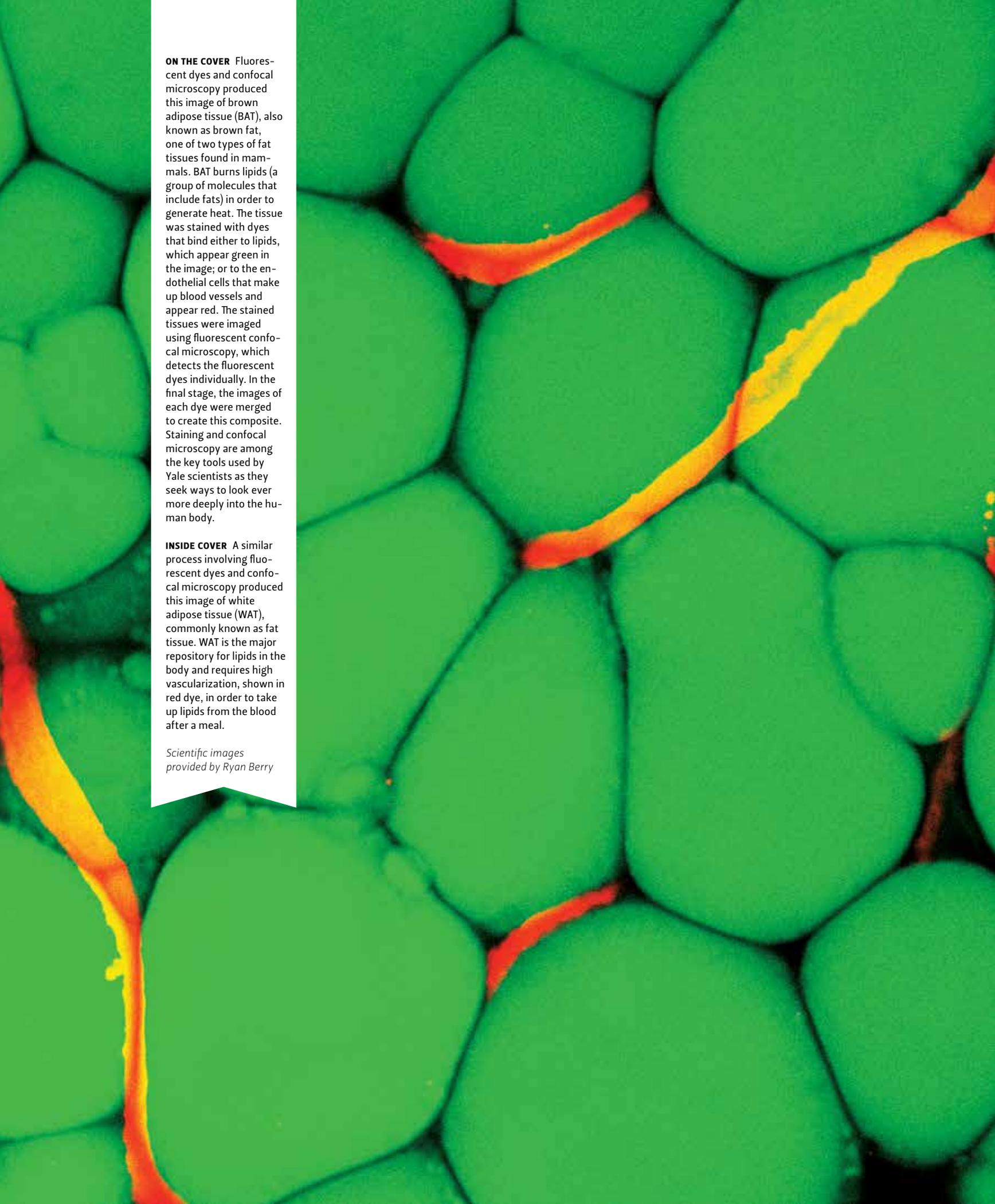
The background of the entire page is a microscopic image of a tissue section. It features a dense network of green, circular or polygonal cells, likely representing a glandular or epithelial structure. Interspersed among these cells are prominent, branching, and irregular structures stained in bright red and orange, which could represent blood vessels, connective tissue, or specific cellular components. The overall appearance is that of a complex biological network.

yale medicine

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imag(in)ing

Scientists at Yale and around the world are challenging the laws of physics as they seek new ways to peer ever more deeply into the workings of the human body.

A fluorescent confocal microscopy image of brown adipose tissue (BAT). The image shows a dense network of adipocytes, which are stained green, and a network of endothelial cells, which are stained red. The adipocytes are arranged in a honeycomb pattern, and the endothelial cells form a complex network of blood vessels. The overall appearance is a vibrant, multi-colored structure.

ON THE COVER Fluorescent dyes and confocal microscopy produced this image of brown adipose tissue (BAT), also known as brown fat, one of two types of fat tissues found in mammals. BAT burns lipids (a group of molecules that include fats) in order to generate heat. The tissue was stained with dyes that bind either to lipids, which appear green in the image; or to the endothelial cells that make up blood vessels and appear red. The stained tissues were imaged using fluorescent confocal microscopy, which detects the fluorescent dyes individually. In the final stage, the images of each dye were merged to create this composite. Staining and confocal microscopy are among the key tools used by Yale scientists as they seek ways to look ever more deeply into the human body.

INSIDE COVER A similar process involving fluorescent dyes and confocal microscopy produced this image of white adipose tissue (WAT), commonly known as fat tissue. WAT is the major repository for lipids in the body and requires high vascularization, shown in red dye, in order to take up lipids from the blood after a meal.

*Scientific images
provided by Ryan Berry*



Features

12/ **From dead cells to live movies**

New light microscopes developed by Yale cell biologists are helping researchers unravel the complexities of human biology. *By Ashley Taylor*

22/ **Can brain scans reveal how we think?**

Scientists debate whether such higher-order processes concepts as consciousness and morality have their own real estate in the brain. *By Jenny Blair, M.D. '04*

33/ **Inside the toolbox**

An inside glimpse at the School of Medicine's five core research facilities that allow biological visualization on the atomic, molecular, and cellular scales. *By Amanda Alvarez*

36/ **A new vision in the lab and in the clinic**

Since the discovery of X-rays in the 19th century, new imaging technologies have helped physicians peer into the causes of disease. *By John Dillon*

winter 2014 departments

2 From the Editor / 3 Dialogue / 4 Chronicle / 9 Round Up / 42 Capsule / 44 Faces / 48 Q&A / 50 Books / 51 End Note

A new vision for Yale Medicine

The magazine in your hands or on your computer screen or tablet is the first that reflects a new design and vision for *Yale Medicine*. As we announced in our Spring issue, in addition to a redesign of the magazine, we focus our feature stories on a single topic in medicine, science, or medical education. The theme for this inaugural issue is biomedical imaging, in which we explore the ways in which biologists have endeavored to peer ever more closely at the molecular underpinnings of life. You can also visit our website at yalemedicine.yale.edu, or download an iPad version at yalemedicine.yale.edu/app or by scanning the QR code at right. We hope you enjoy this issue and those to come.

John Curtis
Editor, *Yale Medicine*

We would like to offer our thanks to these faculty and alumni who have served on the *Yale Medicine* Advisory Board for their counsel and insights over the past few years. We have benefited greatly from their participation in the planning of the magazine:

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How to reach us

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SECOND OPINION
BY SIDNEY HARRIS



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Abbreviations used in *Yale Medicine*
include HS to denote the final year of
residency for house staff, FW for the
final year of a fellowship, and YNHH
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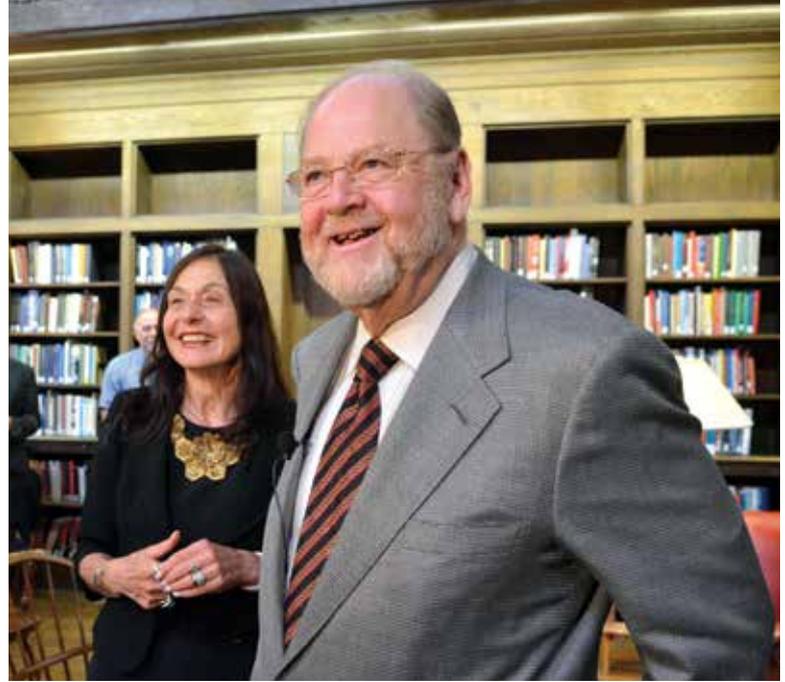
THIS ISSUE OF YALE MEDICINE is devoted to bioimaging technology. In our feature section we explore the history of biomedical imaging and examine how scientists at Yale are developing new ways of seeing smaller and smaller structures. Advances in imaging technology over the last several decades have enabled an ever more detailed view of the internal structures of organisms as well as their functions, from the level of molecules and cells to that of tissues, organs, and body. The pace of technological development is rapidly increasing, with the result that we can now see, in high resolution, things that we could only guess at before. Below, Dean Robert J. Alpern, M.D., discusses the importance of imaging in science and medicine and what the future may bring.

When we talk about imaging at Yale School of Medicine, what does that encompass? Imaging includes everything from crystallography to microscopy at the highest resolution, to X-rays, CT scans, MR, SPECT, and PET. It's any technology that allows us to visualize biologically relevant structures and processes.

Why is it important? A rate-limiting factor in science now is access to the latest technology. For instance, our ability to understand how the brain functions is presently limited by the availability of technologies that allow us to measure the function of individual neurons, parts of neurons, and even molecules within each specific neuron. Traditionally, the best medical schools invest in the best scientific talent and then equip those individuals with the best available technology. We're investing in a third element, which is research in new technologies, so we can advance the state of the art. We want our scientists to invent the new technologies, which is already happening.

How will imaging change science and medicine in the future? One way is the example that I alluded to earlier. I think the functional definition of all the circuits in the brain will come only from improved technologies in brain imaging. Another area where it might help is the use of imaging to identify new biomarkers. For instance, right now we're very good at using imaging to see if someone has prostate cancer. But can we tell if the prostate cancer is the type that needs to be taken out because it's going to metastasize? Or if it's the one that's going to sit there and not metastasize? If you combined spectroscopy with imaging, by studying the metabolism of those cells, could you find something that determines whether they'll metastasize? If you could, you would spare many patients unnecessary treatment, and you would lower health care costs at the same time. These are benefits that would accrue across medicine and science.

chronicle





A Nobel Prize for studies of cell trafficking

AS HE NEARED THE END of the day in October that began with an early-morning phone call from Sweden, James E. Rothman, Ph.D., recalled before a gathering of his colleagues, students, and university leaders what he described as an “out-of-body experience”—the news that he had shared in the 2013 Nobel Prize in physiology or medicine.

CLOCKWISE FROM TOP LEFT

Dean Robert Alpern and President Peter Salovey praised Nobel laureate James Rothman's persistence in pursuing research into vesicles that transport material between and within cells.

At a press conference in the Historical Library, James Rothman, shown with his wife, Joy Hirsch, described receiving the news that he had won a Nobel as an “out-of-body experience.”

Rothman told assembled journalists that vesicle trafficking is essential to many biological processes, including cell division and insulin secretion. It is also important in the nervous system, passing on messages that govern movement, perception, cognition, memory, and mood.

“I have gotten close to 1,000 e-mails,” said Rothman, the Fergus F. Wallace Professor of Biomedical Sciences and professor and chair of cell biology, speaking at a reception in the Beinecke Rare Book and Manuscript Library on the evening of October 7.

“You hear from all kinds of people: someone who practiced medicine with my father, a third grade classmate.”

Earlier in the day at a press conference he said he was still absorbing the news. “It’s a little hard to believe all this is happening,” he said. Rothman noted his good fortune in having studied at Yale and learning “to appreciate science and intellectual activity at its highest, to have matured and started my career as a researcher when your idea was the only limit. Any risk could be

taken, no matter how difficult. I was fortunate to have taken a few of those risks and today’s Nobel Prize recognizes the success that came out of that.”

The prize acknowledged his contributions to the understanding of membrane trafficking, the means by which proteins and other materials are transported within and between cells. Rothman, a 1971 Yale College graduate who previously shared in the Albert Lasker Award for Basic Medical Research, the Louise Gross Horwitz Prize of Columbia University, and the Kavli Prize in Neuroscience, is one of the world’s foremost experts on exocytosis, a form of trafficking in which cargo-bearing spheres called vesicles fuse with cell membranes to deliver their contents.



PHOTOS, CLOCKWISE FROM TOP LEFT: TERRY DAGRADI, MICHAEL MARSLAND, TERRY DAGRADI



NOBELS AT YALE

George E. Palade, Ph.D., who served on the Yale faculty from 1973 to 1990, and was the first chair of cell biology, shared the 1974 Nobel Prize in physiology or medicine with Albert Claude, Ph.D., and Christian de Duve, Ph.D. The prize was granted for “for their discoveries concerning the structural and functional organization of the cell.” Palade’s innovations in electron microscopy laid the foundations of modern cell biology.

Sidney Altman, Ph.D., Sterling Professor of Molecular, Cellular, and Developmental Biology and professor of chemistry, shared the 1989 Nobel Prize in chemistry with Thomas R. Cech, Ph.D., “for their discovery of catalytic properties of RNA.”

Thomas A. Steitz, Ph.D., Sterling Professor of Molecular Biophysics and Biochemistry, professor of chemistry, and investigator, Howard Hughes Medical Institute, shared the 2009 Nobel Prize in chemistry with Venkatesan Ramakrishnan, Ph.D., and Ada E. Yonath, Ph.D., “for studies of the structure and function of the ribosome.”

This process is essential to such processes as cell division and insulin secretion, for example, but also plays a crucial role in the nervous system. Vesicles carrying neurotransmitters fuse with cell membranes at synapses and pass on chemical messages that govern movement, perception, cognition, memory, and mood. For three decades, Rothman has performed experiments that have revealed the molecular machinery of membrane trafficking in fine detail. In much of his work Rothman sidestepped the complexities of working with complete cells by using a “cell-free” approach—isolating the intracellular components crucial to membrane trafficking.

Rothman and the two scientists who shared in the \$1.2 million award—Randy W. Schekman, Ph.D., of the University of California–Berkeley, and Thomas Südhof, M.D., of Stanford University—all faced skepticism within the scientific community when they began their research. Each went on to solve a different piece of the puzzle.

While Rothman figured out the machinery underlying membrane trafficking, Schekman discovered a set of genes essential for vesicle traffic, and Südhof determined how vesicles know when and where to release their cargo.

Rothman began his research career after receiving his Ph.D. from Harvard in 1976. From there he went on to the Massachusetts Institute of Technology, Stanford,

Princeton, Memorial Sloan-Kettering, and Columbia before coming to Yale in 2008.

“When Jim started his career, a number of successful biochemists were recognizing the importance of studying molecular processes in cell-free systems, but no one imagined that you could study vesicle trafficking in a cell-free system,” said Robert J. Alpern, M.D., dean and Ensign Professor of Medicine, at the press conference. This bold approach revolutionized the field.”

“Yale is absolutely thrilled to have one of our most distinguished faculty—who is also one of our most distinguished alumni—receive this great honor,” said President Peter Salovey, Ph.D., the Chris Argyris Professor of Psychology.

—Charles Gershman
and John Curtis



Yale celebrates its 23rd president

In his more than 30 years at Yale, Peter Salovey, Ph.D. '86, has been a student, scholar, psychologist, and scientist. Over those years he has risen through the university hierarchy—serving as chair of psychology, Yale College dean, provost, and now, president.

His tenure became official on October 13 with his inauguration as Yale’s 23rd president. In the week leading up to the ceremony, Yale celebrated with a reception for faculty and staff,

a parade of dogs including Yale mascot Handsome Dan, an open house, and a panel of university presidents who offered their cumulative wisdom. Salovey, the Chris Argyris Professor of Psychology, also visited 27 schools and departments across Yale. His penultimate visit brought him to the medical school, where he has longstanding collaborations in research in cancer and HIV/AIDS. As a social psychologist, he is best known for developing the concept of emotional intelligence with colleague John D. Mayer, Ph.D.

The roles he has filled throughout the university have given him a sense of community that infused his inaugural address, in which he vowed to continue to “bring Yale to the world and the world to Yale.” On the stage in Woolsey Hall, flanked by his predecessor Richard C. Levin and dignitaries including New Haven Mayor John DeStefano Jr., Salovey noted the achievements of Levin’s 20-year tenure. Many reflect engagement with the broader world: a strong partnership with the city of New Haven, more than 900 faculty members engaged in overseas projects, and Yale’s emerging status as a global institution.

Salovey said that he would develop a university that is both more unified and more global and ensure that Yale remains accessible to “brilliant, hard-working, and committed applicants who would invigorate our campus and improve our world.” He promised to “support, expand,

Yale's new president, Peter Salovey, greeted well-wishers during a procession from Woolsey Hall to a block party on

Hillhouse Avenue. The university's task, he said in his inaugural address, is to "retain the ties that bind us together ..."



and celebrate basic and problem-driven research in the fields of today and those of tomorrow.

"Our task," he said, "even while we grow in size, even while we commit to being a more diverse faculty, staff, and student body; more cross-disciplinary; and more global, is to retain Yale's focus on the ties that bind us together, the sense of being a small, interdependent community, but one with an impressively broad scope. This intimacy and shared sense of purpose is what generates Yale's distinctive spirit."

Glad tidings added to that sense of unity in the days leading up to his inauguration: A 1954 Yale College alumnus gave Yale College the largest gift in its history—\$250 million; James Rothman, Ph.D., chair of cell biology and an alumnus of Yale College, shared in the 2013 Nobel Prize in physiology or medicine, and the football team kicked off the season with a 3-0 winning streak. (The day after his inauguration Salovey had more

good news to share: Robert J. Shiller, Ph.D., Sterling Professor of Economics, had won the Nobel Prize in economic science.)

But being part of a larger community allowed him to *birg*, a term from social psychology that stands for bask in reflected glory. "You identify with something bigger than yourself, like Yale; then, when something good happens to someone else in that organization, your self-esteem goes up. Jim Rothman won the Nobel Prize—that reflects on me!" Salovey said. "If you can identify with something bigger than yourself and 'bask in reflected glory,' you can feel pretty good about other people's successes. This is the key to happiness. ... This is what makes this place so wonderful."

—John Curtis



How Cold War nuclear testing launched the field of DNA repair at Yale

In the 1950s, U.S. Senator Prescott Bush, a Republican from Connecticut and father and grandfather to future presidents, approached Yale's biophysics faculty with a request. Could they determine whether radiation causes irreparable damage to human DNA?

