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Medical electronics

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Contributing Editor

THE MYSTERY SHROUDING THE INTERIOR OF A PATIENT'S body lifted a little further last year. One noteworthy step was the simultaneous application of a pair of imaging techniques (not just one) to internal organs and tissues. Another was a virtual approach to endoscopy, enabling physicians to inspect organs for disease without invading the body with a catheter or other device.

The most dazzling revelations, though, arose from the race to decode the human genome—that is, to uncover the sequence of each gene's four chemical bases: adenine, thymine, guanine, and cytosine. Late last year, researchers said they had decoded most of the genes on Chromosome 22, and by the spring, 90 percent of the human genome will be decoded.

Other cellular techniques have also borne fresh fruit. For example, an application of mass spectroscopy has made it possible to inspect the proteins of a cell all at once, instead of picking them out and analyzing them individually.

THE WORKING DRAFT

The labyrinthine task of mapping human genetic material has been under way for about a decade, with a consortium comprising some 1100 scientists of the Human Genome Project in 18 countries currently at work. The largest efforts are being mounted by the Sanger Centre in Cambridge, England; Washington University in St. Louis, Mo.; and the National Institute of Health's National Human Genome Research Institute in Bethesda, Md. Fighting diseases more effectively is the chief goal.

"The intention of the enterprise is to find out how differences in the genes of individual humans lead to a risk of disease," observed Francis Collins, the genome institute's director. At the 49th Annual Meeting of the American Society of Human Genetics, in San Francisco last October, he announced that all but 10 percent of the three billion bits of information composing some 100 000 human genes will have been decoded by the spring of 2000.

Using large, automatic gene-sequencing machines, the remaining gaps could be closed within three more years, he said. The present state of knowledge of the genome is referred to as a draft, in the sense that gaps still have to be closed and many of the base sequences are

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still short of the 99.99 percent accuracy desired.

As for Chromosome 22, it is one of 23 pairs of human chromosomes and is on the small side. But its genes are associated with such maladies as schizophrenia, movement disorders, and DiGeorge syndrome, which causes facial abnormalities and heart problems. The chromosome has 43 million bases, of which only 33.4 million have been sequenced so far. But the bases decoded in some 545 genes are thought to include regions containing protein-making genes, which are of most interest.

Notably, 60 percent of the three billion human bases will be sequenced in just six or seven months, whereas only 30 percent were sequenced in the first nine years. (The other 10 percent are going slowly because they are inherently difficult to decipher.) The speedup is due in part to a new generation of heavy-duty automated sequencing machines, and in part to the emergence of competition to be first. In June 1998, a private company, Celera Genomics of Rockville, Md., jumped in with its own sequencing technique. Backed in part by scientific instrument maker Perkin-Elmer Corp., Norwalk, Conn., it aims not only to sequence the genome but to patent those genes of commercial interest. The consortium, on the other hand, wants to prevent the patenting of human genes, and so has presented its data daily on the World Wide Web. Once made public, the genes cannot be patented.

Sequencers used by the consortium, for example, separate DNA fragments in thin capillary tubes instead of on large flat gels. The separation principle stays the same: tell-tale fluorescent markers—one for each of the four bases—are attached systematically to the end of all the fragments derived from an unknown piece of DNA. Placed in an electric field, these fragments move, or migrate, at speeds that vary with their size. At the "finish line," a laser detects the markers, whose order of arrival reveals the sequence of bases within the piece of DNA.

In traditional sequencing with large gels, the limiting factor was the relatively slow speed at which the DNA fragments migrated. But in the capillary tubes, whose inside diameter is 75 μm, the DNA fragments separate faster, although in the same way. What's more, the process of loading the DNA fragments into the tubes can be automated so the sequencers can run day and night.

The technology was developed largely with funding from the U.S. Department of Energy, one of the major sponsors of the Human Genome Project. The licensee is Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, United Kingdom.

"Clearly the advent of capillary sequencers has been a boost: it saves time and money," said Collins. "Now we are looking forward to even more savings by using smaller volumes of material."

MICROARRAYS REVEAL GENE ACTIVITY

Decoding the human genome is just the beginning. "Next we must learn how genes are switched on and off and how they work and interact," said Claus Bertram, director of the Institute for Human Genetics, University of Heidelberg, Germany. The idea is to study the activity of genes before and after something has been done to their cells—say, a drug has been added or the cells irradiated.

