May 2000

Science & Technology REVIEW

U.S. Department of Energy's
Lawrence Livermore
National Laboratory

DNA Signatures Defend Against Bioterrorism

Also in this issue:

- Uses of Laser Isotope Separation
- Imaging Catheter for Less Invasive Surgery
- JanUSP, a New Ultrashort-Pulse Laser

About the Cover

A multidisciplinary scientific team from Livermore's Biology and Biotechnology Research Program is developing DNA-based signatures for detecting biological warfare agents. The unique characteristics of disease-causing microbes can be incorporated in detection systems and used to quickly and accurately identify the causes of biological outbreaks. The full story of this work, which is supported by the Department of Energy's Chemical and Biological Nonproliferation Program, begins on p. 4.

Cover design: George Kitrinos

About the Review

Lawrence Livermore National Laboratory is operated by the University of California for the Department of Energy. At Livermore, we focus science and technology on assuring our nation's security. We also apply that expertise to solve other important national problems in energy, bioscience, and the environment. *Science & Technology Review* is published 10 times a year to communicate, to a broad audience, the Laboratory's scientific and technological accomplishments in fulfilling its primary missions. The publication's goal is to help readers understand these accomplishments and appreciate their value to the individual citizen, the nation, and the world.

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May 2000

Lawrence Livermore National Laboratory

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Abstracts

Elusive physics problem is solved

Collaborating scientists from the Lawrence Livermore and Lawrence Berkeley national laboratories and the University of California at Davis reported in the December 24, 1999, issue of *Science* that they have solved a fundamental but elusive problem in atomic physics. They have calculated the final energies and directions of three charged particles that collide, break up, and scatter when a hydrogen atom ionizes. Being able to predict what happens during ionization is important because the phenomenon pervades a wide range of physical processes. For example, ionization by electron impact is responsible for the glow of fluorescent lights and for the ion beams that engrave silicon chips.

The collaborating team—physicist Tom Rescigno of Lawrence Livermore and computer scientist Bill McCurdy of Lawrence Berkeley, along with doctoral candidate Mark Baertschy of UC Davis and postdoctoral fellow William Isaacs of Lawrence Berkeley—were dealing with the simplest example of electon-impact ionization, which still is an intrinsically difficult problem. It requires calculation of energies and directions for a final state in which all three particles are moving away from each other. The problem had resisted solution for some 40 years.

Earlier, others had developed analytic solutions for particle interactions in an isolated hydrogen atom; they helped to establish the new quantum theory in the early part of the 20th century. In the 1950s, the bound states of the helium atom, with its two electrons, were computed accurately and established the theoretical framework for modern quantum chemistry. But scattering problems are much more difficult, and complete solutions for three or more scattering particles seemed intractable until large-scale computing power became available.

The ionization of a hydrogen atom begins with an electron incoming at a certain velocity. It interacts with the atom so that two electrons fly out at an angle to each other, leaving a proton behind. In their breakthrough calculations, the scientific team employed a transformation of Schrödinger's equation, which yields probabilities of finding particles in a certain state. Using computationally intensive processes, they extracted all the dynamic information of the particle interactions, which was then used to obtain specifics about the energies and directions of outgoing electrons.

In solving the problem, the collaborators noted that the large-scale computers they used, which are usually found tackling problems concerning very large and therefore very complex systems, had instead been used "to answer a basic physics question for one of the simplest systems imaginable in physics and chemistry."

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The Lab's contribution to an orbiting observatory

One of the world's most powerful x-ray telescopes, the X-Ray Multi-Mirror Newton Observatory (XMM), was launched in December 1999 by the European Space Agency. From space, the telescope has been sending back images and spectra of stars exploding and spewing out materials to form new stars, or twin stars chasing one another at such great speeds that they generate a dynamo that twists the stars' magnetic fields, causing intense stellar flares and storms.

The XMM has two spectrometers that split incoming x radiation into different wavelengths. The resulting x-ray spectra reveal the elemental makeup of objects being observed as well as the temperatures, densities, and velocities of the emitting material.

Reflective grating arrays inside the spectrometers perform the x-ray splitting. They were designed, prototyped, and fabricated by Lawrence Livermore researchers. The arrays function like a glass prism that splits ordinary visible light into a rainbow of hues. They consist of 182 gold plates with extremely tiny grooves (on the order of millionths of centimeters) that must be precisely constructed and aligned. The plates must remain fixed to within 20 millionths of a centimeter in order to diffract x rays to a common detector. The delicate arrays must be mounted in the spacecraft in a way that can withstand the tremendous shock and vibration of launch without losing alignment.

"It was an incredible challenge to design and develop not only the reflective grating plates in the arrays but also the large, lightweight support structures that hold the grating arrays in the spacecraft," said Todd Decker, project engineer on Livermore's work for the XMM.

The work came to Livermore via Columbia University physics professor Steve Kahn, principal investigator for the reflection grating array, which is funded by NASA. Kahn came to Livermore for help because of the Lab's expertise in precision engineering.

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Good, Bad, or Ugly, Microbes Deserve Our Respect

WE live as guests in a world dominated by microbes. They provide about half of the oxygen we breathe, are the basis for the food web that is essential to all higher species, and help balance the delicate global environment that has sustained our planet for almost 4 billion years.

Microbes were discovered only about 300 years ago—relatively recently. Antoni van Leeuwenhoek, a Dutch merchant, made microscopes with high-quality lenses that made it possible to observe microbes. He was the first to see microscopic living systems in blood, skin, plants, and pond water. But it wasn't until the 20th century that we began to appreciate the role of microbes in maintaining the health of our ecosystem.

Many microorganisms, such as *Prochlorococcus marinus*, are good. The *P. marinus* bacterium is the primary biomass producer in 75 percent of the world's oceans and is the smallest, most abundant photosynthetic organism. It is said to be equivalent in mass to all humans on our planet. DOE's Joint Genome Institute is currently completing the DNA sequence of this organism.

Other examples of "good" microorganisms are those used in industrial processes, including paper making, leather tanning, food processing, and alcohol fermenting. Others are found in products like laundry detergent, cheese, and yogurt. Some bacteria are responsible for keeping us healthy. These include the bacteria in our intestines that assist digestion and make essential B vitamins, or bacteria that can be made into vaccines to ward off other microbes.

"Bad" microbes are usually associated with human disease and are called pathogens; they have been responsible for many of the world's major diseases. *Yersinia pestis* is a bacterium that causes plague. Although relatively rare in the U.S., plague was responsible for the death of nearly one-third of the European population in the 14th century and is still a problem today in some developing countries. Another microbe, the influenza virus, killed more than 20 million people worldwide in 1918–1919, more deaths than occurred in World War I.

Bad microbes not only infect humans but also can attack crops, wildlife, and farm animals. They could cause major agricultural losses or lead to infection of people through contact or ingestion.

Microbes get ugly in at least two ways. First, some microbes are known to mutate (change) the sequence of their DNA quite rapidly. Mutation has the consequence of making vaccines and other treatment regimes less effective, such as those for AIDS, influenza, and hepatitis. Second, microbes can be abused for the purpose of bioterrorism. History shows that biowarfare has existed since the time of the Romans and remains a threat today.

The plethora of microorganisms, both good and bad, leaves one in awe of their genetic diversity, of the roles they play in maintaining our ecosystem, and of the elaborate but utilitarian machinery that has evolved over billions of years to allow them to maintain their niche in life. From the metabolic pathways that regulate the utilization of carbon to the biochemical pathways that precisely control the mechanisms of infection, these organisms deserve our respect.

Lawrence Livermore scientists are increasing their emphasis on microbial research. As members of the Joint Genome Institute, we are sequencing the DNA of several microbes important for carbon sequestration, nitrogen fixation, and bioremediation. In addition, as part of our national security role, we are focusing on those microbes that could be used in bioterrorism. With support from the Department of Energy and with partners at Los Alamos and the Centers for Disease Control and Prevention, we are using our expertise in molecular biology and biotechnology to sequence selected pathogens and develop the molecular tools to detect them. We are now beginning to unravel the mechanisms by which these pathogens become infectious in host systems. The article beginning on p. 4 illustrates some of the technologies and results of this work.

■ Tony Carrano is Associate Director, Biology and Biotechnology Research Program.

Uncovering Bioterrorism

DNA-based signatures are needed to quickly and accurately identify biological warfare agents and their makers.

WITH the end of the Cold War, the threat of nuclear holocaust faded but another threat emerged—attack by terrorists or even nations using biological agents such as bacteria, viruses, biological toxins, and genetically altered organisms. The former Soviet Union once had a formidable biological weapons program. Now, several countries and extremist groups are believed to possess or to be developing biological weapons that could threaten urban populations, destroy livestock, and wipe out crops.

Even terrorists with limited skills and resources could make biological weapons without much difficulty, says Tony Carrano, Lawrence Livermore's associate director for Biology and Biotechnology Research. "It's not complex, it's not expensive, and you don't need a large facility." For these reasons, biological weapons have been dubbed the poor man's atomic bomb.

Contributing to the ease of making and concealing biological weapons is

the dual-use nature of materials to produce such weapons, because they are found in many legitimate medical research and agricultural activities as well. CIA Director George Tenet touched on this topic in Congressional testimony in February when he noted the overlap between manufacturing vaccines and producing biological weapons.

The agents used in biological weapons are difficult to detect and to identify quickly and reliably. Yet, early detection and identification are crucial for minimizing their potentially catastrophic human and economic cost. Lawrence Livermore scientists are participating in the Department of Energy's program to improve response capability to biological (as well as chemical) attacks on the civilian population.

A major part of DOE's program is developing better equipment, both fixed and portable, to detect biological agents (see *S&TR*, June 1998, pp. 4–11). However, any detection system is dependent on knowing the signatures of organisms likely to be used in biological weapons. These signatures are telltale bits of DNA unique to pathogens (disease-causing microbes). "Without proper signatures, medical authorities could lose hours or days trying to determine the cause of an outbreak, or they could be treating victims with ineffective antibiotics," says Lawrence Livermore's Bert Weinstein, deputy associate director of Biology and Biotechnology Research.

Because of the importance of biological signatures, DOE has launched a biological foundations program as a key thrust of its effort to improve response to terrorist attacks. The program involves experts at the Lawrence Livermore, Brookhaven, Los Alamos, and Sandia national laboratories, as well as colleges and universities. Researchers from the four national laboratories get together at least quarterly to share information and yearly for a formal review of their work. Weinstein reports that important progress has been made since the program began in early 1997, and new signature sets are being transferred to the Centers for Disease Control and Prevention and the DOE.

Over the next several years, DOE scientific teams expect to produce species-level signatures for all of the most likely biological warfare pathogens. The teams also expect to have an initial set of species-level signatures for likely agricultural pathogens, because an attack on a nation's food supply could be just as disruptive as an attack on the civilian population.

Several Levels of Signatures

The teams also aim to develop strain-level signatures for the top suspected agents. Strains are a subset of a species, and their DNA may differ by about 0.1 percent within the species. A species, in turn, is a member of a larger related group (genus), and its DNA may differ by a percent or so from that of other members of the genus.

Characterizing pathogens at the strain level requires significantly more work than recognizing a species. But strain-level signatures are essential for determining the native origin of a pathogen associated with an outbreak; such information could help law enforcement identify the group or groups behind the attack.

The biological foundations work aims to provide validated signatures useful to

public health and law enforcement agencies as well as classified signatures for the national security community. In developing these signatures, biological foundation researchers are also shedding light on poorly understood aspects of biology, microbiology, and genetics, such as immunology, evolution, and virulence. Increased knowledge in these fields holds the promise of better medical treatments, including new kinds of vaccines.

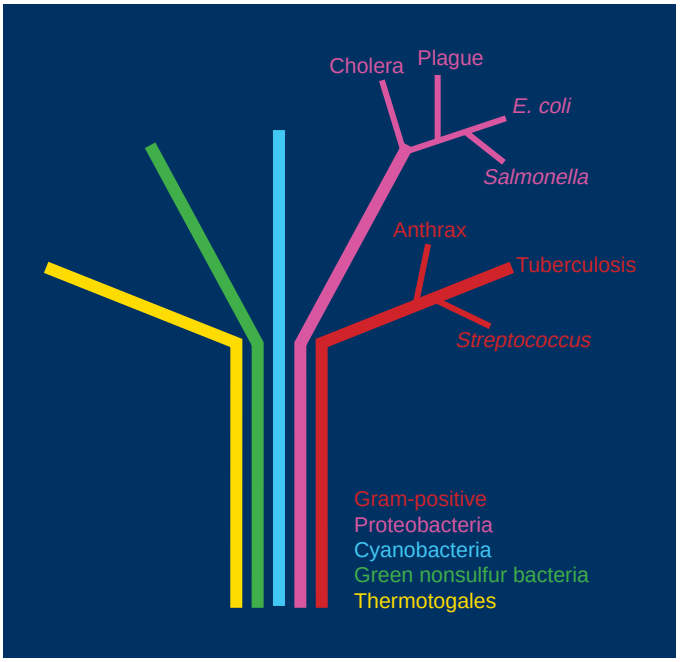
The biological foundations work is one element in DOE's Chemical and Biological Nonproliferation Program. Livermore's component of this work is managed by its Nonproliferation, Arms Control, and International Security Directorate. Other components of the overall program include detection, modeling and prediction, decontamination, and technology demonstration projects.

Livermore researchers were among the first to recognize, in the early 1990s, the tremendous potential of detectors based on DNA signatures. "We knew that a lot of work was necessary to develop the signatures the new detectors would need," says Weinstein. In particular, the researchers recognized several pitfalls. For example, if signatures are overly specific, they do not identify all strains of the pathogen and so can give a false-negative reading. On the other hand, if signatures are based on genes that are widely shared among many different bacteria, they can give a false-positive reading. As a result, signatures must be able, for example, to separate a nonpathogenic vaccine strain from an infectious one.

