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Science & Technology REVIEW

November/
December 1995

Lawrence
Livermore
National
Laboratory

Sperm's Chromosomal Contribution to Embryo and Infant Health

Also in this issue:

The 1995 R&D 100 Award Winners

About the Cover

This month, *S&TR* features a report on the Laboratory's 12 years of research into genetic defects in sperm and our more recent studies of paternally transmitted defects that alter normal embryo development and may cause birth defects. Pictured is Xiu Lowe, a research scientist on the Biology and Biotechnology Program's sperm and embryo research team. She is performing an important step in the fluorescence *in situ* hybridization (FISH) process, the method by which we identify chromosomal abnormalities in sperm and embryos. Developed at Lawrence Livermore as a gene-mapping tool, FISH is now the key method our research team uses to detect and characterize chromosomal errors using several different fluorescence dyes to tag different chromosomes simultaneously. In the background are fluorescing sperm as FISH reveals them. Our report on the Livermore team's sperm and embryo research begins on page 6.

Cover photo: Jacqueline McBride

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About the Review

The Lawrence Livermore National Laboratory, operated by the University of California for the United States Department of Energy, was established in 1952 to do research on nuclear weapons and magnetic fusion energy. *Science and Technology Review* (formerly *Energy and Technology Review*) is published monthly to communicate, to a broad audience, the Laboratory's scientific and technological accomplishments, particularly in the Laboratory's core mission areas—global security, energy and the environment, and bioscience and biotechnology. 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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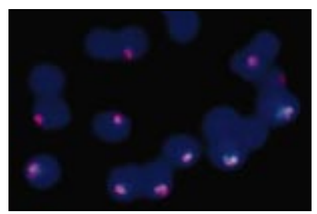

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President affirms all three DOE weapons labs essential for confidence in nuclear arsenal

Livermore Laboratory employees were gratified to learn on September 25 that President Clinton had decided to maintain nuclear weapons research at Lawrence Livermore, Los Alamos, and Sandia National Laboratories to preserve confidence in the nation's nuclear weapons stockpile and ensure a sound technical foundation for seeking a Comprehensive Test Ban Treaty (CTBT).

In a signed directive, the President stated: "To meet the challenge of ensuring confidence in the safety and reliability of our stockpile, I have concluded that the continued vitality of all three DOE nuclear weapons laboratories will be essential." The President said his decision in August to seek a "zero" yield CTBT "was based on assurances by the Secretary of Energy and the Directors of the Department of Energy's nuclear weapons labs that we can meet the challenge of maintaining our nuclear deterrent under a CTBT through a Science-Based Stockpile Stewardship program without nuclear testing." President Clinton added that the laboratories "can help change the course of history with respect to nuclear weapons."

The Presidential decision, announced by Energy Secretary Hazel O'Leary in a Washington, D.C., press conference, was the first result of a major review begun May 4 of the federal labs belonging to the Departments of Defense and Energy and the National Aeronautics and Space Administration.

"I am extremely pleased with the President's confidence in the capability of the three Laboratories to provide for the national security through the stockpile stewardship program," said LLNL Director Bruce Tarter. "The Laboratories and the Department of Energy have worked very hard to develop the program that made this decision possible. It will be an extraordinary challenge to all our employees to meet the program's objective in the coming years."

In her press conference, Secretary O'Leary cited the DOE weapons laboratories' significant contributions to winning the Cold War and predicted their contribution to a permanent cessation of nuclear testing. "Providing confidence in our stockpile in the absence of nuclear testing will rank among the most important scientific and technical challenges of our times. This work will need and deserves strong, bipartisan support," she said.

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>
> *Bruce Tarter, Director*
> *Lawrence Livermore National Laboratory*

O'Leary said the administration examined the implications of removing one of the weapons labs from the stockpile stewardship program. "Our conclusion was that the savings from consolidation would be insignificant compared with the impact that such action could have over time on our ability to maintain confidence in the nation's nuclear weapons stockpile." She added that Bill Perry, Secretary of Defense, strongly opposed the idea of transferring DOE nuclear weapons work to the Department of Defense, as some had proposed.

She said the Administration's vote of confidence in LLNL was also influenced by the breadth of work conducted at Livermore, citing "firm cornerstones" in lasers, DOE's Human Genome Project, and environmental technology. O'Leary pointed out that Brad Allenby, AT&T's research vice president for technology and environment, recently joined Livermore, "indicating that the Laboratory itself understands there's a great future there."

She said that the President "provided a powerful validation of the importance of the Department of Energy National Laboratories to the future security, prosperity, and well-being of our Nation." DOE's reform efforts, she said, are aimed at making the laboratories "more efficient and effective, while preserving their capacity for excellence in science and technology."

Lab helps unravel Mammoth Mountain mystery

Working with the U.S. Geological Survey (USGS), Laboratory scientists have been helping unravel the mystery of trees dying in California's Mammoth Mountain area and the near-asphyxiation of a Forest Service Ranger in 1990.

In the August 24, 1995, issue of *Nature* magazine, members of the USGS group and Laboratory scientist John Southon wrote that they believe seismic activity deep inside the mountain is causing emissions of magmatic carbon dioxide (CO_2) similar to those observed in other volcanic areas like Mt. Etna and Mt. Vesuvius in Italy.

Southon, a member of the Laboratory's Center for Mass Spectrometry, said his principal role in the investigation has been to provide the expertise of LLNL's radiocarbon group to confirm the conclusions reached by the primary USGS researchers.

Laboratory scientists, for example, aided the USGS team by distinguishing emissions of magmatic CO_2 from those resulting from naturally occurring biological activity. Recently, Laura Hainsworth, a post-doctoral fellow in the LLNL radiocarbon group, has been using carbon-14 measurements on individual rings in the tree remains to determine if there is a correlation between CO_2 emissions and seismic activity at Mammoth. She plans to extend this work to other volcanic sites like Mt. St. Helens.