The development of machines smaller and cheaper than the ones used currently in big and well-funded laboratories plays a role here. For example, at around US $53 000, the GMS 417 arrayer from Genetic Microsystems Inc., Woburn, Mass., is within the affordability range of relatively small research facilities, which can devote the smaller machines to individual projects.

The arrayer relies on a DNA chip, or array, basically a glass microscope slide roughly 25 by 75 mm in area. On this slide thousands of droplets of DNA fragments can be placed [Fig. 1]. Fabricated chemically, these are short pieces of single-stranded DNA containing parts of the genes under study; they are not in the typical double-stranded form where every base in a sequence has an opposing partner.

Extracts then made from the original "untainted" cells also contain single-stranded copies of the DNA. Rather as in sequence analysis, researchers couple fluorescent dyes to these DNA copies.

The next step is to lay the dye-marked cell extract on top of the chip. When the bases on the DNA chip and their matching bases in the cell extract make contact, they form double strands of DNA. After a rinsing process, only these double strands of fluorescent-marked DNA copies remain on the slide.

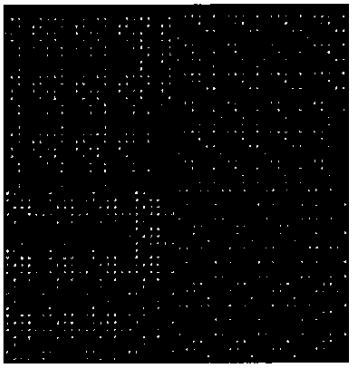
A view through a microscope reveals a fluorescent mosaic of spots, each representing a matched pair of genetic strands. Their colors indicate what bases have joined. Today no one need stare through a microscope counting spots and noting their color. Instead, a laser scanner reads the mosaic and passes the data on to a computer, whose algorithms may eventually answer a question such as which genes have been switched to a new state by a certain added drug.

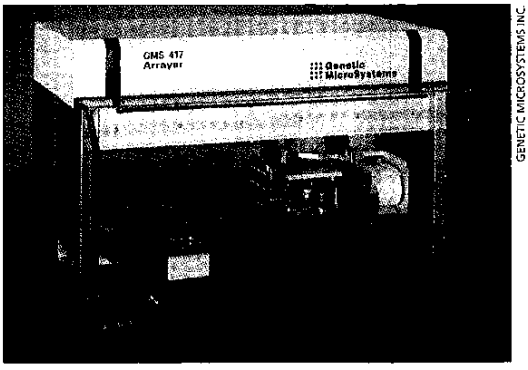
Progress has been made in miniaturizing the underlying chemistry—the spots on a DNA chip and the spaces in between are 50–300 μm across. But the cost of the laser scanning equipment is steep. Hoping to both reduce costs and improve performance, Genetic MicroSystems last year introduced a new laser-based GMS 418 Array Scanner to work with its GMS 417 Arrayer. The $60 000 system's focused laser beam is scanned by means of an oscillating lens weighing a fraction of a gram, while a motor moves the stage holding the slide. The "flying objective" microscope can scan a 22-by-75-mm slide in about four minutes with 10-μm resolution.

"This concept is extremely attractive for capturing a high-resolution image of a large field of view such as a DNA array," explained Jean Montagu, the company's chairman and president.

A more elegant way to detect the double-stranded DNA on the chip relies on direct electronic detection, under study by Rainer Hintsche at the Fraunhofer Institute for Silicon Technology, Itzehoe, Germany. Since the DNA bases are charged molecules, the electric field surrounding them changes as the cellular extract is added to the chip and as some bases move from single- to double-stranded DNA. This change can be detected by microcapacitors attached to the chip, with the magnitude of the effect depending on the base pair. The use of a laser scanner would be unnecessary.

"This procedure would really merge DNA chip technology





GENETIC MICROSYSTEMS INC.

[1] Researchers can design experiments of their own using an instrument such as Genetic MicroSystems' desktop GMS 417 Arrayer. It can put down precise spots of DNA or other materials on sample slides of about 22 by 75 mm. A companion laser scanner [not shown] then rapidly detects the presence and quantity of the fluorescent signal at each spot on the array [slide, near right]. A variety of software packages are available for gene mapping on the chip and other applications.

with electronics and make a pocket-sized machine possible," noted Jörg Hoheisel, a researcher at the German Cancer Research Center, Heidelberg.

Others are also developing better and cheaper ways to prepare the single-strand DNA chips. The market leader in such chips—that is, the glass slides to which DNA is already attached—is the GeneChip from Affymetrix Inc., a Santa Clara, Calif., equipment maker that recently acquired Genetic MicroSystems. Affymetrix relies on photolithography techniques borrowed from semiconductor fabrication. Instead of putting down circuit patterns, the photosensitive technology deposits DNA strands and then defines them in tiny spots of material.