World War II had just ended with the first strategic use of atomic weapons. The Soviet Union and the United States were locked in a Cold War arms race. Scientists and the public alike feared that radiation from nuclear testing would cause irreparable DNA damage and cancer—a belief that grew out of the illness and death in the aftermath of the atomic bomb blasts in Japan.

The Atomic Energy Commission (AEC) countered that people exposed to equivalent doses of radiation exhibited different outcomes; while some developed cancers, others didn't. It was premature, the AEC concluded, to say that the effects of radiation were irreversible.

The search for answers was on. Yale biophysics and radiobiology researchers began to study the effects of radiation on living cells. "The time was ripe and the situation was ideal," recalled Philip Hanawalt, Ph.D. '59, then a graduate student in the biophysics department. "Watson and Crick had just reported the structure of DNA,



ONLINE EXCLUSIVES

Med school alum nominated as surgeon general.

Med student's research recognized at a cancer conference.

A lesson from a Nobel laureate.

How the medical school is recruiting minority faculty.

Full stories and event photo galleries, as well as other online-only content, can be found on our homepage at yalemedicine.yale.edu.

and biophysicists at Yale decided it was important to learn what radiation did to DNA.”

Hanawalt and other researchers reflected upon those heady times in May, when the Department of Therapeutic Radiology hosted a symposium to commemorate 50 years of DNA repair research at Yale.

Hanawalt, who holds the Dr. Morris Herzstein Professorship in Biology at Stanford, recalled an all-hands-on-deck mentality among the biophysics faculty. “We met weekly for informal research discussions. We were like a large family sitting around the table discussing science. We were

all focused on a common goal, figuring out what radiation did to cells, and particularly to DNA.”

In their search for answers Yale scientists made many of the pioneering discoveries in the field of DNA repair. Researchers in the Radiology Department, a precursor to the Department of Therapeutic Radiology, and in biophysics discovered not only DNA repair mechanisms but also their genetic control. The research that began at Yale led, Hanawalt said, “to an understanding of the multiple DNA repair mechanisms required for the maintenance of genomic stability in all living cells.”

The formal discovery of DNA repair occurred in three laboratories simultaneously. Hanawalt’s graduate research with Richard Setlow, Ph.D. ’47, initiated studies on the inhibition and recovery of DNA synthesis in bacteria following irradiation with ultraviolet light. Then Setlow subsequently found, mutant cells that were sensitive to ultraviolet light retained damage in their DNA, while normal cells cut out the damage. Hanawalt had moved to Stanford, where he showed that repair patches were inserted into DNA, presumably replacing the damaged parts that had been removed. At Yale, Paul Howard-Flanders, Ph.D., isolated mutant bacteria sensitive to ultraviolet light and reported that while normal bacteria removed the damage, the mutant bacteria could not. Damage in DNA, the researchers concluded, can be cut out and the missing parts replaced correctly through a process called nucleotide excision repair.

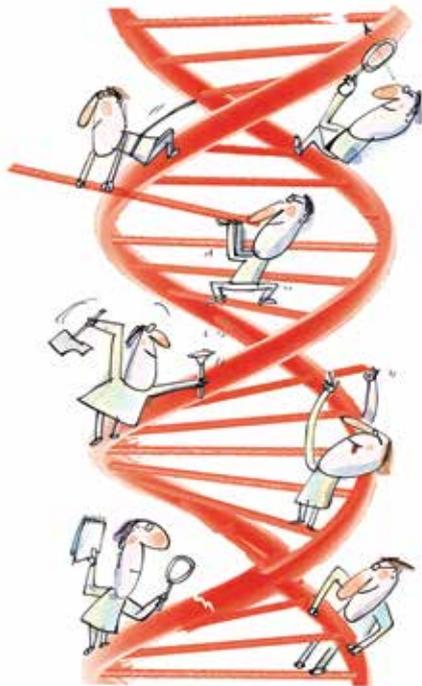
Joann Sweasy, Ph.D., professor of therapeutic radiology and

of genetics, pointed out that DNA repair occurs naturally in our cells every day. “But if the repair isn’t good, or there’s a faulty gene, that’s when you get suboptimal mutations that lead to cancer,” she said.

Peter Glazer, M.D. ’87, Ph.D. ’87, chair and Robert E. Hunter Professor of Therapeutic Radiology, professor of genetics, and member of the faculty advisory committee of the Cancer Biology Institute at West Campus, has overseen a \$9 million grant from the National Cancer Institute titled “DNA repair in cancer biology and therapy.” The title suggests an important goal for the field of DNA repair. The grant, which ended June 30, was to take advantage of knowledge of DNA repair pathways in order to treat cancer. The interdisciplinary effort brought together more than a dozen investigators to focus on fundamental and translational cancer biology.

“The DNA repair field is getting more and more exciting in its complexity and its relevance to human health,” said Hanawalt. “If you Google DNA repair, you’ll get more than 18 million hits. It’s alive and well, and the early insights of radiation biologists at Yale got it started, while current scientists at Yale help to keep it in orbit.”

—Jennifer Kaylin



round up

a collection of recent scientific findings



INFLAMMATION & DISEASE

Everybody knows what happens when we get hurt or infected. The injured part of our body swells. That's our innate immune system offering a short-term fix by attempting to restore the proper physiological balance. But that quick fix may also be a root cause of many serious disorders, according to Ruslan M. Medzhitov, Ph.D., and Richard A. Flavell, Ph.D. The two immunobiologists have proposed a unifying theory to describe how inflammation can affect the body's homeostatic control mechanisms to trigger the onset of disease. Now Medzhitov, the David W. Wallace Professor of Immunobiology and a Howard Hughes Medical Institute (HHMI) investigator, and Flavell, chair of the department of immunobiology, Sterling Professor of Immunobiology, and an HHMI investigator, will have a chance to test their theory of inflammation and chronic disease, thanks to a \$10 million grant from the Blavatnik Family Foundation, the charitable organization headed by American industrialist and philanthropist Len Blavatnik. The grant will support the scientists' efforts to define the molecular links among inflammation, commensal microorganisms, and chronic disease.



MELANOMA SURVIVORS USING TANNING BEDS

About a quarter of those who have survived melanoma leave the sunscreen at home on sunny days, and some survivors still use tanning beds, according to research by Yale Cancer Center (YCC) that was presented at the American Association for Cancer Research Annual Meeting 2013 in Washington, D.C. Both tanning beds and unprotected sun exposure raise the risk of life-threatening skin

cancer. Using data from the National Health Interview Survey, conducted annually by the Centers for Disease Control and Prevention, the Yale team found that most of a sample of 171 melanoma survivors were taking precautions, but 15.4 percent said that they rarely or never stay in the shade; 27.3 percent said that they never wear sunscreen when they're outside on sunny days for more than an hour; and 2.1 percent reported using an

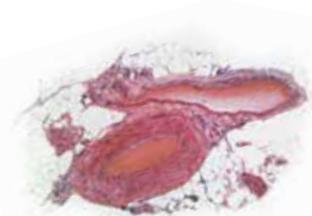
indoor tanning bed during the previous year. "It's incredibly disturbing that even after getting the disease once, some survivors continue these practices, which would put them at greater risk of getting it again," said author Anees B. Chagpar, M.D., M.P.H., associate professor of surgical oncology at YCC and director of the Breast Center at Smilow Cancer Hospital at Yale-New Haven.

CANCER PATIENTS: QUIT SMOKING

It may seem like a no-brainer, but Yale Cancer Center experts and the American Association for Cancer Research (AACR) are calling on doctors to advise their patients to quit smoking after being diagnosed with cancer—any cancer. Patients who smoke have worse outcomes than those who quit, and quitting smoking improves the efficacy of cancer treatments, reduces

risk for future cancers, and enhances rates of survival. This is true for many cancers, not just lung cancer. The experts made their call in a statement released at the AACR Annual Meeting 2013 in Washington, D.C. "It is crucial that all oncologists in any setting both assess tobacco

use and take ownership of ensuring that their patients receive appropriate treatment for their tobacco use," said Benjamin A. Toll, Ph.D., associate professor of psychiatry, director of the smoking cessation program at Yale Cancer Center, and chair of the committee charged with writing the AACR policy statement.



PATHWAY TO NEW ARTERIES

Scientists at the School of Medicine and University College London (UCL) have found a molecular pathway that can bypass blocked arteries and help form new arteries after heart attacks, strokes, and other acute illnesses. The Yale-UCL team reported in the April 29 issue of *Developmental Cell* that in order to make new arteries, which can form in adults when organs become oxygen-deprived, three molecules must work together. The oxygen-starved organs must first release a molecular signal called VEGF. That signal must then bind with two molecules known as VEGFR2 and NRP1. NRP1 transports the other two molecules to a signaling center in the walls of blood vessels. Mice that lacked part of that transporter had poorly constructed arterial branches in their internal organs and could not repair blood vessel blockage by forming new arteries. "This opens new therapeutic opportunities for developing drugs that would either stimulate or inhibit blood vessel formation," said corresponding author Michael Simons, M.D. '84, professor of medicine and cell biology, and director of the cardiovascular research center at the School of Medicine.



imag(in)ing

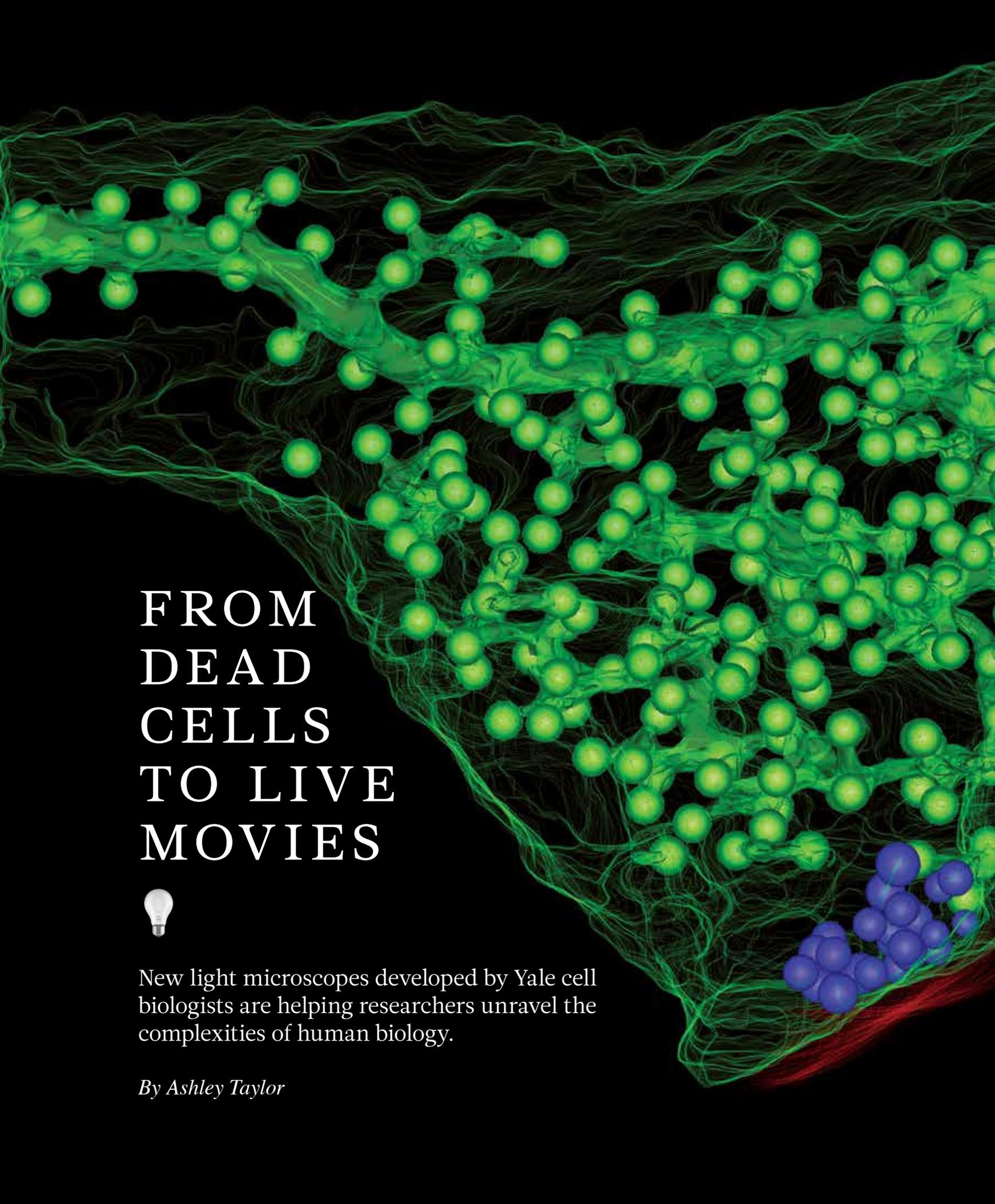
Scientists at Yale and around the world are challenging the laws of physics as they seek new ways to peer ever more deeply into the workings of the human body.

SINCE THE INVENTION OF THE MICROSCOPE in the 1700s, scientists have struggled to find new ways to peer deeper and deeper into the human body to look at smaller and smaller cells and organelles. Constrained for centuries by a law of physics, they began to find ways to overcome what is known as the diffraction limit only in the 20th century. New technologies now allow scientists to see things on a scale once thought impossible, and the knowledge gleaned from those views is helping clinicians find new ways to care for patients. In this issue of *Yale Medicine*, we explore the ways in which Yale investigators and physicians are pushing the frontiers of imaging technology in a search for answers to human biology and disease. Our lead story by Ashley Taylor, “From dead cells to live movies,” looks at super-high-resolution microscopy, which has advanced cell biology by offering real-time glimpses of what one scientist calls “cellular fireworks.” John Dillon interviewed a neurosurgeon, a diagnostic radiologist, and a urologist who work with scientists to improve care for their patients through new imaging techniques. Jenny Blair delved into the debate in neuroscience over the mind and the brain: Can scientists determine your political, moral, or philosophical leanings based on an fMRI scan of your brain? And Amanda Alvarez described core imaging facilities at the School of Medicine that provide access to new technologies.



“Valentine,” by Elizabeth Jameson, is a Solarplate etching on paper that shows a coronal view of her brain stem, cerebellum, and lateral ventricles. In her art, Jameson said in her artist’s statement, she celebrates the beauty, complexity, and mystery of the brains of individuals who, like herself, have one of the most common neurological diseases of the human body, multiple sclerosis.

/ 33 Inside the toolbox / 36 A new vision in the lab and in the clinic

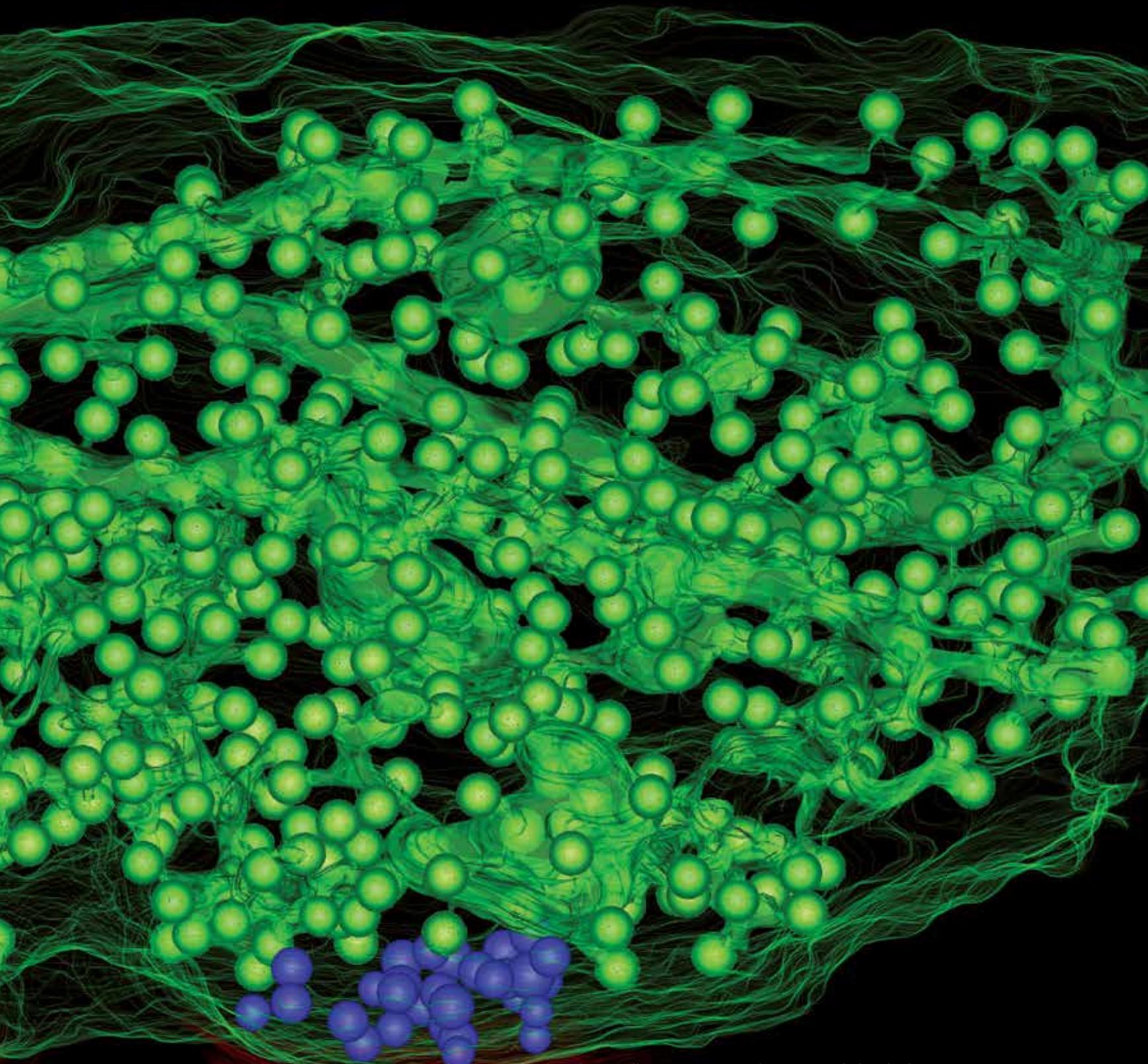


FROM DEAD CELLS TO LIVE MOVIES



New light microscopes developed by Yale cell biologists are helping researchers unravel the complexities of human biology.

By Ashley Taylor



Pietro De Camilli, who studies how brain cells package neurotransmitters, used electron tomography to capture this 3-D image that shows what happens when the process goes awry. Vesicles about 40 nanometers in diameter (blue) deliver neurotransmitters between cells by fusing with the outer membranes of neurons. Normally, after they release their

cargo, vesicles are recycled and take on more neurotransmitters for another shipment. When the protein dynamin is mutated, this recycling does not occur and vesicle buds (green) that cannot be released to generate new vesicles accumulate at the cell surface and its infoldings.



IN 1974, THE LATE GEORGE E. PALADE, PH.D., chair of Yale's newly formed cell biology department, shared a Nobel Prize in physiology or medicine for using electron microscopy to elucidate the inner workings of cells—ground-breaking findings that some say ushered in the modern field of cell biology. But although the electron microscope opened new avenues of research, it had a huge drawback as a tool for studying life: it can observe cells only after they are dead, treated with special fixatives, and sliced into thin sections or coated in a layer of metal. The grayscale world pictured in such detail in electron micrographs, while powerful, is “a cellular cemetery,” in the words of Pietro De Camilli, M.D., FW '79, the Eugene Higgins Professor of Cell Biology, professor of neurobiology, and director of the Yale Program in Cellular Neuroscience, Neurodegeneration and Repair.