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Lab assesses offshore seismic hazards

The Laboratory is conducting an earthquake hazard assessment of the eastern portion of California's Santa Barbara Channel, a region of high seismicity containing 17 oil platforms.

The work was requested by the California State Lands Commission and the U.S. Minerals Management Service as part of a project to reevaluate seismic response of offshore platforms. There are 31 oil platforms off the coast of California. Most date back to the 1960s and 70s—before earthquake design methods and standards were established for the offshore oil industry.

Scientists and engineers from the Laboratory's Geologic and Atmospheric Hazards Project in the Environmental Programs Directorate were asked to determine what earthquake faults could affect the channel region and what earthquake forces might be expected in the area. While detailed seismic hazard analyses already exist for onshore areas, there are currently no comparable analyses for offshore regions, such as the Santa Barbara Channel.

By the end of this year, Lab scientists should finish gathering information on faults in the Santa Barbara Channel area and on the seismic forces those faults could unleash. Next year, researchers will refine the data and develop a seismic hazards map of the region.

Contact: Bob Murray (510) 422-0308 (murray6@llnl.gov).

Shock compression used to study Jupiter

What astronomers believe to be the boundary between Jupiter's molecular mantle and its metallic core may not exist. This is one of the possibilities outlined by Livermore physicist Bill Nellis and two Livermore coauthors, Neil Holmes and Marvin Ross, in an article entitled "Temperature Measurements of Shock-Compressed Liquid Hydrogen: Implications for the Interior of Jupiter," published September 1 in *Science* magazine.

"The only way on Earth you can achieve both the high temperatures and high pressures comparable to those inside Jupiter is by shock compression," Nellis said. For their research, Nellis and his colleagues used the Laboratory's two-stage light-gas gun. High pressures and temperatures were generated by slamming tantalum or aluminum projectiles (accelerated to 7 kilometers per second, or 16,000 miles per hour) into sample holders containing liquid deuterium.

According to the team's findings, the molecular envelope of Jupiter (which is 90% hydrogen) is cooler and has much less temperature variation than previously believed. Their data suggest there is no sharp change at what astronomers now commonly believe is a distinct core-mantle boundary. The analog on Earth is the distinct boundary between the rocky mantle and the iron core.

Nellis sees the publication of the *Science* article as the beginning of a new line of inquiry in the ongoing debate over the nature of the Jovian interior. The findings also will lead to new databases affecting weapons and laser fusion programs, both of which use isotopes of hydrogen. Laser fusion scientists use the compressibility of hydrogen to tune laser pulses to obtain a maximum energy yield. These new results indicate that the equation of state of hydrogen is such that higher fusion yields are expected.

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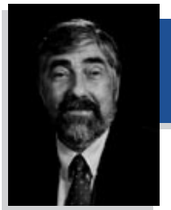
Patents

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.

Patent issued to	Patent title, number, and date of issue	Summary of disclosure
Daniel M. Makowiecki Philip B. Ramsey Robert S. Juntz	**Process for the Fabrication of Aluminum Metallized Pyrolytic Graphite Sputtering Targets** U.S. Patent 5,428,882; July 4, 1995	A method for fabricating and contouring pyrolytic graphite sputtering targets with superior heat transfer ability, longer life, and maximum energy transmission, and for the low-temperature joining of these metallized targets to a high thermal conductivity metal backing.
Martin Vanderlaan Larry H. Stanker Bruce E. Watkins Peter Petrovic Siegbert Gorbach	**Method for Immunodiagnostic Detection of Dioxins at Low Concentrations** U.S. Patent 5,429,925; July 4, 1995	A method for the detection of dioxins and dibenzofurans, using monoclonal antibodies, and a method of sample preparation to optimize detection of dioxins by immunoassay in contaminated samples at concentrations in the range of a few parts per trillion.
Chol K. Syn Donald R. Lesuer	**Laminated Metal Composite Formed from Low-Flow Stress Layers and High-Flow Stress Layers Using Flow-Constraining Elements and Method of Making Same** U.S. Patent 5,429,879; July 4, 1995	A laminated metal composite of alternating low-flow stress layers and high-flow stress layers, with each layer of low-flow stress material surrounded by an individual flow-constraining element, such as a ring. Pressure applied to the top and bottom surfaces of the resulting stack bonds the dissimilar layers together.
John F. Cooper	**Continuous-Feed Electrochemical Cell with Nonpacking Particulate Electrode** U.S. Patent 5,434,020; July 18, 1995	An electrochemical cell with a tapered cavity containing a quasi-stationary, permeable bed of electrochemically reactive particles. The dimensions of the cell cavity promote the bridging of particles across the cavity and the formation of voids to maintain a highly permeable bed. The cell provides 100% consumption of the particles.
Rex Booth	**Lumped Transmission Line Avalanche Pulser** U.S. Patent 5,434,456; July 18, 1995	A lumped transmission line pulse generator, utilizing the summed power output of a series of stages wherein each stage, consists of at least one avalanche transistor, a decreasing capacitance per stage, and a power source for the transistor stages coupled through a respective resistance and zener diode network.
William A. Brummond Ravindra S. Upadhye Cesar O. Pruneda	**Molten Salt Destruction of Energetic Waste Materials** U.S. Patent 5,434,335; July 18, 1995	A method for destroying energetic materials, including high explosives, propellants, and rocket fuels, in a molten salt reactor by side stream delivery of a preblended fuel mixture into a high-temperature molten salt bath, with optional continuous molten salt recycling.
Gary R. Dreifuerst Bernard T. Merritt	**High Voltage Power Supply with Modular Series Resonant Inverter** U.S. Patent 5,434,770; July 18, 1995	A high-voltage power supply incorporating a plurality of phase-controlled, series-resonant half bridge inverters (modules) for minimizing harmonic distortion and for maximizing high voltage and current output. Any number of such modules may be connected for easy power scaling.
Jim J. Chang	**Injection-Controlled Laser Resonator** U.S. Patent 5,434,882; July 18, 1995	A self-filtering and self-imaging laser resonator that converts a low-divergence laser signal injected into the resonator to the desired resonator modes before the main laser pulse starts. The laser cavity improves the quality of the injection signal through self-filtering before the main laser pulse starts, while the self-imaging property of the resonator reduces cavity-induced diffraction effects to improve laser beam quality.
Sol P. Dijaili Frank G. Patterson Robert J. Deri	**Cross-Talk-Free, Low-Noise Optical Amplifier** U.S. Patent 5,436,759; July 25, 1995	A segmented optical cavity oriented off-axis to suppress parasitic lasing modes. The laser cavity is segmented along its length with optically isolated regions. Amplifier crosstalk is reduced.



