors may be more vulnerable to addictive behaviors including compulsive food intake," the researchers concluded. "We speculate that … decrements in D2 receptors perpetuate pathological eating as a means to compensate for the decreased activation of reward circuits, which are modulated by dopamine."

Just because a person is vulnerable, however, doesn't necessarily mean he or she will become addicted. Exercise, for example, has been shown to increase the level of D2 receptors and dopamine release in rats.

Volkow believes it may be possible to identify protective factors in humans, particularly in the age group most vulnerable to drug addiction – the adolescent.

"Very much the initiation of experimenting with drugs occurs in social settings, in group settings, in adolescents that want to actually be part of groups," she says. "It's a very important area of research to develop, so we can better understand the needs of kids and come up with strategies to overcome situations where this response is going to be elicited."

Volkow became a U.S. citizen in 1993. While her group published their findings prolifically, she rose through Brookhaven's administrative ranks: director of the Nuclear Medicine Program (1994); chair of the Medical Department (1996); first director of the NIDA Regional Neuroimaging Center (1997); and the first woman to serve as Associate Laboratory Director for Life Sciences (1999).

"Nora is a dynamic, creative person with a broad vision of her field and a passion for science," says former Brookhaven director John H. Marburger III, Ph.D., Science Advisor to the President and director of the Office of Science & Technology Policy.

"I asked her to be the Associate Director for Life Sciences at Brookhaven because I thought her dynamic style and clear vision for the work that could be done there would bring focus and energy to the division," he says. "I think it prepared her well for her current position."

Wolf died in 1998, but Volkow's extraordinary partnership with Fowler and the other Brookhaven scientists continued to churn new scientific ground.

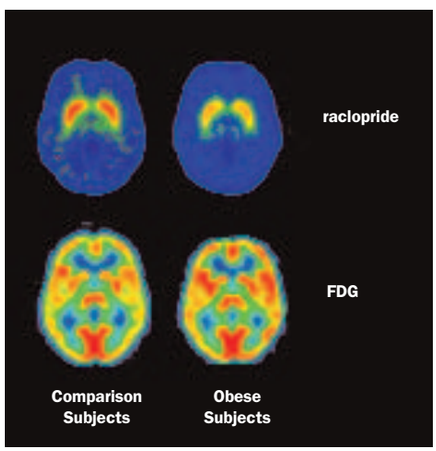
"It's a very unusual relationship," Fowler says. "We're personally very close and also scientifically close, and we talk all the time … Every time I read something interesting, and the same with her, we call and we talk about it."

As Volkow's perspective on addiction was broadening, so too were her opportunities. In the fall of 2002, Elias A. Zerhouni, M.D., director of the National Institutes of Health, asked her to lead NIDA, which funds the bulk of research conducted nationally (and internationally) on the health aspects of drug abuse and addiction.

Volkow, the scientist, wanted to continue her research. Volkow, the visionary, saw an opportunity to apply that research to improve the lives of people. Armed with Zerhouni's promise that she could continue her research (she spends a long weekend every month at Brookhaven), in April 2003 she became the fifth person and first woman to direct the 30-year-old institute, which now has an annual budget of more than $1 billion.

Championing science

As a leader, Volkow is more revolutionary than bureaucrat. Her agenda is diverse and far-ranging, but its central theme is making connections – uniting physicians and pharmaceutical companies, drug



Obesity and the brain

PET scans taken at Brookhaven National Laboratory suggest that the brains of obese individuals have fewer dopamine D2 receptors than do brains of normal controls. A radiolabeled compound (raclopride) that binds to the receptors was used to determine receptor concentration. Red spots in an averaged image of the control brains (top left) indicate a greater concentration of raclopride, and thus more D2 receptors, than are present in an averaged image of the obese group (top right). Overall brain metabolism, measured by the concentration of FDG, a radiotracer for glucose, did not differ significantly between the two groups (bottom panel). Since dopamine modulates motivation and reward circuits, the researchers concluded that dopamine deficiency in obese individuals may perpetuate pathological eating as a means to compensate for decreased activation of these circuits.


RHODA BAER

courts and community groups to improve the treatment and prevention of addiction.

Among her top priorities: understanding the interactions between drug abuse, mental illness and AIDS.

Experimenting with drugs often begins during the novelty-seeking, peer pressured years of adolescence, and is a major contributor to the continued rise in HIV/AIDS in the United States. Drug use can lower resistance to risky behaviors like unprotected sex or sharing needles.

Drug abuse complicates treatment of other diseases, such as diabetes and cancer, yet addiction among patients with "co-morbid" conditions often is ignored, Volkow asserts. Limited access to drug treatment also contributes to the disproportional impact of AIDS and incarceration on minority groups.

African-Americans make up only 13 percent of the U.S. population, yet account for half of all HIV infections and more than 40 percent of jail and prison inmates. "These numbers," she says, "are unacceptably high, embarrassingly high."

Volkow's blunt approach has been a "breath of fresh air" to retired Judge Karen Freeman-Wilson, CEO of the National Association of Drug Court Professionals. According to association statistics, treatment of drug-addicted criminal offenders can reduce by at least half the rate of recidivism – the relapse to criminal behavior.

"She has been a tremendous help to us to really educate people on the science of addiction … but more importantly, the science of treatment," explains Freeman-Wilson, a former Indiana attorney general who established that state's first drug court in 1996.

During Volkow's presentations, "I can see the lightbulbs going on in the heads of judges who obviously operate on evidence," she continues. "With that information, people have been able to say, 'Well, maybe this isn't just a bad person. Maybe this is a bad disease. And maybe we have to look at it and address it in a totally different way.'"

While many find Volkow's frank speech refreshing, former Robert Wood Johnson Foundation President and CEO Steven A. Schroeder, M.D., wishes she and others in government would advocate more forcefully for treatment programs and policies – such as methadone and smoking cessation programs – that are already known to work.

"Unless I'm missing it … there is no federal champion for that. And that's a shame. It's a missed opportunity to make our country healthier," says Schroeder, who directs the Smoking Cessation Leadership Center at the University of California at San Francisco.

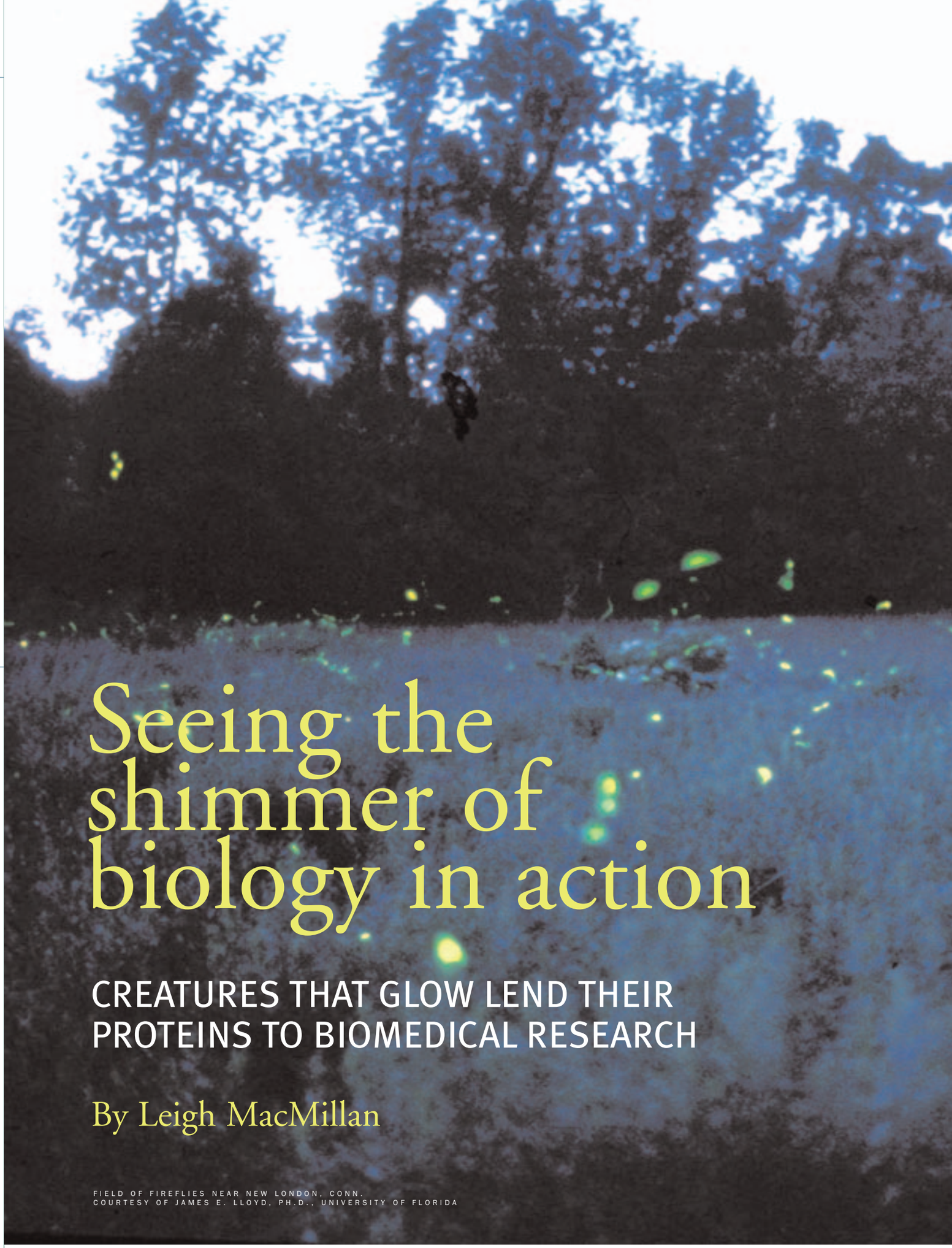
Schroeder's point is well taken, Leshner responds, but Volkow's responsibility is much broader than advocacy. "She actually does advocate," he says, "but ... her job is to make sure that the science is as good as it can be and then to bring the science to the attention of policy makers."