Several Levels of Identification

To enhance their detection development effort, researchers are exploring advanced methods that distinguish slight differences in DNA. They are using the multidisciplinary approach that characterizes Livermore research programs. In this case, DNA signature development involves a team of microbiologists, molecular biologists, biochemists, geneticists, and computer experts. In addition, the Livermore work benefits from collaborations with experts worldwide, extensive experience with DNA sequencing, and affiliation with DOE's Joint Genome Institute (see *S&TR*, April 2000, pp. 4–11).

Much of the work is focused on screening the two to five million bases that comprise a typical microbial genome to design unique DNA markers



This phylogenetic tree is a simple representation of the bacterial kingdom. All human bacterial pathogens belong to the Gram-positive (red) or Proteobacteria (magenta) divisions. The other divisions consist of nonpathogenic bacteria associated with diverse environments. Biological signatures must be able to differentiate infectious bacteria from hundreds of thousands of harmless ones. Each genus of bacteria has many species, and each species can have thousands of different strains.

DNA signature development involves a multidisciplinary team of microbiologists, molecular biologists, biochemists, geneticists, and computer experts. Here, biomedical scientists Peter Agron and Lyndsay Radnedge are performing suppressive subtractive hybridization to distinguish DNA of various species of virulent organisms.

that will identify the microbe. The markers, called primer pairs, typically contain about 30 base segments and bracket specific regions of DNA that are a few hundred bases long. The bracketed regions are replicated many thousands of times with a detector that uses polymerase chain reaction (PCR) technology. Then they are processed to unambiguously identify and characterize the organism of interest.

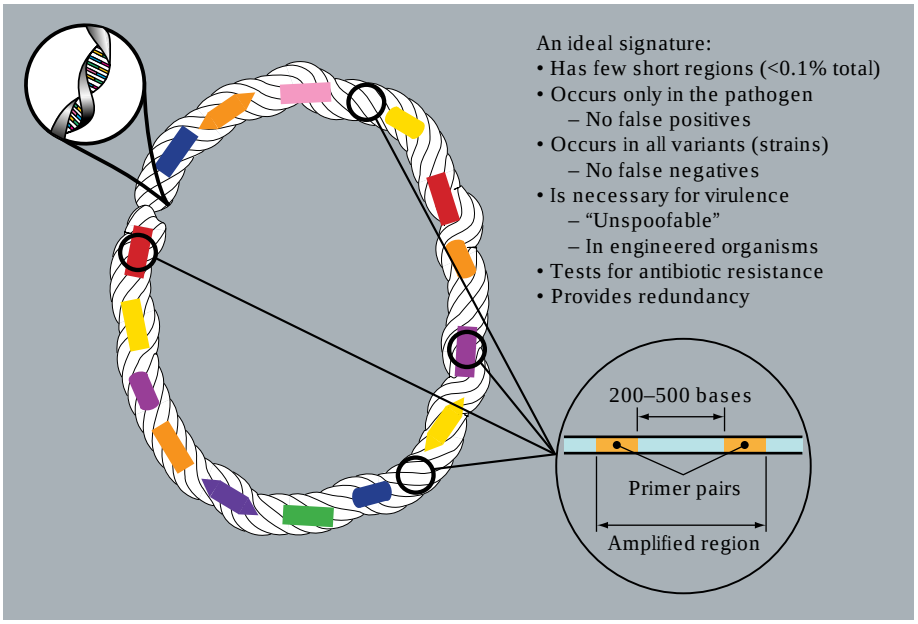
Weinstein notes that different signatures will be needed for different levels of resolution. For example, authorities trying to characterize an unknown material or respond to a suspected act of bioterrorism will begin with fairly simple signatures that flag potentially harmful pathogens within a few minutes. Typically, such a signature would encompass one or two primer pairs and be sufficient for identification at the genus level (*Yersinia* or *Bacillus*, for example) or below.

A signature in the next level of resolution is needed for unambiguously identifying a pathogen at the species level (*Yersinia pestis*, for example). This signature involves about 10 primer pairs. Currently, it takes several days to obtain conclusive data for a species-level signature. The goal is to reduce that time to less than 30 minutes.

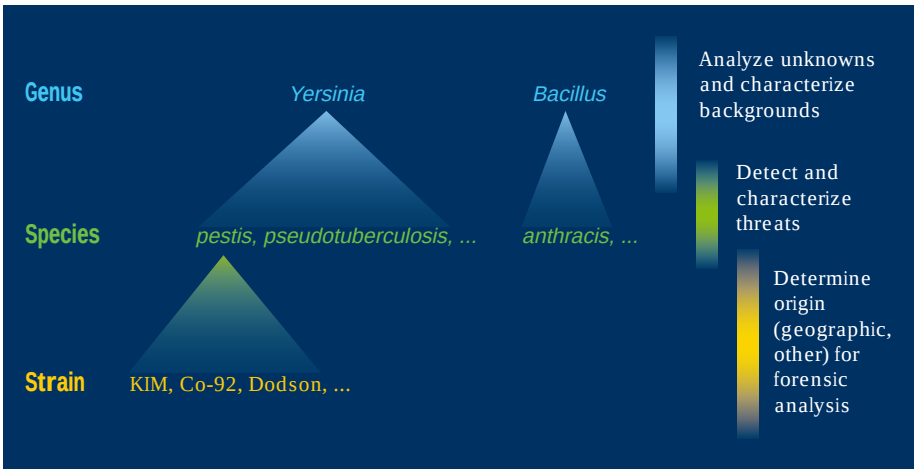
The third signature level is used in pathogen characterization, identifying any features that could affect medical response (for example, harmless vaccine materials versus highly virulent or antibiotic resistance pathogens). This signature level involves some 20 to 30 primer pairs. Together, the primer pairs offer a certainty of correct identification. Currently, providing such a high level of confidence requires several days; the goal again is to reduce the time to less than 30 minutes.

The final signature level, intended primarily for law enforcement use, will permit detailed identification of a specific strain of a pathogen (for example, *Yersinia pestis* KIM) and correlate that strain with other forensic evidence. Such data will help to identify and prosecute attackers. The present typical time lag for results is currently a few weeks, and the goal is to reduce that to a few days.

Biological foundations program scientists have worked with DOE and other agencies to assemble a list of natural pathogens most likely to be used

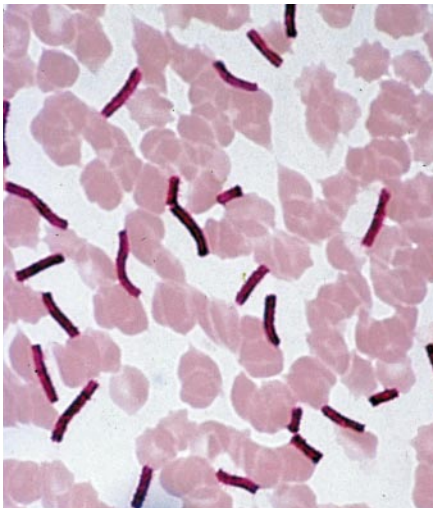


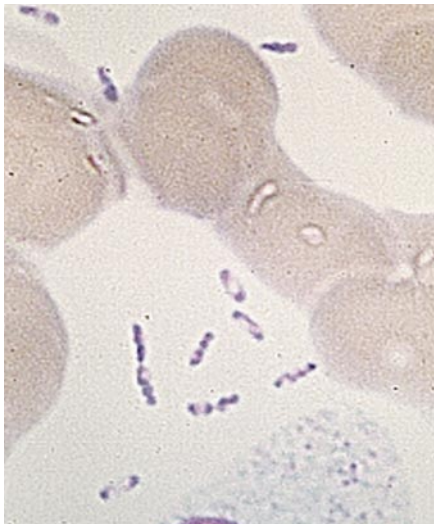
Bacterial chromosomes (DNA) form loops, unlike human chromosomes which form strands. In the loop, between two to five million bases of bacterial DNA are screened to locate unique regions (circled), which are marked with primer pairs. The marked regions are amplified thousands of times using polymerase chain reaction technology and then processed to identify and characterize an organism.



Different levels of signature complexity provide different problem resolutions. A simple signature, involving only one or two unique DNA marker regions, provides genus-level identification. Signatures for species- and strain-level identifications involve more marker regions and take longer to process, but provide more detail and accuracy. Law enforcement uses require signatures that provide strain-level identification.

in a domestic attack. The list includes bacteria, viruses, and other classes of threats, such as agricultural pathogens. Two extremely virulent pathogens head the list: *B. anthracis* and *Y. pestis*, which cause anthrax and plague in humans, respectively. *Bacillus anthracis* has few detectable differences among its strains, whereas *Y. pestis* strains can vary considerably in genetic makeup. Unraveling the significant differences between the two organisms will give national laboratory researchers experience vital for facing the challenges of the next few years, as they develop signatures for a wide spectrum of microbes.





Two extremely virulent organisms head the list of pathogens most likely to be used by terrorists: *B. anthracis* (top) and *Y. pestis* (bottom), which cause anthrax and plague in humans, respectively.

Livermore Focuses on Plague

Research has been divided and is carefully coordinated among laboratories to avoid duplication. Livermore researchers are focusing on *Y. pestis*, *Francisella tularensis* (a bacterium causing a plaguelike illness in humans), and several other microbes that threaten human and animal health. They are working in collaboration with the U.S. Army Medical Research Institute of Infectious Diseases, the Centers for Disease Control and Prevention, the California Department of Health Services, Louisiana State University, Michigan State University, and research centers in France, China, and Russia. “We want to be prepared for the most likely pathogens from throughout the world,” says Weinstein.

Eleven species and many thousands of strains belong to the *Yersinia* genus. The most notorious species, *Y. pestis*, causes bubonic plague and is usually fatal unless treated quickly with antibiotics. The disease is transmitted by rodents and their fleas to humans and other animals. Although rare in the U.S., cases are still reported in the Southwest.

Livermore researcher Emilio Garcia notes that the seemingly subtle DNA differences among many *Yersinia* species mask important differences. One species causes gastroenteritis, another is often fatal, and a third is virtually harmless; yet all have very similar genetic makeup. Garcia’s team is using a technique called insertion-sequence-based fingerprinting to understand these slight genetic differences. Insertion sequences are mobile sections of DNA that replicate on their own. Analyzing for their

Biological Warfare Has a Long History

The use of biological agents as weapons is not a new phenomenon, Lawrence Livermore’s Tony Carrano points out. The Romans, for example, used corpses of diseased animals to poison the drinking wells of their enemies. During the horrific Black Death of the Middle Ages, the bodies of bubonic plague victims were catapulted over fortress walls of besieged cities.

During the French and Indian wars, 1754–1763, the British gave smallpox-infested blankets as gifts to the Indians because of their suspected alliance with the French. During World War II, Germany and Japan produced bacteria capable of infecting humans.

Biological attacks in the United States have been few and isolated. One occurred in 1984, when followers of Baghwan Shree Rajneesh poisoned several salad bars in Oregon with salmonella bacteria. In Europe, terrorist groups in Germany began producing botulinum toxin. In the late 1980s in Japan, the Aum Shinrikyo cult acquired anthrax bacteria and botulinum toxin and attempted to collect samples of Ebola virus.

Following the 1991 Persian Gulf War, United Nations inspectors revealed the vast scope of Iraq’s biological arsenal. Iraq was found to possess more than 150 bombs and 25 missile warheads filled with botulinum toxin, anthrax, or aflatoxin. What’s more, Iraq had built sophisticated laboratories to study and produce a wide range of biological agents and toxins.

presence will not only help refine signatures for *Y. pestis* but also shed light on how microorganisms evolve into strains that produce lethal toxins. This understanding, in turn, should give ammunition to researchers seeking an antidote or vaccine.

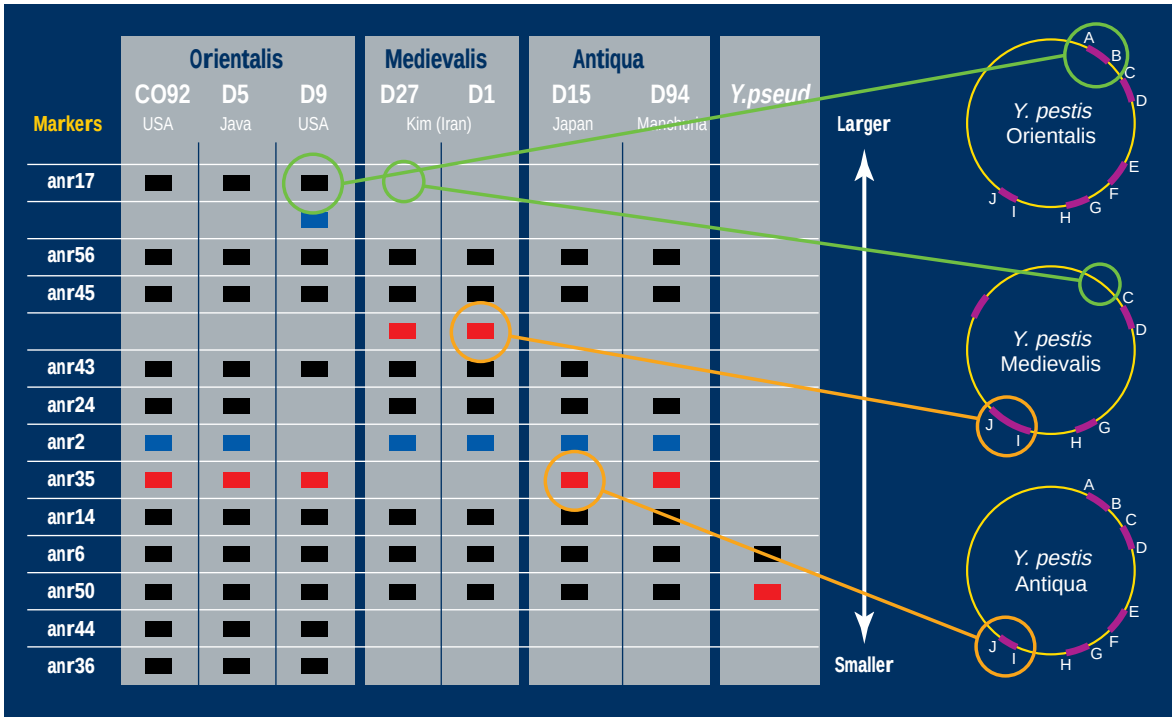
Garcia's team is collaborating with other world-renowned research centers to better understand the genetic differences among species and strains. A collaboration with France's Pasteur Institute is comparing the genetic complement of *Y. pestis* with another member of the *Yersinia* group (*pseudotuberculosis*) that causes an intestinal disease. "They are closely related, and yet they cause such different diseases," Garcia says.