Hal Graboske
Principal Deputy Associate Director,
Physics and Space Technology

Technology "Spin-Offs" and "Spin-Backs"

THE Laboratory is proud of its five R&D 100 award winners this year. The winners represent a cross section of our expertise in lasers, mass spectroscopy, electron beam processing, and aerogels and are an indicator of the technological vitality of our programs.

Our nation's security needs drive the Laboratory's missions, which in turn determine the technical questions and issues that our scientists must resolve. As they look for answers to difficult questions, they find themselves inventing new products, new processes, and even entirely new technologies. Our scientists do not set out to be inventors; rather, they look for the best way to deal with the problem at hand. Then, once a program has developed a new product that helps it carry out its mission, the product is often picked up by another program, modified, and put to a whole new use. This "spin-off" sometimes becomes a "spin-back," returning to the original program where it is put to a new use altogether. Because of the Laboratory's multiple missions, its programs generate more new ideas, products, and processes, and cross-fertilization frequently occurs.

The development of aerogels is an excellent example of the spin-offs and spin-backs that occur within the Laboratory. Aerogels are an ultra-light, solid material invented in the 1930s and studied at Stanford University. They were first put to practical use in the 1970s, when European physicists used them in nuclear-particle detectors. Then, in 1980, a physicist at the Laboratory found that aerogels with some modification were useful in shock-wave studies to explore the physical properties of materials used in nuclear weapons. A few years later, inertial confinement fusion researchers at the Laboratory found that aerogels could absorb and hold liquid hydrogen (as a sugar cube absorbs coffee), which made them excellent fuel capsules in Shiva and Nova fusion targets.

A few years later, aerogels spun back to the weapons program, where scientists found a new use that required the material scientists to expand their synthesis, characterization, and production capabilities for aerogel materials. A new level of precision machining was achieved for these lightweight, brittle materials, some of which were only a few times denser than air.

In the early 1990s, the Department of Energy determined that the know-how of the national laboratories should be made available to improve U.S. industrial competitiveness, and the Technology Transfer Initiative was born. Aerogels became one of the more widely applicable materials, with uses studied for insulation, filters, and batteries—NASA even studied them as a means of collecting micrometeoroides in space. This diversity of uses was largely a result of Laboratory efforts to develop and characterize new aerogel varieties and develop production facilities and machining techniques. About the same time, university researchers studying condensed matter discovered that these improved aerogels could be used to study the properties of superfluids, which are fluids that can flow with no resistance under ultra-cold conditions. Aerogels had spun back to their original use, as a tool for basic research.

Now, in the mid-1990s, aerogels have spun back yet again to the weapons program, where Laboratory scientists are using them in their science-based stockpile stewardship program. Used in improved shock-wave studies, aerogels help scientists explore the properties of materials under the conditions found in nuclear implosions and explosions. Most certainly, a number of our present and previous R&D 100 award winners can tell similar stories about the spin-offs and spin-backs of their inventions.

As products spin off from their original use, so have some Laboratory programs spun off from the core missions. The direction from the Atomic Energy Commission to the new laboratory in 1952 was to develop improved diagnostic techniques and study new types of weapons for our nuclear deterrent. Thus, early studies of the mutagenic effects of radiation on people handling test devices eventually led to our preeminent biomedical research capabilities, which have made us a leading player today in the Human Genome Project. Likewise, early research to understand how radiation migrates through air, soil, and water led to the creation of our environmental programs, which are using this knowledge to develop site remediation technologies. These programs, while much more diverse and more broadly defined today than they were in 1952, still retain the common goal of serving our national needs.

The array of programs sponsored by the Laboratory is a key to our continued success, and R&D 100 awards are an acknowledgment of our continuing technical accomplishments.

The Genetic Contribution of Sperm: Healthy Baby or Not?

Members of the sperm and embryo cytogenetics team at LLNL. Back row, left to right: Francesco Marchetti, Jiri Rubes, Andy Wyrobek (principal investigator), Joyce deStoppelaar, and Paul Van Hummelen. Front row, left to right: Armand Tcheong, Emily Panico, Xiu Lowe, Nancy Oschbach, and Mike Cassell. Not shown: Christina Sanders, Thomas Ahlborn, Adi Baumgartner, Luoann Uelese, Wendie Robbins, Elizabeth Notley, and Janet Baulch.

We are developing powerful molecular methods to visualize individual chromosomes in sperm and to detect genetic defects in embryos. Our research methods, combined with animal models, have broad implications for screening males with chromosomal abnormalities and genetic diseases, for studying the effects of exposure to mutagenic agents, and for assessing genetic risks to the embryo and offspring.