Volkow believes that continued research is the way to overcome many of the challenges to improving treatment of addiction. PET studies, for example, are aiding the development of anti-obesity drugs that may interrupt the conditioned responses reinforcing compulsive drug-taking behavior as well as compulsive eating.

Brain imaging also could be used to measure the effectiveness of non-drug treatments. Since chronic drug abuse weakens the reward and motivation circuitry of the brain so that it only responds to more drug, it may be possible to "exercise" the brain in a way that increases the response to normal reinforcing stimuli and reduces the likelihood of relapse.

Yet Volkow agrees with Schroeder: "We have information we're not using."

"Drugs permeate the medical system," she says. "We're doing a disfavor to the well-being of a wide variety of patients – whether they have lung disease, whether they have cancer, whether they have mental illness, whether they have an infectious disease – by not addressing the problem of addiction." **LENS**

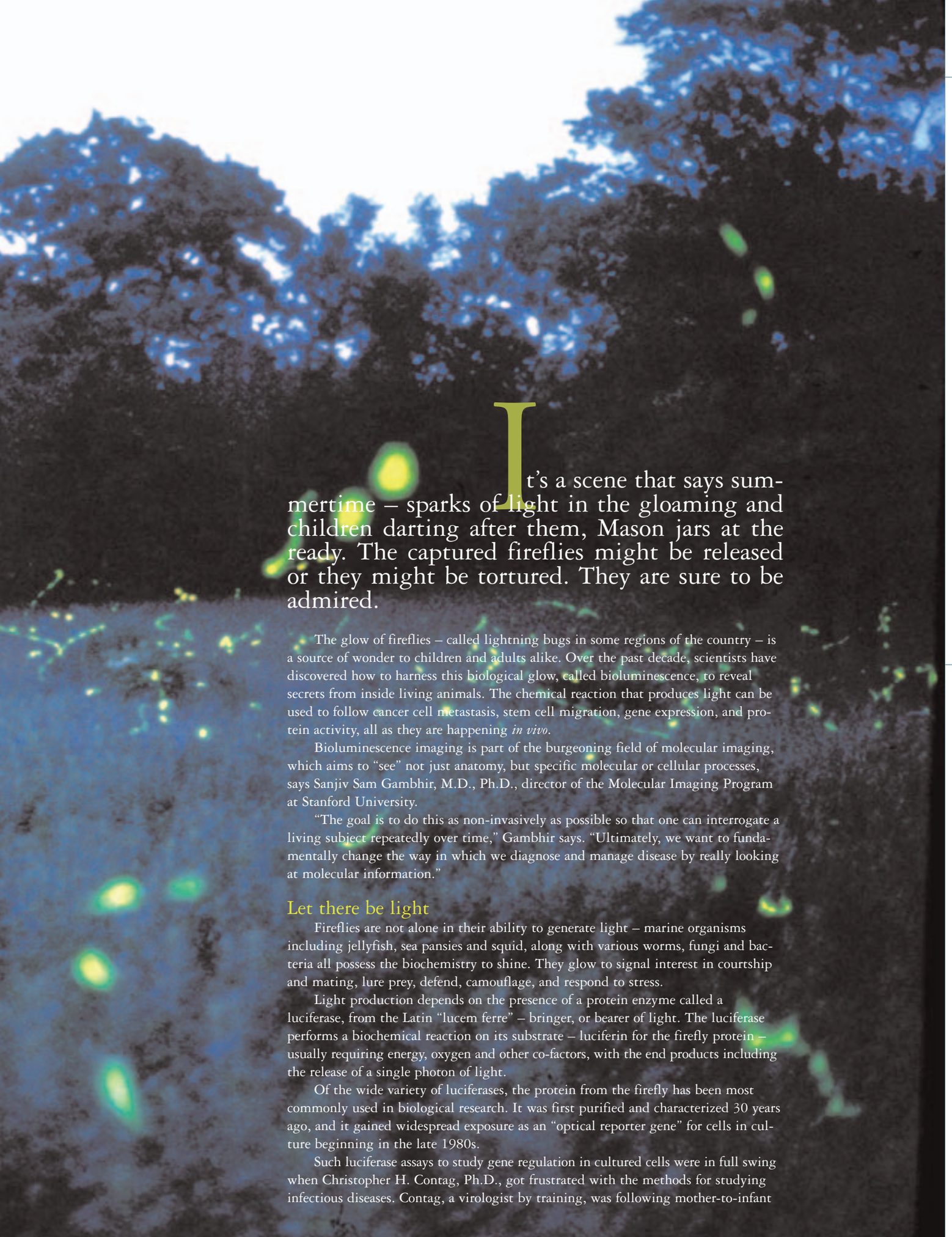


Seeing the shimmer of biology in action

CREATURES THAT GLOW LEND THEIR PROTEINS TO BIOMEDICAL RESEARCH

By Leigh MacMillan

FIELD OF FIREFLIES NEAR NEW LONDON, CONN.
COURTESY OF JAMES E. LLOYD, PH.D., UNIVERSITY OF FLORIDA



It's a scene that says summertime – sparks of light in the gloaming and children darting after them, Mason jars at the ready. The captured fireflies might be released or they might be tortured. They are sure to be admired.

The glow of fireflies – called lightning bugs in some regions of the country – is a source of wonder to children and adults alike. Over the past decade, scientists have discovered how to harness this biological glow, called bioluminescence, to reveal secrets from inside living animals. The chemical reaction that produces light can be used to follow cancer cell metastasis, stem cell migration, gene expression, and protein activity, all as they are happening *in vivo*.

Bioluminescence imaging is part of the burgeoning field of molecular imaging, which aims to "see" not just anatomy, but specific molecular or cellular processes, says Sanjiv Sam Gambhir, M.D., Ph.D., director of the Molecular Imaging Program at Stanford University.

"The goal is to do this as non-invasively as possible so that one can interrogate a living subject repeatedly over time," Gambhir says. "Ultimately, we want to fundamentally change the way in which we diagnose and manage disease by really looking at molecular information."