Because electron microscopy's vision is limited to dead cells, it provides just a snapshot of a cell's inner workings. Derek K. Toomre, Ph.D., associate professor of cell biology, likes to compare an electron micrograph of a cell to a still photograph taken during a football game. If you are trying to learn the rules of the game, Toomre said, a snapshot doesn't get you very far. The same is true in biology. “There are a lot of biological problems that—if you could see them in living cells in action—we would be able to unravel.”

To observe live cells, scientists use light microscopy, which includes the dissecting microscopes familiar from high school biology and extends to high-tech microscopes whose images brighten the pages of scientific journals.

But standard light microscopy too has a major limitation in resolution: scientists have known since the 19th century that it cannot resolve, or distinguish between, structures smaller than about the size of organelles. Smaller structures—the vesicles carrying cellular messages and the protein scaffolding that gives cells their heft and shape—blur together because of what is called the diffraction limit, described in 1873 by Ernst K. Abbe, a contemporary of the microscope manufacturer Carl Zeiss.

A light microscope, even with an excellent lens, cannot resolve structures smaller than about half the wavelength of the light used to illuminate them. That works out to a resolution of about 250 nanometers, around the

size of the measles virus and about 400 times smaller than the width of a strand of human hair.

In trying to learn the rules of cell biology's game, scientists had at their disposal detailed still images from the electron microscope and views of the cell in action from the light microscope, with some of the most interesting players too small to see. Although each type of microscope had its uses, between them lay a large gap.

In the last 20 years, however, scientists have found ways to overcome the diffraction limit and close that gap through what is called super-resolution light microscopy. Using custom-made fluorescence microscopes, some designed by Yale scientists, researchers at Yale are observing the live-cell dynamics of structures that they could previously see only in snapshots. With these new data, they are beginning to answer scientific questions nearly as old as the limit that once held them back.

“This is the direction in which we have to go,” said De Camilli, who studies how brain cells package neurotransmitters, the chemicals that pass along neuronal signals. “Super-resolution microscopy is really the next critical step.” Gesturing toward a photo on his wall of a smiling Palade, who looks as though he's listening in on our conversation, De Camilli continued, “Palade was a pioneer in the use of electron microscopy. We feel like super-resolution microscopy is the next frontier in microscopy, and we think it's appropriate that it happen here, in the heritage of George Palade.”

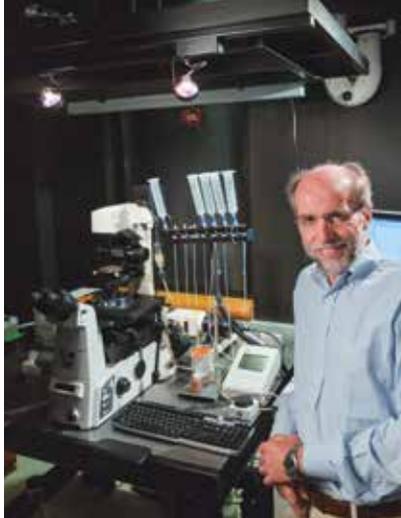
CELLULAR FIREWORKS

Joerg Bewersdorf, Ph.D., assistant professor of cell biology and of biomedical engineering, “stumbled into microscopy” in 1996. Then an undergraduate studying physics at the University of Heidelberg, Bewersdorf wanted to develop technologies that would help scientists in other fields. After taking an optics class with Stefan W. Hell, Ph.D., who, Bewersdorf said, was then “a junior professor, not really known, just a very dynamic person,” Bewersdorf joined Hell’s new lab at the Max Planck Institute for Biophysical Chemistry in Göttingen, which was quite small at the time. “Eight years later, when I left,” Bewersdorf remembers, “the lab had like 35 people, Hell was famous and had won a lot of awards, and this whole field of super-resolution microscopy had taken off.”

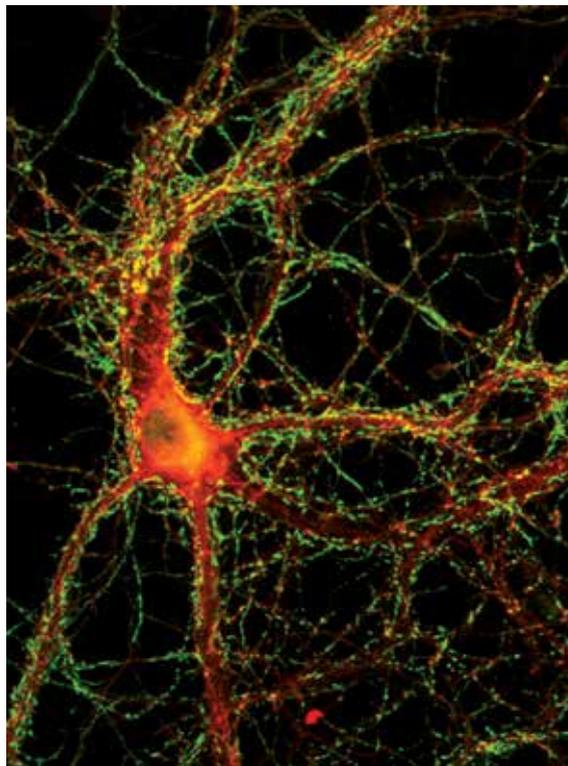
Hell, director of the institute since 2002, had broken the diffraction limit, building a microscope that allowed scientists to see tiny biological structures in a way not thought possible. For years Abbe’s diffraction limit had been considered dogma, with scientists skeptical of attempts to bypass it, Bewersdorf said. Breaking the limit required a change in the way scientists thought about microscopy.

“To really break the diffraction limit, you can’t think of the microscope as just optics,” said Bewersdorf. “And this is what people had done for 150 years—it was always about lenses or it was always about light.” Instead, said Bewersdorf, Hell was thinking about the interaction of the microscope light with the cells or tissue being examined. Hell believed that resolution could be improved, not by modifying the light used to make a sample fluoresce but by altering the fluorescent light as it is emitted. Using this approach, Hell theorized in a visionary 1994 paper in the journal *Optics Letters* that he would be able to achieve a resolution of 35 nanometers—small enough to see not just organelles but structures within them, like the involutions of the mitochondria, the cell’s power plants, or the many layers of the Golgi apparatus, the cell’s protein-processing pipeline.

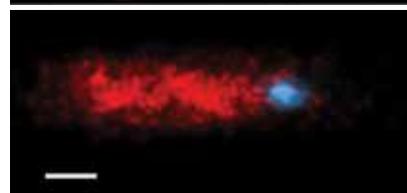
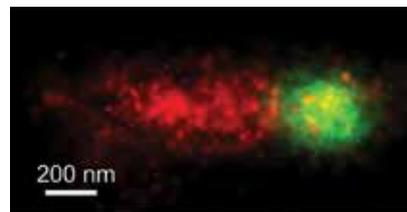
By 1999, Hell had built a super-resolution microscope. His new technique was called stimulated emission depletion (STED) microscopy. A year later his lab showed that STED could work with biological material. In 2006, other research groups independently published papers



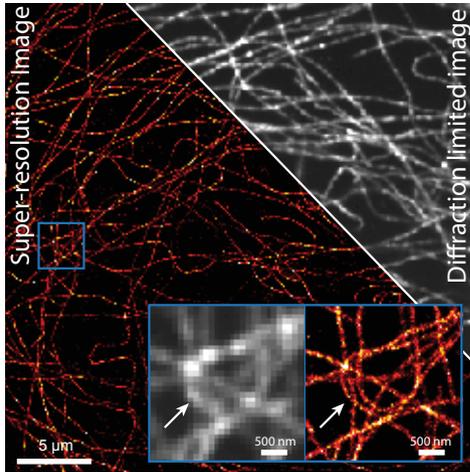
TOP “This is the direction in which we have to go,” said Pietro De Camilli. High-resolution imaging technologies can overcome the diffraction limit that held back advances in cell biology for many years. De Camilli, shown here with a TIRF microscope, uses the new imaging modalities to study how brain cells package neurotransmitters.



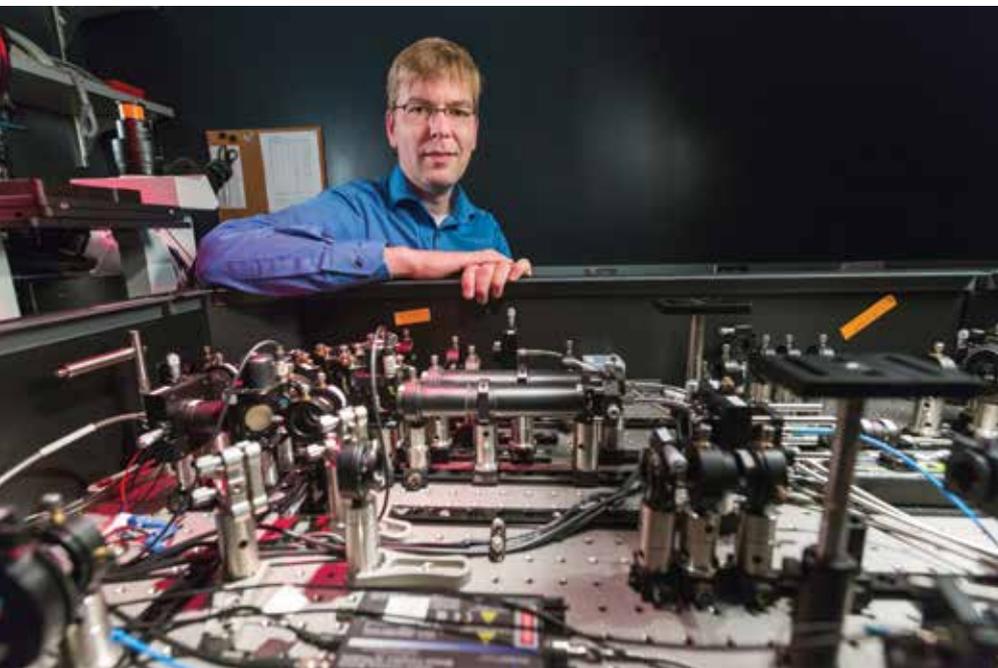
MIDDLE Epifluorescence microscopy produced this image of synapsin (green spots on the surface of two neurons) and adaptin (in red). These two proteins play a role in forming and storing neurotransmitter-filled synaptic vesicles at sites where neurons communicate. Comparing the staining patterns of synapsin and alpha-adaptin—under different conditions of neuronal activity and/or after pharmacological and genetic perturbations—allows researchers to assess the status of synaptic vesicles and their recycling. Shawn Ferguson, who produced this image, noted that it “does not boast a high degree of resolution—hundreds of nanometers.”



BOTTOM De Camilli and his collaborators used super-resolution microscopy to create these images of vesicles being reformed. The color images show the location of two proteins, dynamin and clathrin, involved in that process. “We are zooming in at incredible levels of resolution,” he said. The color images were taken with fluorescence microscopy and the other image was taken with electron microscopy.



describing a different approach to light microscopy that achieved even higher resolution than Hell’s technique. Each group gave its version of the technique a different name: photoactivated localization microscopy (PALM); fluorescence photoactivated localization microscopy (fPALM); and stochastic optical reconstruction microscopy (STORM). All three versions, however, rely on the same principle—imaging a fluorescently labeled sample a few scattered points at a time. By 2012, scientists were using the term “diffraction-unlimited microscopy,” or even “nanoscopy,” to reflect the fact that these new microscopes work on the scale of nanometers. The paradigm shift was complete.



TOP A split image of microtubule structures shows the advantage of a super-resolution pointillism technique called fPALM/STORM over traditional imaging methods. Thousands of images of fluorescent molecules are taken and reassembled by computers. Microtubule structures are important in processes ranging from maintaining cell structure to providing platforms for intracellular transport.

ABOVE This is not your grandparent’s microscope. In their efforts to obtain images of smaller and smaller structures, scientists have moved away from lenses and light to such complex and sophisticated devices as this STED microscope, which relies on fluorescence and laser beams. Joerg Bewersdorf trained in physics, but now works with cell biologists looking for images of ever-smaller cellular structures.

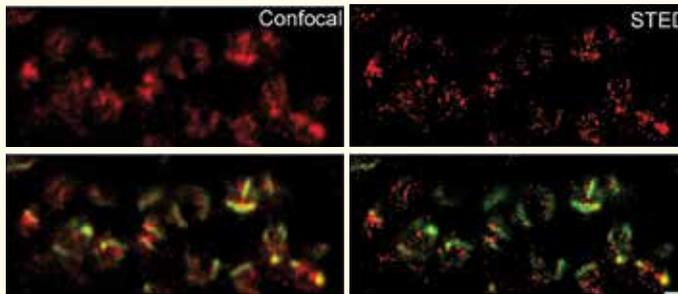
At about the time that Hell was developing his new microscope, from 1997 to 2001, Toomre was also in Germany (though he did not meet Bewersdorf until they both came to Yale) as a postdoctoral fellow at Heidelberg’s European Molecular Biology Laboratory. Toomre was trying to learn about vesicles, the bubble-like structures that cells use to shuttle materials in and out and from one cellular location to another. Vesicles are ubiquitous in biology—they transport everything from hormones to neurotransmitters. In the brains of animals, signals pass from one neuron to another thanks to a process called exocytosis, in which vesicles packed with neurotransmitters fuse with the cell membrane of one neuron and empty the chemicals into the synapse—the space between it and the next neuron—to transmit the message. (James E. Rothman, Ph.D., the Fergus F. Wallace Professor of Biomedical Sciences and professor and chair of cell biology, shared in the 2013 Nobel Prize for physiology or medicine for his studies of vesicles.)

“I was frustrated,” said Toomre, “because we knew biochemically that these things had to go out to the surface, and we could see these little vesicles moving, but we really didn’t see them fuse.” He had tried to simulate vesicle fusion in a test tube, an effort that failed after a year. But he had heard about a microscope called a total internal reflection fluorescence (TIRF) microscope that might help. The TIRF microscope could selectively illuminate objects in a thin 80-nanometer optical section and achieve much higher resolving power than traditional confocal microscopes—but only near the surface of the cell. The technology was perfect for observing the fusion of a vesicle with the cell membrane. Toomre “begged and pleaded” to

Breaking the diffraction limit

The period at the end of this sentence is 1 million nanometers wide. With super-resolution microscopy, scientists can see synaptic vesicles as small as 30 nanometers wide. Imagine taking a picture of the continental United States from the stratosphere and being able to distinguish a single strand of hair.

Fluorescent tags make this possible. Molecules and structures of interest are given a fluorescent



tag—either a dye or a genetically engineered tag like green fluorescent protein (GFP). A laser beam is directed at the target sample, which makes the tag emit light, and the resulting fluorescence is recorded to create an image.

The first fluorescence microscopes scanned an entire sample at once, which produced a fair amount of out-of-focus fluorescence. Today's standard fluorescence microscope—the confocal microscope—scans a sample one point at a time, pixel by pixel, and assembles the pixels to create an image. The size of each fluorescent point in these standard microscopes is determined by how much the laser light diffracts and is limited by the diffraction limit to between 200 and 250 nanometers.

Stefan Hell's innovation, STED, improves resolution by reducing the size of each fluorescent spot. STED targets the light returning from the sample and a second laser blocks out the fluorescence in a donut shape around the center of each fluorescent

spot. Each fluorescent point is reduced to the size of the donut hole. These smaller points of light yield a higher-resolution image. STED microscopy can achieve a resolution of 25 to 80 nanometers, small enough to distinguish cellular vesicles and the folds within organelles.

PALM/fPALM/STORM capture just a few scattered molecules at a time so that they are unlikely to overlap and blur together. Using labels that turn on and off, scientists arrange to have only a few molecules fluoresce at one time; then they take a picture. A computer finds the center of each spot, representing a single fluorescent molecule, on the individual photo. This process is repeated thousands of times, and the photos are then combined. The approach is sometimes called pointillist microscopy, after Impressionist Georges Seurat's painting technique. Pointillist techniques achieve extremely high resolution, about 25 nanometers. However, the technique can also be slow—it requires many photos to generate one image, and it is dependent on high-powered computers to process the data.

TIRF microscopy, developed in the early 1980s, excites fluorescence in a thin layer near the cell surface, which reduces background fluorescence and improves resolution to between 40 and 100 nanometers. TIRF microscopy is faster than pointillist techniques but has lower resolution and can record only the cell surface.

These are only a few of the high-resolution microscopy techniques available today, and Yale is unusual in that it has all these microscopes—STED, PALM/fPALM/STORM, the electron microscope, and others—in one place, said Derek Toomre. Each has its strengths and weaknesses. "If we knew that there was one type that could do everything, we wouldn't be investing in all of them. ... There's no clear winner. We'll see; maybe there will be."



“Cavorting wee beasties”

The magnitude of advances in microscopy becomes clear in the context of its four-century history.

Seeing the unseeable

Starting around the turn of the 17th century, natural philosophers using the light microscope saw things where, to the naked eye, there was nothing to see. The Englishman Robert Hooke observed pockets of air within cork, which he called cells; the Dutch scientist Anton van Leeuwenhoek saw living bacteria in pond water and cells within blood and even found “wee beasties,” as he sometimes called his “cavorting” specimens, in his own semen.



Electron microscope



In the 1930s, the German physicist Ernst Ruska developed a microscope with ultra-high resolution by using electrons, which have a smaller wavelength than light and can distinguish tinier features. Using Ruska’s new electron microscope, scientists could view structures within an individual cell, with the downside that they could not look at live cells.

borrow the TIRF microscope in the lab of cell physiologist Wolf Almers, Ph.D., who was then at the Max Planck Institute. “Within a few hours of imaging,” said Toomre, “we had an amazing result. We could see these vesicles arrive and explode during fusion.” In 2000, Toomre, Almers, and others published their observations in *The Journal of Cell Biology*. This was Toomre’s entrée into super-resolution microscopy, which he would pursue at Yale beginning in 2001.

In his office, Toomre shows a more recent video generated by a TIRF microscope of fluorescently labeled vesicles fusing with the cell membrane. Fluorescent green dots—the vesicles—move around on the screen, then flash brightly as they fuse with the cell membrane. “It’s fireworks,” Toomre said. “Cellular fireworks.”

NEW FRONTIERS ... IN BIOLOGY

Bewersdorf, one of the first physicists recruited to the highly interdisciplinary Department of Cell Biology at Yale, joined the faculty in 2009 because he wanted to collaborate with biologists who were using these new microscopes to answer important questions in biology.

A burning question both within and outside the department: How does the Golgi apparatus, the cell’s protein processing plant, work? A stack of membrane-bound disks, the Golgi processes proteins into their final forms, adding sugar and phosphate molecules as they pass from one end of the stack to the other and are sorted to other areas of the cell. If necessary, the Golgi packages them into vesicles to be released from the cell. For 100 years, Toomre said, scientists have debated whether the Golgi is a stable structure that moves vesicles around or a dynamic structure that transforms itself into the vesicles it releases. The debate continues, as a major roadblock is the inability to see small vesicles trafficking within the highly convoluted Golgi “pancake” in live cells. Now, by labeling both the Golgi and the proteins moving through it, then watching the labeled cells at super-resolution, an international consortium of researchers at Yale, Oxford, and Cambridge are hoping to find the answer.