The arrayer from Genetic MicroSystems lays even smaller amounts on a DNA chip. It passes a pin down through a film of liquid that contains the fragments and is held by surface tension in an open ring. Packard Instrument Co., Meriden, Conn., has developed an ink-jet-like machine for depositing the droplets of DNA fragments. Glass capillary tubes spit out submicroliter volumes under piezoelectric control.

DECODING PROTEINS

For some scientists, there are other things worth decoding besides genes. In an analogy to genomics, these scientists speak of proteomics, which involves analyzing the different proteins making up a given cell. Proteins are made up of 20 different building blocks, the amino acids.

"Proteomics also strives to find out about the differences between diseased and healthy cells, thereby identifying new markers for diagnosis and targets for therapy," Jasminka Godovac-Zimmermann, a researcher at the Centre for Molecular Medicine in University College, London, told *IEEE Spectrum*. She studies how the protein pattern of cells changes under the influence of so-called signal molecules. Every protein in a cell can be characterized by its molecular mass. That's why mass spectrometry (MS) has become the "proteomicist's" dearest toy. Having evolved from studying the chemistry of small molecules within a decade, Maldi MS, for short, is today the key technology in proteomics.

Maldi stands for matrix-assisted laser desorption/ionization mass spectrometry. In this instrument, a chemical helper is needed to get the relatively huge protein molecules to carry a charge and "fly" through the spectrometer's electric field. A laser aimed at the cellular material adds charge to the proteins, which are torn from a cellular extract and accelerated. But the proteins themselves usually show only poor resonance absorption of the laser energy—hence the need for the highly absorbant chemical helper.

When the technique was used in protein research about 10 years ago, the proteins composing the cell had to be reconstructed from ionized fragments because the laser shot destroyed the molecules. Today, the technique has been refined and the proteins are not destroyed; their masses are calculated from the flight times they need to arrive at a detector, often simply an electron multiplier. Once ionized, a so-called time-of-flight (TOF) mass spectrometer speeds up the various proteins to the same kinetic energy. Small proteins are sensed by the detector before the heavier ones arrive.

At present, advances are being made in several areas. First, the methods for providing a "runway" for the proteins are changing, in an analogy to the shift in DNA analysis from gels to chips. For example, "helper" chemicals are not needed by the Protein Chips from Ciphergen Biosystems Inc., Palo Alto, Calif. These are thin strips of aluminum coated with molecules that are capable of latching onto proteins out of a cellular extract. The laser ionizes the proteins directly from the surface of the strips, and researchers now speak of Seldi (surface-enhanced laser desorption/ionization) instead of Maldi.

In fact, with the help of a second mass spectrometer, proteins can be characterized still further. In Tandem-MS, the protein decays into its amino acid components,

An interview with BANU ONARAL

Banu Onaral (r), a professor of biomedical engineering and electrical engineering at Drexel University in Philadelphia, is director of Drexel's School of Biomedical Engineering, Science and Health Systems. She is current president of the IEEE Engineering in Medicine and Biology Society.

***Spectrum:* You are also a founding member of Drexel's Scaling Signals and Systems Laboratory. Can you tell us what its work involves?**

We work primarily with biomedical signals and systems. For example, we study the electroencephalogram (EEG), which reflects the brain's electrical activity, to develop automatic seizure detection algorithms for adults and just born babies. More recently, we introduced a method based on heart rate variability signals to monitor the loss of consciousness experienced by pilots subjected to acceleration stress.

We also modeled the global dynamics of the loss of consciousness induced by the loss of blood to the brain. We did this by combining physiological knowledge at the local microscopic and global macroscopic levels with experimental data and computational tools.

What does "scaling" refer to in the name of your lab?

It refers to the scaling nature of physiological signals. To visualize this, think of the branching pattern of a tree (or the arterial tree of the cardiovascular system or the bronchial of the lungs): trunk to branches, branches to smaller branches, smaller branches to twigs, and so on. If we enlarge the smaller patterns (say, the twigs), they look like the larger patterns (small branches). Thus, in scaling structures, smaller patterns are somehow linked to larger patterns; there is a systematic relationship between scales.

If the patterns at different scales look exactly alike, the object is called self-similar. Likewise, scaling properties are observed in dynamic signals. Here, fast activity or fluctuations are related to slow activity or fluctuations. Upon magnification, the smaller details look like the whole.