Let there be light

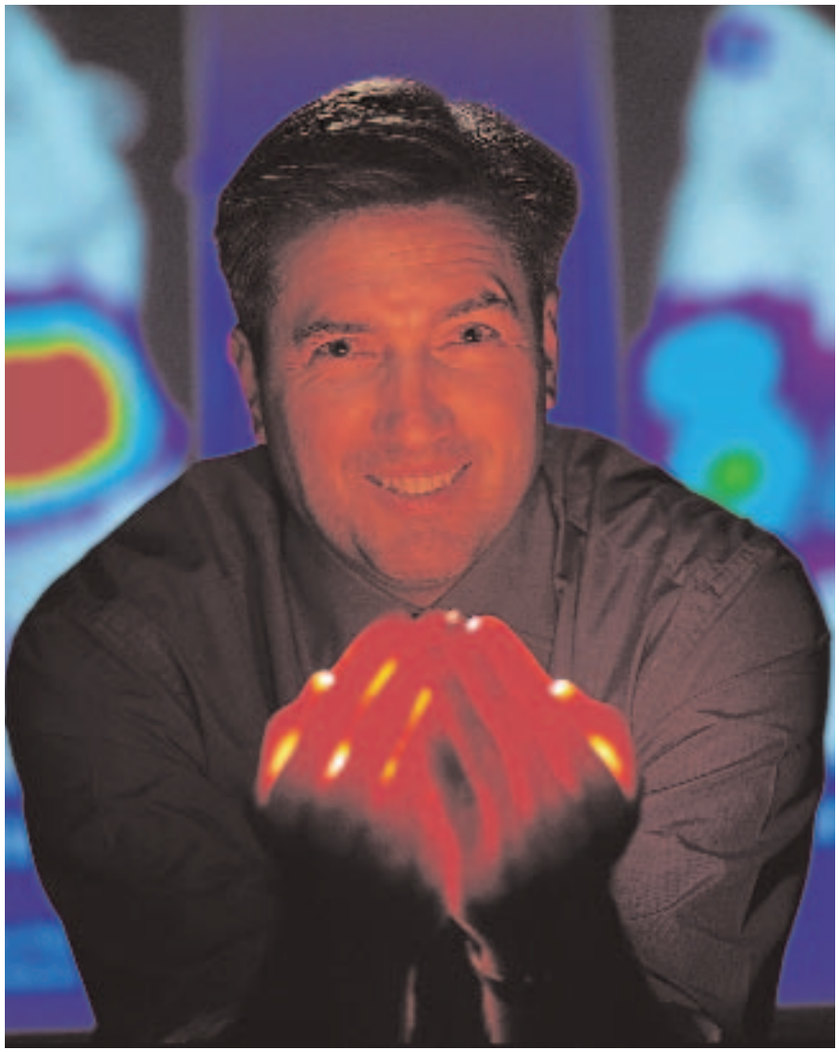
Fireflies are not alone in their ability to generate light – marine organisms including jellyfish, sea pansies and squid, along with various worms, fungi and bacteria all possess the biochemistry to shine. They glow to signal interest in courtship and mating, lure prey, defend, camouflage, and respond to stress.

Light production depends on the presence of a protein enzyme called a luciferase, from the Latin "lucem ferre" – bringer, or bearer of light. The luciferase performs a biochemical reaction on its substrate – luciferin for the firefly protein – usually requiring energy, oxygen and other co-factors, with the end products including the release of a single photon of light.

Of the wide variety of luciferases, the protein from the firefly has been most commonly used in biological research. It was first purified and characterized 30 years ago, and it gained widespread exposure as an "optical reporter gene" for cells in culture beginning in the late 1980s.

Such luciferase assays to study gene regulation in cultured cells were in full swing when Christopher H. Contag, Ph.D., got frustrated with the methods for studying infectious diseases. Contag, a virologist by training, was following mother-to-infant

STEVE FISCH PHOTOGRAPHY



"The greatest contribution to human medicine that probably all molecular imaging approaches, including bioluminescence, will have is in refining and accelerating our animal models of disease, such that we can test and develop drugs more efficiently."

Christopher H. Contag, Ph.D.,
co-director of the Molecular Imaging Program
at Stanford University

transmission of HIV by comparing viral genetic sequences.

"It turned out to be a very complicated analysis, and I said at the time, 'wouldn't this be so much easier if we could just watch the whole process?'" recalls Contag, now co-director of the Molecular Imaging Program at Stanford. "It occurred to us that we should be developing tools for watching complex biological processes in the context of living animals, and at some point in time, living humans."

A search of the available imaging modalities and a fortuitously timed lecture by an environmental microbiologist about luminescent bacterial enzymes pointed Contag and his wife Pamela R. Contag, Ph.D., in the direction of bioluminescence.

"We figured that since animals and people don't glow in the dark, if you put something in the body that does glow in the dark, you should get great signal-to-noise ratios since there should be relatively no background noise," Contag says. We now know there are background signals – from biological processes in animals that produce small amounts of light – but for bioluminescence imaging, he notes, "the signal-to-noise ratio is really extraordinary."

For their first studies, the Contags and their colleagues followed bioluminescent bacteria in a mouse.

"When we saw the first images of glowing bacteria in the intestines of a mouse, I said, 'Every biology lab in the world will want to use this; this will be fantastic,'" Contag recalls. The team published its findings in 1995, and Contag anticipated that use of the technology "would explode." It took a little longer than he expected, with adoption by many laboratories occurring only in the last five years.

To propel what they saw as a powerful technology, the Contags and David A. Baneron, M.D., founded a company called Xenogen to market the technology, along with unique instrumentation and biological reagents that utilize luminescent signals for studying biology in animals. Pamela Contag has served as president of Xenogen since the company's inception in 1995.

Christopher Contag remained at Stanford. "I decided to take a very broad approach and demonstrate the breadth of this technology," he says. "So we've been tracking viruses and tumor cells and stem cells, and in the early days attempted to show how versatile this technology is. Now we're focusing on stem cells and cancer biology."

Lightbulbs inside cells

Investigators at Vanderbilt embraced bioluminescence imaging early on to follow cells and gene expression in living animals. Watching cells as they migrate through a living animal, take up residence, multiply, and in the case of tumor cells, metastasize to new sites, has been the most popular application of bioluminescence to date.

"What bioluminescence gives you is a level of sensitivity of detection that is not attainable by any other current method," says E. Duco Jansen, Ph.D., associate professor of Biomedical Engineering at Vanderbilt.

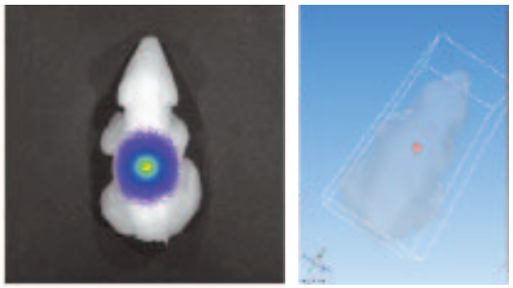
The way it works is conceptually quite simple, Jansen explains. Cells of any sort can be infected with viruses or engineered to incorporate a luciferase gene. After being injected into small animals, usually mice, the cells begin to produce the luciferase protein. Investigators then inject the substrate molecule – such as luciferin – into the animals, and the luciferase acts on it, releasing photons of light.

"So we have effectively a lightbulb inside the cell," Jansen says.

That lightbulb is really quite weak – the mice do not actually glow like fireflies. But some of the photons of light do make their way out of the animal, and sophisticated charge couple device (CCD) cameras, cooled with liquid nitrogen to minimize noise, can capture them. Imaging systems such as those produced by Xenogen, which are available to Vanderbilt scientists via the new Institute of Imaging Science, make the process relatively straightforward. The

New software is improving the spatial resolution of bioluminescence imaging. In these test images, a luminescent bead has been implanted inside a silicon mouse model. On the left, a "standard" planar bioluminescence image shows light scattered as it moves through the model mouse. The image on the right shows the result of bioluminescence tomography using Xenogen's Living Image Software 3D Analysis Package. The red pixel indicates the reconstructed light source location.

Courtesy of Jack Virostko, graduate student in the lab of E. Duco Jansen, Ph.D.



systems manage everything from administration of the inhaled anesthetic to quantitation of the detected light.

Tumor cells were the early front-runners in the "cells-to-watch" category.

"It's a pretty well-established paradigm now to incorporate a luciferase into a tumor cell line, implant those modified cells into animals, and then monitor the luciferase activity to find where the tumor cells become established and to follow the growth of the tumor," says J. Oliver McIntyre, Ph.D., research professor of Cancer Biology at Vanderbilt.

And because of the high sensitivity of bioluminescence, the very early stages of tumorigenesis and of metastasis are open for study.

"Bioluminescence imaging lets us detect very small numbers of cells – in the hundreds – from the internal organs of a small animal," says P. Charles Lin, Ph.D., associate professor of Radiation Oncology at Vanderbilt. "There is no other way right now to detect those cells."