Vesicles are also a focus of De Camilli’s lab, which is studying the way they are made. Vesicles are formed by pinching off from a larger membrane, like the cell membrane or the membrane of an organelle. De Camilli wanted to know which proteins are responsible for cutting the new vesicle off from its parent membrane. Two proteins might be involved, he thought: clathrin and dynamin. He wanted to see where the two proteins are located on the vesicle. In his office, De Camilli draws furiously on a scrap of paper: green for clathrin,

gray for dynamin. Under regular light microscopy, clathrin and dynamin seem to overlap. To demonstrate this overlap, De Camilli draws green and gray swirls, one atop the other. But using super-resolution microscopy, he shows me that dynamin is clearly distinguishable from clathrin.

... IN MEDICINE ...

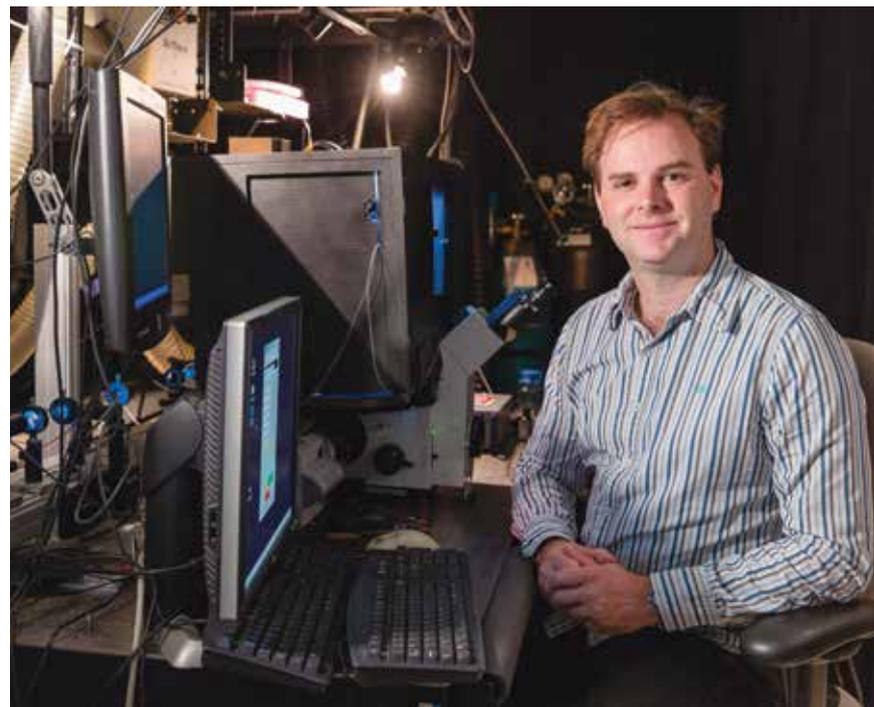
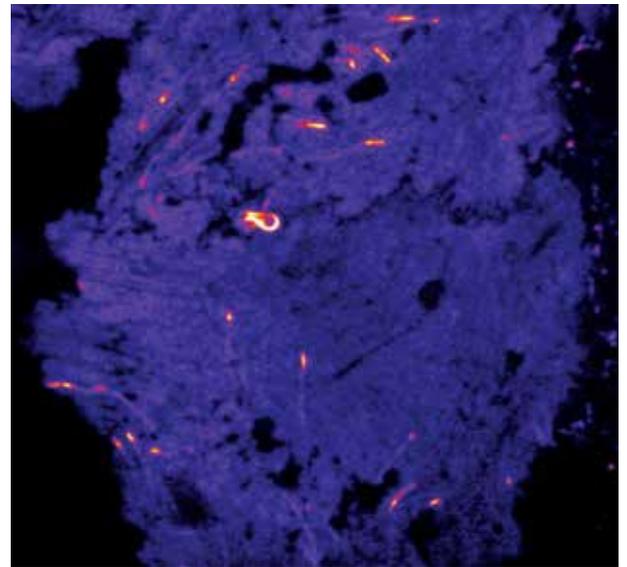
The new information about cell structure revealed by super-resolution microscopy is helping scientists to understand the mechanisms of diseases that affect humans, De Camilli said—in particular, diseases rooted in genetic mutations. Many genetic disorders, he said, result from changes in the distribution or localization of proteins in cells. “In order to understand in which way the mutation affects cell function, it is very useful to be able to localize either the mutant protein itself or organelles and proteins with which it interacts or the organelle on which it is localized,” De Camilli said.

For example, De Camilli is studying Lowe syndrome, a rare disorder that almost exclusively affects males and causes intellectual disability, congenital cataracts, and kidney problems. His previous research had revealed that, on the molecular level, Lowe syndrome causes problems with endocytosis, the process by which a vesicle empties its contents into a cell. Using super-resolution microscopy to monitor the distribution of the normal and mutant Lowe syndrome proteins on endocytic vesicles, De Camilli hopes to better understand the mechanisms of the disease, with implications for therapy.

Toomre is using TIRF microscopy to study diabetes by watching the way fat cells respond to insulin. When fat cells are stimulated with insulin, he has found, vesicles whose membranes contain sugar transport proteins rush to the cell membrane and fuse with it, adding the transporter proteins to the cell membrane and allowing the fat cells to take up more glucose. In diabetes, this process is somehow disrupted, and Toomre hopes to find out how. So far, he said, “Using this TIRF microscopy, we discovered that there were two different types of vesicles arriving at the surface, and until we could see it, we didn’t realize that.”

... AND IN TECHNOLOGY

On the cellular level, the Golgi apparatus is the new frontier. In the lab, this new frontier is studded with giant microscopes enclosed in black boxes to keep out light and prevent temperature fluctuations. Bewersdorf walks me through his lab. His custom-made STED microscope looks in part like other confocal microscopes I’ve seen,



TOP Throughout his career Derek Toomre has tried to learn more about vesicles, structures that move proteins, neurotransmitters, and other cargo in and out of cells and from one cellular location to another. This composite image, taken with dual-color live-cell epifluorescence microscopy, shows vesicles moving along microtubule “highways.”

ABOVE Toomre believes that seeing cells in real time is crucial to understanding human biology. “There are a lot of biological problems that—if you could see them in living cells in action—we would be able to unravel.”

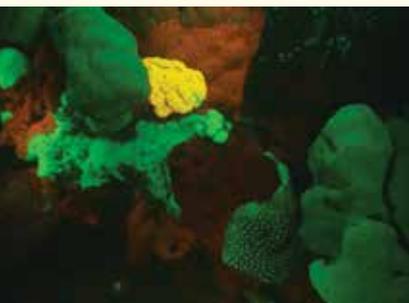
Dyes and stains

In 1873, the Italian physician and scientist Camillo Golgi stained neurons using a silver compound that turned the cells black. The Spanish neuroanatomist Santiago Ramón y Cajal put Golgi's method to fruitful use, making observations that led to the neuron doctrine, the now-accepted idea that the nervous system is composed of discrete cells. In 1886, Paul Mayer invented the hematoxylin and eosin staining procedure. Hematoxylin stains cell nuclei blue; eosin is nonspecifically attracted to proteins and gives the rest of the cell a contrasting reddish hue. The most important dyes used in light microscopy today, however, are fluorescent.



Fluorescence microscope

Though the fluorescence microscope was invented around 1910, fluorescence microscopy did not really take off until the end of the century, spurred by the development of fluorescent labels for specific biological structures.



The most famous of these fluorescent tags is called green fluorescent protein, or GFP, a protein derived from jellyfish that emits green light when stimulated

by blue light. [For more on the use of marine life as a source of fluorescent tags, see “In coral reefs, a treasure trove of tools” on next page.] In the 1990s, scientists isolated the gene encoding GFP, which allowed them to engineer cells genetically so that GFP could be fused to a protein of interest for visualization with the fluorescence microscope. Microscopy's palette expanded as scientists developed variations of GFP that fluoresce in different colors; and by labeling different structures with different fluorescent molecules that can be visualized at the same time, scientists can determine whether those structures are colocalized and potentially interacting. Fluorescent labels are not limited to proteins: they can also label DNA, lipid molecules, and carbohydrates. And efforts to break the diffraction limit would increasingly rely on these fluorescent proteins.

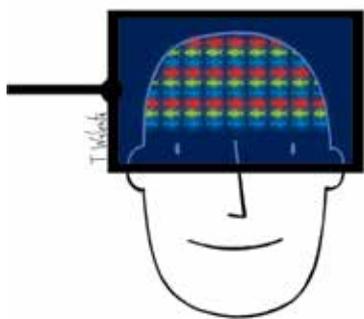
with eyepieces and a computer screen displaying an image. But nearby is a table with black sides reminiscent of a filled casket. Inside are black tubes with white labels, lined-up lenses of different tints, and blue and silver cables. The laser beam, he said, travels through the blue cables; the fluorescence travels to the detector through the silver ones. What's good about these custom-built microscopes, Bewersdorf said, is that he can easily adjust them for different samples.

“A lot of the things at the edge are not commercial. A lot of the microscopes that you'll see in Joerg's lab are custom-made,” said Toomre, “and they're custom-made because that's the only way you can do it.”

Bewersdorf has achieved his goal of working with scientists in other fields, and the number of scientists who can thank him is likely to grow. “Super-resolution is something that just about everyone is trying to jump into,” said Michael W. Davidson, Ph.D., a Florida State University scientist who is collaborating with Bewersdorf and Toomre by providing fluorescent proteins from his large collection. “It's had a huge impact, but I think the impact is just starting. I think almost everybody's going to be doing it within 10 years.”

Bewersdorf and Toomre are working with microscope companies to commercialize the instruments that they have custom-built in their labs. For now, though, this is what Toomre calls “the edge,” the frontier of science. I asked Bewersdorf if he thought the resolution of light microscopy would continue to improve. “No,” he said. The goals are no longer about resolution. Now the challenges are finding compatible fluorescent labels in order to watch multiple structures simultaneously and developing cameras that can capture the images faster and faster to create videos of cellular structures in motion. Perhaps the most important challenge is to apply this technological tool kit to questions of neuroscience, metabolism, and cancers whose answers may be central to human health. Bewersdorf shows me a pointillist microscope, also custom-built in his lab. That microscope, armed with a digital camera, can take photos so fast that they can be used to create high-resolution movies of fluorescently labeled cells, as Bewersdorf and colleagues reported in a *Nature Methods* paper published online in May. I think back to Toomre's analogy about trying to learn the rules of football from a snapshot. At last, a high-resolution movie of cells at play. Now scientists can really learn the rules of the game. ***/yale medicine***

Ashley Taylor is a freelance writer based in New York City.



In coral reefs, a treasure trove of tools

By Amanda Crowe

Few things are as mysterious and captivating as the ever-changing mosaic of colors emanating from the sea life in and around coral reefs. This shifting kaleidoscope of colors comes in part from tiny fluorescent proteins produced by coral and other marine species—part of a creative adaptation that helps them hide from predators, attract friends, and survive in the ocean's battlegrounds. Now scientists are harnessing this evolutionary feat to develop novel, noninvasive ways to detect and monitor neurological and other diseases and, possibly, to tailor treatments.

Vincent A. Pieribone, Ph.D., professor of cellular and molecular physiology and of neurobiology, and other scientists are using these proteins to expose the electrical activity of neurons and other cells, thereby making invisible cellular processes visible in ways that are not possible with conventional imaging techniques.

For more than a decade, Pieribone and his team have been on a quest to find and clone new fluorescent proteins from such far-flung places as Austr-

alia's Great Barrier Reef and the Solomon Islands. In the lab, they insert these proteins into animals to track and monitor brain activity and then decode how and when neural cells fire. When inserted into neural tissue, these fluorescent proteins produce a glow that is visible through the skull and skin, converting the surface of the brain into something akin to a television screen and revealing pictures of the processes within.

Every time the tissue produces an electrical signal, the intensity of light changes. These fluorescent proteins don't just highlight the cells: Under certain conditions they can be stimulated to change the output of light intensity so that scientists can see biologic processes unfolding in real time. Using computers, researchers can record and observe this complex display of light over time to interpret the behavior of the neural tissue. Clinicians may one day be able to use these

proteins to monitor and predict epileptic seizures.

"It's allowing us to get a glimpse into the complex workings of the brain," said Pieribone. "With these proteins, we can generate really powerful probes that we can then put into any cell and they will report the voltage of the cell as a change in fluorescence—it's a real breakthrough to optically look at cells firing at high speeds."

The use of fluorescence in imaging has inherent benefits, too. It avoids the use of invasive electrodes, radiation exposure, or contrast agents. "We currently stick wire electrodes into the brain to touch the cells, and when they give off electrical bursts we can try to figure out what the brain is doing and saying, but it's invasive and causes permanent damage to the very organ you are trying to study," Pieribone explained.

His work was initially inspired by the discovery in the early 1960s of a green fluorescent protein (GFP) in a species of jellyfish known as *Aequorea victoria*, which is indigenous to the Pacific Northwest. This discovery resulted in a Nobel Prize in chemistry in 2008 and has revolutionized biomedical research, he said.

Despite the discovery's promise, Yale is one of the few academic centers in the world to engage in this type of field research. Pieribone has made a dozen trips to search some of the most diverse aquatic ecosystems for new fluorescent proteins. In all, Pieribone has identified over 100 species of fluorescent coral and other ocean life, most recently from

eels and fish. "We have found fluorescent properties in a huge range of animals—far more than was thought possible," said Pieribone. This discovery means greater opportunity and potential applications for medicine.

The irony, he says, is that the very coral reefs that produce these proteins—molecules humans could never invent on their own—are disappearing. The reefs are sensitive to warmer ocean temperatures and pollution, which cause them great stress and leave them vulnerable to disease. "We have these libraries of cool proteins vanishing from the earth as quickly as we can get them, clone them, and study them," he said.

What does the future of fluorescent protein technology hold?

While scientists are now using light to interact with nervous tissue both to observe and to control what the brain is doing, researchers believe that these proteins might lead to better treatments down the line, perhaps even linking mind to machine. For someone with a spinal cord injury, for example, where the brain no longer communicates with the body, the hope is that a computer could convert brain activity—such thoughts as, "I want to pick up that glass"—into action.



CAN BRAIN SCANS REVEAL HOW WE THINK?



Scientists debate whether such higher-order processes as consciousness and morality have their own real estate in the brain.

By Jenny Blair Illustration by Francis Blake



SOME PEOPLE WITH ALCOHOLISM can change their behavior and remain abstinent, while others fight the battle over and over again. And while treatment for alcohol abuse is often effective, many patients wind up backsliding. If clinicians knew which ones are most likely to do so, they could intervene to help them stay sober.

In the not-too-distant future, a clinician in that position might turn to brain imaging for answers. Using functional magnetic resonance imaging (fMRI), a method that maps neural activity to specific locations in the brain, Yale scientist Dongju Seo, Ph.D., and Rajita Sinha, Ph.D. '92, Foundations Fund Professor of Psychiatry, professor of neurobiology, and in the Child Study Center, examined 45 alcohol-dependent patients and compared the scans of those who later relapsed to the scans of those who did not. Surprisingly, when the relapse group tried to mentally relax, the prefrontal cortex failed to settle, and during stressful thoughts, it failed to activate, according to their study in *JAMA Psychiatry* in May. If your brain activity looks like that, you're less likely to stay sober—or so, apparently, says the scan.

fMRI is the closest we can come to watching the brain at work. Its vividly colored images seem to offer snapshots of thought and emotion themselves. The central tool of many brain researchers at Yale and around the world, fMRI holds the promise of illuminating the brain-mind connection.

Hal Blumenfeld //

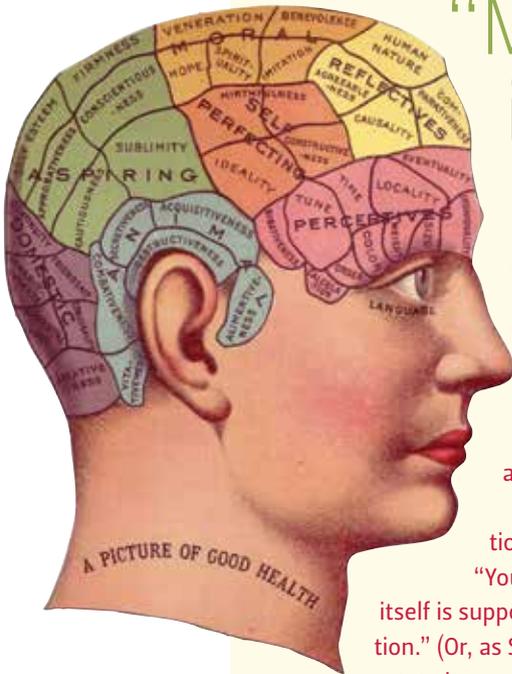
“What [fMRI] does is provide a wonderful initial draft or road map of structures that could be important and that should be investigated through other techniques.”

“It provides information that can't be obtained with any other approach right now,” said Hal Blumenfeld, M.D., Ph.D., FW '98, professor of neurology. The method is noninvasive and shows the whole brain at once, with better resolution in time and space than older methods can offer.

Certainly, fMRI's ability to peek into our heads hasn't been lost on lawyers, advertisers, and entrepreneurs. Brain scan findings have been used in court to defend sociopaths, while “neuromarketers” have used fMRI to measure audience reactions to a Harry Potter movie trailer. A company called No Lie MRI claims to have developed a reliable lie detector test—or “truth verification technology”—based on fMRI.

Yet as the technology comes of age, some observers of the field are calling for caution, and earlier this year two Yale authors published books arguing that fMRI is all-too-often misused. In *Brainwashed: The Seductive Appeal of Mindless Neuroscience*, co-author Sally Satel, M.D., HS '88, a lecturer in the Department of Psychiatry, examines the implications that our hasty embrace of fMRI may have for the concepts of free will and human agency. Amid the popular enthusiasm for brain images, she argues, misunderstandings abound and dubious conclusions are often drawn. For example, when predicting an alcoholic patient's behavior with fMRI findings, she said, we risk falsely concluding that relapse is inevitable.

In his recent book, *Brain Imaging: What It Can (and Cannot) Tell Us About Consciousness*, Robert G. Shulman, Ph.D., professor emeritus of molecular biophysics and biochemistry, questions whether fMRI should be used to study such higher-order cognitive processes as working memory, attention, and consciousness. A biophysicist who pioneered the technique in the early 1990s, Shulman believes that the design and interpretation of many studies that use it have been faulty. The brain, he argues, is best studied just like any other organ—via a physiologic approach that can identify neural



“Mixed-use development” in the brain

The debate over localization long predates the introduction of fMRI. In the early 19th century, Franz Joseph Gall, the founder of the now-discredited discipline known as phrenology, proposed that the brain comprised distinct functional units whose usage was reflected by bumps in the skull. But when experimental physiologists of that era tried to confirm this notion by studying brain damage in birds, they failed to find specific functional losses.

Later, studies of human strokes, the discovery of neurons, and the beginnings of a distinction between localized symptoms and localized functions continued to fuel arguments about whether brain functions are discrete and easily mapped.