Better and Faster, with More Uses

Livermore scientists are using a number of methods that allow more rapid identification and characterization of unique segments of DNA. Each method has advantages and drawbacks, with some more applicable to one organism than another. Weinstein expects that within two years, the Livermore team will have settled on a handful of techniques as the workhorses of signature generation.

In addition to the insertion sequence method, another promising technique is called suppressive subtractive hybridization. The method takes an organism and its near neighbor, hybridizes the DNA from both, and determines the fragments not in common as the basis of a signature. A team headed by Lawrence Livermore biomedical scientist Gary Andersen is working with colleagues at Moscow State University in Russia to advance the technique; one goal is to simultaneously analyze 96 strains of DNA.

Andersen's team has used suppressive subtractive hybridization to distinguish the DNA of *Y. pestis* from that of *Y. pseudotuberculosis*. The team has also used the technique to aid California's poultry industry by providing a handy way to detect *Salmonella enteritidis*. This bacterium can cause illness if eggs are eaten raw or undercooked. Subtractive hybridization results have been so



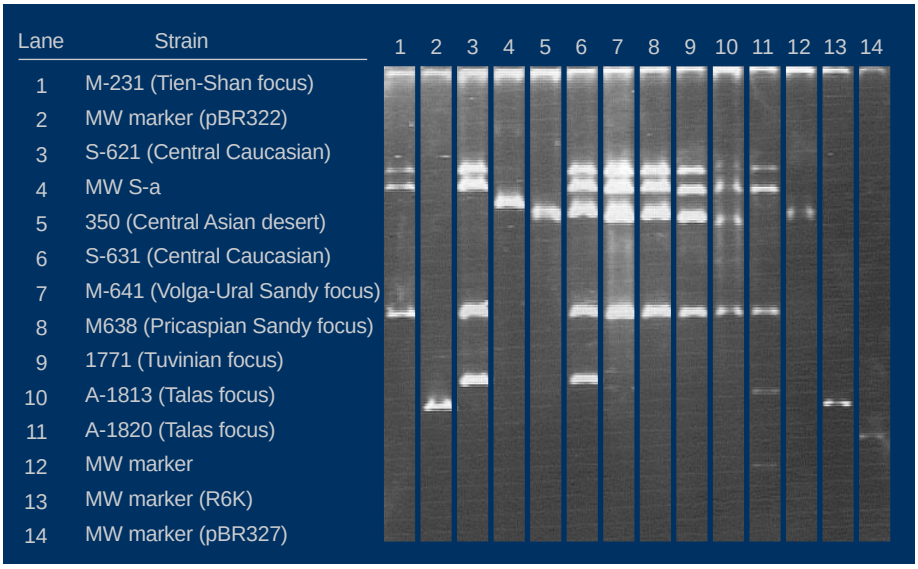
Insertion sequences are repeated sections of DNA whose location in the chromosome varies between different strains. Analyzing for their presence provides information about the type and biological function of a strain. The table at above left shows differences in the insertion sequence "fingerprints" of *Y. pestis* strains associated with the last three plague outbreaks. Red and blue rectangles indicate fragment shifts and changes from strain to strain. Some of these differences are graphically represented in the three strains of *Y. pestis* diagrammed at above right. For example, a fragment found in Orientalis is absent from Medievalis, and a fragment in Antiqua has shifted and become shorter than what it was in Medievalis.

successful that the signature can now be used to distinguish between subtypes of salmonella bacterium.

In addition to the DNA-based pathogen detection methods, researchers are developing detection capabilities using antibodies that can tag a pathogen by attaching to a molecular-level physical feature of the organism. Antibody assays are likely to play an important role in pathogen detection because they are generally fast and easy to use (commercial home-use medical tests use this form of assay).

Biological foundation researchers are working to improve these detection methods as well. For example, a collaboration with the Saratov Anti-Plague Institute in Russia is studying a bacteriophage (bacteria-killing virus) that only attacks *Y. pestis* and none of its cousins. Researchers recently discovered that the virus produces a unique protein component to attach to the bacterium cell wall at a certain site and gain entry. Garcia says that recognizing the distinct site could form the basis of a foolproof antibody signature. "If it's possible to achieve it with *Y. pestis*, we may be able to do it with other pathogens," he adds.

A Lawrence Livermore team has aided California's poultry industry with a biological signature to detect *Salmonella enteritidis*, a bacterium that can cause illness if eggs are eaten raw or undercooked. The signature can distinguish between subtypes of the bacterium and their different pathways to humans and other hosts.



Russia's Saratov Anti-Plague Institute is an important collaborator with Livermore in elucidating the subtle genetic differences among strains of *Y. pestis*. Above are some of the strains isolated by the institute.

Sensing Virulence

As more information about pathogens and their disease mechanisms becomes available and as genetic engineering tools to transplant genes become cheaper and simpler to use, the threat of genetically engineered pathogens increases. Biodetectors must be able to sense the virulence signatures of genetically engineered pathogens, or they will be blind to an entire class of threats. "Our ultimate objective is to identify several specific virulence factors that might be used in engineered biological warfare organisms so that we can detect these engineered organisms and break their virulence pathway," says Weinstein.

One key factor useful for detecting engineered organisms is an antibiotic resistance gene. When transplanted into an infectious microbe, the gene could greatly increase the effectiveness of a biological attack and complicate medical response. Some antibiotic resistance genes are widely shared among bacteria and are easily transferred with elementary molecular biology methods. In fact, a standard biotechnology research technique is introducing antibiotic resistance genes into bacteria as an indicator of successful cloning. "We need to be able to rapidly recognize such genes so that the medical response is appropriate," says Weinstein.

Another telltale indication of genetic tampering is the presence of virulence genes in a microbe that should not contain them. Virulence genes are often involved in producing toxins or molecules that cause harm or that simply evade a host's defense. "If a series of genes is made available to perform their functions at the right time, they could cause real damage," says Lawrence Livermore molecular geneticist Paula McCready. If interfering with the action of one of these genes or its proteins interrupts the virulence pathway, the disease process can be halted. Identifying and characterizing important virulence genes and determining their detailed molecular structure will greatly aid the development of vaccines, drugs, and other medical treatments.

As an example, *Y. pestis* disables the immune system in humans by injecting proteins into macrophages, one of the body's key defenders against bacterial attack. Because the protein acts as an immunosuppressant to disable the macrophage, understanding its structure not only would help scientists fashion a drug that physically blocks the protein but also would shed light on autoimmune diseases such as arthritis and asthma. A Lawrence Livermore team led by Rod Balhorn is working to determine the three-dimensional shapes of toxins such as the one produced by *Y. pestis* (see *S&TR*, April 1999, pp. 4–9).

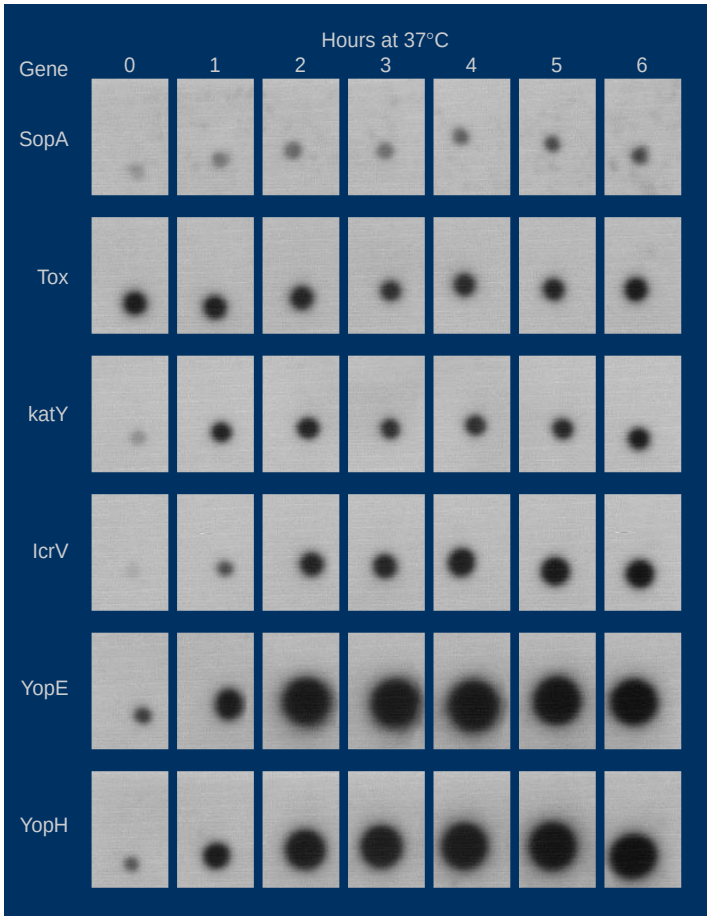
Virulence Genes in Common

Virulence genes spread naturally among pathogens and thus are also found in unrelated microbial species. Therefore, virulence genes alone are not sufficient for species-specific DNA-based detection. "We have to differentiate the virulence genes in natural organisms from engineered organisms," says Garcia.

Livermore researchers are using different methods for differentiating virulence genes from among the thousands of genes comprising the genomes of pathogens. One technique looks for genes that "switch on" (start making proteins) at the internal temperatures of mammals. For example, Livermore scientists are studying genes of *Y. pestis* that become much more active at 37°C. It seems a safe bet that many of these genes are associated with the bacterium multiplying within a warm-blooded host.

In 1998, a Lawrence Livermore team made an important contribution to understanding the genetics of *Y. pestis.* They sequenced the three plasmids (bits of DNA located outside the microorganism's circular chromosome) that contain most of the virulence genes required for full development of the bubonic plague in animals and humans. Plasmids sometimes transfer their genes to neighboring bacteria in what is called lateral evolution. (Antibiotic resistance genes are also located on plasmids.)

Garcia, who led the plasmid sequencing team, says that studying virulence genes can shed light on how new strains develop. The *Y. pestis* strain that causes bubonic plague, for example, may have evolved some 20,000 years ago. Such understanding is relevant to HIV, which may not have become infectious for humans until the 20th century.



As a way of identifying virulence genes, Livermore researchers look for bacterial genes that produce proteins at the internal temperatures of mammals (37°C). Four such genes (katY, IcrV, YopE, and YopH) in *Y. pestis* become much more active over a 6-hour period at 37°C.

Working with End Users

McCready notes that there needs to be a strong relationship between development of biological signatures and detection technologies and their end uses. Livermore researchers work with agencies that will be using signatures from Livermore and Los Alamos for both handheld detectors and field laboratories. "We want to make sure our tools get to the experts and agencies that need them," she says.

McCready is working closely with colleagues at the Bioterrorism Rapid Response and Advanced Technology Laboratory of the federal Centers for Disease Control and Prevention. Livermore is collaborating with the CDC to make diagnostic tools available to regional public health agencies and thus create a national mechanism for responding quickly to bioterrorism threats. Currently, many health agencies use detection methods that are not sufficiently sensitive, selective, or fast. For example, one culture test for detecting anthrax takes two days. Major damage and even death may have occurred in that time.

McCready emphasizes that DNA signatures will be thoroughly validated before being released, because their use might lead to evacuations of subways, airports, or sporting events, and such evacuations cannot be undertaken lightly. As part of the validation effort, Livermore scientists are characterizing natural microbial backgrounds to make sure that the signatures are accurate under actual conditions. To that end, researchers are collecting background microbial samples in air, water, and soil, as well as in human blood, urine, and saliva. McCready points out that *B. anthracis* is related to *B. thruginensis*, a naturally occurring harmless microbe that lives in dirt and can give a false positive reading to anthrax if the signature used is not adequately specific. The characterization effort is being aided by a device called the Gene Chip. Manufactured by Affymetrix Inc. and using technology developed by Livermore, the device simultaneously monitors the expression of thousands of genes.

Livermore researchers are looking ahead to a time when their efforts will have helped to equip federal and state agencies with a robust set of biological signatures crucial for America's response to any biological warfare threat. Equally important, the researchers envision a strong mechanism linking biomedical scientists with public health and law enforcement officials to develop new signatures speedily and cost-effectively to stay several steps ahead of terrorists.

—Arnie Heller

Key Words: anthrax, bacteriophage, biological signatures, biological weapons, Centers for Disease Control and Prevention (CDC), DNA, Gene Chip, plague, plasmids, virulence.