Watching tumor growth and metastasis in real time gives investigators a window to a tumor's molecular environment and to its susceptibility to therapeutic interventions. McIntyre, who works with Lynn M. Matrisian, Ph.D., professor and chair of Cancer Biology at Vanderbilt, describes how the group has used bioluminescence to study the effect of an enzyme called MMP-9, which "chews up" the matrix material between cells, on tumor growth.

Heath Acuff, Ph.D., at the time a graduate student in the group, compared the establishment of lung tumors in mice with and without MMP-9. Luciferase-expressing tumor cells were injected into the tail vein of mice; they then homed to the lung and grew, and the investigators followed their growth by looking at the light being produced.

Mice lacking MMP-9 had fewer tumors at the end of the study, and by following the mice over time, the investigators knew this difference occurred very early, within the first 24 hours.

"The imaging really provides this temporal information from individual animals or groups of animals that is not so easy to obtain by other methods," McIntyre says.

Shining light on diabetes

The high sensitivity of bioluminescence imaging was just what Alvin C. Powers, M.D., professor of Medicine at Vanderbilt, needed to track his favorite cells – those that populate pancreatic islets. Islets – so-named because they appear to be small cellular "islands" within the pancreas – are home to the insulin-producing beta cells and several other hormone-releasing cell types.

Islet transplantation is an emerging experimental therapy for type 1 diabetes and has shown promise in multiple small clinical trials. One difficulty in moving the field forward, Powers explains, is that investigators have no way to follow the islets after transplantation.

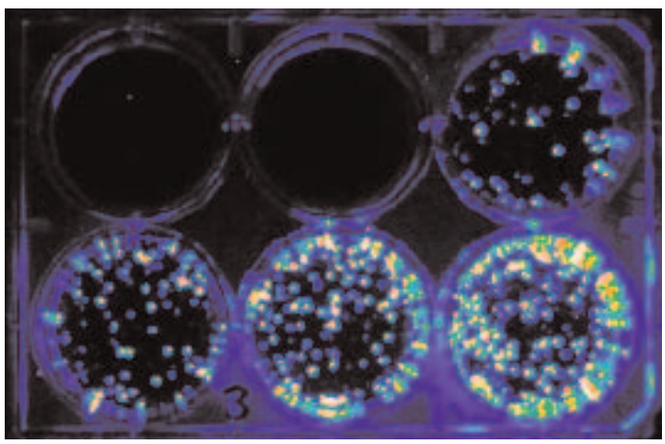
"We really need a way to assess where the islets go after transplantation, how many survive, (and) what kinds of therapies promote islet survival," Powers says.

In collaboration with Jansen, Powers and colleagues have "tagged" islets with luciferase. They have used primarily a strategy of infection: first the investigators harvest islets, both from mice and humans, then they infect the islets with a virus carrying the luciferase gene. A certain percentage of the islet cells incorporate the luciferase, and after transplantation into a mouse the surviving cells can be followed with bioluminescence imaging.

The team is also beginning to use islets from genetically modified mice that have luciferase in all of the beta cells of the islet. These light-emitting islets offer the advantage that all cells permanently express the luciferase.

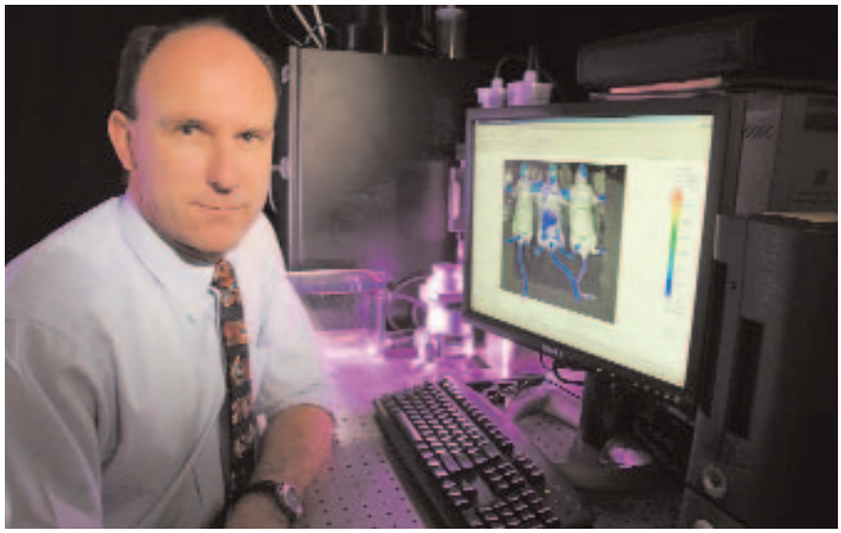
In both models, the investigators are attempting to optimize transplantation parameters, Powers says. What is the best site for survival? Which growth factors best promote survival? Is it best to treat the islets with growth factors before transplantation, to treat the animals after transplantation, or both?

"Bioluminescence is really the only way to non-invasively assess these islets over time," Jansen says. He notes that it would be possible to sacrifice animals to get single time-point snapshots, but that methodology would require a very large number of animals and retain the problem of biological variation between individuals. Non-invasive imaging of any sort, in the



Human pancreatic islets glow after infection with a virus carrying the firefly's light-producing luciferase gene. Number of islets in each well: (top row, from left) 0, 1,000, 50; (bottom row) 100, 500, 1,000. The 1,000 islets in the middle well of the top row were not infected, do not produce the luciferase enzyme, and therefore do not emit light.

Image courtesy of Alvin C. Powers, M.D.



ANNE RAYNER

"Being able to image the biological event is critical in studies of drug development. The advantage here is that bioluminescence imaging can be done *in vivo* and repeatedly in the same animal, which gets you better data."

E. Duco Jansen, Ph.D., associate professor of Biomedical Engineering at Vanderbilt

same animal over time, gives the best statistical results by avoiding interindividual variability, he says.

And as with the luminescent tumor cells, the glowing islets offer a model system for evaluating new pharmaceutical interventions. Suppose, Jansen says, that a drug company has a new immunosuppressant drug candidate. That company can use the transplanted islet model and bioluminescence imaging to quickly assess the drug candidate's efficacy.

"Being able to screen compounds in live animals, with a relatively high throughput readout, is critical for pharmaceutical companies," Jansen says. "If a drug candidate can be eliminated from the pipeline earlier because of *in vivo* molecular imaging, that translates into huge cost savings. And likewise, if a candidate can make it to the market sooner, that means millions of dollars of added revenue. Drug companies are very interested in these small animal *in vivo* molecular imaging approaches."

Blaze of inflammation

Bioluminescence imaging changed the way Timothy S. Blackwell, M.D., associate professor of Medicine at Vanderbilt, thinks about lung inflammation and injury. In addition to tracking cells and bacterial pathogens, Blackwell's group has been following gene expression in the context of inflammation.

To do this, the team engineered a "transgenic" mouse that contains the firefly luciferase gene, linked to a stretch of DNA that responds to a transcription factor – a protein that controls the expression of other genes – called NF-kappa-B. When NF-kappa-B is active in cells, it turns on the production of luciferase and the cells light up. NF-kappa-B is an important mediator of inflammatory processes.

In some of their first studies with these transgenic reporter mice, the investigators studied short-term versus long-term infusions of endotoxin, "with the idea of trying to figure out the parameters of inflammatory signaling that lead to lung injury – was it dose, timing, duration, cellular distribution – those sorts of things," Blackwell says.

The bioluminescence imaging revealed that the duration of the inflammatory stimulus affected the final outcome, he says. A single bolus injection of endotoxin caused a peak of NF-kappa-B activation, as measured by light output, but no lung injury. The same dose of endotoxin given as an infusion over 24 hours caused a progressive and sustained activation of NF-kappa-B in the lung, and resulted in lung injury.

"That was something that would have been very time consuming to try to figure out without the use of bioluminescence," Blackwell says, "and it has led us to other studies now trying to understand which cells are activated over this period of time and identify specific injury-provoking gene products.

"The ability to look non-invasively at NF-kappa-B activity over time in a relatively quantitative way is helping us to define the balance of factors that cause either lung injury or effective host defense against infection," Blackwell says. "Ultimately we might be able to come up with ways to prevent injury and still maintain adequate defenses."

These inflammation-reporting mice have been useful for Vanderbilt's Lin as well. Lin is interested in blood vessel formation – angiogenesis – in the context of diseases including cancer, arthritis and cardiovascular disease.