Some researchers think that the truth lies between the two extremes: simpler functions are localized in modules, or specific areas, while more complex ones are distributed. “You have some modularity,” said Yale’s Douglas Rothman, Ph.D. ’87, “but the modularity itself is supported in networks, not in discrete regions completely responsible for complex function.” (Or, as Sally Satel, M.D., and her co-author, Scott O. Lilienfeld, Ph.D., put it, “most neural real estate is zoned for mixed-use development.”)

processes that are necessary for a person’s behavior.

But many neuroscientists believe that fMRI can indeed get at higher-order functions—especially when combined with other measurement methods—and that research methodologies are improving, reducing the risk of unwarranted conclusions. They say that Shulman’s call to limit themselves to neurophysiology and behavior would do science and patients a disservice.

PRESENT AT THE CREATION

Shulman was among the first physicists to study biological systems with nuclear magnetic resonance, and by the late 1970s, working at Bell Labs, he was using it to study how glucose is metabolized in yeast and muscle. That decade also saw the first magnetic resonance images and the first whole-body MRI scanner. Improvements in MR technology set the scene for the development of functional MR imaging at Yale and the University of Minnesota in 1992 (see sidebar: “BOLD beginnings,” page 26).

MR imaging had been a major advance in revealing anatomical structures. Functional MR went a big step

further by mapping brain activity to specific locations and superimposing that data over the MR image. It exploits the propensity of hemoglobin to behave differently in a magnetic field—depending on whether or not it is oxygenated—a principle called blood-oxygenation-level-dependent (BOLD) imaging. Because active neurons consume oxygen, the brain compensates by sending oxygen-rich blood their way; fMRI can map areas of neuronal function by tracking the flow of oxygenated hemoglobin.

After the initial studies in 1992, scientists rushed to adopt fMRI, finding it a faster, more accurate, and more accessible way to image brain activity than such older technologies as positron emission tomography, or PET. Early experiments yielded detailed, reproducible maps of brain areas corresponding to sensory and visual stimulation. The technique has been central to the advent of cognitive neuroscience, a developing field that studies the neural basis of higher brain functions. Cognitive neuroscience studies have implicated the amygdala, for example, in evaluating threats and mediating emotional learning. Circuits in the hippocampus



appear to be critically important for relational memory, which allows us to associate names with faces. Parts of the prefrontal cortex seem to power down in schizophrenia, and so on. Previous methods had produced a great deal of information about the functions of these and other brain areas, but fMRI allowed scientists to ask more sophisticated questions and clarify what they previously had only suspected was true.

In 2000, for example, investigators at Yale's Child Study Center found evidence, through fMRI, that subjects with autism don't process faces in the brain's facial recognition center. Instead, they use an area

of the brain normally associated with recognizing objects. The finding caused a sensation in the autism community—it seemed to explain why autistic children tend to show little interest in faces. Their 1997 work followed studies by a group at Yale led by Gregory McCarthy, Ph.D., and by another group at Harvard and Massachusetts General Hospital led by Nancy Kanwisher, Ph.D. She and colleagues including Marvin M. Chun, Ph.D., now a professor of psychology at Yale, showed that this brain region—the fusiform face area—is selectively activated by faces, confirming years of suggestive but ambiguous data from other methods. It is today the most-cited fMRI brain research paper in the scientific literature.

WHERE MORALITY AND MEMORY RESIDE?

Shulman believes that many fMRI studies are too ambitious. Mapping brain areas specialized for sensory or motor systems is one thing. Mapping the life of the mind is quite another. Memory and attention are subjective processes that cannot be experienced by an observer, and they may not be as discrete as we think they are. We talk about remembering to pick up the kids or remembering a phone number, but those two acts may not be as fundamentally similar as our single term for them would imply. “When you start looking for localization of concepts like honor, values, morality, memory, consciousness, you aren't going to find them,” Shulman said, “because we have never learned exactly what they are.” Shulman points to a UCLA study purporting to show that Republicans have higher amygdala activation and are more likely to vote based on fear and other emotions. Such experiments, he said, constitute “phrenological fMRI,” a term critics have used since the early 2000s to dismiss such research.

To grapple with such objections, it's important to understand a few things. For one, the brightly colored images that appear in journals and news reports usually don't represent one brain at one time; rather, they represent highly processed, composite results obtained by processing several individuals' brain data through statistical algorithms (see sidebar: “How functional MRI works,” page 29). Moreover, these algorithms rely on assumptions not everybody agrees on.

Second, BOLD imaging has important limitations. Though increased oxygen-rich blood and its stronger BOLD signal usually flag increased neuronal activity, there's a time lag, since neurons fire thousands of times faster than blood flows. Moreover, sometimes the BOLD signal is positively misleading. Yale's Blumenfeld

BOLD beginnings

The BOLD effect—tracking neuronal activity through blood flow—was first demonstrated in small animals in 1990 by one of Robert Shulman's former Bell Labs postdocs, Seiji Ogawa, Ph.D. Then, Ogawa and another former Shulman postdoc, Kamil Ugurbil, Ph.D., used the method to produce the first functional magnetic resonance images (fMRI) of humans at the University of Minnesota.

Shulman, who was using magnetic resonance spectroscopy to study metabolic changes in the human brain, saw an opportunity to get similar information at much higher spatial and temporal resolution. His Yale postdoc Andrew Blamire, Ph.D., had learned a technique called echo planar imaging that sped up the process of capturing MR images and was considered critical for exploiting the potential of the BOLD effect for mapping brain function. Shulman directed Blamire, along with fellow postdoc Douglas Rothman, Ph.D. '87, and Terry Nixon, now director of facilities at Yale's Magnetic Resonance Resource Center, to soup up the lab's outdated MRI system to incorporate the speed of echo planar imaging.

When Ogawa and Ugurbil heard that the Yale system had rapid imaging capability, they began a collaboration with Shulman. This work, which also included Gregory McCarthy, Ph.D., now a professor of psychology at Yale, led in 1992 to one of the earliest fMRI studies, the first to show the brain responding to individual events, in this case a single visual stimulus. Shulman's team subsequently collaborated with McCarthy to perform the first fMRI measurements of a person performing a cognitive task.

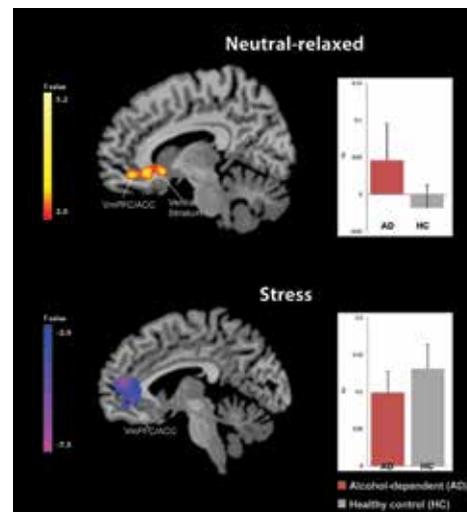


and his colleagues were the first to show that, in some seizures, neurons fire in such a frenzy that they need more oxygen than the brain can deliver and the BOLD signal goes down instead of up. They also found that blood flow sometimes declines in response to neuronal activity. These findings strike at the heart of all BOLD assumptions. “Taking BOLD alone is always going to be potentially risky,” said Blumenfeld. “Everyone is hoping for [better] techniques. ... I’ve been hoping for it my whole career.”

Third, and perhaps most importantly, experimental premises are crucial. Cognitive neuroscience assumes that mental processes like working memory, attention, problem-solving, and decision-making are real, objective, measurable, observable phenomena. Especially in the early years of the field, cognitive neuroscientists believed that these brain functions reside in discrete modules, a school of thought called localizationism. Many researchers have come to believe that these functions are organized in networks. Others posit that the whole brain is involved in all functions—the aggregate field view (see sidebar: “‘Mixed-use development’ in the brain,” page 25).

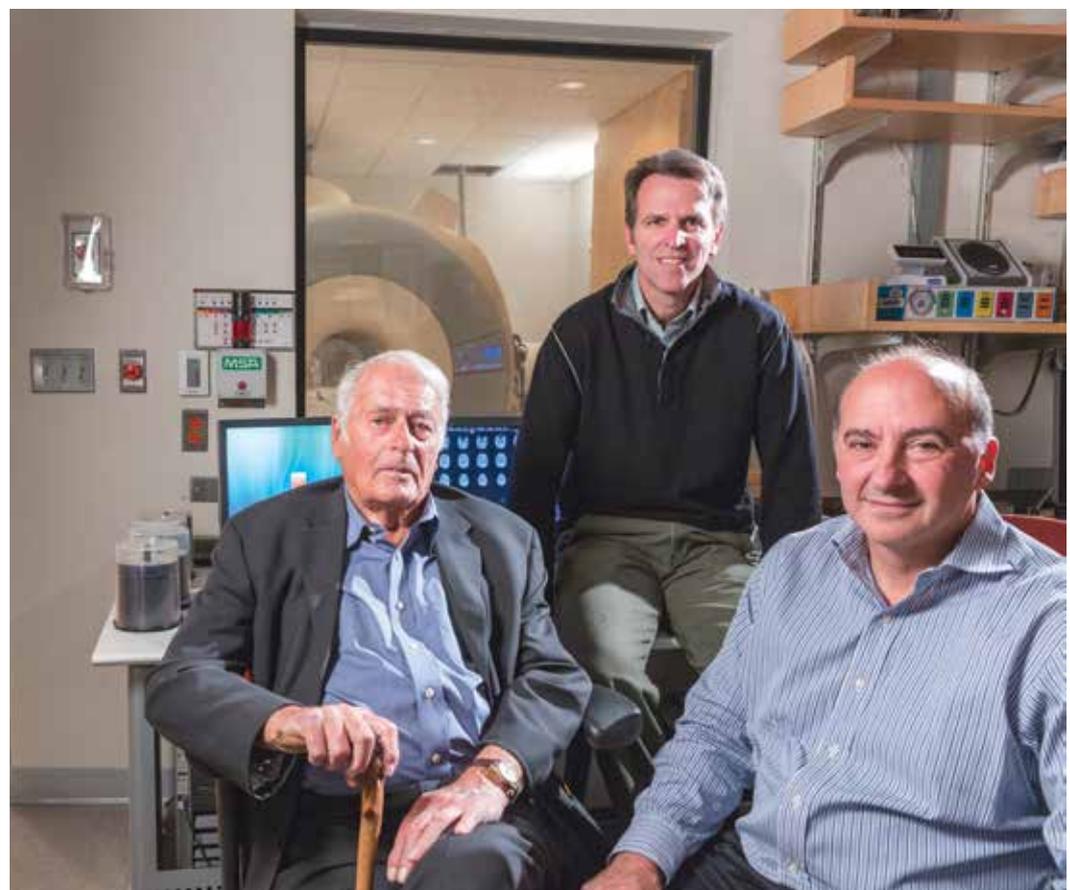
These things matter because they affect how a researcher plans and interprets experiments. A localizationist who expects working memory to reside in one particular spot in the prefrontal cortex will naturally process his data to look for that area lighting up. But there are other ways to analyze the same data set that can lead to different conclusions about which areas of the brain are active during a cognitive task.

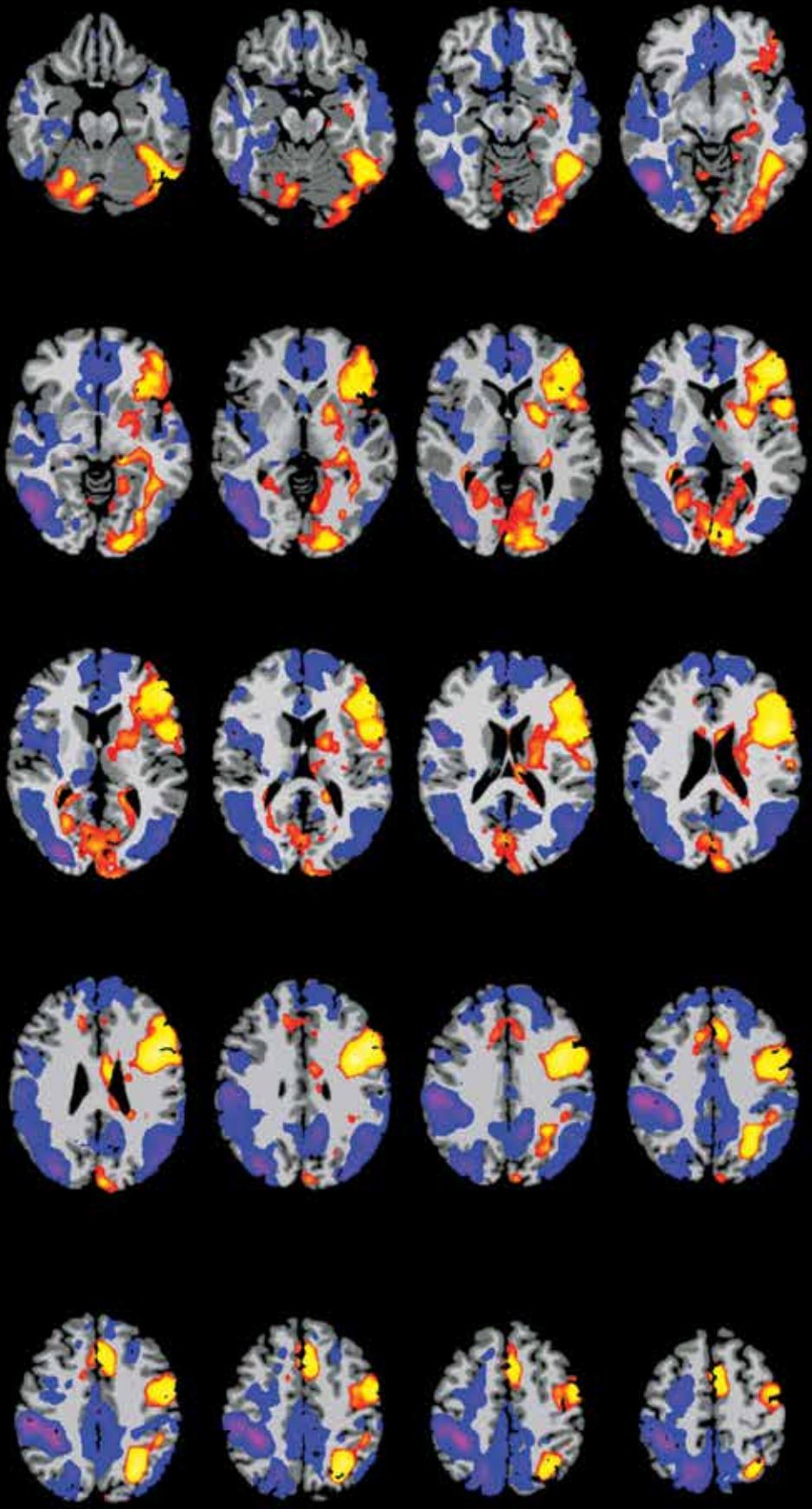
Chun said he believes that Shulman “has appropriately urged caution over the years, but his concerns do not acknowledge all the recent advances in analysis methods that enable more precise interpretation of BOLD signal activity for understanding perception and cognition.” As an example, he points to the work of Jack L. Gallant, Ph.D. ’86, at UC Berkeley.



ABOVE This graphic highlights areas of the brain where Yale researchers found significant differences in responses to stress and relaxation-inducing stimuli between alcoholic patients and healthy controls.

BELOW Robert Shulman (left) with Todd Constable and Douglas Rothman, co-directors of the Magnetic Resonance Research Center at Yale.





How functional MRI works

Don't let that detailed image throw you. Unlike, say, X-rays, a functional magnetic resonance image (fMRI) isn't a snapshot. It's a statistical map, the colorful end product of massive calculations.

During an fMRI experiment, researchers typically scan the brain at rest and then during a series of such tasks as recalling a string of numbers. This technique yields hundreds of images, each containing information about changes in metabolism and blood flow. These changes are associated with neuronal activity, measured in voxels by the tens of thousands. (A voxel is a cube-shaped data unit analogous to the 2-D pixel.) Long after the person being scanned has gone home, the researchers must contend with gigantic amounts of raw data.

First, in a step called preprocessing, researchers correct the data. There is slice-timing correction, since not all "slices" of the brain are imaged simultaneously. There is motion correction, since subjects tend to move during scans. Low-resolution fMRI data are superimposed onto a standard "template brain" image obtained by regular MRI (coregistration), but these have to be corrected because not everyone's brain neatly matches the template (normalization).

Then comes data analysis—researchers try to isolate those areas and networks that sent a stronger signal during a specific mental task. Subtraction is a common approach, in which the signal obtained at rest is "subtracted" from the one obtained during the task. To further localize the mental process being studied, images obtained during the study task may be subtracted from images captured during a control task. Whatever

signal remains may show the area of brain involved in that task, though this deduction contains many pitfalls—correlation does not imply causation, and seemingly simple cognitive tasks may comprise multiple simpler processes. Yet the signal from a single task can be extremely faint amid the brain's busy baseline activity. Without subtraction the two scans might look nearly identical.

Combining data from multiple research subjects is crucial, but it, too, can be dicey. The statistics may not take into account the anomalies that often dog complex experiments, such as missing data or a subject with a truly unusual brain signal. And individual subjects' anatomical differences are often blurred during normalization, which means losing potentially important information.

Because of an fMR image's colors, we say that the brain "lights up" in response to some mental task. But in reality nothing "lights up"; these colors are simply a code for the relative strength of the signal within each voxel. To judge whether that signal is random or real, researchers must also choose a threshold, or p-value, that represents the likelihood of a particular result being due to chance. A common p-value in statistics is 0.05, or 5 percent, meaning that an acceptable result is no more than 5 percent likely to be due to chance alone. But some fMRI researchers call for p-values as strict as .005, reasoning that they'll find fewer false positives that way. False positives are a real danger, as one group famously demonstrated by "finding" areas of brain activation during an fMRI analysis of a dead salmon. Unfortunately, stricter p-values might eliminate important data from consideration.

Statisticians are working on ways to refine all these analyses in hopes of ensuring that what "lights up" during a cognitive task reflects real, significant, and specific brain activity. But in the meantime, it's best to bear in mind that an fMR image is more like a graph than a photo. Like any image born of statistics, it can both enlighten and mislead.

The fMR images in this series show the brain's response to changing oxygen levels by overlaying results from a group of participants in a study of genetic influences on reading ability. The results from all 179 subjects were combined into a whole-brain statistical map of areas showing significant BOLD signal changes.

stimuli.) Shulman came to believe that the very philosophical underpinnings of such experiments are shaky, since they assume a modularity that isn't neatly borne out by the findings. Context is all-important: how working-memory tasks look under fMRI varies widely, depending on the nature of the task.

A more effective use of fMRI, argues Shulman, would be to characterize the brain's activity during observable behaviors in brain imaging studies. "For example, the total brain activity necessary for a person to perform the act of memory can be observed," he said. "The location of the psychological concept of memory cannot."

Critics' concerns aren't limited to experimental method; like Satel, some argue that results are being exaggerated. In an opinion piece in *The New York Times* the authors of the UCLA politics-related study claimed that fMRI results revealed how 20 voters felt about Hillary Clinton and Mitt Romney. Exasperated neuroscientists at a dozen universities responded in a letter to the *Times*—it's not possible, they said, to determine a person's mental state by looking at a brain scan.

Contrary to hopes, Satel argues, the technology cannot sway voters, sell products, sniff out lies, or reveal the causes of crime and mental illness. "To regard research results as settled wisdom," she writes, "is folly."