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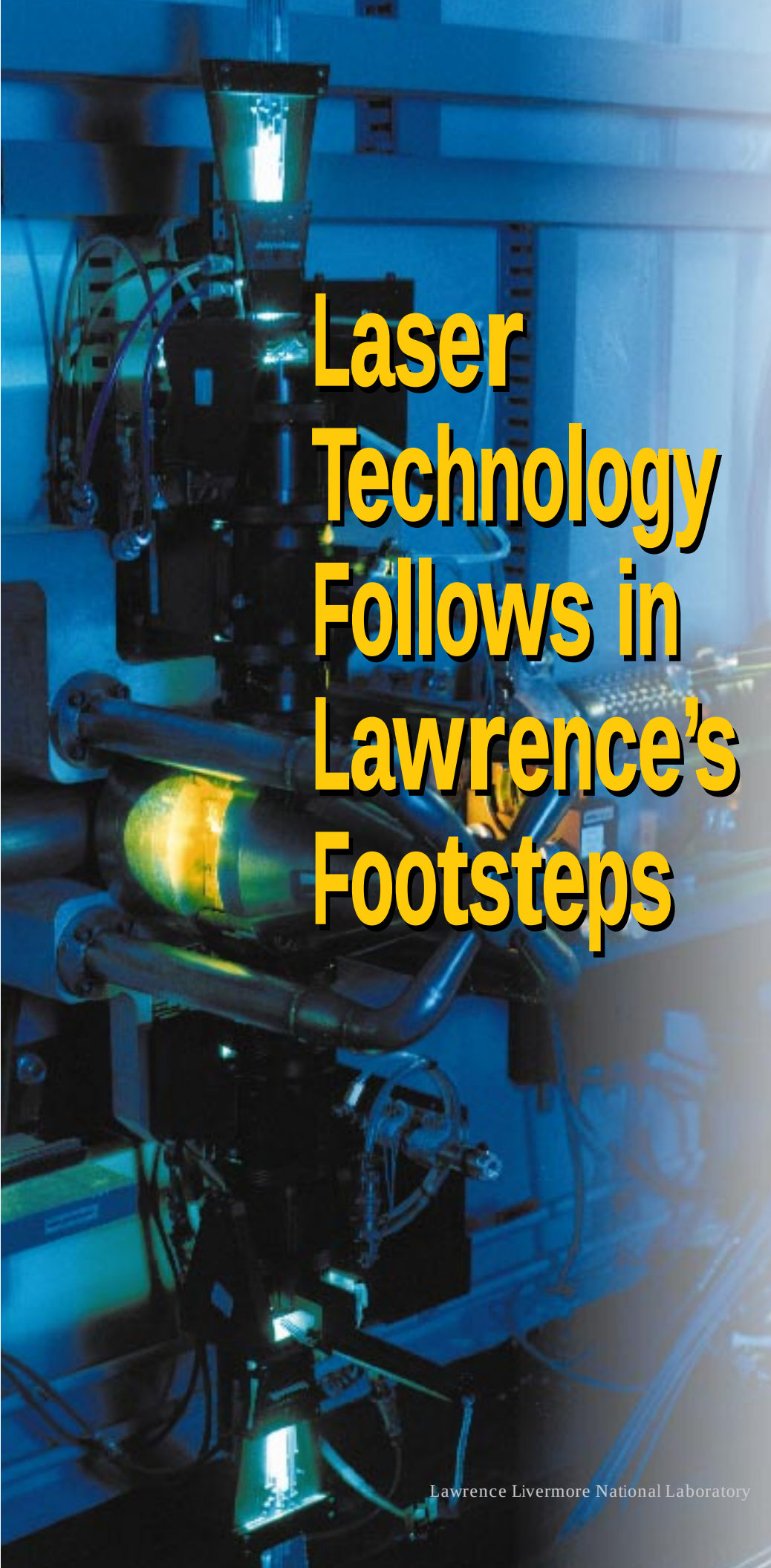
About the Scientist

BERT WEINSTEIN is the deputy associate director of Livermore's Biology and Biotechnology Research Program (BBRP) Directorate. He received his B.S. in physics and mathematics from Brigham Young University and his M.S. and Ph.D. in physics from the University of Illinois at Urbana. He currently serves as leader of the biological foundations thrust area for the DOE Chemical and Biological Nonproliferation Program and as liaison for BBRP with the DOE Joint Genome Institute in Walnut Creek, California. At the Laboratory since 1974, he has held both research and leadership positions in four major programs: inertial confinement fusion, nuclear design, intelligence and national security, and the biology and biotechnology research program. He also served as a member of the Science Council for the Department of Energy's Office of Nonproliferation and National Security (now the Office of Defense Nuclear Nonproliferation).



Laser Technology Follows in Lawrence's Footsteps

Precisely tuned lasers light the way to advances in energy, medicine, and astronomy.

ONE of the toughest scientific challenges has been to effectively—and inexpensively—separate a desired isotope of a chemical element from the remaining isotopes for uses ranging from medicine to energy to weapons applications. Traditionally, isotope separation has been performed through the techniques of gaseous diffusion and gas centrifuge. Over the past two decades, scientists and engineers at Lawrence Livermore have developed another technique, fundamentally different and much more efficient, called laser isotope separation (LIS). The technique is based on the fact that different isotopes of the same element, while chemically identical, absorb different colors of laser light. Therefore, a laser can be precisely tuned to ionize only atoms of the desired isotope, which are then drawn to electrically charged collector plates.

LIS was originally developed in the 1970s as a cost-effective, environmentally friendly technology to supply enriched uranium for nuclear power plants and special nuclear materials for national security needs.

Over the years, funding of about $2 billion was invested to develop the technology at Lawrence Livermore and to successfully demonstrate it with an integrated, full-scale pilot plant. This step was achieved in the early 1990s for special nuclear materials separation and in the late 1990s for commercial uranium enrichment applications.

The uranium enrichment process was being tested successfully at Livermore's pilot plant when the United States Enrichment Corporation (USEC) suspended funding for the project in mid-1999. LIS technology, however, is currently finding other important applications in energy, medicine, astronomy, and industry. Livermore scientists are also proposing its use for tapping the energy value remaining in the tailings left from decades of government uranium enrichment activities.

Lawrence Led the Way

It seems appropriate that a national laboratory named for Ernest O. Lawrence should have developed a technologically superior solution for enriching uranium. During World War II, Lawrence led the effort to enrich the uranium used in the Manhattan Project to build the world's first nuclear weapon. He pioneered an electromagnetic enrichment technique based on research at his cyclotron facility. This Calutron separation technology, named after the University of California, was transferred to production facilities at Oak Ridge, Tennessee, where it separated uranium isotopes in a slow and expensive process.

In the early 1970s, as part of the Laboratory's growing effort in laser technology research, Livermore scientists began experiments using lasers to enrich uranium. By the end of the decade, scientists were reporting important progress, and the project was attracting talented physicists and engineers from around the nation.

Development of process science, technology, and hardware was so impressive that in 1985, the Department of Energy selected LIS (also called Atomic Vapor Laser Isotope Separation, or AVLIS) as having the best potential to provide a low-cost, environmentally sound method to enrich uranium for the U.S. and its international trading partners. DOE's goal was to replace, in an orderly way, the aging and energy-inefficient gaseous diffusion plants in Ohio and Kentucky.

Plant-scale laser and separator hardware began operation in 1986, while researchers were continually improving their performance and reliability. The early 1990s marked the first tests with full-sized components in integrated systems producing enriched uranium over many tens of hours. The Energy Policy Act of 1992 transferred the U.S. government's uranium enrichment activities to USEC, which at that time was a government corporation charged with supplying the nuclear fuel industry with enrichment services through gaseous diffusion technology.

In July 1994, after a two-year period during which Livermore's LIS activities were on a standby status, USEC gave the green light for advanced development. The technology was then transferred from DOE to USEC for commercialization, representing the largest technology transfer in the Laboratory's history.

The separator demonstration facility at Lawrence Livermore's laser isotope separation (LIS) pilot plant tested full-scale equipment. One of three separator units shown for enriching uranium was operational for demonstrations. Also visible are beam tubes for transporting precisely tuned laser light to the separator units.

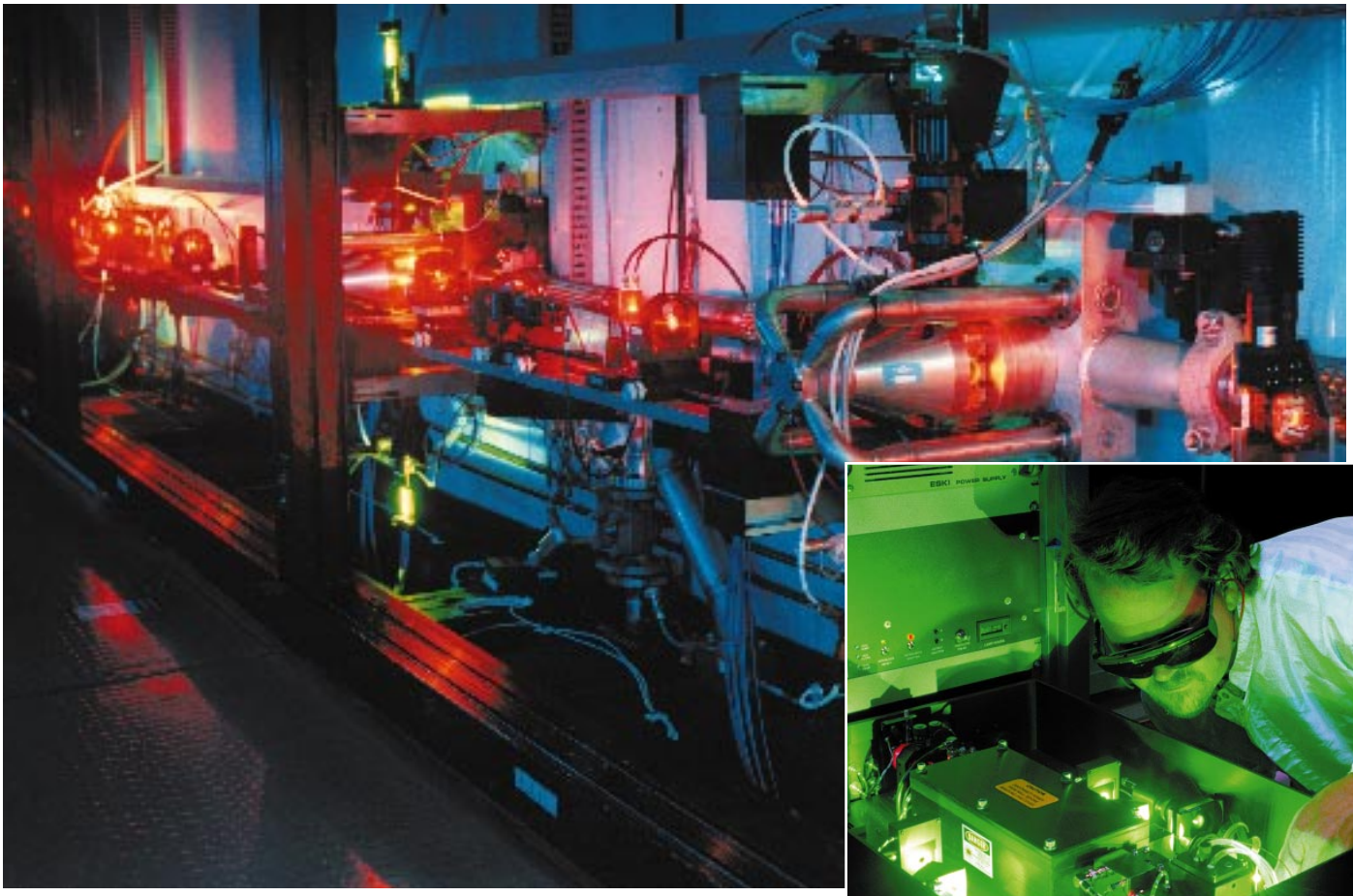
Technology Was Proven

A pilot enrichment plant, completed at Livermore in the fall of 1997, became the focal point for the LIS team composed of experts from Livermore, Bechtel National Inc., Duke

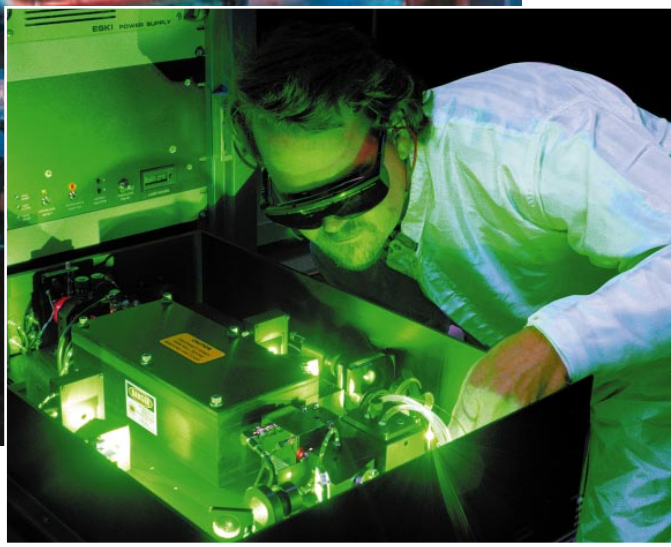
Engineering, On-Site Engineering, Babcock and Wilcox, Lockheed Martin, AlliedSignal Corp., and USEC. The team moved forward with testing commercial-scale equipment. Important improvements over time included making key separation upgrades for better performance and reliability, introducing deformable optics for laser beam uniformity, and substituting compact diode-pumped solid-state lasers for copper lasers to energize the all-important dye lasers. This latter change significantly reduced costs and space requirements.

The pilot plant operated for more than one-and-a-half years, processing several thousand kilograms of uranium in a series of tests aimed at verifying component performance, operational lifetime, and economics. "Running the pilot facility was not an experiment," notes Livermore physicist Steven Hargrove, a former senior LIS scientist. "This was a 24-hours-a-day demonstration of industrial capability using full-scale hardware." In tandem with the long-term tests, scientists and engineers began the first stages of designing and licensing a commercial plant.

By December 1997, the plant had achieved its target for separator lifetime, which was 400 hours of continuous operation before needing refurbishment. However, the next three



LIS plant-scale dye laser chains absorb green light from solid-state lasers and reemit it at a color that can be tuned to the isotope of interest. For uranium enrichment, the green light was converted to red–orange light of three different wavelengths that are absorbed only by uranium-235.



The introduction of solid-state lasers to energize the dye lasers with green light made a significant contribution to meeting the pilot plant's cost and operating efficiency goals.

tests during 1998 fell well short of 400 hours because of unexpected component corrosion. The LIS team traced the problem to a minor impurity in the uranium, verified this diagnosis in a 290-hour test, and moved the separator effort back on track.