THE global population explosion would seem to suggest that human reproduction functions quite well. However, reproductive failures, abnormalities during pregnancy, and birth defects are more common than many people realize. Every year in the U.S., more than 2 million couples who want to have children are infertile, and over 2 million conceptions are lost before the twentieth week of gestation. In addition, about 7% of newborns have low birth weight, and up to 7% of babies, or about 210,000 children per year in the U.S., are born with some birth defect. Half of these birth defects are major, affecting the health and viability of the individual.

The social and medical costs of reproductive abnormalities are formidable, yet their causes are not well understood. Abnormal reproductive outcomes include a wide variety of problems listed in Figure 1. Different molecular mechanisms, diagnoses, and treatments are typically involved in the different conditions. The cause of almost any reproductive abnormality can be the result of genetic and physiological events that occurred in any one (or in some combination) of three people—the mother, father, and child. Because of such complexities, pinpointing the cause of a specific reproductive abnormality may be even more difficult than determining the cause of cancer.

Certain abnormal reproductive outcomes can be caused by events that occurred in the germ cells (sperm or egg) of one of the parents before fertilization. Some abnormal reproductive outcomes, such as Down syndrome, have been traced to abnormalities in the eggs of the mother. Historically, the picture has been much less clear for the father. Now, we have compelling evidence that the male parent can be the source of detrimental effects on the genetic makeup and health of the embryo and child.

Geneticists estimate that about 40% of the cases of human infertility are due to male factors. About 80% of chromosomal aberrations (structural defects in chromosomes seen at birth) originate from the father. Furthermore, almost all new gene mutations seen in offspring and most abnormalities in the numbers of the sex chromosomes come from the father's sperm. Nevertheless, we have only had a limited understanding of the details underlying the father's contribution to reproductive problems and failures.

Biomedical scientists at the Laboratory are now conducting research on chromosomal defects in sperm and their effects on the developing embryo. Until recently, little was known about such defects because no practical method was available for detecting abnormal chromosomes in sperm and early embryos.

Three Kinds of Evidence

Three primary lines of evidence form the basis for our research.

Declining Sperm Count

First, the sperm count of men has been declining over the last five decades, and we still do not know exactly what accounts for the decline. In 1983, we conducted studies for the U.S. Environmental Protection Agency (EPA) on the effects of nearly one hundred different types of exposures on sperm production in human males.[1] About half of the agents we studied, including alcoholic beverages, cigarette smoke, and lead, lowered the production of sperm or affected sperm motility or morphology.

Occupational Exposure

A second important line of evidence suggests that certain jobs and workplace and environmental exposures of the father are linked to spontaneous abortion and problems in their offspring, including birth defects and cancer. Some occupations seem to be repeatedly associated with abnormal reproductive outcomes; however, findings are variable, and actual exposures are often poorly defined. We still do not have conclusive links between specific exposures, mechanisms of transmission, and an increased frequency of birth defects or childhood cancers.

At least two different models can account for many of the epidemiological findings. It is possible that some fathers might bring home potentially damaging agents on equipment, skin, or clothing, thus affecting the wife, offspring, or both. On the other hand, the route of exposure could be more direct, via the father's sperm. LLNL researchers have been developing and applying improved sperm assays to help distinguish between these two models.

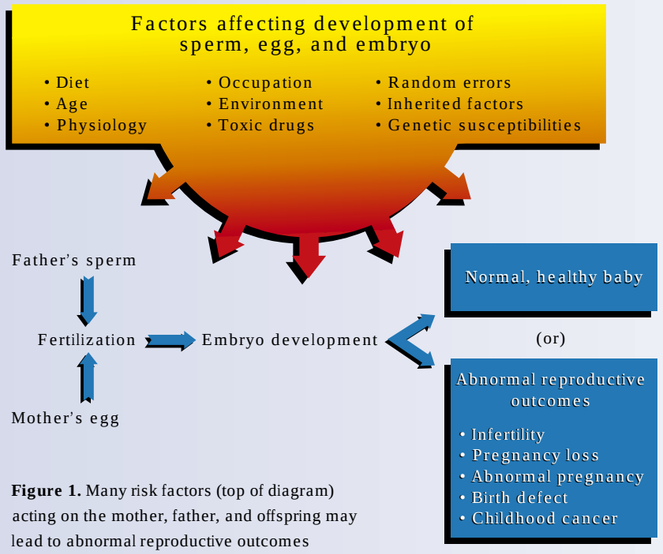


Figure 1. Many risk factors (top of diagram) acting on the mother, father, and offspring may lead to abnormal reproductive outcomes (bottom, right). Some risks can date to shortly after the time of conception of either parent. Livermore researchers are focusing on defects in sperm that lead to abnormal outcomes.

Genetic Mutations in Infants

A third line of evidence for male-mediated reproductive effects comes from studies of babies when some of the newest molecular methods are applied. Such studies show that when entirely new gene mutations occur in an offspring—ones never seen before in either the mother's or the father's family—they are almost always associated with the father's genes. Several defects also predominantly occur in the father's chromosomes. For example, about 80% of the chromosomal aberrations seen in the chromosomes of babies—defects such as chromosome breaks—come from the father.

Confusing Evidence

For decades, we have known that male animals—especially laboratory mice and rats—that are exposed to certain damaging agents can suffer adverse reproductive effects and other

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health problems. The effects can include reduced sperm production, diminished quality of sperm, and reduced libido.

Researchers can systematically study rodents to gain a better understanding of the links between exposure and reproductive effects. However, for humans, we must rely on the few sources of evidence that are available to us, including exposed individuals and their offspring. Studies since the 1950s have consistently shown that exposures of human males to environmental, occupational, or therapeutic agents can have detrimental effects on sperm count, motion, or shape. In contrast, although many environmental agents clearly have mutagenic potential in animals, experts have disagreed on whether environmental exposure of human males contributes very much to genetic disease or to adverse effects in their offspring.