Satel views neuroscience and its tools as nothing short of remarkable. But she thinks that we are too quick to believe that this young science has at last illuminated the mind-brain relationship. Our rush to explain complex behaviors via brain activity alone fails to take psychological or social factors into account—and can lead us astray. She is skeptical, for example, of the way that fMRI findings have been used to argue that addiction is a purely a brain disease. Moreover, she writes, "the fact that addiction is associated with neurobiological changes is not, in itself, proof that the addict is unable to choose." Recovery programs that make use of incentives and consequences work for addicts, she pointed out, but would never help a Parkinson patient.

THE BRAIN'S INTEGRATED NETWORKS

Like psychologist Chun, Marc N. Potenza, Ph.D. '93, M.D. '94, believes that our understanding of brain organization is outgrowing its initial simplicity. Potenza is a professor of psychiatry, neurobiology, and child study who uses fMRI to study behavioral addictions like compulsive gambling.

Conventional wisdom, he said, held until recently that the amygdala processed fear, the ventral striatum provided drug-induced rewards, and so on. But these brain regions have been implicated in other processes as well, pushing cognitive neuroscience toward a network-based model. "The way in which these regions work together in networks or functionally integrated activations that some MRI data can identify, that's really important," Potenza said. The Human Connectome Project, in which research universities share fMRI data on brain networks, is a first attempt at mapping such connections.

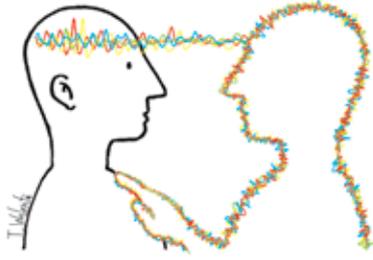
Adoption of a network model isn't the only shift in thinking. Researchers are using fMRI results to break traditional concepts like working memory into such smaller and more isolable components as encoding, shifts of attention, and retrieval. They are also studying the default mode network, brain activity when a person is awake but not doing anything in particular, using both cognitive and physiological approaches.

There is also brain plasticity to consider. Existing functional connections in the brain can be readily altered through learning and experience—and we can see those changes on fMRI.

Potenza rejects the idea that only behaviors observable by others constitute the proper subject of fMRI study. "There are some conditions like major depression where subjective accounts are very important to understand," he said. "If we were to omit looking at subjective responses, motivational states, emotional states, we would be limiting ourselves with respect to our understanding of the human condition in multiple clinically relevant states."

Perhaps some of today's popular fMRI applications will recede into history, taking their place alongside early 20th-century electrical healing gadgets and shoe store X-ray machines. Satel believes this burst of exuberance, if sometimes troubling, is normal in these early days of contemporary brain science. "You start out a little more crude, and then you perfect and perfect and perfect," she said. "Wherever we are in 20 years, I doubt we'd be there had we not gone through this phase first." */yale medicine*

Jenny Blair, M.D. '04, a freelance writer based in Austin, Texas, is a frequent contributor to Yale Medicine.



How neurofeedback helps patients tamp down their fears

By Bruce Fellman

Tiny parts of the brain, School of Medicine researchers are discovering, can have a huge impact on our lives. Michelle Hampson, Ph.D., and Judson A. Brewer, M.D., Ph.D., are leading teams that use real-time fMRI and what's known as neurofeedback to try to teach people how to control brain activity and combat such problems as anxiety, addiction, Tourette syndrome, and post-traumatic stress disorder (PTSD), as well as more mundane self-imposed roadblocks to success.

Hampson, an assistant professor of diagnostic radiology, in collaboration with Christopher Pittenger, Ph.D., M.D., associate professor of psychiatry, has been working to develop treatments for people afflicted with obsessive-compulsive disorder. One common symptom of this disorder is contamination anxiety (CA). Sufferers can be crippled by fears of infection by anything from microbes to moral turpitude. Previous research suggests that the problem arises from hyperactivity in the orbitofrontal cortex (OFC). Existing therapies are not always reliable, and known drugs are not always

effective, so Hampson tried using real-time images of the overwrought brain and neurofeedback methods to enable subjects to calm things down.

The team worked with 20 healthy adults, all of whom had CA. While in an fMRI scanner, subjects were shown a series of such anxiety-provoking photos as moldy fruit and skin infections, along with such neutral pictures as a backpack and a tranquil countryside. The subject's task was to try to control hyperactivity in the OFC. Half the group saw a genuine real-time line graph that provided neurofeedback by depicting OFC activity; the control group watched a sham graph. Both groups attempted to manipulate the graph—and control their OFC activity—by using anxiety-lowering strategies. There was no one-size-fits-all approach—some participants tried to tamp down their feelings in response to a picture, while others invoked religious beliefs,

said Hampson, whose study was published online on April 30 in *Translational Psychiatry*.

“Not only were the subjects in the treatment group significantly better at controlling their CA, but the changes persisted for at least several days.”

Hampson is also working with real-time fMRI and neurofeedback as a way to enable patients to control the tics associated with Tourette syndrome, and she and other researchers are investigating their use as a treatment for PTSD. “We look for mental disorders with a distinctive brain signature—either hyper- or hypoactivity—that can be spatially localized,” Hampson explains. “If we know what's unhealthy in terms of brain activity, we can try to come up with specific neurofeedback strategies that move the pattern in a healthy direction.”

Of course, one group of people—experienced meditators—has been using neurofeedback techniques for thousands of years. Brewer, assistant professor of psychiatry and a veteran meditator, has studied practitioners of this ancient discipline. Using real-time fMRI and neurofeedback, Brewer and his colleagues have figured out how meditation can control an oddly fundamental brain behavior called mind-wandering. “This must have been adaptive at one point in our evolution, but we no longer know how to turn it off,” he explains.

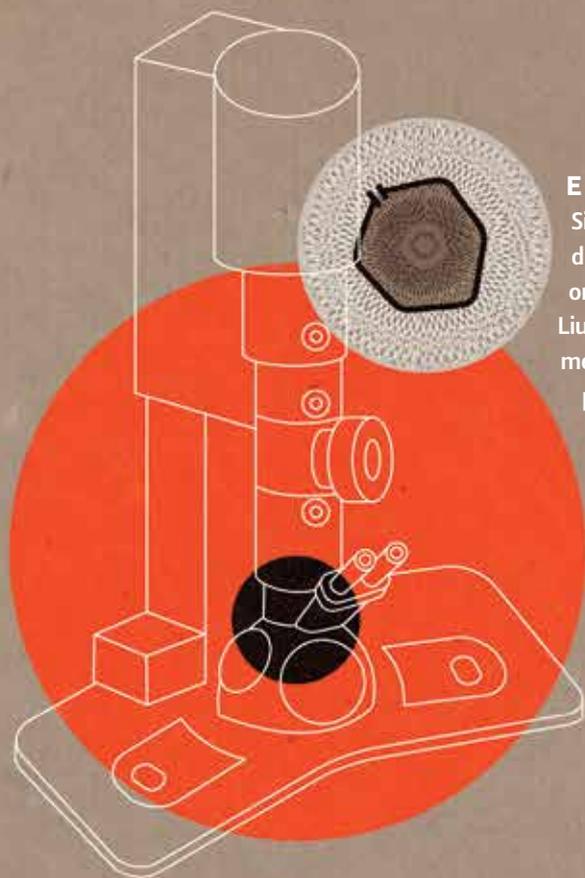
Run-of-the-mill daydreaming can be counterproductive enough, but the former soldiers he works with at the Veterans Administration Healthcare System in West Haven may be

utterly disabled by their inability to stop recurrent war visions. To help vets and others, Brewer and his team are especially interested in the posterior cingulate cortex (PCC), one of the major hubs of a mind-wandering circuit called the default mode network. “The PCC is activated when you're anxious, when you're thinking about yourself, when you're planning for the future, and when you're experiencing cravings—any time you get caught up in something,” said Brewer. “It's the big mover and shaker.”

But when it moves and shakes too much, one untoward result, he continues, is that “we tend to get in our own way.”

In an investigation using real-time fMRI and neurofeedback soon to be published in *NeuroImage*, Brewer and his team compared the ability of meditators and non-meditators alike to dampen the PCC. Not surprisingly, the meditators were more than up to the challenge. “Meditators, through years of practice, have learned how to get caught up in the moment and, maybe more important, how to let go,” said Brewer, who directs the Yale Therapeutic Neuroscience Clinic at the VA.

“A wandering mind is an unhappy mind,” Brewer explains. “We can help people get out of their own way.”

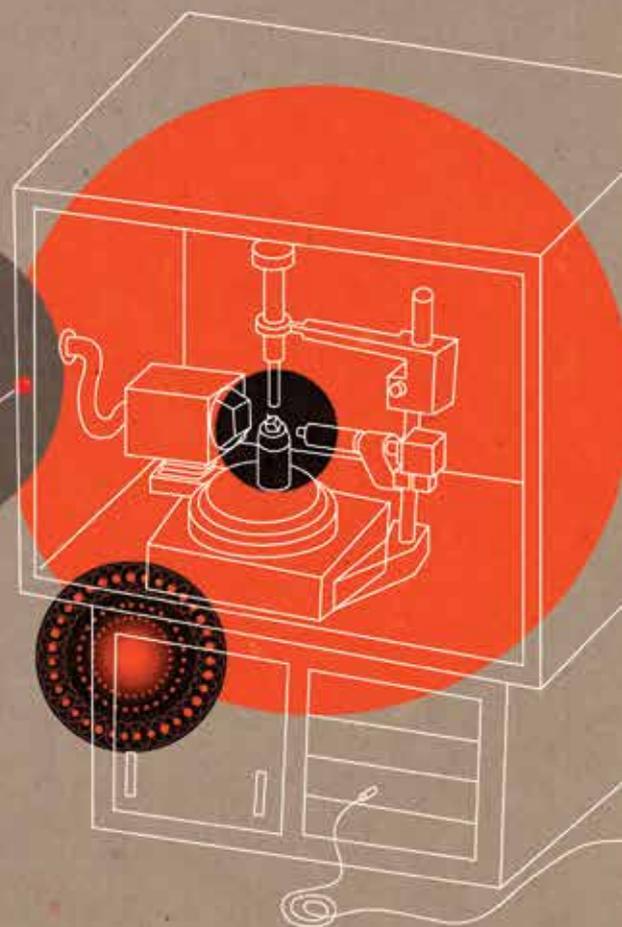
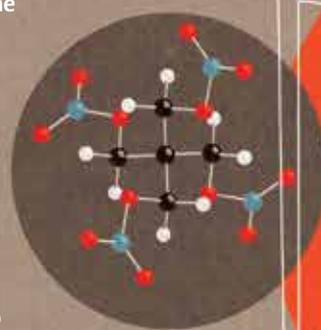


ELECTRON AND CRYO-ELECTRON MICROSCOPY

Since joining Yale at the end of 2011, electron microscopy (EM) facility director Xinran Liu, M.D., Ph.D., has brought biological and cryo-EM under one roof. The facility takes on about one new project per day, estimates Liu, including imaging animal and plant samples from nonmedical departments like forestry. With traditional EM, for example, the changing morphology of genetically manipulated cells can be tracked, and fluorescing agents can be introduced to localize proteins within cells. Cryo-EM uses frozen samples that are unadulterated by staining or fixation to get a clearer view of native protein structures. By tilting samples systematically as they are being bombarded with electrons, researchers can create volumetric reconstructions of samples that reveal 3-D structures of individual proteins and receptors, down to the resolution of ångströms.

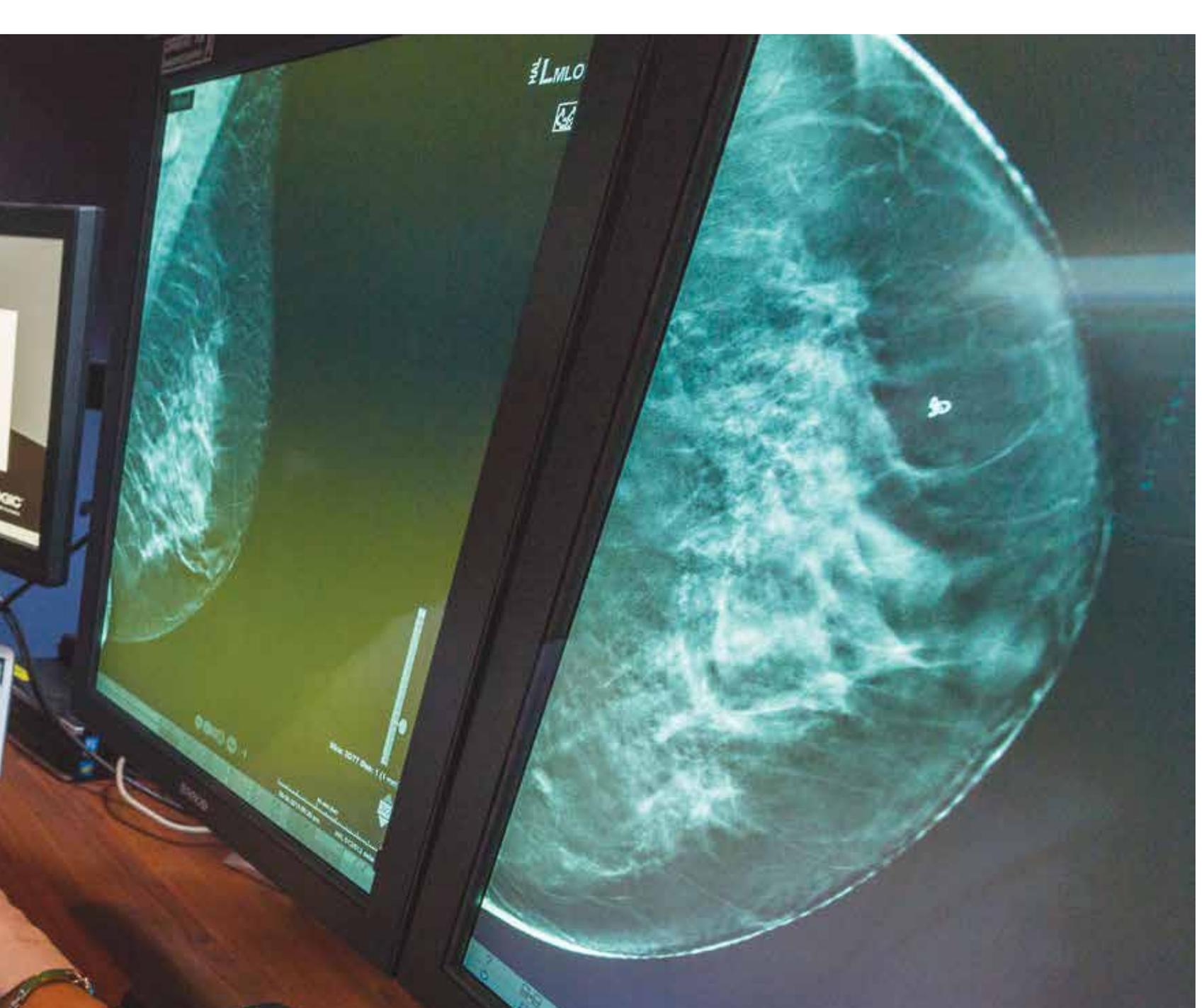
X-RAY CRYSTALLOGRAPHY

Using X-ray diffraction to visualize the structure of molecules is essential to modern drug discovery research, says macromolecular X-ray core facility director Ya Ha, Ph.D., associate professor of pharmacology. The interactions of drugs with cellular target sites are no longer a black-box process, as the diffraction pattern of a crystallized sample can reveal the atomic blueprint of how and where drugs bind with protein. Another facet of research involves looking at how molecular structure changes in cancer, Alzheimer disease, or cerebral cavernous malformations. Only about 1 percent of the protein structures in the human genome are known, so a substantial effort of X-ray crystallographers at Yale also goes toward the basic research needed to solve these structures. A fundamental life science discovery was solving the structure of the ribosome's subunit using X-ray crystallography, a feat that earned Sterling Professor of Molecular Biophysics and Biochemistry and Professor of Chemistry Thomas Steitz, Ph.D. the Nobel Prize in chemistry in 2009.





Liane Philpotts uses digital breast tomosynthesis to cut down on false positives and make more accurate diagnoses for breast cancer. Suspicious-looking images can bring back about one patient in 10 for more tests—usually false alarms. But, Philpotts says, “anybody who gets called back thinks the worst.” Tomosynthesis, which was approved by the FDA in 2011 after trials at Yale and other medical centers, is the first technology to deliver three-dimensional images in mammography.



A NEW VISION IN THE LAB AND IN THE CLINIC



Since the discovery of X-rays in the 19th century, new imaging technologies have helped physicians peer into the causes of disease and provide better clinical care.

By John Dillon Photos by Robert Lisak



WHEN DENNIS SPENCER, M.D., RECALLS HIS FIRST DAYS as a neurosurgeon in the early 1970s, he doesn't wax nostalgic about the way he imaged a patient's brain. "We weren't that far from Harvey Cushing," he says, referring to Yale's renowned "father of neurosurgery" and X-ray pioneer, who died in 1939.

Spencer then had two X-ray imaging tools at his disposal. One was angiography, introduced in 1927 but still widely used, in which an injected dye illuminates a patient's blood vessels on X-ray. The other was pneumoencephalography, a painful invasive procedure dating back to 1919 that involves draining fluid from the brain and injecting air into its ventricles to prepare for an X-ray. "It was a pretty crude field at the time," says Spencer, chair and the Harvey and Kate Cushing Professor of Neurosurgery.

This antediluvian period ended by the mid- to late-1970s with the advent of computer-assisted tomography (CAT or CT) X-ray scans, which enabled Spencer to see blood, soft tissue, and some tumors noninvasively. Imaging capabilities accelerated in the 1980s with the development of magnetic resonance imaging (MRI or MR), which provides even sharper internal images without exposing the patient to radiation; and then positron emission tomography (PET) imaging, which detects changes at the cellular level. These and other devices—boosted by increasingly powerful computers and often used in combination—have radically improved the detection and treatment of disease.

Today Spencer can remove or stimulate parts of the brain responsible for intractable Parkinson or epilepsy and leave vital parts intact. "I can put an electrode within two millimeters of any part of the brain," he says.

Yale clinicians and scientists are seizing on the imaging boom to improve patient care. Advanced techniques have moved from the lab to the clinic and the operating room and have become key tools for quicker, more accurate diagnoses and better outcomes. Surgeons can now see inside a patient's body in three dimensions in real time while they operate. Imaging technology can identify the places to avoid during brain surgery, whether a cancerous prostate should be left alone, or whether a breast mass is normal or cancerous. Imaging modalities can not only make surgery more precise but may also help a patient sidestep an operation or biopsy altogether.

"When I started, I thought I was getting in on the tail end of the developments of MR," says R. Todd Constable, Ph.D., professor of diagnostic radiology. "It turns out, 23 years later, that it hasn't matured yet." Constable, a physicist, is often called on to find the best device (or devices) for other specialists, and his team uses those modalities to develop a clinical map of the inside of a patient's body. Everyone thought CT scanning "was done" by 1990, he says, but the development of multi-detector, multi-slice imaging extended its warranty. "There's still a revolution in imaging for modalities we discovered years ago that we thought were mature," Constable says.

FROM X-RAY TO FMRI

The first great step in medical imaging came in 1895, when Wilhelm Röntgen took an X-ray of his wife's hand, famously displaying her bones and wedding ring. Cushing, then only 26 and a newly minted M.D., recognized the significance of the device and put X-rays to clinical use within months. But X-rays went only so far because they allowed medical professionals to see bones or teeth but little else.