In the case of arthritis, inflammation appears to trigger excessive angiogenesis, which facilitates tissue growth and eventually causes bone damage, Lin explains. Anti-angiogenic therapies may be effective in preventing the tissue growth. Lin's group is using multiple imaging technologies – an increasingly common approach known as multi-modality imaging – to probe the arthritic joint: bioluminescence to see the inflammation, X-ray to look at bone damage, and fluorescence techniques to visualize the blood vessels.

"We think this is a very powerful way to study how the blood vessel affects disease progression as well as what kind of therapy we can use to stop this process," Lin says. "Imaging has really moved from traditional modes of looking at structure into functional imaging, from static into kinetic. We're no longer satisfied looking at single point snapshots of dynamic processes."

A cornerstone technology

While bioluminescence imaging offers excellent sensitivity for tracking cells and seeing gene expression in living animals, it suffers from poor spatial resolution. Because light is absorbed and scattered by tissues as it makes its way out of the animal, images become "fuzzy." Jansen likens it to having a pencil in a glass of water and adding a few drops of milk – you can still make out an image, but it's no longer clear that it's a pencil.

"All of the imaging modalities have strengths, and they all have weaknesses," Jansen says. "Bioluminescence is great at sensitivity, but it's lousy at resolution. So in many cases we combine it with something like CT or MR, or even fluorescence."

There are current attempts to improve the spatial resolution of bioluminescence imaging by collecting data at different wavelengths of light, taking a surface map of the animal and then computing the three-dimensional source distribution, Jansen says. Other attempts include using a rotating stage to image the animal from different planes.

Absorption of light by tissues poses another problem for bioluminescence. Although it is a very useful imaging technique for small animals, where it has to travel only a short distance to reach the surface, it's not likely to translate to humans except in niche areas. One of those areas could be encapsulated cell therapies – therapeutic cells, like glucose-sensing, insulin-producing beta cells that are "contained" within a membrane of some sort and implanted just under the skin.

"Developing encapsulated cell therapies will be greatly enhanced by building into those cells markers that tell us if the cells are alive and doing what they're supposed to be doing," says Stanford's Contag. "Since the cells would be under the skin, bioluminescence imaging should work. I think that's a perfect scenario for its first clinical application."

Another place bioluminescence imaging might find clinical use is in breast cancer detection, Contag says. Proteins tagged with luminescent markers would have the advantage of great signal to noise for detecting very small numbers of cells.

"The question is, is it going to be better than anything else out there, and we won't really know until someone tries it," Contag says.

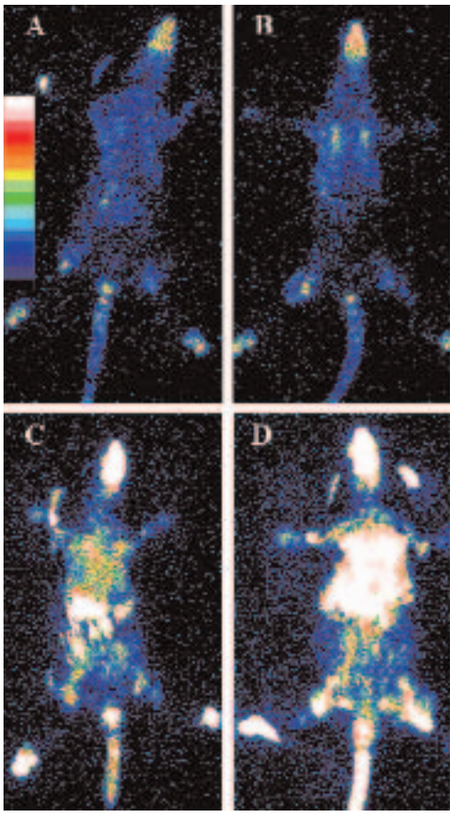
Whether or not bioluminescence imaging makes it to the clinic, "my guess is that optical imaging in small animals will become the mainstay for many laboratories using animal models," Contag says, "and bioluminescence will be one of the cornerstone technologies for developing new ways to treat disease and to visualize biology as it occurs in the living body."

That's bright stuff for those flashing summertime lights. **LENS**

Fighting infection – the glow from within

Bioluminescence imaging reveals inflammation-related gene expression in mice infected with the pathogen *Pseudomonas aeruginosa*. The images show increasing intensities of bioluminescence, from deep blue to bright white, in mice before injection of the pathogen (A), and 24 hours after injection of increasing doses of *P. aeruginosa*: (B), (C) and (D). Studies like this could lead to new treatments aimed at augmenting the host defense, particularly in critically ill patients.

From Sadikot RT, et. al., *The Journal of Immunology*, 2004, 172:1801-1808.



Firefly's glow reveals stem cell role in mending fractures

A bone fracture appears to be a relatively simple medical problem to solve: cast it, wait, and everything will be fine. For about 10 percent of fracture patients though, healing doesn't come easily. These patients, 600,000 people every year in the United States, require bone grafts or synthetic prostheses to mend their breaks.

Anna Spagnoli, M.D., assistant professor of Pediatrics and Cancer Biology, has another idea. She wants to use stem cells, harvested from a patient's bone marrow, to aid fracture healing.

"As a pediatrician, I am especially interested in using stem cells to treat children with severe bone diseases like osteogenesis imperfecta," a genetic disorder characterized by bones that break easily, Spagnoli says.

Investigators have known for 30 years that bone marrow contains two types of stem cells – the better known variety that repopulates the blood and another sort called mesenchymal stem cells that can become bones, cartilage, muscle, and even neurons, she explains. The potential of these cells to form cartilage is of particular importance for fracture healing, since cartilage grows as a "template" for new bone growth.

Spagnoli and colleagues turned to bioluminescence imaging to track mesenchymal stem cells in a mouse fracture model. They watched as the cells migrated through the animal and homed to the injured site after three days.

"This was an extremely important observation because homing of mesenchymal stem cells had never before been clearly demonstrated," Spagnoli says. Bioluminescence imaging has also allowed the investigators to quantitate the number of cells that migrate to the fracture site, she says.

"What we want to do in the long term is to engineer these cells with growth factors that promote cartilage formation and fracture healing – to combine stem cell therapy with gene therapy," Spagnoli says.

For mesenchymal and other stem cells, the only way the research will eventually generate therapies "is if we have ways to follow cells after they're put into humans," says Sanjiv Sam Gambhir, M.D., Ph.D., director of the Molecular Imaging Program at Stanford University. "If you label these cells with a reporter gene, they're permanently tagged – bar-coded – for life. And we can keep finding the status of these cells over time."

Bioluminescence imaging likely won't be the strategy to follow stem cells in humans, Gambhir says, but it is extremely valuable as a starting point for small animal studies.

"The technologies that are having the most impact in biological models right now are the optical technologies – because of their relative ease of use and suitability for small animals," Gambhir says. "Bioluminescence and fluorescence are the main workhorses for solving certain problems before moving to the more complex technologies."

– LEIGH MACMILLAN

Q & A

BRAVE NEW VISIONS

The promises and perils of imaging the brain

In separate interviews with *Lens* editor Bill Snyder, Richard Hargreaves, Ph.D., Vice President, Imaging at Merck Research Laboratories in West Point, Penn., and Judy Illes, Ph.D., Director of the Program in Neuroethics at the Stanford Center for Biomedical Ethics in Palo Alto, Calif., describe recent progress in the use of imaging technologies, their potential and challenges to further development of the field.

Hargreaves has a Ph.D. in physiology from King's College London. At Merck since 1988, he led the biology core for the discovery of Maxalt, an anti-migraine medication, and Emend, which helps prevent chemotherapy-induced nausea and vomiting. Illes earned a doctorate in Hearing and Speech Sciences from Stanford, specializing in experimental neuropsychology. She has conducted research on human neuroimaging, language and cognition, and co-founded the Stanford Brain Research Center in 1998

Interview 1:
Richard Hargreaves, Ph.D.

Imaging technologies are playing an increasingly important role in drug discovery.

Radiotracer imaging such as positron emission tomography (PET) allows scientists to see whether a molecule "engages" its target sufficiently to test for therapeutic efficacy. If the molecule doesn't bind to its target, or saturates the target and yet fails the efficacy test, "then we can move on to a new therapeutic concept," Hargreaves says.