For example, the research literature is consistent in confirming the adverse genetic effects of ionizing radiation in the male mouse and its offspring. However, the human offspring of atom-bomb survivors have no measurable increase in induced mutations. Similarly, exposures to various agents used in chemotherapy and to radiation therapy do not yield clear-cut results for genetic effects in the offspring of treated male patients.

Some of the puzzling inconsistencies between humans and mice may be due to individual variation and species differences. Other explanations involve the role of DNA repair processes or the possibility that some chemical or physical agents (mutagens) may have limited or short-term effects on sperm. Human doses are often small compared to those used in research on mice, and the number of human offspring that have been studied for induced genetic effects remains relatively small. Finally, it is possible that the types of genetic damage (called "endpoints" by geneticists) assessed in studies of exposed humans are not sensitive enough to always reveal a significant effect.

A Review of the Basics

The body (or somatic) cells of humans and other mammals contain pairs of chromosomes. Except for the sperm or egg cells and red blood cells, human somatic cells carry 46 chromosomes (the diploid number). Normal human somatic cells have 22 pairs of autosomes (nonsex chromosomes) and one pair of sex chromosomes, either XX or XY. Of the sex chromosomes, a normal female carries two X chromosomes, and a normal male carries one X and one Y chromosome.

(a) Normal human sperm carrying 22 autosomes and a Y chromosome.

(b) Normal human sperm carrying 22 autosomes and an X chromosome.

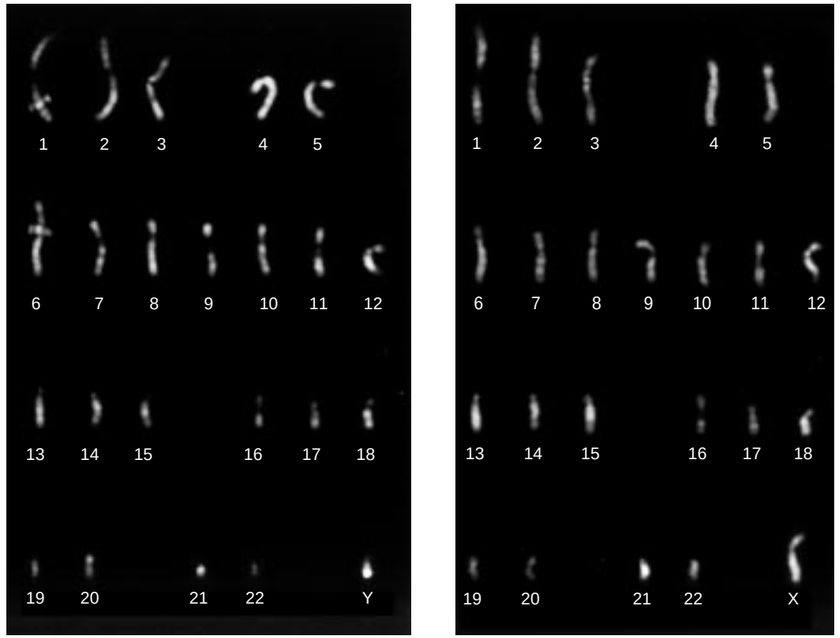


Figure 2. Normal human somatic cells carry 46 chromosomes, but each sperm and egg carries only 23 chromosomes (the haploid number). The photomicrographs show the normal complement of 23 chromosomes from two human sperm that were specially prepared to make the chromosomes visible under a microscope (ref. 4). One sperm (a) carries the Y chromosome and would produce a male; the other (b) carries the X chromosome and would produce a female. (Photographs courtesy of L. Gordon and B. Brandriff of LLNL.)

In contrast to somatic cells, each sperm and egg contains 23 chromosomes (the haploid number in humans). Each normal sperm and egg carries one copy of chromosome 1, one copy of chromosome 2, and so forth. Figure 2 shows the haploid number of chromosomes from human sperm that were specially prepared by a technique developed at the Laboratory.

If either of the germ cells carries an abnormal number of chromosomes or some other genetic defect, major hazards may arise for the offspring. A fetus resulting, for example, from fertilization with a genetically defective sperm would carry a mutation not only in the germ tissues but also in all somatic cells. An embryo's survival and quality of life through birth and beyond depend on the specific chromosomal defect it may carry. An embryo carrying major chromosomal defects will die during development. Thus, a validated method for detecting chromosome abnormalities in sperm has broad implications for maintaining or improving human health.

About Aneuploidy

The measure (or biological marker of male reproductive risk) we have chosen to study in depth is sperm aneuploidy. Aneuploidies in general are an important category of chromosomal damage that can be transmitted to an offspring from either the father or mother. The word "aneuploidy" refers to cells carrying the wrong (thus the prefix "an") number of chromosomes ("euploid"). Aneuploidy is one of the most common and serious chromosomal abnormalities recognized in humans. It is responsible for a large portion of infertility, pregnancy loss, infant death, malformations, mental retardation, and behavioral abnormalities.

Human embryos with an abnormal number of sex chromosomes or with an extra chromosome 13, 18, or 21 can survive to birth and beyond. An extra chromosome 21 causes Down syndrome and is a familiar example of aneuploidy involving one of the nonsex chromosomes. However, the most common aneuploidies in humans at birth involve an abnormal number of X or Y chromosomes. This condition, sex-chromosome aneuploidy, can be diagnosed prenatally through amniocentesis, and the incidence is about 1 in 250.

Table 1 shows different types of sex-chromosome aneuploidies together with other abnormalities involving the autosomes. A male child who inherits, say, an extra Y chromosome from the father would have a total of 47 chromosomes and a sex-chromosome aneuploidy (XYY). We know that human fathers are responsible for 100% of 47, XYY cases because the mother carries no Y chromosome. Another aneuploidy involving the sex chromosomes is Turner syndrome (45, XO), in which a paternal chromosome is lacking about 80% of the time. Other conditions are Klinefelter syndrome (47, XXY) and a triplet of X chromosomes (47, XXX).