Ultrasound, which creates images through sound waves and is familiar to any expectant parent, came into use in the 1950s. Constable says ultrasound didn't—and still doesn't—deliver clear images, but it has the advantage of being safe, affordable, and nowadays, highly portable. The next huge advance came in the 1970s, when the London-based Electric and Musical Industries developed the CT scan. The CT scan was the first to deliver X-ray images of the body in cross sections, and the images could be viewed either as individual slices of bread or an entire loaf. By the next decade, MRI, which uses magnetic fields and radio waves to capture images of internal organs, began providing even clearer shots of soft tissue.

Further breakthroughs in medical imaging came from unexpected sources—computer graphics and the

film industry. “We can all thank Hollywood and the gaming industry,” says Xenios Papademetris, Ph.D., an associate professor of diagnostic radiology, who prepares surgeons for procedures by mapping a patient’s brain or other body part ahead of time. Graphics cards were “designed to let kids play games,” he says. “We’re using them to do other things.”

Constable works with Spencer and other researchers by preparing images for their research or surgical procedures and says that the technology has made Yale a leader in providing surgical treatments for patients with epilepsy who don’t respond to drugs. “Things are moving from the research lab—where we can image these different aspects of brain function or brain metabolism or what have you—and into the sort of real-time intraoperative mapping,” Constable explains. When, for instance, epilepsy patients are being prepared for surgery, they’ll first get an fMRI. “When you speak in the magnet, or read, we can isolate your language cortex,” Constable says. During an operation, a surgeon can see where that spot is and knows not to “cut that cortex because [the patient’s] not going to be able to speak afterwards.”

Epilepsy is Spencer’s specialty, and the technology helps him track the origin of seizures. He’ll cut open a patient’s skull and—with the help of people like Constable and Papademetris—implant a grid over the brain, leaving it there for 10 days while he monitors brain activity. During that time the patient’s epilepsy drugs are withdrawn, and the monitor lets Spencer see which parts of the brain are initiating seizures. Information is collated with the patient’s CT scans to find the problem spot, which Spencer can locate on the axes of the grid as a player might do in a game of electronic *Battleship*. Electrical stimulation, again guided by imaging, identifies such critical function regions as language. He’ll go back into the patient’s brain and resect diseased areas, sparing function.

Two operating rooms at Smilow Cancer Hospital at Yale–New Haven have MRIs specially built for surgery, including the world’s first combination intraoperative MRI/endovascular suite. There, Spencer’s neurovascular faculty uses the MRI and a biplane angiography device that produces 3-D images of the blood vessels in the brain. He says the improved images have drastically changed the treatment of brain aneurysms. Ten years ago, 90 percent of arterial bulges were controlled by placing clips on them. Today, aneurysms are more often secured internally by coils inserted by a microcatheter—a safer, less-invasive method—and the use of clips has fallen to between 30 and 40 percent.

PREVENTING FALSE POSITIVES

While Spencer often uses imaging as a tool during an operation, Liane Philpotts, M.D., chief of breast imaging at Yale, will happily employ it to prevent a false positive. In addition to ultrasound and MR, she says that Yale has the best mammography technology yet: digital breast tomosynthesis.

Tomosynthesis, approved by the Food and Drug Administration in 2011 after trials at Yale and four other medical centers, is the first technology to deliver three-dimensional images in mammography. When used in conjunction with traditional 2-D images, tomosynthesis cuts down false positives by 30 to 40 percent, Philpotts says. It has also increased the rate of cancer detection by up to 20 percent. “Tomosynthesis is a game-changer,” she says. “It’s a win-win.”

Traditional mammograms can’t always distinguish cancerous cells from harmless ones. This lack of clarity is especially problematic in patients—usually younger women—with dense breasts, which have more glandular than fatty tissue. “Fat we can see through,” Philpotts explains. Glandular tissue, however, appears as white on an image, as do cancer cells. “This is one of the limitations of mammography.”

Philpotts shows the difference in the images of a patient who underwent both a standard mammogram and tomosynthesis. The procedures are roughly the same for the patient: the breast is compressed in the machine and the 3-D device takes a series of images through an arc of 15 degrees, which are then reconstructed as 1-millimeter slices instead of just a top or side image of the entire breast as is done in a routine mammogram.

Philpotts calls up a 2-D image of a whole breast on one of two adjacent monitors. It shows a mass of white in the middle. Philpotts is suspicious of the mass but the image’s blurriness won’t let her draw any conclusions. She switches the display on the monitor, which then shows the individual images, like a high-tech zoetrope. Each slice shows an area deeper within the tissue. “It’s as if you can see through the breast,” she says. As Philpotts progresses, she spots a telltale spidery lesion that indicates cancer.

In 2009, the U.S. Preventive Services Task Force (USPSTF), a group of outside advisors to the Department of Health and Human Services, recommended that women over 50 have mammograms every two years instead of yearly. Citing the cost of false positives and the radiation younger women are exposed to, the panel suggested that women in their 40s not get screened unless they are in a high-risk group. Those



recommendations were “controversial,” Philpotts says, conflicting with those of groups like the American Cancer Society, which continued to back yearly mammograms for women between 40 and 49.

Philpotts thinks that 3-D imaging can help bridge the gap between the conflicting recommendations. A team at Smilow Cancer Hospital reviewed the mammograms of 14,684 patients and found that the cancer detection rate was 5.7 per 1,000 patients in those who underwent both 2-D and 3-D screening compared to 5.2 per 1,000 among those who had only a standard mammogram. Subsequent ongoing data collection has shown an even greater difference in cancer detection. Moreover, 54 percent of those whose cancer was detected with the combined imaging had dense breasts; of those whose cancer was identified by 3-D imaging only, 21 percent had dense breasts. In 2009, Connecticut became the first state in the nation to

mandate that women be notified if a mammogram shows that they have dense breast tissue and that their insurance pay for additional screening.

With 3-D imaging, Philpotts said, the risk of false positives is reduced. “We’re saving on the costs of unnecessary diagnostic workups and possibly biopsies.”

At the start of her career 20 years ago, “when you had a finding, you had to go to the OR,” Philpotts says, but today “very few patients need to be taken to surgery.” The 3-D machine can reduce the number of callbacks, but those who must return also benefit from better imaging, which guides doctors through a real-time core needle biopsy to remove small pieces of dubious tissue. Ultrasound is used as a complement to mammography to find the extent of disease, if any, since cancerous cells appear dark on an ultrasound image. Patients fear a biopsy, but they’re relieved by the minimal invasiveness of the procedure.

The prostate presents special problems in imaging. Ultrasound guides clinicians to the prostate but can't image tumors, so clinicians use a combination of MRI and ultrasound to create a 3D model. Peter Schulam (standing), chair of urology, and clinician Preston Sprenkle discuss a case with a patient.



SEEING WHAT CAN'T BE IMAGED

Improved imaging systems are also helping Yale physicians treat cancers specific to men. Prostate cancer is even harder to find than cancer of the breast, because the prostate is the only solid organ in which cancer cannot be imaged. Ultrasound—the modality that guides clinicians to the prostate—alone cannot see tumors, says Peter Schulam, M.D., director of the Cancer Center's Prostate and Urologic Cancer Program. His team, like those of other specialists, uses a combination of imaging modalities that work better together than separately.

When Schulam arrived at Yale from UCLA last year, he recruited a team of doctors, engineers, and radiologists. He also brought a 3-D imaging navigation system called the Artemis Device, which he says is the best available to identify and monitor the progress of prostate cancer.

"Every man with prostate cancer doesn't need to be treated," Schulam says. "The question is: How do you differentiate?" Prostate cancer kills roughly 30,000 American men every year—more than any other malignancy except lung cancer, according to the American Cancer Society. Most men diagnosed with the disease, however, die of some other cause.

High levels of prostate-specific antigen (PSA) may signal cancer, but an enlarged but healthy prostate can also raise PSA levels. In 2012, the USPSTF recommended against PSA screening for cancer, saying that men are too often treated when the disease isn't causing symptoms. CT scans aren't beneficial in detecting possible cancer, so doctors increasingly use MRI. "The problem is that once you see something suspicious on an MRI, it's hard to biopsy" because the powerful magnets prevent the use of needles, Schulam says. A prostate biopsy is often educated guesswork, with doctors taking a dozen or so passes with a 1-millimeter-thick needle into the walnut-sized gland. Not only are biopsies often painful procedures and recovery can lead to such complications as sepsis, but "you can miss cancer," Schulam says. "Or you can detect cancer but not know the volume of cancer."

Because prostate cancer generally progresses very slowly, treatment options range from radiation or removal of the prostate to watchful waiting, in which doctors take no significant action unless the diseased organ causes problems. Active surveillance—careful monitoring for signs that the disease is progressing—is a relatively recent approach that falls somewhere in between. It is usually recommended for men at low risk of developing symptoms from the disease. Artemis, which combines MR and ultrasound images to improve the detection and treatment of prostate cancer, is the key

tool in the image-guided approach to active surveillance of the disease.

Artemis uses a multiparametric MRI—which also measures chemical concentrations and blood flow in tissue—to identify regions of the gland that may be cancerous. "The machine takes the MRI image and an ultrasound image and puts them together in a 3-D model," says Preston Sprenkle, M.D., a urologist on Schulam's team. The real-time ultrasound feature then "helps us guide where our needles go," so biopsies aren't as blind as they have been in the past.

The team can then determine how diseased the prostate is through what's called a Gleason score—which predicts whether the cancer will grow and spread to other organs—and what action comes next. Men with a low Gleason score can prevent or postpone unnecessary radiation therapy or a prostatectomy, which can leave patients incontinent or impotent. "If you lose one or both of those, your quality of life is dramatically changed," Sprenkle says.

Artemis promotes active surveillance because it "records exactly where the biopsy was taken from," Schulam says. When the team members examine the gland a second time, they have a superimposed image so "we can biopsy the exact same place as before. If something changes, you intervene such that you haven't lost your window of opportunity to achieve cure."

A REVOLUTION IN EVERY FIELD

Advances in imaging, from X-rays to CT scans to fMRI, have taken a lot of the guesswork out of diagnosis and treatment. They have reduced inaccuracies in testing, spared patients anxiety from false positives, and improved outcomes. Spencer is happy with the progress he's seen since his early days when neurosurgical imaging was in its infancy. "Imaging is important to everything we do every day," he says. "It's revolutionized every field. Our understanding of the brain—and our understanding of brain disease and the future of treating it—is just so tied to our imaging." */yale medicine*

John Dillon, a New Haven-based journalist, has been writing on health and medical issues for 15 years.



Reducing the risk of CT scans

By Steve Kemper

For patients, computerized tomography (CT) scans are simple and painless. The machine looks like a big doughnut standing on its side. The patient lies on a slab that slides through the doughnut's hole until the part of the body to be scanned is positioned beneath a rotating ring that contains an X-ray camera. Each rotation scans a "slice" of the body part. The patient feels nothing. As the camera rotates around the patient, the bed slides through the doughnut taking pictures. The procedure takes less than 10 minutes and generates data that a computer combines into a portrait of the area scanned, yielding images far superior to a shadowy two-dimensional X-ray.

CT scans have saved countless lives by allowing doctors to detect injuries and diseases that don't show up on standard X-rays. The scans also have prevented misdiagnoses and unnecessary surgeries. It's not surprising that their use by doctors has surged over the last two decades.

Yet this diagnostic power carries a cost: Each of the rotations exposes patients to doses of radiation that accumulate. Each CT scan exposes patients to between 100 and 500 times the amount of radiation in an X-ray. "The same doses that people got from the Hiroshima bomb drop, which we now know increases the risk of cancer development," said Rob Goodman, MB BChir, interim chair and professor of diagnostic radiology and chief of pediatric imaging.

When Goodman came to Yale from Oxford in 2003, he was alarmed by the excessive use of CT scans here. Americans were getting three times more medical radiation than were Europeans. Goodman was especially worried about the effects on children, because their smaller, rapidly changing bodies are more susceptible to

ionizing radiation and hence to the risk of cancer from it.

Goodman began a campaign to shrink the radiation exposure of children who came to Yale-New Haven Hospital (YNHH). He conducted grand rounds for pediatricians on reducing radiation doses and urged them to consider such alternatives to CT scans as ultrasound. He also worked with the hospital's medical physicists to tweak the CT scanners to give children the lowest possible dose while still making images acceptable for diagnosis.

Goodman's efforts coincided with a growing national awareness about the risks of medical imaging, particularly for children. In January 2008 the Alliance for Radiation Safety in Pediatric Imaging, representing more than 70 medical organizations, launched the Image Gently campaign to educate doctors and the public about cumulative radiation exposure. Manufacturers began building scanners that automatically adjusted the dose based on the patient's age and weight, as well as the sensitivity of the area to be scanned.

These steps have raised consciousness and lowered CT use, but children are still getting too much radiation. In June, a study published in *JAMA Pediatrics* reported that of the estimated 4 million CT scans given every year to children in the United States under age 15, a third are unnecessary and may lead to 5,000 cases of cancer. In the same month, a study published in *The Lancet* found that children who get multiple CT scans have a

slightly higher risk of developing leukemia or brain cancer.

At YNHH, Goodman's efforts have paid off. In data compiled by the American College of Radiology's Dose Index Registry, which tracks and categorizes the radiation given by CT scanners in U.S. hospitals, YNHH recorded the lowest doses of any hospital in the country in many age groups and types of pediatric CT studies.

The hospital's own statistics tell a similar story. In 2003 YNHH had three CT scanners and did 4,844 CT scans on children. In 2012, despite now having seven scanners, the hospital did only 2,344 studies on children. Those numbers took even Goodman by surprise.

Goodman expects the numbers to drop further as MRI, which emits no radiation, replaces CT scans for many diagnoses. His campaign to lower radiation doses at YNHH has been so successful that he now sometimes finds himself urging clinicians and parents not to avoid CT scans in the correct clinical setting. "If the suspicion is high that your child may have a significant lesion in the lung," he said, "be reassured that the CT radiation doses at Yale are the lowest in the country and doing the scan is what's best for the patient."

Brain imaging in the era of bell bottoms

Arrival of the CT scanner at Yale

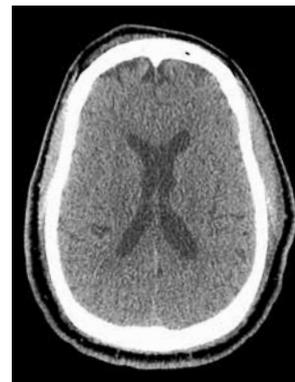
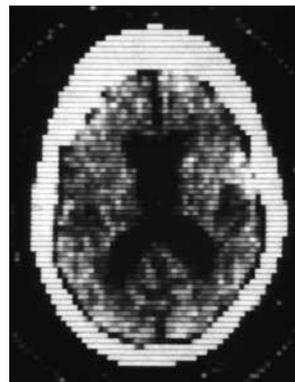
By Amanda Alvarez

“We are proposing that the Yale-New Haven Hospital acquire the EMI scanner, [which] represents the most revolutionary advance in neuroradiologic evaluation of patients. ...” So begins the December 1973 letter from E. Leon Kier, M.D., HS '66, that would lead to Yale-New Haven Hospital's acquisition of Connecticut's first computed tomography (CT) scanner. The first-of-its-kind scanner that Kier refers to in the letter was named after the company where it was developed, the EMI record label, which also had an independent electrical and computer engineering arm. (Profits generated by the British label's top artists—four young Liverpudlians named John, Paul, George, and Ringo—supported EMI's investment in the CT machine.) Kier, then chief of neuroradiology, was writing to the chair of the diagnostic radiology department, Richard H. Greenspan, M.D., to convince him of the necessity of acquiring the new machine, which was

first used on human patients in October 1971 in England.

In the early 1970s, there were two main methods for diagnosing patients with neurological problems: cerebral angiography and pneumoencephalography. The resulting images could tell a well-trained viewer what part of the brain was affected and whether the lesion in question was a tumor, vascular malformation, or hematoma (bleeding). “These procedures were time-consuming and risky for the patient and required hospitalization,” Kier says today.

If a patient came into the emergency room with a stroke, angiography would be performed: A catheter was inserted into a large artery and threaded from the neck to the carotid, where a contrast agent was injected. Radiographs taken while the contrast agent circulated would help identify the location of constriction or



bleeding. During a pneumoencephalogram, oxygen was injected into the spinal subarachnoid space (a cavity filled with cerebrospinal fluid that contains the blood vessels that supply the brain and spinal cord), which permitted the visualization of the brain's ventricular system and subarachnoid spaces on X-rays. Kier recalls that these procedures were dangerous for the patients if not done properly.

Compared to these methods, Kier says, CT was “a quantum jump. It was a revolutionary development, and we felt that we had to get the machine to move into a whole new phase of medical care.” The scanner that was acquired was actually the ACTA, a CT scanner developed at Georgetown University whose gantry size allowed imaging of the whole body, not just the head, as with the EMI scanner. There was a research scanner on campus as early as 1972, and this was used to image both healthy volunteers and patients with trauma. Because it wasn't yet known whether

CT images were sufficient or useful for diagnosis, these initial studies helped justify the clinical protocol and need for a dedicated CT scanner for patient care. By 1978, the CT facility at the hospital was open 24 hours a day, and by 1990, when Kier stepped down as chief of neuroradiology, there were four scanners.

Kier's radiology technician at the time, Cathy Computaro, recounted the early CT scanning procedure. “Imaging the head from the bottom of the chin to the crown took an hour, or six minutes per image slice. Now the entire body can be done in 14 seconds.” Patients were sent away while computers reconstructed the images, which were then printed as Polaroids or on X-ray film. “When the first images came out, I was stunned that I could see the ventricles. It was incredible. There was no other way at that time to do

OPPOSITE Side-by-side images of a CT scan from the 1970s and a more recent scan show how the technology has advanced.

THIS PAGE Early CT scans could provide images only of the head.

neurological diagnoses except with cerebral angiography or pneumoencephalography.” Camputaro, who still manages 3-D CT and MR imaging at the hospital, says that not only did CT simplify image interpretation, but it also changed how anatomy and physiology are taught. Since almost all ER patients are automatically scanned, and those scans are available on the hospital server, students are now trained with CT images rather than with textbook images.

Summing up the sea change, Kier, who is still one of the hospital neuroradiologists, says, “We went from painful, dangerous procedures to painless and safe procedures. Of all the changes that the diagnosis of neurological disorders has gone through prior to the modern era of MRI scanning, the biggest change was at the time CT was introduced.”





From selling used car parts to studying organ transplants

FROM THE TIME PARWIZ ABRAHIMI STARTED SCHOOL until he was 13 or 14, weekend mornings often meant getting up at 1 a.m. to help his father deliver *The Seattle Times*. Then, before sunrise, Parwiz and his brother and two sisters would help their parents load used car parts into a rickety station wagon to sell what they could at the Midway Swap & Shop in suburban Seattle.

“I don’t think my dad really understood labor laws,” said Abrahimi with a laugh. During the week, his father did odd jobs, and the family lived in public housing.

In a roundabout way, it was that flea market that set Parwiz Abrahimi on the road to what he is today: an M.D./Ph.D. student at Yale and a 2013 recipient of a Paul & Daisy Soros Fellowship for New Americans.