In the early stages of drug discovery and development, imaging "actually increases the attrition in the number of molecules you test," he says. "You actually throw more molecules away to get the best one."

Can imaging reduce the cost of developing drugs?

Built into the cost of any drug are all the failures of the molecules you test along the way. If earlier on you can select the best development candidates, you improve your chances of moving through the development process in a more informed and efficient way.

Remember, too, that making early "go/no-go" decisions also has ethical implications for the human subjects who take part in clinical trials, since fewer will be exposed to potentially ineffective therapies.

If you get it right, you'll bring good medicines to the market faster.

Is imaging improving treatment?

In oncology, PET radiotracers can be used to assess different aspects of tumor physiology, such as glucose metabolism, cell proliferation, angiogenesis (growth of new blood vessels), apoptosis (cell death) and oxygenation.

PET tracers exist for some of these and are well embedded in clinical care. An example is 18F-fluorodeoxyglucose (FDG), which provides a measure of glucose metabolism. Others, such as 18F-fluorodeoxy-L-thymidine (FLT), which may reflect cell proliferation, are still being validated.

The beauty of being able to track aspects of tumor growth and viability means that you can see very early on after administering an experimental therapy whether it is impacting tumor physiology in any way at all. If it is, then you have a very good reason to carry on and look for tumor regression.

In our oncology projects, we're also looking for molecular imaging agents that can tell you whether people are likely to respond to targeted therapies and how well our drugs engage them.

I think that imaging truly has the opportunity to revolutionize and personalize care in oncology. We're going to see the fruits of that ripen in the near future.

How has imaging improved our understanding of the brain?

Functional imaging has fundamentally changed the way we can question brain systems because we can see them in action. It has given whole new dimensions to many areas of neuroscience such as pain, basic sensory systems, psychiatric disorders and their co-morbidity with other disorders of the central nervous system, cognition, language and neural mechanisms that underlie developmental plasticity and recovery of function.

Imaging studies that help us understand neurodegenerative brain disease can also help pave the way to better health care in the future.

At Merck, we have participated in the Alzheimer's Disease Neuroimaging Initiative (ADNI), a collaborative consortium between industry, academia and the National Institute on Aging. The goal is to validate imaging tools such as PET and magnetic resonance imaging (MRI) and fluid biomarkers – alongside neuropsychiatric scores – for tracking the progression of Alzheimer's disease in people currently on the best therapies available for this condition.

This is an important study because it will tell us what an aging Alzheimer's disease population looks like in North America today. Once we have an understanding of that, then we have a reference library or baseline that we can use to evaluate whether our new medicines produce true improvements in clinical care by modifying disease progression.

It would be brilliant if you could prevent Alzheimer's disease totally, but just think of a therapy that delays its onset by 10 years. That too would be a phenomenal achievement.

Can these collaborations overcome concerns about disclosure and conflicts of interest?

It's in the interest of the patient, the National Institutes of Health, the FDA, health care providers, and all of us in the pharmaceutical industry to move safe and effective new medicines forward efficiently.

We will attempt internally to make imaging agents that validate target engagement for our new therapies and have the desired mechanistic effects. That's proprietary to drug discovery programs. Eventually, when appropriate, we will try to make these agents available to all for research.

When we move into evaluating disease and finding imaging endpoints that chart progression, the story is somewhat different. Here the imaging endpoints are valuable to everybody trying to treat that disease – independent of the mechanism they are using to do so.

In this case there is a very good case for a consortia approach, which combines the efforts of interested pharmaceutical companies with academia and perhaps also diagnostic companies. The goal: defining new surrogates that speed drug assessment and approval, particularly in areas where medical need is poorly met.

RICHARD HARGREAVES, PH.D.

ILLUSTRATION BY DAVID JOHNSON

A Closer Look at Drugs

Just as imaging technologies are guiding and, in some cases, replacing the scalpel, they are revolutionizing the evaluation of new cancer drugs.

Traditionally, a drug's effectiveness has been determined by its impact on patient survival, or by measuring the diameter of a tumor on a series of X-rays or CT scans taken over the course of several weeks.

That's too slow for oncologists like Craig Lockhart, M.D., M.H.S., assistant professor of Medicine in the Vanderbilt-Ingram Cancer Center. If there was a way of telling, within 48 hours, that a patient's tumor was not shrinking or otherwise responding to the drug, "we'd be able to save that patient a lot of time and potential toxicity," he says.

John Gore, Ph.D., director of the Vanderbilt University Institute of Imaging Science, is equally impatient. "We want to have more quantitative, more sensitive and more specific measures" of drug response, he says. "Imaging is the only way to do that."

Gore envisions radiologists and imaging scientists from the Institute sitting down with oncologists at the Cancer Center to plan "what imaging should be done for every clinical trial."

That's also the goal of the National Cancer Institute (NCI), which is funding the establishment of Image Response Assessment Teams, composed of radiologists and imaging scientists, who would contribute to the design of clinical studies at cancer centers across the country.

"We're trying to get oncologists and imagers to work on the same team … to prove the value of therapeutic maneuvers," explains C. Carl Jaffe, M.D., chief of the NCI's Diagnostic Imaging Branch.

"We don't mean to imply the imaging community doesn't care about oncologic trials," Jaffe adds. "They're leading the development of new methods … (But) cancer trials are a special situation. You have to have greater uniformity, greater communication and greater discipline in order to produce quality data that can be trusted."

Advances in imaging technology will continue to refine – and challenge – the testing of new drugs. "For now, we have pretty darn good technology, but it's relatively undisciplined," Jaffe says. " … It just needs to be stabilized. That's where we're headed."

– BILL SNYDER

I think the ADNI has the potential to be a wonderful example of this approach. We're going to set the "goal posts" for imaging biomarkers and the evaluation of therapies in Alzheimer's disease.

Will imaging increase the emphasis on using drugs to treat disease, or will it help validate behavioral/cognitive approaches as well?

I think the two go together.

The industry spends its time making highly selective pharmacological agents. But if you combine those together with the amazing imaging of brain function, then you can understand the functional/chemical neuroanatomy of health and disease as well.

Functional brain imaging should help you design better therapies – be they pharmacological or non-pharmacological. Indeed, it's already begun to help us understand what the placebo component of responses really is, and to segregate responders from non-responders in the field of pain research.

If brain scans become a practical, reproducible method of predicting risk for disease, what kind of ethical issues will it raise?

Clinically, brain structural imaging is used to assess disease and to make treatment decisions based on prediction of long-term sequelae. I think functional brain imaging has a way to go before it reaches this point.

One concern that has been raised about functional brain imaging in the past is that we may find things that are unexpected, or image things that may be predictive of events or behaviors for which we have no solution or control. This is clearly an ethical issue, and we need to think how to respond in advance.

Do you want to bury your head in the sand and not know, or do you want to develop a strategy or set of rules that can help you deal ethically with the issues that could be raised as you strive, through imaging, to understand brain disease and provide a platform for discovery of better therapies?

The bottom line for me is that many new prognostic and diagnostic medical technologies raise these types of issues. You need to think about them and plan how to respond in advance, or reconsider what you are doing. It's not just science for science's sake.

Is there concern that the field is over-hyped?

It's very visual and so it's very powerful. It tends to get jazzed up. Scientists need to be central in communicating what the functional MRI technique is.

What are its limitations? What are we actually measuring, and how certain can we be about what we see? How has it been validated? If we do the same thing twice, do we get the same answer?

Does the field need standards? Yes, undoubtedly. Let's be sure to use this tool to ask the unique questions that can actually be answered with it, rather than as a more complicated and expensive route to answers that could be obtained more simply and reliably another way. Otherwise, it can be neo-phrenology, can't it?

At the end of the day, we need to use functional brain imaging carefully, and manage our excitement to make sure that we give clear context and boundaries to what we do and what we see.

Interview 2:
Judy Illes, Ph.D.

Imaging is at the intersection of neuroscience and bioethics, says Illes. Technology like functional MRI can provide measurements of cognitive phenomena ranging from fear and addiction to learning and memory. Imaging allows scientists to probe the deep recesses of the human mind, she says, "romance and hatred and prejudice, existential thinking." Even the fetal brain is being imaged.

What are some of the ethical concerns about imaging?

What if we could use those data to predict behavior in the future? What if we could in an adolescent predict propensity to aggression or sociopathy or suicide? How are we going to handle people in whom we are able to predict potentially devastating behaviors?