By March 1999, the pilot demonstrations had verified projections that the technology could achieve enrichment at costs comparable to those of current U.S. gaseous diffusion, even without the planned pilot improvements. Project managers were preparing both final engineering improvements and additional 400-hour enrichment tests intended to address two remaining economic factors: separator lifetime and enrichment efficiency. The goal was to conclusively demonstrate within a few months' time the basis for projecting that the LIS plant could enrich uranium at costs equal to or below those of all existing technologies.

Development efforts to achieve high enrichment efficiency centered on improving laser beam uniformity and uranium vapor conditions. Eighty percent of the plant's enrichment efficiency goal was achieved in several tests, including the 290-hour demonstration tests in March 1999 that had laser systems operating at record power levels. Engineering upgrades were in place to address about half the remaining shortfall in forthcoming tests and to fully meet the 100-percent target during final design of the commercial plant.

However, the tests were interrupted by USEC's decision in June 1999 to halt development of LIS technology because a combination of near-term factors limited its funds. These factors included market-driven price declines for enriched uranium, significant cost increases to operate U.S. gaseous diffusion plants, and the need to continue shareholder dividends. USEC also judged expected investment returns were insufficient to outweigh the usual anticipated risk of new technology.

The decision halted the efforts of more than 500 people, including some 300 Livermore employees. In making the announcement, USEC President and Chief Executive Officer William Timbers, Jr., said, "The Lawrence Livermore team has displayed outstanding dedication, creativity, and responsiveness in its efforts to develop and commercialize AVLIS."

Enriched uranium produced at the Livermore pilot plant was collected as nuggets the size and thickness of several quarters. The nuggets were packed in 55-gallon drums for shipment to commercial fuel fabricators.

LIS Offers Advantages

Livermore physicist Bruce Warner, former LIS program leader, notes that USEC's decision was no reflection on the strong advantages and technical capability of LIS. For example, the LIS process uses only 5 percent of the electricity consumed by existing gaseous diffusion plants, and LIS facilities would cost substantially less to build than those for other enrichment techniques such as centrifuge technology.

The enrichment of uranium, from natural levels of 0.7 percent uranium-235 (^{235}U) to between 3 and 5 percent ^{235}U, is achieved in a few passes with LIS, a great improvement over the hundreds to thousands of passes required by other processes. This translates into a much smaller plant and production costs substantially below those of either gaseous diffusion or gas centrifuge technology. "With LIS, it

An operator removes enriched product from the separator withdrawal canister.

doesn't take much real estate to make a lot of product," says Warner.

Indeed, the system is remarkably compact. A vacuum chamber holding one separator unit produces output equivalent to that of several thousand of the best commercial centrifuges. A commercial LIS plant would use 84 enrichment units, compared to more than 150,000 centrifuge machines.

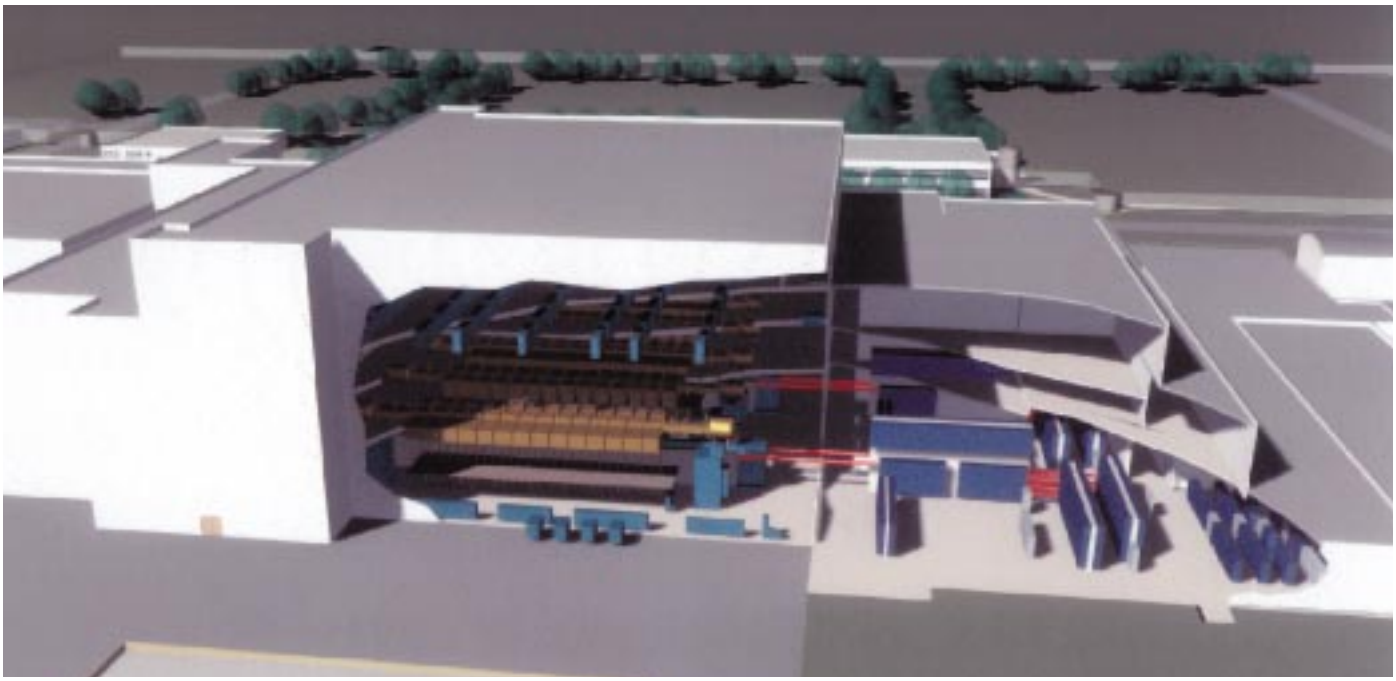
Hargrove notes that LIS also offers strong environmental, safety, and health advantages. Instead of using uranium hexafluoride, the starting material required by other processes, LIS uses uranium metal, which is less hazardous. Compared to centrifuge or gaseous diffusion, the laser process requires about 30 percent less natural uranium ore to produce a comparable amount of enriched product, which also minimizes the amount of uranium tailings by about 30 percent.

"LIS technology is the future of uranium enrichment, whether or not the U.S. commercializes it," says Warner. He notes that France and Japan also have LIS development programs and that the nation first deploying the technology for uranium enrichment will enjoy important economic advantages in the world marketplace. "We want to be sure our nation has a reliable and commercially attractive production source of enriched uranium in the 21st century, preferably one based on the best U.S. technology," says Warner.

Spinning Off a Star

A natural outgrowth of Livermore's successful LIS development effort was research into other isotopes that could be supplied efficiently using similar technology. More than 60 potential spinoffs were identified by Livermore scientists in a study for the National Academy of Sciences and the National Academy of Engineering. One spinoff, called the laser guide star, has been of immense use to astronomers because it removes the effects of atmospheric turbulence that blurs the images taken by Earth-bound telescopes. The technology has been installed at several leading telescopes worldwide.

The laser guide star uses technology originally developed for the LIS effort. The guide star begins with green light from flashlamp-pumped, solid-state lasers beneath the main floor of the telescope dome. The light travels through fiber-optic lines to a compact dye laser similar to that used in uranium enrichment. The dye laser converts the light from green to yellow, and a beam projector mounted on the telescope directs the yellow light up through the upper atmosphere.



An engineering drawing of a commercial uranium enrichment plant using LIS technology. The cutaway shows banks of separators on the left, lasers on the right, and beam tubes (red) connecting the two systems.

How LIS Works

Laser isotope separation (LIS) is based on the fact that different isotopes of the same element, while chemically identical, have different electronic energies and therefore absorb different colors of laser light. The isotopes of most elements can be separated by a laser-based process if they can be efficiently vaporized as atoms. For those elements to which it can be effectively applied, LIS is likely to be the least expensive enrichment technology.

The best-known laser isotope process is for uranium enrichment because the ^{235}U isotope is fissile; that is, it can sustain a nuclear chain reaction to provide energy. Uranium ore is usually processed to increase the natural concentration of ^{235}U from 0.7 percent to about 4 percent to fuel the commercial nuclear power plants that are in widespread use today.

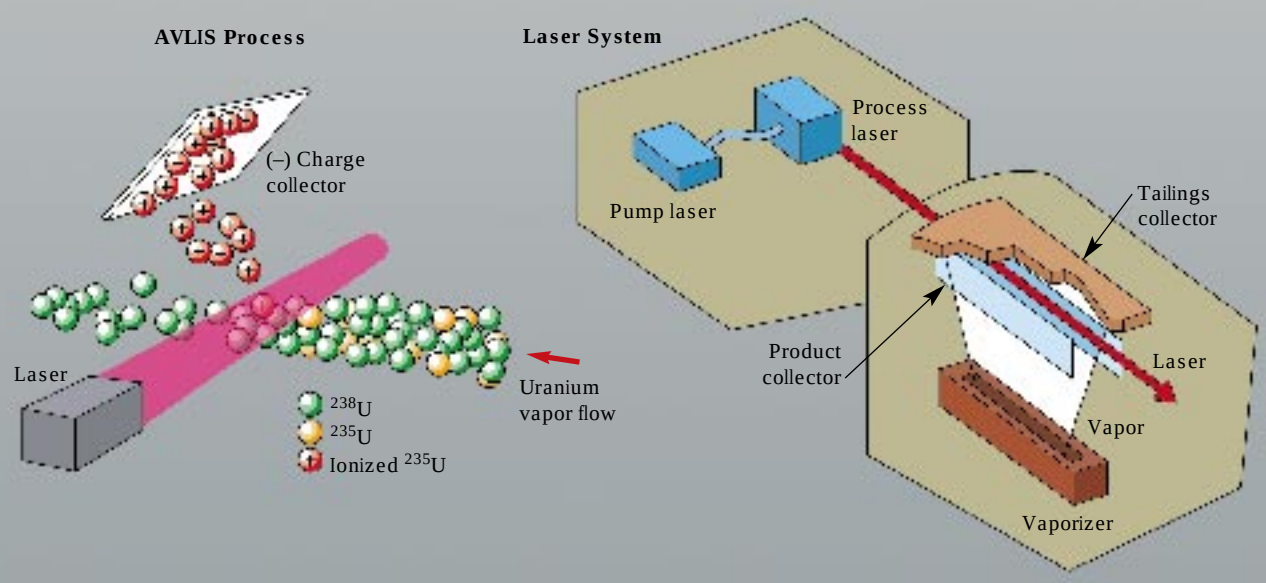
In LIS enrichment, uranium metal is first vaporized in a separator unit contained in a vacuum chamber. The vapor stream is then illuminated with laser light tuned precisely to a color at which ^{235}U absorbs energy.

The generation of laser light starts with diode-pumped, solid-state lasers providing short, high-intensity pulses at high repetition rates. This green light from the solid-state lasers travels via fiber-optic cable to energize high-power dye lasers. The dye laser absorbs green light and reemits it at a color that can be tuned to the isotope of interest. In uranium enrichment, the light converts to three wavelengths of red–orange light, which is absorbed only by ^{235}U.

Each color selectively adds enough energy to ionize or remove an electron from ^{235}U atoms, leaving other isotopes unaffected. "The uranium atoms are subjected to a razor-sharp beam," notes Livermore physicist Steve Hargrove. "Given the several kilowatts of high average power of the dye laser beam, it's a significant achievement that the wavelengths are stable to better than 1 part in 10 million and that the beam's ability to travel long distances is nearly perfectly preserved."

Because the ionized ^{235}U atoms are now "tagged" with a positive charge, they are easily collected on negatively charged surfaces inside the separator unit. The product material is condensed as liquid on these surfaces and then flows to a caster where it solidifies as metal nuggets. The unwanted isotopes, which are unaffected by the laser beam, pass through the product collector, condense on the tailings collector, and are removed.

In the laser system used for the LIS uranium enrichment process (right), electrons from the ^{235}U atoms are separated (left), leaving positively charged ^{235}U ions that can be easily collected for use.



At an altitude of about 95 kilometers, the laser beam hits the layer of sodium atoms that are continuously produced by burning micrometeorites. The light excites the sodium atoms, causing them to emit yellow light in all directions and create a sharply defined guide star. An adaptive optics system at the telescope corrects the guide star image for atmospheric distortions. The corrections made to the guide star's light sharpen the image of all of the celestial objects in the same patch of sky under the telescope's view.

Enrichment for Fuel Efficiency

Another promising spinoff of LIS applications, endorsed by the National Academy of Sciences, is enriching gadolinium for use in boiling-water nuclear power plants. Fuel rods now contain about 8 percent of natural gadolinium, which is a mixture of seven isotopes. The odd-numbered isotopes, gadolinium-155 and gadolinium-157, are of interest because they absorb neutrons to control excess reactivity of burning fuel rods, which translates to more efficient use of the fuel and reduced reactor waste.

LIS technology can be used to double the concentration of odd-numbered gadolinium isotopes, so reactor fuel would need only half its current amount of gadolinium, further contributing to fuel burnup efficiency. Hargrove says that enriched gadolinium could be an important constituent in future nuclear fuel designs because it could permit significant increases in fuel burnup time. Enriched gadolinium could also play an important role in mixed oxide reactor fuels that burn surplus plutonium from nuclear weapons; DOE is considering this concept. An LIS plant for gadolinium would be similar to a uranium enrichment system, but it would require only about one-tenth the hardware to economically meet market-demand projections.

Another product with similar benefits is enriched erbium-167, currently used in its natural form in reactor fuel for pressurized-water nuclear power plants. Test runs at Lawrence Livermore have verified the enrichment process for both gadolinium and erbium and have produced kilograms of each material. Together, they could potentially command a 6,000- to 9,000-kilogram annual world market of about $50 million per year.

Isotopes for Medical Diagnostics

Livermore scientists have also investigated the technical and economical feasibility of laser isotope separation for medical diagnostic tests and research. In 1998, a research team supervised the completion of a facility constructed for separating the isotopes of a wide range of metals for medical use.