Abrahimi and his family came to the United States from Afghanistan in 1990, when he was four. Fighting against the invading Soviet army had turned into a civil war a year earlier after the Soviet Union withdrew its troops from the country. Three of Abrahimi’s uncles had died in the violence, and his father, who had worked for the previous government, had twice been arrested. A smuggler led the family—members of the persecuted minority group, the Hazara—through the mountains to Pakistan. They were granted asylum in the United States 18 months later.

When Abrahimi was 12, his father bought him a manual for the programming language C++ at the swap meet. As Abrahimi struggled to learn the program on a clunky Intel “286,” he discovered that “I was a technical guy.” It opened up the world of quantitative reasoning. The gift also conveyed a message: that learning was a priority. “We took the interpretation of the American dream as obtaining an education and achieving social mobility.”

At the University of Washington, Abrahimi studied biomedical engineering because it was new and multidisciplinary and addressed problems in medicine. He graduated in 2007 and worked for a year at the National Institute on Aging in Baltimore, Md., trying his hand at laboratory research “to see if it was something for me.” It was.

In 2008, before starting the M.D./Ph.D. program, Abrahimi taught science for a year at the American University of Afghanistan in Kabul and at Marefat High School in his family’s former home, the impoverished Dashti

A former refugee from Afghanistan finds hope in the American dream and a career as a physician-scientist.



Barchi neighborhood. He moved to New Haven a year later to begin his medical studies. Three years later, before beginning his doctoral research, he returned to Afghanistan to study the safety of donated blood.

Now doing his doctoral research in the laboratory of Jordan S. Pober, M.D. '77, Ph.D. '77, professor of immunobiology, Abrahimi is testing ways to modify foreign proteins in a transplanted organ so that

received one of 30 fellowships, which provide \$90,000 over two years to fund living expenses and tuition. Fellowship director Stanley J. Heginbotham said that Abrahimi stood out for his achievements in academia and for “his commitment to moral solutions in Afghanistan, and his commitment to transplant medicine. ... This guy’s going to make a real difference in some aspect of American life.”

In his spare time Abrahimi serves as a director for social services at the student-run HAVEN Free Clinic in New Haven. He envisions a career that combines care of transplant patients with



Patience pays off for Yale neuroscientist-turned-inventor

Medical students who’ve studied on an iPhone while waiting in line for a latte have Mark Williams, Ph.D. '96, to thank for helping break the chains that once bound them to the library.

After completing his Ph.D. in neuroscience at the School of Medicine and postdoctoral research at Duke University, Williams found that basic science research wasn’t for him. With interests spanning neuroanatomy, art, design, communications, and business, he asked, “What’s a career that you can build from that?”

Somewhere between the time that a computer cropped up in every home and a smartphone appeared in every pocket, Williams found the answer. He first developed educational materials on CD-ROM for medical students in the late-1990s. Eventually he was designing apps for the video iPod before there was a way for consumers to buy such apps. In 2008, when Apple launched the App Store—the platform through which iPod touch and iPhone users now buy software—four of Williams’ products were among the first 500. Williams’ company, Modality, produced two Frommer’s travel guides, Netter’s *Anatomy Flash Cards* and Netter’s *Neuroscience Flash Cards*.

Williams’ inspiration came when he started teaching neuroscience to first-year med students

Parwiz Abrahimi //

“This is a country that took my family in when we had nothing, and here I am studying at Yale.”

they won’t set off alarm bells in the recipient’s immune system. Those warning proteins come from the endothelium, the thin layer of cells that lines blood vessels in the organ.

“We want to change the gene expression of the endothelium to make it less stimulating to the immune system,” said Abrahimi. While most efforts to prevent rejection focus on dampening the host’s immune response, Abrahimi is looking at modifying the transplanted organ.

Pober describes Abrahimi’s ability to design and carry out experiments as “remarkably well-developed for someone this early in their graduate training.”

In May the Soros Fellowship announced that Abrahimi had

research on transplantation. “My end goal is to become a physician-scientist, for the clinical care to inform my research.”

Abrahimi became a U.S. citizen in 1997. “This is a country that took my family in when we had nothing, and here I am studying at Yale,” he said. “That said a lot about the country, that it would provide an opportunity to a person like me. This is a country of immigrants, and my generation of Afghan Americans is slowly being integrated into this country and is able to contribute back to the society.”

—Cathy Shufro

at Duke in 1997. He wanted to tackle an educational challenge. “How could we reinforce the basic concepts outside the classroom so that class time is really about problem-solving, collaboration, and building relationships between student and mentor?”

In the late 1990s, at a time when every textbook came with a supplemental CD-ROM, Williams developed a neuro-anatomy reference on CD-ROM through startup company Pyramis. Users could select the name of a part of the brain, see images of it, then slice it and rotate it in 3-D. “It’s silly today, but the Internet was only just emerging. This was a great way to see an image and learn where it’s located in the brain,” Williams said.

Williams left Duke in 2005 to focus on software development and to start a company which eventually became Modality. He remains in Chapel Hill, where he continues to develop new technologies.

As personal technology moved from the desktop to the palm of the hand, so did Modality.

When the market shifted toward digital flashcards, textbooks, and guides, Williams and his team learned to transform already digitized content into applications for handheld devices. They could format any digital book for use on an iPod. The only problem was that Apple did not allow software development for the iPod at this time. Williams and his team waited

for a day when Apple would recognize their worth.

“That day came. We got the call,” Williams said. They made a deal for Modality to sell its products to video iPod users.

This was in 2007, when users had to go to an Apple store—in person—to buy a code on a card so they could download the app to their computers and transfer it to their iPods. Drove of publishers clamored to get onto the gizmos that their campus reps were seeing plugged into the ears of college students everywhere.

Modality transformed a number of titles, but Williams was most excited about medical illustrator Frank Netter’s products, which are published by Elsevier. “Netter revolutionized medical illustration, so when we could bring that to the iPod, it was really exciting,” he said.

The users were fans, too.

“A student said to me, ‘Dr. Williams, I learned five new brain terms while I was waiting in line for my latte today.’”

In 2008, at the Apple Worldwide Developers Conference, former Apple CEO Steve Jobs announced the launch of the App Store, which allowed users to download apps directly from their phones. Onstage beside him were the dozen developers whose products would stock the store. Among them was Williams.

Over the next two years, Modality launched more than 150 educational apps, including one that allowed users

to cross-reference images in countless anatomy atlases with their own CT and MR images. “Clinicians saw the value of having their device in the clinic and showing the anatomy to a patient. It was a real opportunity for patient engagement.”

In 2012, shortly after Epocrates bought the company, Williams left Modality to pursue projects on his own. He wants to develop apps to maximize relationship-building opportunities for patients and doctors the way his educational software does for students and teachers. “Technology should be clearing the way for these relationships to take hold,” he said.

—Sonya Collins



An alumnus was building educational apps for the iPod before Apple was ready for him. The company finally saw the light.



ONLINE EXCLUSIVES

A project to build over the Route 34 Connector reverses an urban renewal project of the 1960s and vies to bring two parts of the city together.

Humility was the word of the day at the Commencement Ceremony held May 21 for the 91 members of the Class of 2013.

An impressive 32 members of the Class of 1963, along with their guests, celebrated their 50th Reunion at the Quin-nipiac Club on June 1!

A new scholarship gives aspiring surgeons a boost.

Full stories and event photo galleries, as well as other online-only content, can be found on our homepage at yalemedicine.yale.edu.

Ellen Matloff was right— the Supreme Court said so

ON JUNE 13, 2013, in the case of the Association for Molecular Pathology v. Myriad Genetics, the United States Supreme Court ruled, in a unanimous decision, that genes cannot be patented. The news was both a shock and a relief to Ellen T. Matloff, M.S., who started Yale's Cancer Genetic Counseling Program in 1995. For 14 years Matloff had argued that something occurring in nature should not merit patent protection and that the patents were harming patients and medical researchers. When the American Civil Liberties Union filed a suit against Myriad Genetics, which held patents on the BRCA1 and BRCA2 genes that are linked to breast and ovarian cancers, Matloff joined the case as a plaintiff. Two weeks after the Supreme Court decision, Matloff spoke with *Yale Medicine*.



watch an interview
with Ellen Matloff at
yalemedicine.yale.edu

How did you get into a profession that didn't exist when you were born?

I first learned about the field of genetic counseling when I was a sophomore at Union College, taking a course in genetics. I enjoyed the course so much that I did an internship at Albany Medical School. At that time, cancer genetics was not an option because the field hadn't

even cracked open yet. My first job out of graduate school was doing pediatric and adult genetics at SUNY Upstate Medical Center in New York. It was then, in 1995, that significant discoveries began being made in cancer genetics.

What has changed since you started?

When the genetic counseling program was started in 1995 by Vincent DeVita, who was director of Yale Cancer Center, I was everything. I was the secretary;

I scheduled appointments. They gave me a supply closet that they had emptied out for my office. Now we have two secretaries, a phlebotomist, and six and a half genetic counselors, and we are still growing.

In 1995, BRCA1 and BRCA2 hadn't been cloned, so we didn't have genetic testing for that condition. Patients would come in,

{ To nominate a subject for Q&A, contact
Yale Medicine, 1 Church Street, Suite 300, New Haven, CT 06510 or e-mail yymm@yale.edu

we'd take their family history, and determine, based on that history, if it looked hereditary. If it did, we would make estimates of their risk to develop cancer. I'd been at Yale for about three months when BRCA testing became available and the phones were ringing off the hook. With BRCA1 and BRCA2, genetic testing really became available to the masses.

With her decision to go public on her double mastectomy, has Angelina Jolie done a service to the cause of genetic counseling? For a movie star whose living is based on her body and her looks, as well as her talent, to put this out there was a really brave thing to do. Since the story broke, our referral rate has increased by 40 percent. A lot of people have asked me if Angelina Jolie made the right choice. This is a very individual decision. It varies based on the person, the family history, and personal preferences. Do I think Angelina Jolie made a reasonable decision? Absolutely. She's reduced her risks tremendously. She's reduced her worry.

How did you get involved in the Myriad case? I'd been very outspoken about the danger of gene patenting since the late 1990s. I had written many editorials to prestigious medical journals and they told me my letter was so preposterous they weren't even going to send it out for review. I couldn't get anyone to take it seriously. Someone suggested I contact a very well-known bioethicist, Arthur Caplan, and see if I could get him interested in becoming a co-author. Lo and behold, he was interested. Because of his reputation, we landed the cover of a prestigious bioethics medical journal. Later on, when the ACLU decided to sue and was looking for plaintiffs, I got a phone call.

Why shouldn't genes be patented? First of all, patents are supposed to be protection for innovation. The human gene is nothing that was discovered by Myriad Genetics. There was nothing new invented. Second, we can now see what kind of damage can be done to patients and researchers if a company holds the patent to the letter of the law. When BRCA1 and

BRCA2 were first discovered, there were many labs across the country that were offering testing. We were offering testing here at Yale for \$1,600. Over time the cost of genetic testing has gone down, down, down, yet the cost of BRCA testing went up, up, up. Before the patent was overturned, the cost of BRCA testing was \$4,000.

Where were you when you heard the Supreme Court decision? The decision came on my daughter's last day in preschool, and I went for the parent sing-along. It was pouring rain. We ran from preschool to the car and were soaking wet. It was then that I heard my cell phone going crazy. I had a million text messages, and they all said the same thing: unanimous decision by the Supreme Court banning gene patents. I started crying. I was shocked and relieved and overwhelmed. For me this had been a 14-year battle. To have it go all the way to the Supreme Court and to have it be a unanimous decision were just overwhelming. My 3-year-old daughter was alarmed that mommy was crying. I said, "We won today. These are happy tears."



What should we believe?

In a new book, a public health professor helps the public understand what's behind health reports in the media.

By Cathy Shufro

News about health deluges us daily: On the nightly news, in the newspaper, on blogs, and even at parties, we hear pronouncements about what to eat, what pill to take, which screening test we absolutely must schedule—or might be better off avoiding. And what's recommended one day seems laughable the next. So what health news should we heed? What should we ignore?

Most of us lack the tools to judge, said Michael B. Bracken, M.P.H. '70, Ph.D. '74, the Susan Dwight Bliss Professor of Epidemiology at the School of Public Health. "We don't educate children and adults in anything to do with understanding risk or probability," Bracken said in a recent interview.

Bracken offers a remedy in his new book, *Risk, Chance, and Causation: Investigating the Origins and Treatment of Disease*. In this accessible, lively, and often witty book, Bracken explains how epidemiologists understand the world. He shows why good study design is crucial in distinguishing between chance and causation. Bracken discusses the reasoning behind ethical guidelines and, surprisingly, explains how they can sometimes cause harm. In "Celebrity Trumps Science," Bracken cites a rock star's misinformation about the effects of marijuana and an ex-Playboy bunny's inaccuracies about the cause of autism.

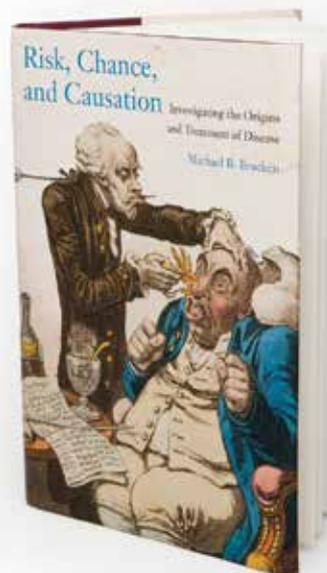
In addition, Bracken devotes a chapter to the benefits and the limitations of using animals for research—animals often fail as proxies for human beings. For example, a potential

leukemia drug worked well in monkeys but nearly killed six healthy humans.

Bracken lays out the principles of a good study by describing one from China that tested whether women who examined their breasts for lumps reduced their chance of dying from breast cancer. The study enlisted 250,000 women and compared a study group (women taught self-examination) with a control group (women not instructed in self-exam). All were Shanghai textile workers living similar lifestyles, which minimized influences of other variables. The 10-year study found that the women trained in self-exam found more breast lumps than the controls did, but deaths from breast cancer were identical in both groups.

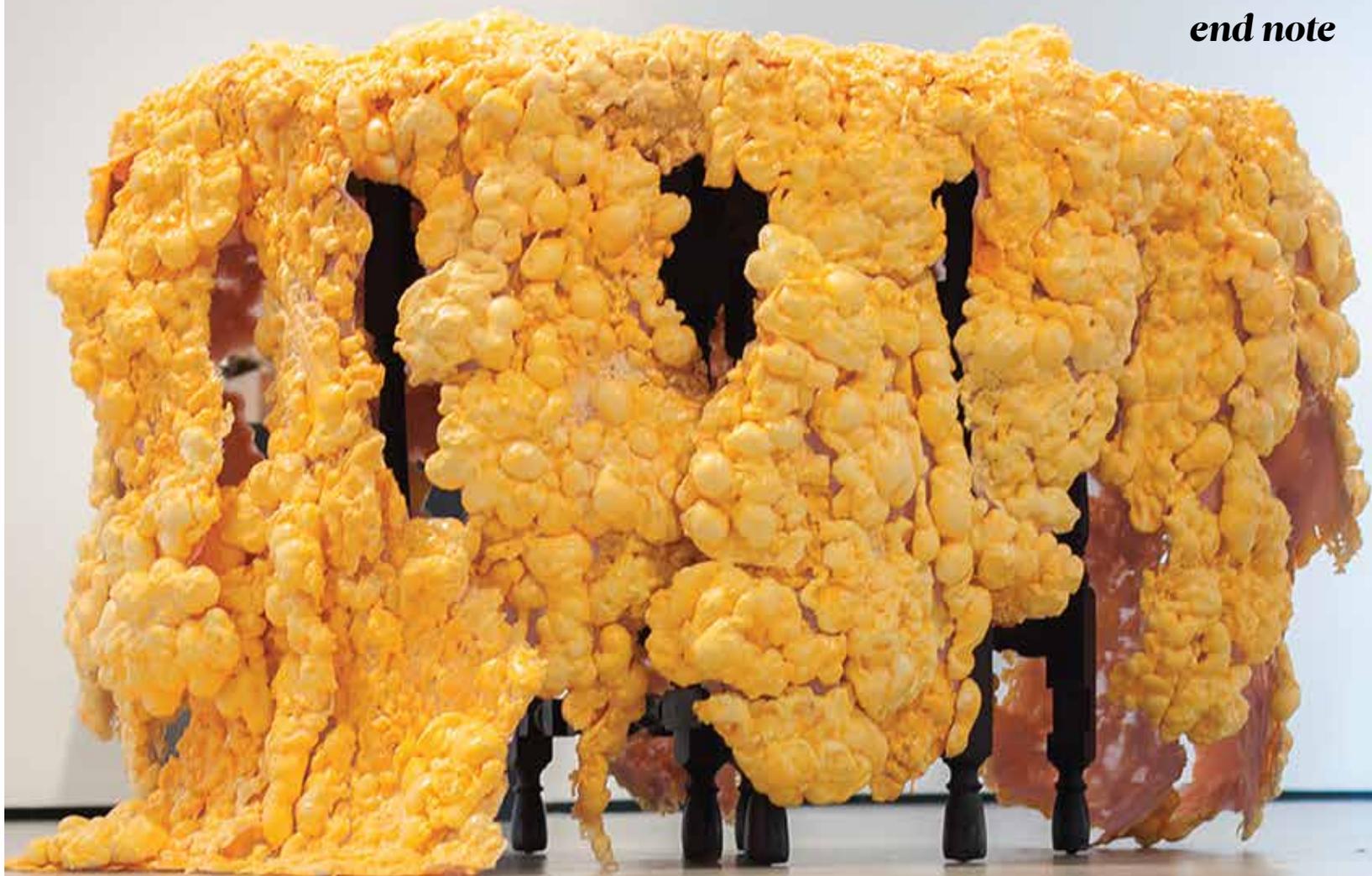
An example from Bracken's own research on the effects of early childhood illness on later chronic disease illustrates the complexity of study design. Does giving antibiotics to very young children make them more likely to develop asthma? Antibiotics might limit the development of the child's immune system. On the other hand, children prone to asthma may get more antibiotics because they wheeze. Even careful study design could not totally eliminate uncertainties about which came first: the antibiotics or the asthma.

The deluge of health news will remain relentless, he said. "The 24-hour news cycle has to be continually fed. They jump on everything." Unless people learn how to evaluate news on their own, he said, "the net effect will be that the real health messages get lost in this quagmire of misinformation."



Risk, Chance,
and Causation
BY
Michael Bracken
PUBLISHED BY
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{ Send notices of new books to
Yale Medicine, 1 Church Street, Suite 300, New Haven, CT 06510 or e-mail yymm@yale.edu



Art and the human body

DURING A WEEK IN JUNE visitors to the Yale School of Art's Green Gallery were treated to glass representations of the human anatomy, everything from wombs to gall bladders including this depiction of human fat. The exhibit, "Looking In," was assembled by physicians, glass artists, and Reintegrate: Enhancing Collaborations in the Arts and Sciences and coincided with New Haven's annual International Festival of Arts & Ideas.

The exhibit closed with a talk by David Yuh, M.D., professor and chief of cardiac surgery at the School of Medicine, a member of the doctor/artist team. Others on the team included medical student Lucinda Liu; G. Kenneth Haines III, M.D., associate professor of pathology; and glass artists Michael Skrtic and Daryl Smith, a scientific glassblower in Yale's chemistry department. Sinclair Marber, a student at Yale College, curated the exhibit.

—John Curtis