Slightly more than half of the sex-chromosome aneuploidies at birth

Table 1. Examples of aneuploidy in the sperm of humans. Abnormal chromosomal conditions arise in the embryo when a sperm contributes an abnormal number of chromosomes to the embryo. The normal complement of sex chromosomes is shown in the shaded area at the top for comparison.

Father's contribution in sperm	Mother's contribution in egg	Embryo or offspring	Syndrome
X	X	46,XX	Normal female
Y	X	46,XY	Normal male
XY	X	47,XXY	Klinefelter syndrome • Hypogonadism • Sterile
No sex chromosome	X	45,XO	Turner syndrome • Characteristic physical features • Hypogonadism • Sterile
YY	X	47,XYY	XYY male
XX	X	47,XXX	XXX female
21,21	21	47,+21*	Down syndrome • Mental retardation • Characteristic physical features
18,18	18	47,+18*	Edward syndrome • Mental deficiency • Anomalous hands, face • Often fatal
13,13	13	47,+13*	Trisomy 13 • Severe anomalies • Often fatal

*The extra autosome is often contributed by an aneuploid egg, but sperm are also known to be responsible for the genetic defect.

are of paternal origin. The effects of such aneuploidy depend on which combination of X and Y chromosomes is involved. The health effects of XYY, for example, are minor; however, the effects of Turner and Klinefelter syndrome include physical, behavioral, and intellectual impairment as well as sterility.

Among human babies, the frequency of known chromosomal abnormalities, including aneuploidies and structural aberrations, is about 0.6%. In addition, about 1% of newborns carry a mutation for a genetic disease.[2] When an inherited error occurs, we need some way to ascertain when the condition is due to the father, what factors can cause the condition, and what prevention strategies might be effective.

Assessing Damage

The DNA complex forming the chromosomes (chromatin) in sperm is typically rigid and so dense that it occupies nearly the minimum possible volume. The high degree of condensation makes it nearly impossible to visualize and identify individual chromosomes by standard light or electron microscopies. LLNL researchers have been involved in the development of several techniques that allow sperm chromosomes to be visualized and assessed for anomalies.

The Hamster Technique

In 1978, Rudak and colleagues working in Hawaii pioneered a way to analyze the chromosomes in human sperm after fusion with hamster eggs.[3] Until recently, this "hamster technique" was the only method available for characterizing chromosomal defects in human sperm.

During the 1980s, LLNL researchers were the first to get the hamster technique to reliably work for a variety of applications, a considerable challenge because biologists elsewhere in the world were having major difficulties in obtaining usable results. (The March 1984 issue of *Energy and Technology Review* provides a more complete description of this highly useful tool.)[4] We showed that the hamster technique gives valuable baseline information on the normal burden of damage in healthy men. Using the method, we found that a small proportion of sperm in otherwise healthy males carries aneuploidies or other types of structural aberrations.

The hamster technique has become a highly reliable tool, and we consider it to be the "gold standard" against which we evaluate any new methods. However, the technique is difficult to apply and is both labor-intensive and inefficient, so it is costly to perform.

Fluorescence *In Situ* Hybridization

By about 1990, the Laboratory had developed a new biological procedure that could detect aneuploid sperm more efficiently than the hamster technique. Fluorescence *in situ* hybridization (FISH) has been previously described in *Energy and Technology Review* (see the April/May 1992 issue) as a gene-mapping tool.[5] The method is illustrated in the context of our sperm research in the box on page 12. In essence, we prepare chemically labeled DNA probes and bind (hybridize) them to target chromosomal DNA within the sperm head.