Laser light beamed from California's Lick Observatory hits a layer of sodium atoms at an altitude of about 95 kilometers to create a guide star, which is used to see celestial objects more clearly through a telescope. The guide-star technology was a spinoff from the laser isotope separation effort.

Last year, that team developed the capability to access the 250- to 450-nanometer wavelength range (required for many isotopes of interest) with tunable, high-power, and high-repetition-rate dye lasers. The team also successfully demonstrated a master oscillator that produces more than 10,000 pulses per second. Such pulse rates are critical for missions that require narrowly tuned and high-repetition-rate dye laser light.

Livermore physicist Karl Scheibner says that LIS technology would help revitalize DOE's medical isotope production program, which uses World War II–era Calutron technology. DOE's sales of medical isotopes have fallen significantly as a result of competition from Russia's modernized electromagnetic separation plants and Europe's centrifuge separation facilities. A study conducted last year for DOE found that small machines using electromagnetic, plasma-separation, and LIS technologies could all have an important role in providing medical isotopes at substantially reduced costs.

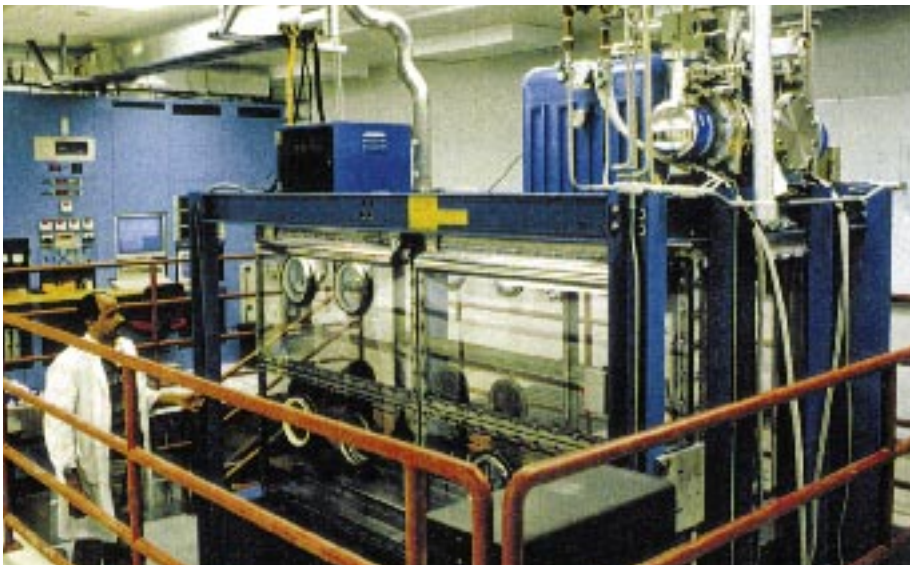
For example, calcium-43 used in medical research costs about $400 per milligram when produced on Calutrons but would cost just a few dollars per milligram using LIS technology. Scheibner believes that LIS will permit significant new contributions to health care, especially in the area of new diagnostic procedures employing isotopes. Scheibner says that commercial production of enriched isotopes would probably not take place at Lawrence Livermore. Instead, Livermore engineers would transfer the process to industry.

High Value in Tailings

Lawrence Livermore scientists also propose using LIS technology for another DOE mission, one closely related to enrichment of natural uranium. Their idea is to use LIS to recover the energy value remaining in the tailings from several decades of uranium enrichment activities at U.S. gaseous diffusion plants, activities that were undertaken for both commercial and defense purposes. "LIS could clean up as much as 60 percent of DOE's tailings inventory with full cost recovery as well as generate significant profits. It would also provide important environmental, energy conservation, and national security benefits," says Hargrove.

In all, over 700 million kilograms of depleted uranium hexafluoride (DUF_6), containing 475 million kilograms of uranium, have been generated by the U.S. government. The material is stored in nearly 60,000 steel cylinders at Oak Ridge National Laboratory and at the Ohio and Kentucky gaseous diffusion plants. Although DUF_6 does not present a serious radiological threat, it is a potential chemical hazard and is under safe management by DOE's cylinder surveillance and maintenance program. DOE is considering long-term plans to convert DUF_6 into a more environmentally acceptable and nonhazardous form (either an oxide or uranium metal) before final disposition of the tailings.

Lawrence Livermore laser scientists note that an LIS plant could convert the tailings first to metal (required for the LIS enrichment process), enrich the uranium to recover its energy value, and finally convert the leftover tailings to oxide for burial. Livermore projections show that LIS can profitably reenrich about 60 percent of the tailings inventory (the proportion containing more than a quarter of a percent of ^{235}U). By recovering energy



A medical laser isotope enrichment facility was built for demonstrating cost-effective separation of isotopes of interest to the medical research and medical treatment communities. The facility's separator is shown above. The dye and solid-state lasers are housed in an adjoining room.

from existing tailings, the LIS plant would dispose of over 11 million kilograms of uranium tailings every year and provide enough fuel to power 40 gigawatt-class nuclear power plants (about 8 percent of America's electric power needs).

Hargrove says that revenue from enriched uranium produced by the plant, even at prices well below current market levels, would more than pay for both the plant's construction costs and all operating costs. The market is currently at about $80 to $85 per SWU (separative work unit, the standard measure of enrichment services). Even at $60 per SWU, DOE could recover all of its costs as well as generate a profit of $2.4 billion over the 25-year life of the LIS plant. This profit could be used to more than offset costs to clean up the remaining 40 percent of tailings not economical for LIS.

"There's obviously a lot of value in the tailings that's waiting to be exploited," says Hargrove. As an advanced, low-cost U.S. enrichment technology, LIS could be used to clean up a significant portion of the depleted uranium inventory and do so profitably, he says. But of more importance, the LIS depleted uranium plant would also preserve technology fundamentally important to both national and energy security, technology that would otherwise be lost.

Hargrove emphasizes that LIS is a demonstrated engineering capability. "We're prepared to move ahead and apply it to uranium enrichment applications for the government as well as to a host of scientific, medical, and energy applications."

—Arnie Heller

Key Words: Atomic Vapor Laser Isotope Separation (AVLIS), Calutron, diode lasers, erbium, E. O. Lawrence, gadolinium, gas centrifuge, gaseous diffusion, laser guide star, laser isotope separation (LIS), Manhattan Project, uranium enrichment, United States Enrichment Corporation (USEC).

For further information contact Steven Hargrove (925) 422-6178 (hargrove2@llnl.gov).

About the Scientist

STEVEN HARGROVE received his B.S. and M.S. in physics from the University of California at Los Angeles. He joined the Laboratory in 1975 in the Laser Programs Directorate, where one of his first responsibilities was to identify and develop laser and electrooptic technologies for strategic materials and commercial isotope separation applications. That investigation broadened in scope and responsibility over the years, culminating in his work on the atomic vapor laser isotope separation (LIS) process to enrich uranium to fuel commercial nuclear power plants. Hargrove has been involved in the LIS effort since its beginnings, starting with work on laser systems design and cost and process analysis, to developing isotope separation for naval propulsion, commercial nuclear fuel, and industrial laser materials processing. Hargrove was the senior scientist supporting the director of the uranium enrichment commercialization effort and the program leader.

Imaging Catheter Gives Surgeons the Inside Picture

NOT so long ago, a blocked artery called for major surgery. That meant patients spent many hours under anesthesia, endured large and potentially traumatic incisions, and required months of recovery time.

Today, surgeons are using minimally invasive medical procedures that are less traumatic and more cost-effective. Most of these newer procedures incorporate thin catheters—hollow flexible tubes—that surgeons insert in small incisions in a major artery, such as the femoral artery in the thigh. The surgeon snakes the catheter through the arterial network to the problem area. Millimeter-size tools can then be guided through the tube to fix the medical condition.

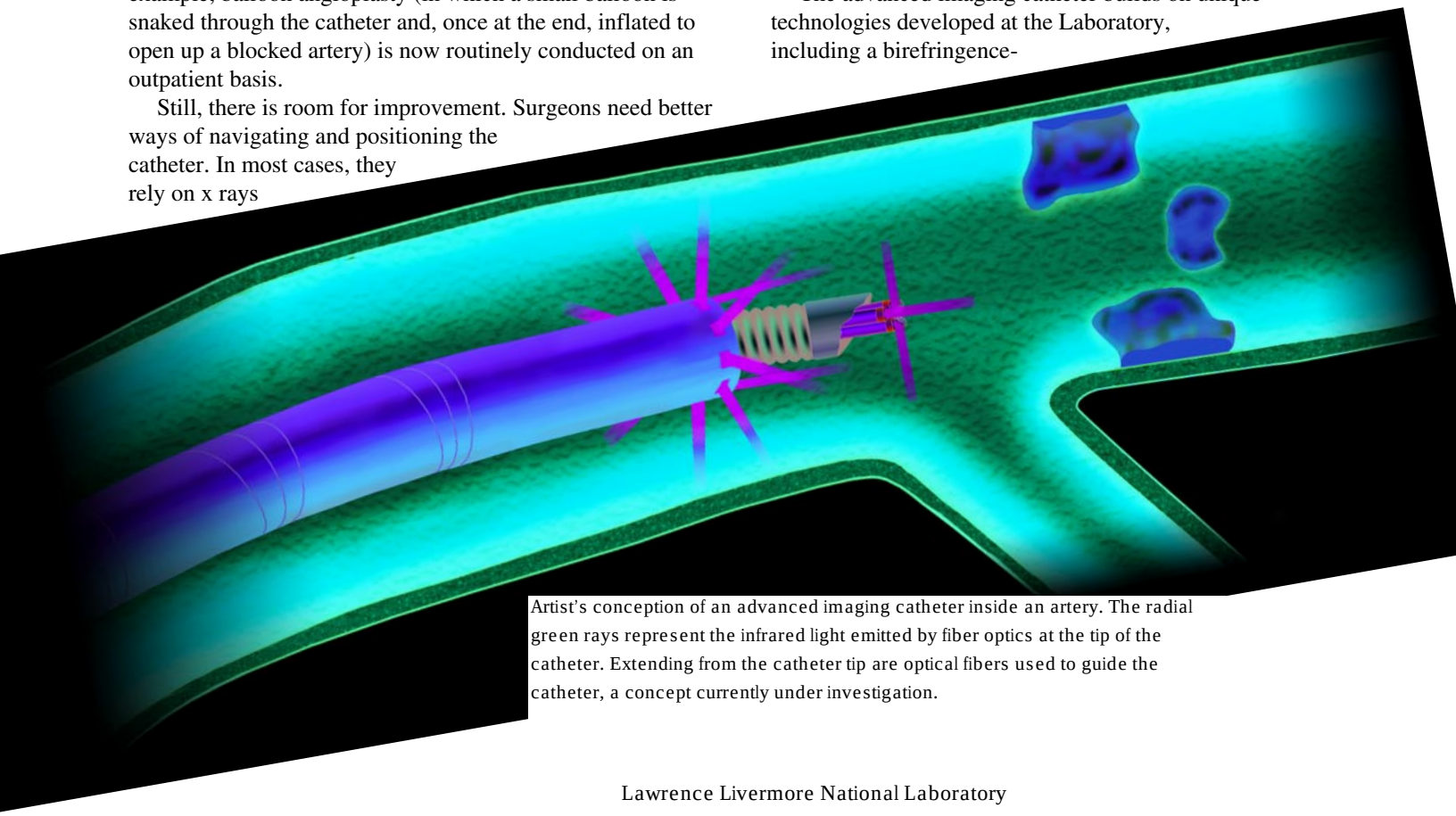
Use of catheter-based procedures is growing rapidly. In the United States alone, over 700,000 of these minimally invasive surgeries are performed annually. A key advantage of this technique is that patients recover in days, not months. For example, balloon angioplasty (in which a small balloon is snaked through the catheter and, once at the end, inflated to open up a blocked artery) is now routinely conducted on an outpatient basis.

Still, there is room for improvement. Surgeons need better ways of navigating and positioning the catheter. In most cases, they rely on x rays to provide a snapshot of the arterial system as they manually push and pull the catheter into position. In addition to this limited view, there's also the problem that the catheters can be 2 meters long and 800 micrometers across, and maneuvering these thin soft tubes of plastic is like pushing on a string.

At Lawrence Livermore, a team of researchers (backed by three directorates—Laser Programs, Engineering, and Defense and Nuclear Technologies—and by the Laboratory Directed Research and Development Program) has developed a prototype catheter that shows promise in meeting these challenges. The advanced imaging catheter, as envisioned by physicist Luiz Da Silva and his team, will have a number of optical fibers embedded in the catheter wall to produce a stream of images—essentially a video—of the surrounding fluid and arterial wall.

3D Brought Inside

The advanced imaging catheter builds on unique technologies developed at the Laboratory, including a birefringence-



Artist's conception of an advanced imaging catheter inside an artery. The radial green rays represent the infrared light emitted by fiber optics at the tip of the catheter. Extending from the catheter tip are optical fibers used to guide the catheter, a concept currently under investigation.

insensitive optical coherence tomography (OCT) system. OCT is a noninvasive, noncontact optical technique that uses infrared light to image through highly scattering media such as blood and the vascular wall. "OCT is similar to ultrasound imaging, but it can achieve significantly higher spatial resolutions and is sensitive to differences in optical rather than acoustic properties of tissue," explains Da Silva.

This same technology lies at the heart of an R&D 100 Award-winning system that images teeth and dental tissue with near-infrared light. (See *S&TR*, October 1998, pp. 10–11, for more information about OCT and the optical dental imaging system.)

The three-dimensional imaging makes it easier for the physician to identify the location of the medical condition in an artery and guide the catheter to it. For instance, the figure at bottom right shows an x-ray image of a cerebral aneurysm. Because an x ray is a two-dimensional image of a three-dimensional structure, its views often leave the surgeon unsure which way to navigate the catheter. Moreover, the amount of chemical dye injected to provide contrast in the x ray must be limited, so the dye is not used continuously and the surgeon at times must work "blind" without even this two-dimensional visual clue.