What will it mean to be able to predict the onset of a disease may occur 30 years down the line, especially when there's no treatment? Alzheimer's a perfect case of that. The issues of prediction are immense.

Some hardcore MR (magnetic resonance) physicists who developed this technology would say, 'Nah! Nah! Never!' But

I don't know. I think that the evidence is that we haven't been stopped yet in our innovations. It's just a matter of time.

Are there places we shouldn't go?

Is everything allowable as long as it's done ethically? Should there be boundaries imposed on our science because of their new potential real-world applications?

I will argue that limitations on ethically conducted science are not appropriate. It's just part of the human condition to be curious and innovate and push the envelope. But now we have every good reason to couple our ethical thinking with our neuroscience.

I think part of the ethical construction of research is not only good protocols and protection of human subjects, but actually thinking about the downstream implications of the research.

What if you're doing a study of people with schizophrenia and you find a mass? How do you handle that with that kind of population? What about children? What if something comes out and they end up with a result that may be stigmatizing? That could have a lifelong implication.

What if somebody develops a drug that can check addictive behaviors? What kind of interventions are developed and become available? All the more reason to start thinking about the ethics of it. If in fact we can use an imaging technology to predict addiction, then we definitely want to have a response ready.

And to the extent that we continue to do these studies that probe personhood, it's not enough just to say, 'I'm not going to hurt somebody in my experiments.' But it is becoming, I believe, a requirement to think, 'If I find out that there is a locus or loci or network for making race judgments in the human brain, this is how I'm going to handle the information in terms of its dissemination.'

Without being too alarmist and certainly without being negative, I do feel that unless we start to introduce a reasonable – not a heavy, but a reasonable – ethical component to the kind of work that we're doing, there are risks of adverse effects on people that then have the adverse effect of potentially slowing down the progress of research.

And so by being proactive, and by trying to address these issues jointly from within the neuroscience and bioethics community, we can get a very good handle on what the issues are and what are the ways that we can empower our science ethically so that we can either prevent the adverse events down the road or at least be able to manage them very efficiently.

What are the limitations of these technologies?

They start at the beginning with the design of an experiment. Any experiment, especially one that probes complex phenomena such as existential decisions, takes some pretty clever design and invariably will reflect – and I don't see how it could not – the cultural orientation and biases of the experimenters developing it.

The way I value something might be very different from the way you value something at Vanderbilt. When we start to probe personhood, we're unequivocally invoking issues of values and culture, ethnicity, so there's a limitation right there.

Another limitation is, as we know, in the statistical processing of the data. The different kind of statistics you do may affect the results, and along with that we don't have a very good handle yet on individual neuro-functionality in terms of blood flow, for example. We're still using group averages, although people are really making some fantastic strides in that domain.

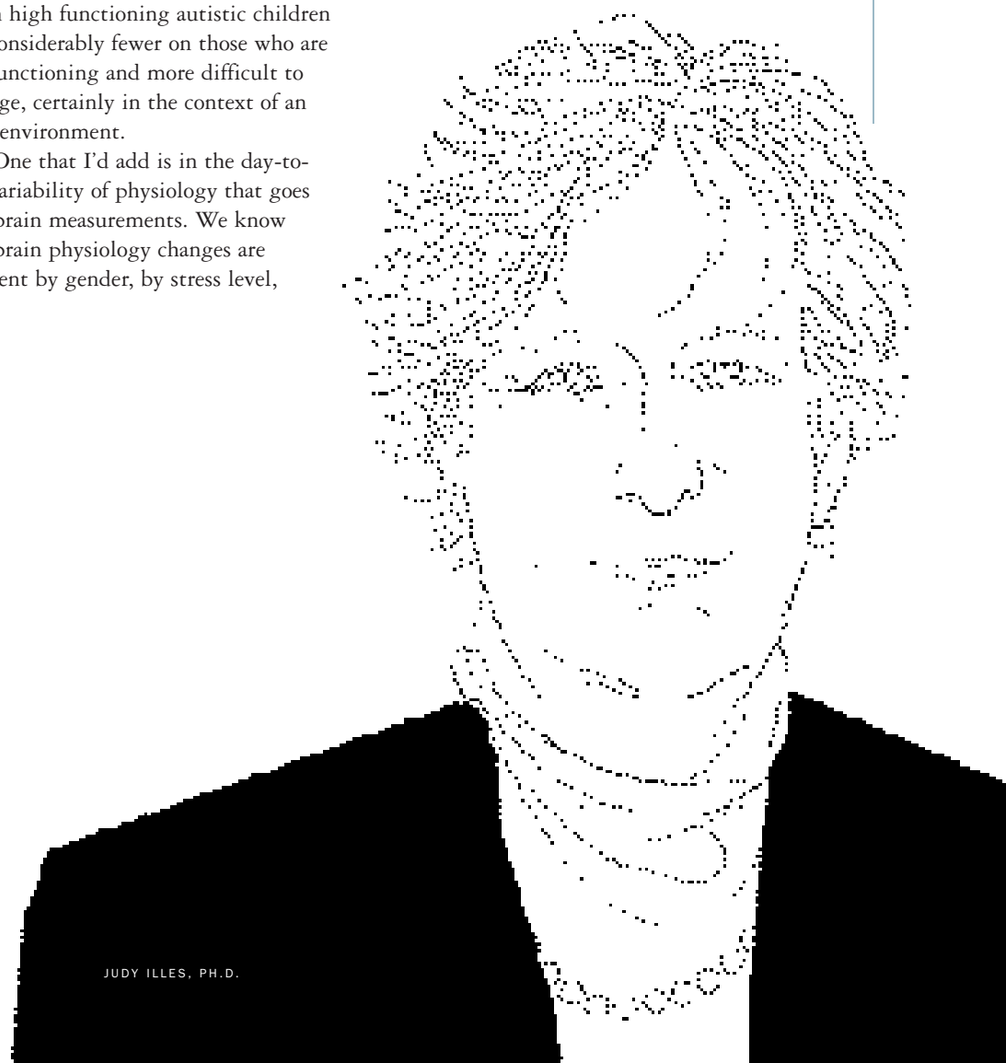
Some investigators are concerned that we're only able to study subsets of certain disease populations. I think of autism, for example, where we have quite a few studies on high functioning autistic children but considerably fewer on those who are low functioning and more difficult to manage, certainly in the context of an MRI environment.

One that I'd add is in the day-to-day variability of physiology that goes into brain measurements. We know that brain physiology changes are different by gender, by stress level, by food level. Again, understanding individual variability is really crucial.

There are definitely limitations to using the knowledge we gain in the laboratory, especially about these incredibly complex phenomena in the real-world setting. But nonetheless, we are measuring them in the laboratory. These laboratory studies are being covered by the press and the word is out in the public domain.

And so we as neuroethicists really have to work closely with our neuroscience colleagues in the trenches to be proactive about aligning the ethical considerations of some of these studies up front in the research design and really trying to anticipate what kind of social impact or legal impact or ethical impact it might have down the road.

We're accustomed to, in many ways, living in a very privileged environment where we publish in science journals and we speak with our colleagues at meetings, and a little bit filters out to the public. But I really believe and our data suggest that we're in a different place now and we have to consider these ethical, legal, social issues to a far greater extent than we ever did before. **LENS**



JUDY ILLES, PH.D.

Peeking into the womb

By Bill Snyder

Pictured here: Fetal magnetic resonance images with structures noted with arrows.
A) Normal brain and bladder.
B) Hydrocephalus – abnormal accumulation of fluid in the brain.
C) Congenital diaphragmatic hernia – hole in the diaphragm that allows the liver and bowel (marked) to enter the chest cavity.
D) Myelomeningocele – open neural tube defect (spina bifida).

Courtesy of Sharon M. Stein, M.B., Ch.B.

One of the most powerful applications of imaging science is the diagnosis of fetal abnormalities.

"Imaging provides an ability to depict things that previously were only able to be diagnosed by surgery," says Marta Hernanz-Schulman, M.D., director of Pediatric Diagnostic Imaging at Vanderbilt University Medical Center.

Some conditions can be treated before birth.