In nature, and particularly in biology and physiology, many systems derive their global collective behavior from the interplay of a multitude of locally interacting components. Such complex systems often exhibit scaling properties, or a "scaling signature." In turn, scaling measures can be used to capture and to characterize the essence of complexity, that is, the interplay between local elements or events. In addition to scaling, we now enjoy powerful tools to analyze, model, process and control the global behavior of complex physiological systems—though we're not quite where we want to be yet with these capabilities.

Is this mainstream biomedical research?

It is spearheaded by several frontier laboratories around the world. With biological information exploding, we're facing the ultimate scientific challenge: to put the pieces of the amazing biological and physiological puzzle together, from molecular to systemic levels, across many scales and degrees of complexity. The term "physiome" has been coined for this concept: "physio" for life or nature, "ome" for the entity, the whole. This can be viewed as the systemic counterpart of the "genome," which integrates biology with quantitative and computational methods to determine the basic building blocks of life.

Also, in other emerging areas such as neuroengineering, this

which can be detected in the second spectrometer. Not only the mass but also the detailed composition of the protein can be found. In still another technique, the proteins are thrust into a magnetic chamber for analysis. Happily, this technique is gentle: investigators can analyze both the mass and the chemical composition of the proteins.

SEEING DOUBLE

Computed tomography (CT) and magnetic resonance imaging (MRI) are routine for imaging organs within the human body, whether for diagnosing illness or for monitoring the progress of a prescribed therapy. The techniques generate images that slice up the patient virtually (nonsurgically), and that provide a physician with three-dimensional information of the patient's anatomy. But seeing images of just the size, shape, and location of the body's organs and tissues can be inadequate for gauging their condition. Critical diagnostic questions might include: Is the blood flow to the myocardium adequate? Or has a tumor metastasized to other organs?

Answers are often obtained using diagnostic methods collectively known as functional imaging, which looks at physiology and metabolic processes in tissues and organs. The most versatile and common functional imaging methods are PET and Spect, acronyms for positron emission tomography and single-photon emission computer tomography, respectively.

In PET, a positron-emitting tracer is injected intravenously and spreads rapidly through the body. A physician looking for an answer to a specific physiological or diagnostic question would pick a radionuclide tracer for its biochemical characteristics whereby it accumulates in specific tissues.

The most common radiolabeled agent imaged with PET is fluorodeoxyglucose labeled with fluorine-18. This agent is metabolized using the same pathway as glucose and, therefore, homes in on metabolically active tissue such as the brain, the myocardium, or malignant tumors.

The radionuclide emits a positron that annihilates, resulting in the simultaneous emission of two antiparallel 511-keV gamma rays. These are detected in coincidence by one of two means: either a ring of bismuth germanate scintillation crystals coupled to photomultiplier tubes placed around the patient or a pair of sodium iodide scintillation cameras at opposite sides of the patient.

Upon absorbing the gamma rays, the scintillation detectors generate electronic signals indicating the photon energy and location of the events. Those signals are digitized and read into a computer, where a tomographic reconstruction algorithm turns them into one or more cross-sectional images. Between them they comprise a 3-D distribution of the radionuclide in the patient. More specifically, the picture indicates the tracer's regional concentration, thus delineating the metabolic activity of a specific tissue, hence how it is functioning and whether it has been affected by disease.

Spect relies on the detection of gamma-emitting radiopharmaceuticals labeled with technecium-99m or other radionuclides that are used, for example, to determine the extent of metastatic disease in the skeletal system, perfusion in the myocardium, kidney function, blood flow in the brain, and a wide variety of other physiological parameters.

Today, CT, MRI, and Spect are on hand at most U.S. and western European hospitals. PET is available at many major medical centers and is becoming more widely available. However, "PET or Spect examinations typically are performed in different rooms of a clinic and often on different days than CT or MRI examinations," noted Bruce Hasegawa, a faculty member at the University of California, San Francisco (UCSF), Department of Radiology. "Thus it can be difficult to correlate the anatomical and physiological information in a precise way."

To overcome this problem, Hasegawa and his colleagues combined two machines: they placed a Spect scintillation camera adjacent

s becoming the defining approach. Here, if you can capture information on the brain cellular level and connect it with physiologic levels through computational models, you might be able to determine how thoughts are turned into action. The feasibility of this was demonstrated in animal models by our colleagues at MCP Hahnemann University (in Philadelphia) this summer. This gives us hope that we may eventually develop brain-machine interfaces that use neural data to control prosthetics and robotics.