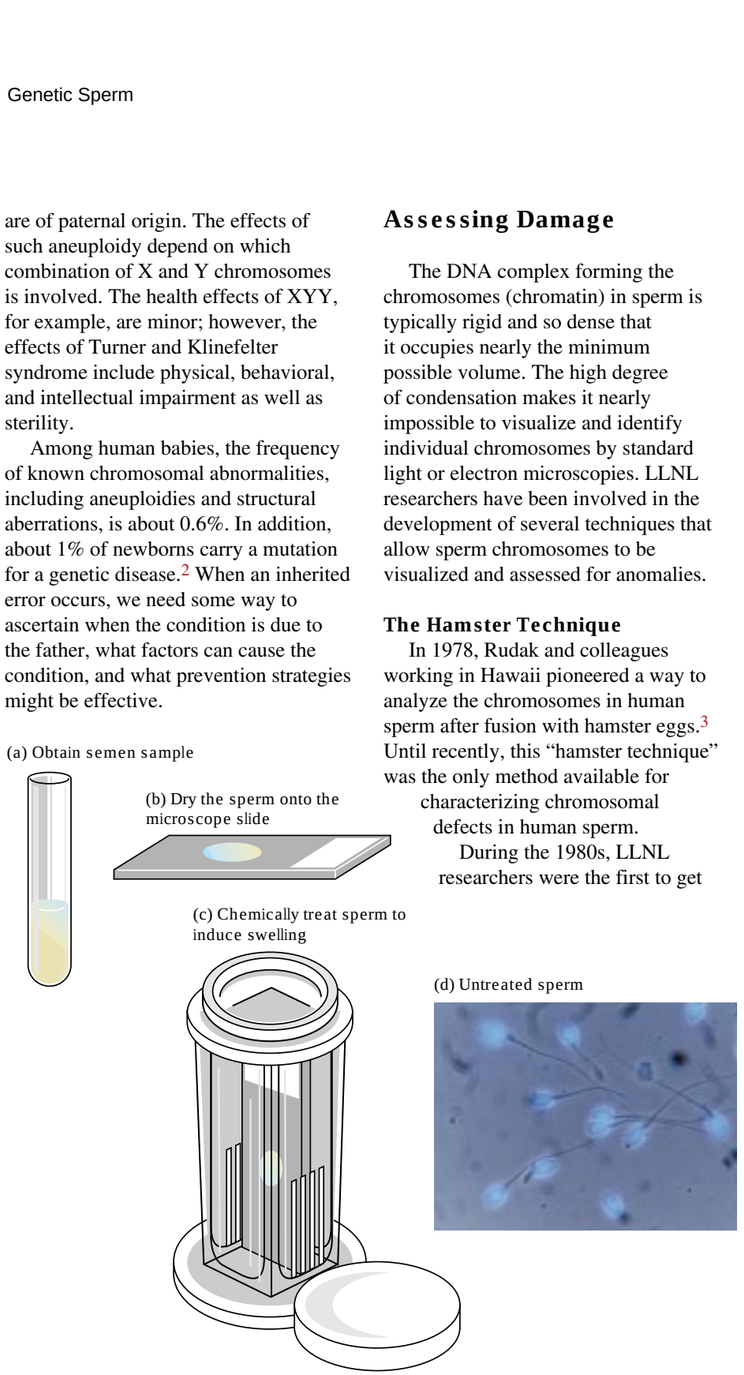


Figure 3. The high degree of condensation of the DNA–protein complex (called chromatin) in sperm makes it nearly impossible to identify individual chromosomes by standard microscopy. The two photos show how our chemical treatments (a–c) swell the sperm head, which is shown before (d) and after (e) treatment. The scale is the same in (d) and (e).

One of the challenges we met in applying FISH to human sperm was finding the right chemical treatments needed for introducing probes with fluorescent tags into the dense sperm head to penetrate the DNA. We learned how to control the amount of swelling (see Figure 3) that occurs during the process while maintaining the integrity of the nuclear material in sperm. FISH is especially useful because it provides vivid fluorescent signals, it allows us to distinguish between several probes with different colors, and it is reliable.

First Use of FISH in Sperm

In our first demonstration of FISH in human sperm, we applied a fluorescently labeled DNA probe to the Y chromosomes of sperm from human volunteers. As shown in Figure 4a, the Y chromosomes were tagged with a fluorescein label, a green-fluorescing dye that can look yellow, for example, on a red background. The Y chromosomes are easily recognized as bright yellow spots, called "domains." We counterstained the sperm nuclei with the red-fluorescing dye propidium iodide, which produces the bright red background color.

After examining and scoring 11,500 sperm nuclei, we found that 50% of sperm showed fluorescent domains consistent with the presence of a Y chromosome. The proportion is what we would expect, because about half of all sperm carry a Y chromosome, and half carry an X chromosome. This finding is also consistent with the proportion of sperm containing Y chromosomes as determined by the hamster technique. As anticipated, FISH proved to be a direct and reproducible method for monitoring the chromosome constitution of sperm, and it allows us to visually analyze thousands of cells rapidly. In subsequent studies, we expanded the number of DNA probes we can apply to sperm nuclei, allowing us to tag two or three different chromosomes simultaneously, and we extended the method for use in laboratory animals.

Extending the Human Assay

Two-Probe Assays

Because aneuploidy at birth frequently involves the two sex chromosomes, we initially extended the FISH assay to include the second sex chromosome (chromosome X). Figure 4b shows normal sperm carrying either a single X chromosome, which fluoresces blue-green due to the dye FITC, or a single Y chromosome, which fluoresces red due to the dye Texas Red. In the photograph, the red domains are larger than the blue-green ones because the DNA regions we targeted on the Y chromosome had longer repetitive sequences. In this and subsequent photos, the precise color of a fluorescent dye can vary as a function of counterstains used to highlight the sperm nucleus.

Beyond studies of normal sperm, the two-probe assay gives us a method for detecting sperm carrying an abnormal number of chromosomes X and Y. This type of assay can be applied to study sperm that give rise to Turner syndrome, Klinefelter syndrome, and other inherited sex-chromosome conditions. When such aneuploid sperm are produced, our two-probe assay can differentiate among sperm containing two red domains (YY), two green domains (XX), or both colors (XY).

Three-Probe Assays

Next, we added a fluorescently labeled DNA probe for one of the autosomes in sperm. Whereas any autosome would suit the purpose, we selected a probe for chromosome 8, which was our best DNA probe available at the time. Adding one autosome to FISH is a major advantage because it allows us to distinguish among three possibilities: duplication of a sex chromosome only (sex-chromosome aneuploidy), duplication of a single autosome only (autosomal aneuploidy), and duplication of the