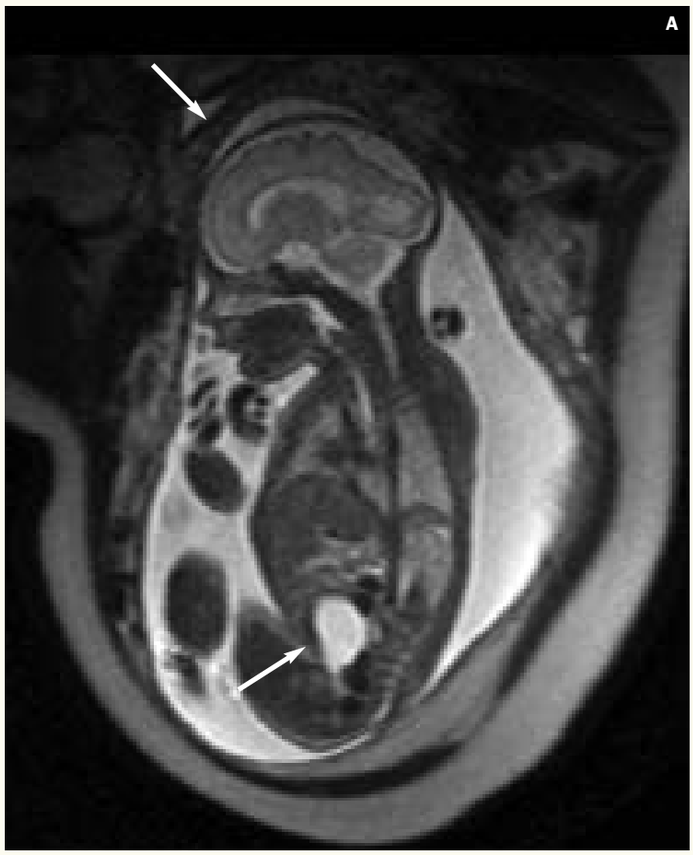
For example, when the male urethra – the tube that carries urine from the bladder to the outside – is obstructed, the bladder can become massively dilated. Urine may back up the ureters, causing severe kidney damage. By inserting a needle through the uterine wall into the fetal bladder, under the guidance of ultrasound, the obstetrician can drain the bladder into the amniotic fluid surrounding the baby, thereby relieving pressure on the kidneys.

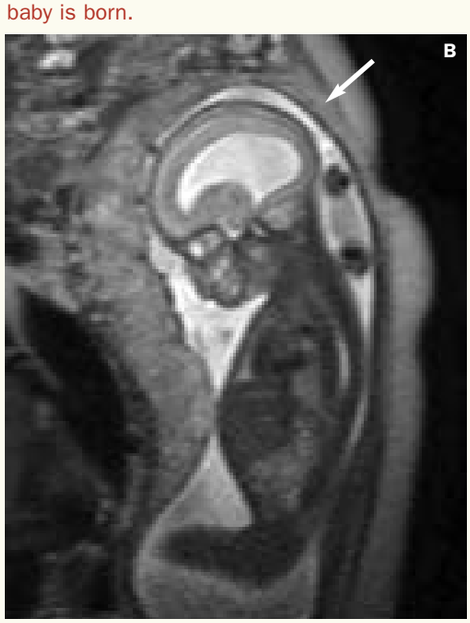
Usually pregnant women are referred for further imaging studies when a routine ultrasound indicates there may be a problem, says Sharon M. Stein, M.B., Ch.B., associate professor of Radiology and Radiological Sciences, and Pediatrics.

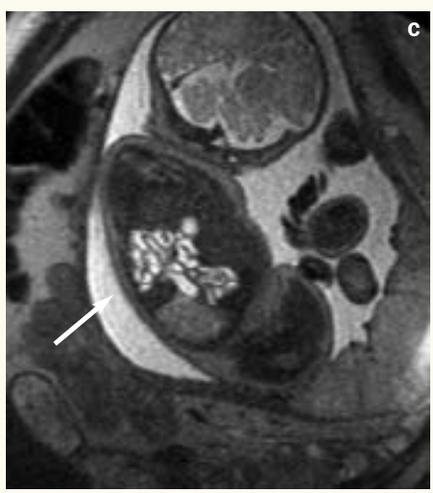
Computed tomography (CT) is not used because of the need to protect the fetus from exposure to X-rays. Instead, remarkably detailed pictures can be obtained using ultrasound and magnetic resonance imaging (MRI), which have no known harmful effects.

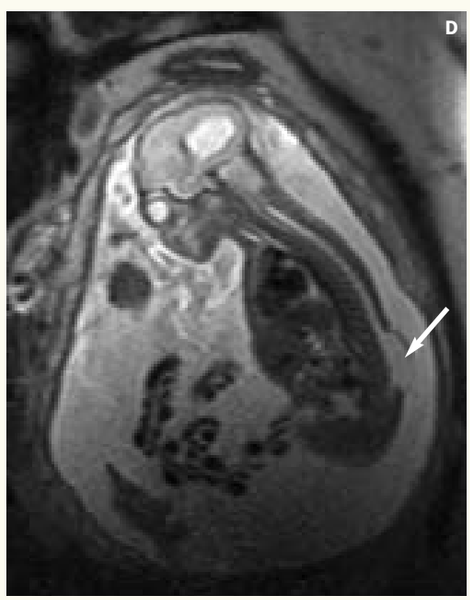
During a weekly fetal therapy conference, Vanderbilt specialists in radiology, neonatology, surgery, urology, genetics and medical ethics review the images, and use them to guide decisions about the management of the pregnancy, including the timing of delivery.

The aim, says Stein, is to ensure "that everything is well orchestrated and planned for optimum care" once the baby is born.









Through our Lens

LETTERS TO THE EDITOR

The empty medicine chest

Regarding the Summer 2005 issue, the medicine chest is about to be emptied. My guess is that more than one pharmaceutical company will collapse as the result of their failure to abide by the same rules that guide good medical practice. The requirement to "do no harm" has been ignored due in part to what appears to be a real lack of understanding of the connection between prescribed drugs and insulin resistance.

Approximately 85 percent of us are genetically predisposed to developing one or more of the components of metabolic syndrome (related to insulin resistance): obesity, coronary artery disease, gallbladder disease, type 2 diabetes, hypertension, colon cancer, breast cancer or other related problems. Providing medications for those disorders that alter one or more biochemical pathways related to insulin resistance without taking into consideration what impact it might have on other metabolic pathways is not only irresponsible but also reprehensible.

Both the pharmaceutical industry and the university also need to take into consideration Peter Drucker's Theory of S-Curve Discontinuity. Drucker states that all products and processes have a defined life cycle that can be graphed as an "S" curve.

At the outset of the development of a product or process, the effort and/or funds expended are high, while the results are quite low (the lower horizontal part of the curve).Then things begin to click, with productivity and profitability increasing at an exponential rate (the vertical portion). At some point, however, no amount of new effort or funds produces the kind of results previously experienced (the upper horizontal part of the S).

What happens when we reach the top of the S-Curve? We establish a totally new product or process that is based on a completely different concept, a new S-Curve, what Drucker calls a "discontinuity." Individuals who don't understand or get on the new S-Curve find themselves in great turmoil, chaos and confusion.

In health care, S-curve discontinuity began approximately 20 years ago, when we began to experience the transition from the "illness" curve to the "wellness" curve.

Prescription drugs used to modify insulin resistance-related diseases are doomed to failure, as they are on the illness S-Curve. These diseases are preventable using the following formula: routine exercise + adequate sleep – stress – nicotine – high glycemic index calories + omega 3 essential fatty acids – unnecessary prescribed medications – excessive alcohol intake = reduced insulin resistance + improved health.

Although Vanderbilt is renowned for its research on the old illness S-Curve, it is time for it to spend more time, energy and money on promotion of health and prevention of disease. And it is time for publications like yours to begin to disseminate information on this "non-glitzy" subject.

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Colorized image of the head of an adult female *Anopheles gambiae* mosquito, the insect that transmits malaria. Because malaria kills more than 1 million people a year, most of whom are children in Africa, *An. gambiae* is considered to be "the most dangerous animal on the planet."

Scanning electron microscope image courtesy of Larry Zwiebel, Ph.D.; colorization by Dominic Doyle

IN THE NEXT ISSUE:

The world is flat, but bumpy
International research collaborations are transforming global health care. But they are easier said than done.

Tricking the mosquito's "nose"
Basic biological research suggests a new, environmentally friendly approach to thwarting an ancient scourge: malaria.

Skipping the 20th Century
How export of the latest information technology is aiding the fight against AIDS in Africa.

Lens

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