"I observed an operation where the surgeon refused to proceed because he was uncertain that the catheter was in the correct position," said Da Silva. "Physicians told us that if a compact catheter could produce three-dimensional images and allowed them to actively guide it in the body, these procedures would be quicker, less traumatic, and have a much higher success rate."

Three Areas to Tackle

The team is focusing on three areas of development for this next generation of catheters: the fabrication technology needed to place optical fibers within the thin polymer wall of a catheter, the materials and techniques required for actively controlling the catheter, and radiation transport modeling.

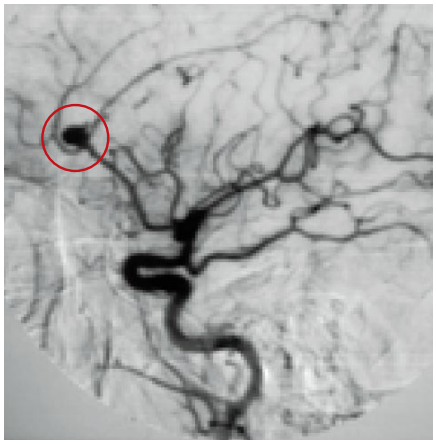
"There are two ways to place optical fibers in the walls of the catheter," says Da Silva. "One way is to embed the optical fibers into existing commercial catheters. A key question regarding this approach is how it would affect the flexibility of the catheter. Another possibility is to extrude the catheter polymer with the optical fibers already embedded in the walls. We're pursuing both possible solutions." Along with a commercial collaborator, the team has developed tubing that can hold 10 optical fibers and is 1.7 millimeters in diameter with a wall 0.2 millimeters thick.

To find a better way of "pushing on a string," the team looked at Laboratory-developed microactuators and so-called smart materials. One possible solution might come from a shaped-memory polymer material being investigated at Livermore that can be activated by optical heating. "By using multiple wavelengths and different polymers," says Da Silva, "it may be possible to actively control and manipulate the tip of the catheter." Alternatively, shaped-memory alloy materials that can be made to move when heated could be placed near the catheter tip. A surgeon could then guide or position the tip by altering the temperature of the shaped-memory alloy, causing the catheter tip to bend. A microrudder scheme has also been proposed, which would steer the catheter tip using blood flow as a means of propulsion.

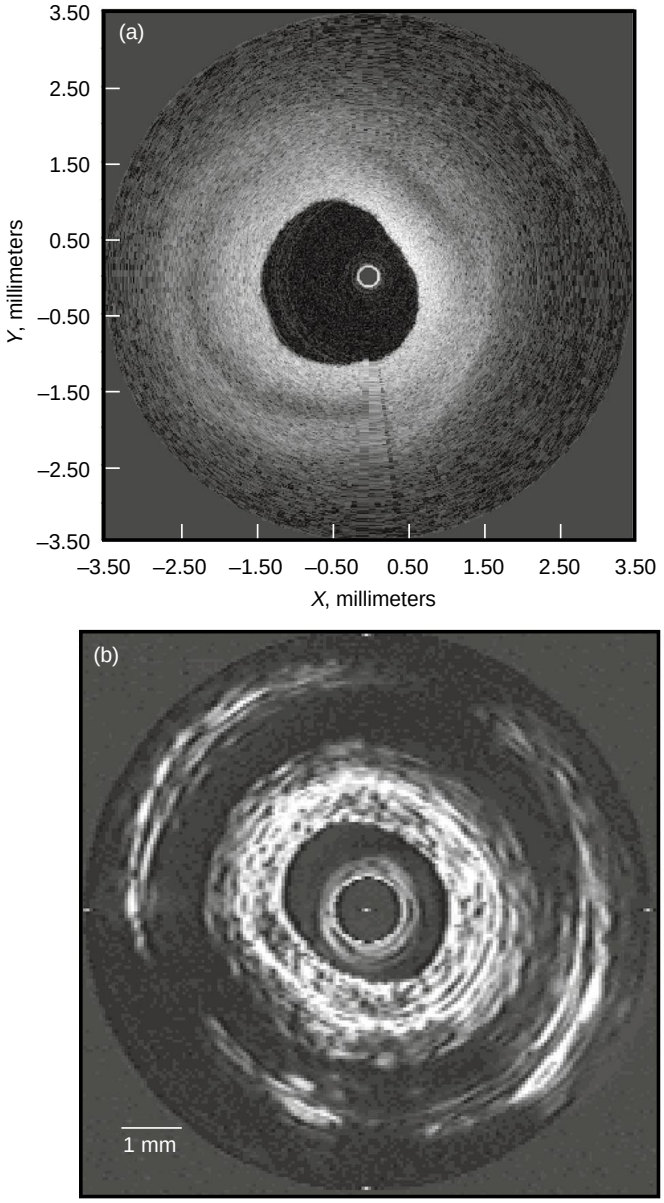
Radiation transport modeling is key to understanding OCT and developing simulation and analytical tools for optimizing the catheter's imaging system. Laboratory researchers recently adapted the Livermore-developed code LATIS (a two-dimensional laser–tissue interaction code) to simulate what occurs when photons from the fiber optics scatter from blood and biological tissue and return to the catheter's collecting lens. (For more information about LATIS, see *S&TR*, March 1999, pp. 23–25.) Besides helping in the design of the final system, the modified LATIS code can help researchers interpret OCT imaging in a variety of applications.

Results Are Promising

The team has manufactured and tested a prototype single-fiber catheter with encouraging results. "The OCT imaging system has an order of magnitude higher resolution than state-of-the-art ultrasound," says Da Silva. The team has also been working on miniaturizing the electronics and has managed to



Two-dimensional angiogram (x ray) showing a cerebral aneurysm (circled). An advanced imaging catheter would provide a perspective from inside the artery in three dimensions.



(a) Images obtained with optical coherence tomography (OCT), a technology developed at the Laboratory, can resolve details as small as 12 micrometers in size, whereas (b) state-of-the-art ultrasound systems can resolve features down to about 150 micrometers in size.

shrink the device from a large (60 by 90 by 90 centimeters) rack-mounted electromechanical device to a box about half a meter on each side—a size much more compatible with operating room conditions.

In the process, the team made significant improvements to the system, increasing its sensitivity by a factor of 30. It can now penetrate through 2 to 3 millimeters of biological tissue such as arteries.

The team sees other possible uses for the catheter as well. For instance, it could be used to examine the structures of composite materials and to search for signs of delamination in plastic explosives. The team is also evaluating whether the imaging catheter might be used to characterize wall- and ice-layer thickness in laser targets for the National Ignition Facility.

"The other challenge we must keep in mind, if our imaging catheter is to become a commercial reality, is cost." continues Da Silva. "The added cost of the device needs to be reasonable. Right now, we're looking at adding $10 or less to catheters that normally cost between $500 and $1,000. We're talking with the National Institutes of Health, industry, and physicians as we continue our development. Everyone's interested and sees cost-effectiveness as an important step toward the next-generation catheter."

—Ann Parker

Key Words: advanced imaging catheter, LATIS, medical technology, optical coherence tomography (OCT), radiation transport modeling.

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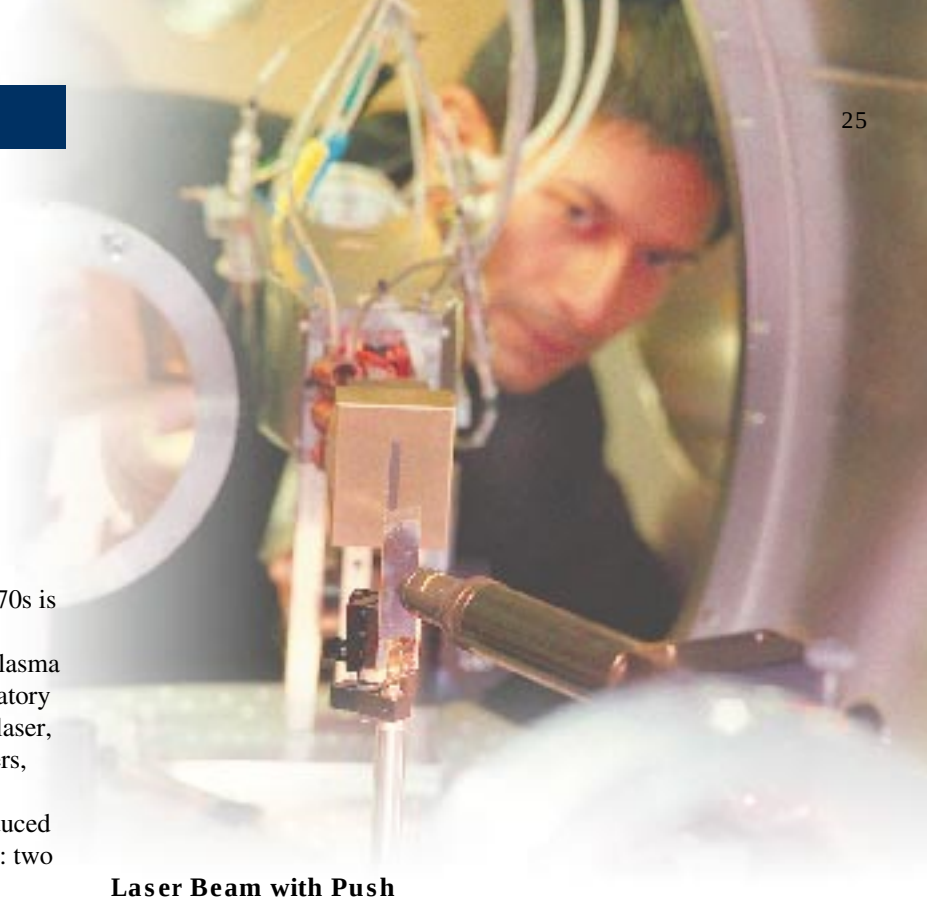


JanUSP Opens New World of Physics Research

A legacy from the Livermore lasers program of the 1970s is helping Laboratory researchers achieve the world's brightest laser, thereby making possible a new world of plasma physics experiments. In a project funded under the Laboratory Directed Research and Development Program, the Janus laser, a milestone in the Laboratory's development of glass lasers, has been incorporated in JanUSP (for Janus-pumped, ultrashort-pulse laser). This new instrument recently produced the highest irradiance (power per unit area) ever recorded: two sextillion (2×10^{21}) watts per square centimeter.

Achieving this long-sought level of brightness is a requirement for exploring plasmas present only in the interiors of stars and detonating nuclear devices. The characteristics of such extreme plasmas include electric fields 100 times stronger than those binding electrons to atomic nuclei, magnetic fields like those found on the surface of white dwarf stars, electron oscillatory ("quiver") energies similar to gamma-ray bursts, and pressures one trillion times that of Earth's atmosphere at sea level.

JanUSP is a significant upgrade to Lawrence Livermore's longstanding ultrashort-pulse laser facility. Research on ultrashort-pulse lasers (with pulse lengths lasting from a billionth to a trillionth of a second) has been the focus of intense activity at Livermore since the mid-1980s. The work arrived at a major milestone in the late 1990s when the Laboratory's Petawatt laser achieved record-breaking levels of power (more than a quadrillion, or 10^{15}, watts) and irradiance (approaching a sextillion, or 10^{21}, watts per square centimeter) at full energy of about 680 joules before it was shut down (see *S&TR*, March 2000, pp. 4–12).

At 200 terawatts (2×10^{14} watts) and 15 joules, JanUSP has a fraction of the power and energy, respectively, of the Petawatt. However, with its shorter pulse length (85 femtoseconds, less than a tenth of a trillionth of a second) and smaller spot size (2 micrometers), it can access much different regimes of matter.

The machine's front end is a commercial oscillator that produces 75- to 80-femtosecond pulses of 800-nanometer light. The low-energy laser pulses are passed through diffraction gratings, made by Livermore's Diffractive Optics Group. The gratings drastically stretch pulses out in time so that they do not distort and eventually damage the laser optics.

Laser Beam with Push

The stretched pulses are energized by a series of amplifiers using increasingly larger titanium-doped sapphire crystals. The final amplification stage features a 10-centimeter-diameter, 5-centimeter-thick, titanium-doped sapphire crystal, the largest in the world and one that required three years to be produced commercially. Energizing this crystal is 130-joule green light from the Janus laser. The fully amplified light is recompressed to its original duration and focused onto a target inside a 2-meter-diameter chamber.

"We want to use JanUSP to explore the uncharted regime of matter subjected to irradiance above 10^{21} watts per square centimeter," says physicist Paul Springer. To meet that goal requires heating a relatively thick (several micrometers) sample of metal extremely fast, and researchers are tapping the enormous pressure of JanUSP's laser light to do so. Physicist Jacques Denavit first proposed the technique of using light to literally push ions from an aluminum or gold foil some 500 nanometers thick onto a target of uranium or other dense metal. "We don't typically think of light as having a noticeable pressure, but JanUSP's laser beam is so intense that it can generate hundreds of petapascals of pressure at the surface of a target," observes physicist Scott Wilks, who has been using the two-dimensional Zohar computer code (written by physicist A. Bruce Langdon) to model JanUSP–plasma interactions.