' ARTIFICIAL HEARTS, ONCE ELECTROMECHANICAL DEVICES, ARE NOW "SCAFFOLDS" WITH CELLS GROWING INTO THEM, SO THAT THEY HAVE MANY PROPERTIES OF NATURAL BIOLOGICAL SYSTEMS.'

What developments in biomedical engineering will have the biggest impact in the next decades?

Clearly, the genome sciences and related technologies—like DNA chips—will soon connect with information processing and the quantitative and systems-integrated approaches of engineering. Drugs and therapies will be tailored to the basic program of life—our genetic code. The Blue Gene supercomputer announced last month by IBM Corp. will simulate the folding of proteins, a routine process for the molecular workforce of life but daunting to fathom. Once harnessed, however, it will inspire the design of human-made systems that take their cues from the brilliance of nature.

The emerging field of "biomedical information engineering" is aiming to seamlessly integrate tools and techniques across the spectrum of gene and protein sciences up to the functional levels, through medical and clinical informatics and health care management. Its success will depend on how effectively we can leverage the talents of life, information, and computational scientists with the systems know-how and integrative skills of engineers.

What are the biggest hurdles, then, to overcome?

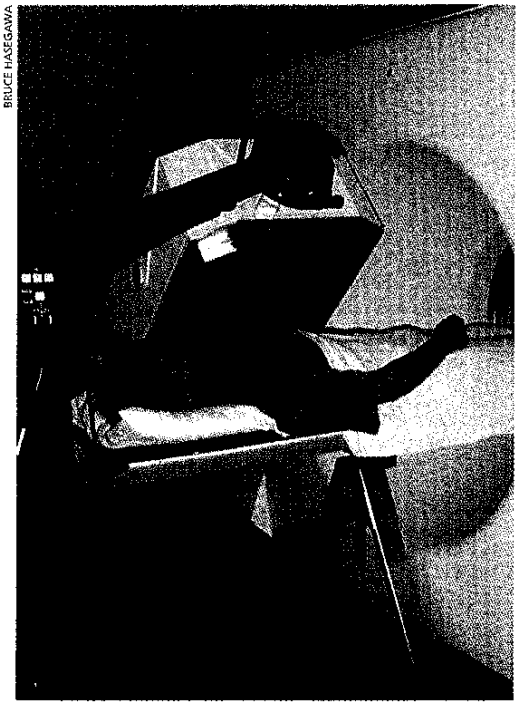
Learning to bring to bear on health problems everything we know about life from sensing, coding, quantifying, monitoring, processing, imaging, visualizing, modeling, managing . . . The huge amount of information produced by decoding the genome needs to be dealt with before bioinformatics is well linked to what happens at a hospital bedside. Everything we learn at the bench of genome and physiome sciences must be integrated into clinical practice, and insights gained from patient data must be fed back to the research bench.

Is integrative systems engineering having effects outside the genome sciences, drug design, and gene therapy?

Yes. In all areas of physiological systems engineering, involving, say, the cardiovascular or respiratory or vision systems and in tissue engineering. We can regenerate skin, have made progress with bone, and we're working on the liver and other organs. Artificial organs have new meaning. Artificial hearts, once electromechanical devices, are now "scaffolds" with cells growing in them so that they have many properties of natural biological systems.

And neuro-engineering?

When we truly understand how the brain works, we will figure out how to mimic it. This will inspire us to design systems that will impact profoundly how we deal with information, how we interact with our physical environment and with each other, and how our communities and societies form and function. Life and living as we know it today will change. —*A. R.*



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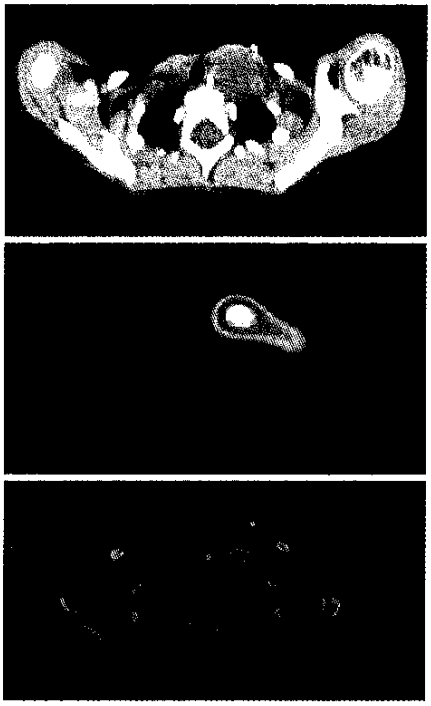

[2] A scintillation camera [to left of patient] in a system at the University of California, San Francisco, is used in single-photon emission computed tomography (Spect). The camera generates radionuclide tomograms for evaluating the functional or physiological status of a part of the body. A picture of the patient's anatomy is gained after a scan with computed X-ray tomography (CT) [shown at patient's feet].