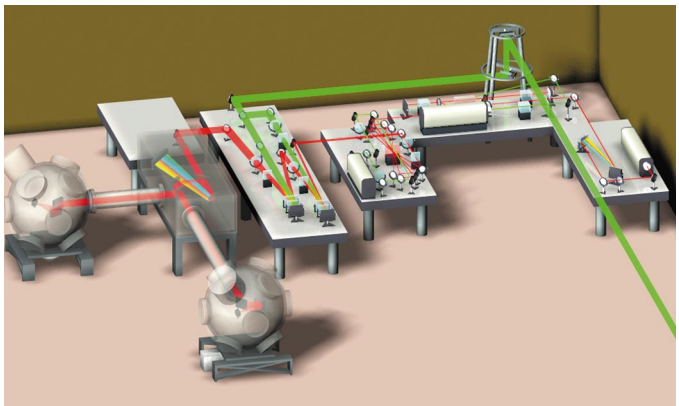
If all goes according to plan, later this summer a laser pulse from JanUSP will literally push electrons forward and out of the foil in less than a trillionth of a second. The negatively charged electrons, in turn, will drag positively charged protons and uncharged neutrons with them. The heavy ions, traveling at 8,000 kilometers per second (about 3 percent of the speed of light), will be deposited onto a target made of a heavy metal such as uranium.

In less than a billionth of a second, the impinging ions will travel 5 to 10 micrometers into the target, rapidly creating a superhot plasma of 1,000 volts (about 10 million kelvins). The ions, with about 1,800 times the mass of electrons, are much slower and therefore travel only one-thousandth the distance. As a result, the plasma they create will be confined to a thickness of several micrometers. The plasma will be in thermal equilibrium, meaning that all ions and electrons will radiate at the same temperature. "It's the same kind of plasma that is found at the center of a star or an exploding nuclear device," Springer says. In those cases, thermonuclear energy is transferred through ions, not electrons.

Heating Matter with Ions

Springer notes that although lasers have been used for years to create plasmas from solid targets, the interactions have been with targets' electrons, not their neutrons and protons. "Lasers with prepulse—the less intense, first part of a pulse—couple their energy to hot electrons, which heat up materials to lower temperatures than what we want," says Springer. "Heating with ions is a better way to create the high-energy-density plasmas we're looking for."

Typical laser pulses have what's known as a pedestal, or a slow rise time before the pulse achieves its full intensity. As a result, the prepulse boils electrons off the target's surface. This initial impact creates relatively low-density plasma that interacts with the main pulse, which arrives an instant later. The key to creating plasmas with ions is making the pulse rise time extremely fast. "You don't want the target to know the laser is there until the main pulse arrives," explains Wilks. "The main pulse can then couple its energy directly to the ions and not into the electrons."



JanUSP's front end produces 75- to 80-femtosecond pulses of 800-nanometer light. The low-energy pulses are passed through diffraction gratings that drastically stretch them out in time. The stretched pulses are energized by amplifiers using increasingly larger titanium-doped sapphire crystals, including the largest in the world. Energizing the final crystal is laser light supplied by Janus, built in the late 1970s. The fully amplified pulses are recompressed and focused onto a target inside a 2-meter-diameter chamber.

The Petawatt laser achieved its record power level with a much longer pulse length (440 femtoseconds) and a much larger aperture (57 centimeters). As a result, its prepulse was a million times larger than the one Livermore scientists hope to see with JanUSP. Indeed, the goal for the so-called pulse contrast (ratio between the main pulse and prepulse) is an unprecedented 10 billion.

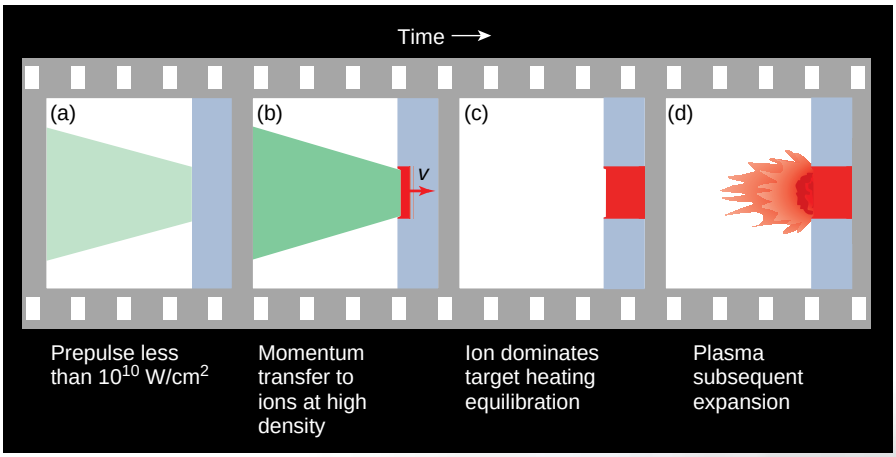
Alternatives for creating extreme states of plasma with ions don't exist. Gas guns are too slow by a factor of 1,000. Much more energetic lasers like the National Ignition Facility (now under construction at Lawrence Livermore) heat vastly more mass, but they were not designed to achieve the temperatures of JanUSP. And typical ultrashort-pulse lasers don't have the light intensity or pulse contrast necessary to couple energy to ions.

Springer also notes that the rapid time scales involved in JanUSP experiments are driving improvements in diagnostics for measuring the fleeting plasmas. The JanUSP team has improved upon the x-ray streak camera that was successfully used on Lawrence Livermore's Nova laser, which was decommissioned last year. The new camera achieves 50 times greater resolution than its predecessor. The techniques used to improve Nova's diagnostics can also be applied to those being built for the National Ignition Facility, Springer says.

New Plasma Regimes to Help Stockpile Stewards

JanUSP's high-temperature, high-density plasmas will shed new light on a wide range of astrophysical studies. Such plasmas, especially those caused by strong shocks in solid materials, are also important to the Department of Energy's Stockpile Stewardship Program to ensure the reliability and safety of the nation's nuclear arsenal. A major goal of the Stockpile Stewardship Program is gaining a better understanding of materials under extreme pressures and temperatures.

Another possible application for JanUSP is testing the concept of an ion "lens." The lens involves curving the foil target to focus ejected ions into a 0.3-micrometer-wide, intense beam at the back of the target. Wilks, who's done computer simulations on the concept, says the ion lens could be useful for radiation therapy and for integrated circuit manufacturing



(a) JanUSP's laser pulse will have an extremely low prepulse to prevent low-energy plasma from interacting with the main laser pulse. (b) The main pulse will push electrons out of a thin metal foil. (c) The negatively charged electrons will drag positively charged protons and uncharged neutrons with them, depositing the ions onto a heavy metal target such as uranium. (d) The ions will travel several micrometers into the target, rapidly creating a superhot plasma.

(for doping materials onto silicon substrates). In both cases, he says, "you're focusing substantially more ions on a tiny spot than is possible with conventional methods."

The facility is currently attracting researchers from within Lawrence Livermore (including those who worked on the Petawatt), other national laboratories, and other nations for investigating new concepts. In February, a team from Germany's Max Planck Institute conducted experiments designed to accelerate electrons to 1,000 megaelectronvolts, an energy never before achieved outside a particle accelerator. At that energy, it is possible to create subatomic particles called pi-mesons, which are responsible for nuclear interactions.

Physicist Dwight Price notes that several research institutions worldwide are developing facilities similar to JanUSP because of its unique plasma-generating capabilities. "Other research centers are rushing to catch up to us," he says. For at least the next few years, Springer expects JanUSP to provide a wealth of new data to confirm—or contradict—models of extreme states of matter that until recently could not be tested in the laboratory.

—Arnie Heller

Key Words: ion lens, Janus, JanUSP, Nova, Petawatt, stockpile stewardship.

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Patents and Awards

Each month in this space we report on the patents issued to and/or the awards received by Laboratory employees. Our goal is to showcase the distinguished scientific and technical achievements of our employees as well as to indicate the scale and scope of the work done at the Laboratory.

Patents

Patent issued to	Patent title, number, and date of issue	Summary of disclosure
Alan D. Conder Bruce K. F. Young	**Three-Dimensional Charge-Coupled Device** U.S. Patent 5,981,988 November 9, 1999	A monolithic three-dimensional charge-coupled device (3D-CCD) that uses the entire bulk of the semiconductor for charge generation, storage, and transfer. It vastly improves current CCD architectures that use only the surface of the semiconductor substrate. The 3D-CCD is capable of developing a strong electric field vector (E-field) throughout the depth of the semiconductor by using parallel (bulk) electrodes buried deep in the substrate material. Using backside illumination, the 3D-CCD architecture enables a single device to image photon energies from the visible, to the ultraviolet and soft x-ray, and out to higher energy x rays of 30 kiloelectronvolts and beyond. The buried, bulk electrodes are electrically connected to the surface electrodes, and an E-field parallel to the surface is established with the pixel in which the bulk electrodes are located. This E-field attracts charge to the bulk electrodes independent of depth; it confines the charge within the pixel in which it has been generated. Charge diffusion is greatly reduced because the E-field is made strong by the proximity of the bulk electrodes.
Dennis L. Matthews Peter M. Celliers Lloyd Hackel Luiz B. Da Silva C. Brent Dane Stanley Mrowka	**High-Removal-Rate, Laser-Based Coating Removal System** U.S. Patent 5,986,234 November 16, 1999	A compact laser system that removes surface coatings (paint, dirt, and the like) at a rate as high as 93 square meters (1,000 square feet) per hour or more without damaging the surface. A high-repetition-rate laser with multiple amplification passes propagating through at least one optical amplifier is used, along with a delivery system consisting of a telescoping and articulating tube that also contains an evacuation system for simultaneously sweeping up the debris produced in the process. The amplification beam can be converted to an output beam by passively switching the polarization of at least one amplified beam. The system also has a personal safety system that protects against accidental exposures.
Henry N. Chapman Donald W. Sweeney	**Deformable Mirror for Short-Wavelength Applications** U.S. Patent 5,986,795 November 16, 1999	A deformable mirror compatible with short-wavelength (extreme ultraviolet) radiation that can be precisely controlled to nanometer and subnanometer accuracy. Actuators are coupled between a reaction plate and a face plate with a reflective coating. A control system adjusts the voltage supplied to the actuators. By coordinating the voltages supplied to the actuators, the reflective surface of the mirror can be deformed to correct for dimensional errors in the mirror or to produce a desired contour.

Awards

Technology Review, a magazine of innovation published by the Massachusetts Institute of Technology, has named **Christine Smith** of the Laboratory's Industrial Partnerships and Commercialization office as one of its **100 Young Innovators**, those under 35 who "exemplify the spirit of innovation in science, technology, and the arts."

Smith was featured in the November/December issue of the magazine, which cited her for efforts that "paved the way for productive research collaborations among thousands of people." While a graduate student at the University of California at Davis, Smith developed a laser-based method of designing nanocrystals. In doing so, she realized she needed facilities at Livermore, so she initiated a partnership between students at Davis and researchers at the Laboratory. She ended up doing some of her work in researcher Howard Lee's laboratory in H Division, while also linking other students with Lab researchers. That partnership endures to this day.

After graduating, Smith became director of the University of California's Campus–Laboratory Collaboration program, overseeing a $2-million budget to foster work among the university's campuses, the three national laboratories it manages, and government agencies and corporations.

The magazine noted that "partnerships established through Smith's programs have led to advances in polymer design and human genomics. Equally at home with high-level scientists and venture capitalists, Smith could be an important catalyst in the formation of many future collaborations."

Abstracts

Uncovering Biological Warfare Agents

A growing threat to the U.S. is an attack by terrorists or even nations using biological weapons such as bacteria, viruses, biological toxins, and genetically altered organisms. A team of Livermore scientists, supported by the Department of Energy's Chemical and Biological Nonproliferation Program, is developing signatures of those organisms that would likely be used in biological warfare. The signatures, telltale bits of DNA unique to virulent (infectious) organisms, are needed for timely detection and identification of an attack. The signatures will be provided to public health, law enforcement, and national security agencies. In developing the signatures, researchers are also shedding light on poorly understood aspects of biology, microbiology, and genetics, such as immunology, evolution, and virulence. Increased knowledge in these fields holds the promise of better medical treatments and vaccines.

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Laser Technology Follows in Lawrence's Footsteps

Over the past two decades, Lawrence Livermore researchers have developed laser isotope separation (LIS), a technology fundamentally different and superior to conventional methods for separating isotopes of an element. LIS uses lasers that are tuned to ionize a desired isotope so that it can be easily separated and collected. Livermore scientists took the technology to the pilot demonstration stage in the early 1990s for special nuclear materials separation and in the late 1990s for commercial uranium enrichment. LIS technology is currently finding important applications in energy, medicine, astronomy, and industry. Lawrence Livermore scientists are proposing that LIS be used to tap the energy value remaining in tailings left from decades of government uranium enrichment activities.

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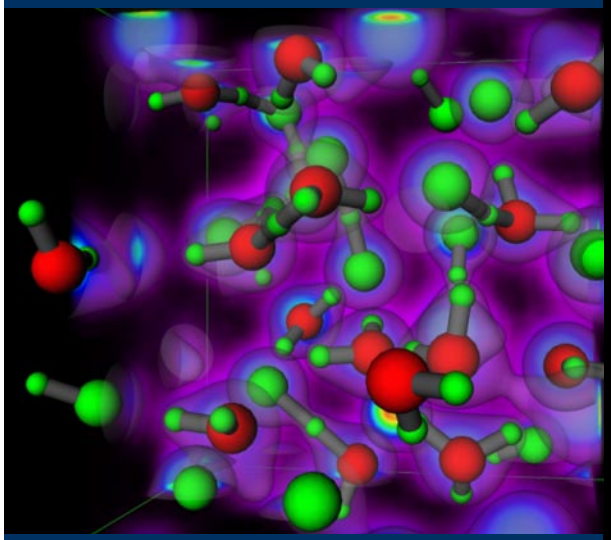
Supercomputing Takes Another Giant Step



When the ASCI White supercomputer comes on line at Livermore this summer, it will carry forward the achievements of its predecessor, the Blue Pacific machine, toward the ultimate goal of DOE's Accelerated Strategic Computing Initiative—full-scale simulations of nuclear behavior in support of stockpile stewardship.

Also in June

- *The many rewards of Livermore's extensive, long-term research of the actinide elements.*
- *Improving organic aerogels by controlling polymerization at the molecular level.*
- *Accelerator mass spectrometry helps to reveal what led to the continental collisions raising the Himalayas and the Tibetan Plateau.*

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