For a patient with a neuroblastoma (a childhood cancer), a CT image of the anatomy [top] is combined with a Spect image [middle] after the patient is injected with a radioactive tracer that accumulates in the tumor. The combined image pinpoints the site of the tracer uptake [bottom].

to a CT, so that both can image a patient without requiring the patient to be moved between scans, according to their report last October at the IEEE Medical Imaging Conference in Seattle [Fig. 2]. The combined imaging system has multiple clinical applications, especially for diagnosis of cancer and coronary artery disease.

The combination not only made it easier to correlate anatomical and physiological information, according to Hasegawa, but also provided a framework for obtaining more accurate functional information than is possible with Spect alone.

For example, after emission, a large fraction of radionuclide photons are absorbed by the body. The effect is known as photon attenuation and can have enough of an effect on the apparent density of photons in the radionuclide image to mislead the physician into a misdiagnosis. However, CT produces a 3-D map of attenuation coefficients in the body, information that can be incorporated into the reconstruction of the Spect image to correct it for photon attenuation artifacts.

Similarly, a PET/CT imaging system has been developed by a research group headed by David Townsend of the department of radiology at the University of Pittsburgh. Some 70 patients have been imaged with the PET/CT system, according to Townsend, with more patients being imaged daily.

These combined imaging approaches have drawn the attention of industry. GE Medical Systems, Milwaukee, Wis., has installed two CT/Spect prototypes, one at Vanderbilt University, Nashville, Tenn., and the other at Rambam Medical Center in Israel. Both General Electric and the joint venture CTI/Siemens, in Knoxville, Tenn., are interested in the CT/PET approach.

Not yet usable on patients, but with great promise for experimental studies, are combinations of smaller versions of these imaging tools. Over the past four years, researchers at the University of California at Los Angeles (UCLA) Crump Institute for Biological Imaging have developed an experimental method that combines PET and MRI. This approach is complicated because MRI systems rely on strong magnetic fields that are perturbed by foreign metallic objects. "Our big problem was to avoid electromagnetic interference between the PET scanner and the MR tomography system," recalled Arion Chatziioannou of the UCLA team led by Simon Cherry.

The group solved this problem by coupling the scintillation detectors to optical fibers. These transmitted an optical signal to photomultiplier tubes placed at some distance from the resonance imager—far enough away to avoid perturbations either in the magnetic resonance system or in the radionuclide signal generated by the photomultipliers. The group is now working to enhance the sensitivity of the PET detector.

"Our new system will have two or three concentric detector rings," said Chatziioannou, to improve the overall detection probability of gamma rays.

PAINLESS TRIP WITHIN THE BODY

While researchers experiment with dual-mode imaging, the classic solo CT technique is not fading away. One of its promising applications is in virtual endoscopy. To examine the bowel for precancerous lesions, for instance, it is customary to insert an optical-fiber probe into the organ and then look through the probe. Besides being uncomfortable, this procedure may cause an infection or or even perforate the bowel.

Three-dimensional images of the colon, reconstructed from computerized two-dimensional data, were obtained some years ago at the State University of New York at Stony Brook. Looking at these images on a computer screen, a physician can examine the inside of the colon just as if a camera were inside the body. Details for study can be chosen by virtually navigating through the 3-D images. But this can be done faster by predesigning the path through the colon.

"Designing such a virtual path is a relatively simple task, because the colon resembles a twisted tube, which is not a complex structure," Taosong He, of the Bell Laboratories division of Lucent Technologies Inc., Holmdel, N.J., told *Spectrum*.

Far trickier than imaging the colon is imaging a branching organ like the lungs. Via the virtual CT camera on the computer screen, the physician must examine each and every section of the organ where anomalies may lurk. As with the colon, too, unnecessary "surfing" of the lung can be eliminated. Taosong He calls the solution "reliable navigation"—a concept he described at October's Seattle imaging conference. The physician's control of the virtual camera is limited to an automatically computed path. Taosong He hopes to evaluate his concept through clinical trials "in the near future." ◆

Spectrum editor: Alfred Rosenblatt