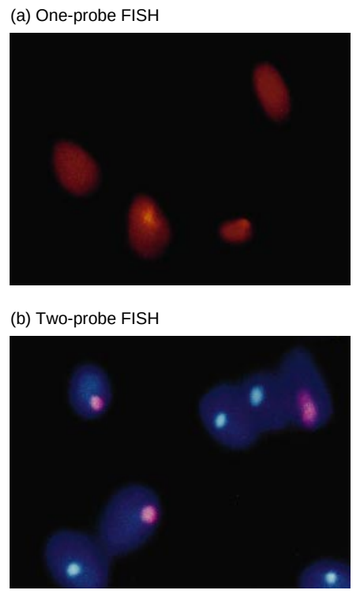


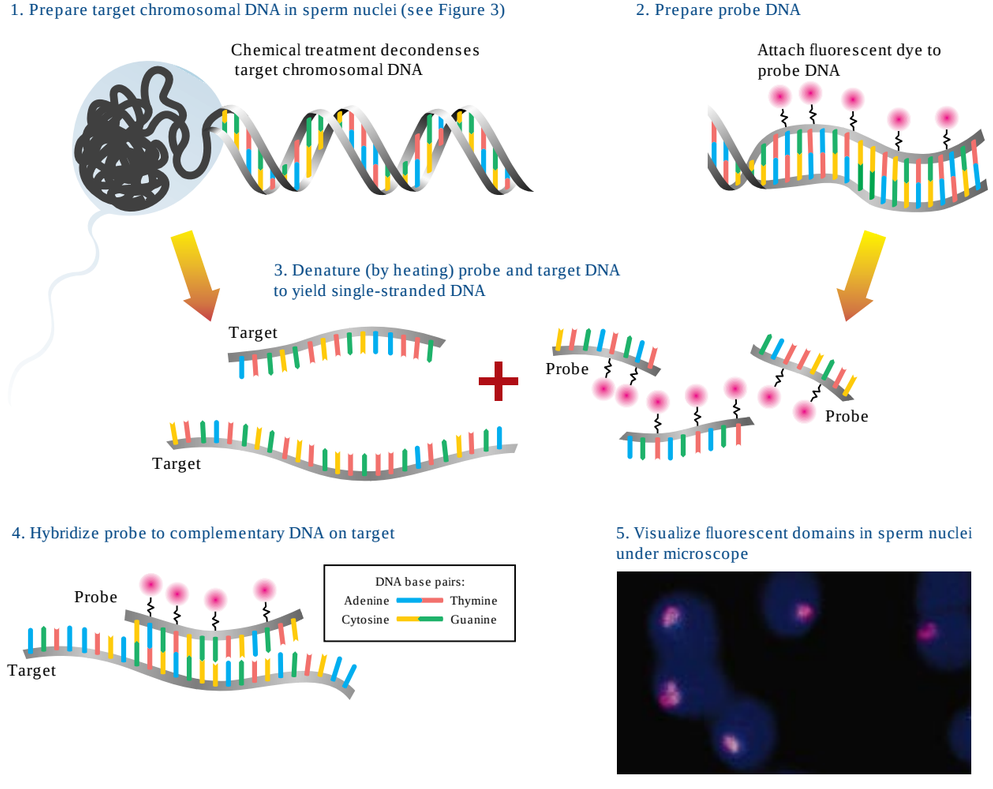
Figure 4. (a) The fluorescent dye fluorescein is applied to the Y chromosomes in human sperm. When they are present (50% of the time), the Y chromosomes are easily recognized as bright yellow areas. The sperm nuclei are counterstained with propidium iodide, giving a red background color. (b) This example of our two-probe FISH procedure shows how we can differentiate among human sperm carrying a single Y chromosome (red fluorescence) or a single X chromosome (green fluorescence).

How FISH Is Used to Detect Aneuploid Sperm

Abnormal human sperm can contain two X chromosomes, two Y chromosomes, neither X or Y, both X and Y, and an abnormal number of autosomes (either more or less than 22). How can we tell the difference between normal and defective sperm when chromosomes are packaged so tightly in the sperm head?

Our technique, called fluorescence *in situ* hybridization (FISH), uses two starting DNA materials: target sperm chromosomes and probe DNA. Sperm carrying the target chromosomes are placed on glass slides. The sperm chromatin is chemically treated, or in technical terms, decondensed, so that our probe DNA can penetrate the chromatin to reach the target chromosomes. The probe consists of DNA fragments (hundreds of copies of a specific region of a particular chromosome) prepared by attaching a fluorescent dye and heating to yield single-stranded DNA. At Livermore, we have DNA probes for most of the human chromosomes, including X and Y, and for many rodent chromosomes. We use several different dyes to differentiate among different chromosome types. When the labeled probes hybridize (bind) with the complementary single strand of target sperm chromosomal DNA, the dyes vividly "light up" the specific region of the chromosome under investigation. We then count and record (score) the fluorescent spots, called domains, which appear as vivid signals through a light microscope.

Our methods are equally successful in studies of human and rodent sperm, and they are far more efficient and less costly than any other assay developed to date, including the hamster technique. Ten thousand cells can be scored in less than two days.



entire genome (diploidy). In other words, if we detected the condition XX88 or YY88, then it is highly likely that all the other chromosomes are duplicated as well (the diploid condition).

Figure 5 illustrates our three-probe assay for chromosomes 8 (yellow), X (green), and Y (red). Figure 5a shows normal human sperm, which carry either X8 or Y8. All such normal sperm fluoresce in only two colors and show two domains (two discrete fluorescent areas). Abnormal human sperm in Figures 5b through 5j show more than two domains (for example, XX88 has four domains) or more than two colors (for example, XY8 has three colors and three domains).

As more FISH probes become available, we will add them to the assay. As soon as an excellent probe for chromosome 21 is developed, we will include it in the FISH assay so that we can look for this important marker for sperm that may lead to Down's syndrome. Similarly, we will soon add DNA probes for chromosomes 13 and 18 because these trisomies, like chromosome 21, survive to birth and beyond in humans.

Recently, we developed another useful tool that has important implications. By adding the technique of phase-contrast imaging to fluorescence microscopy, we can now detect the tails of sperm and distinguish them from somatic cells that are normally present in semen (Figure 6). Differentiation is critical when we detect sperm carrying XY88, for example. Such an arrangement could represent either a diploid sperm or a normal somatic cell (which always carries two sex chromosomes and copies of each autosome). Phase contrast allows us to differentiate clearly between the two possibilities because somatic cells have no tail.

Validating the Method

To demonstrate the utility of the FISH method for assessing sperm chromosomes in humans, we needed to address the issue of validation. How would we know whether the values we obtain—for example, the baseline frequency of aneuploid sperm in healthy males—are actually correct? Fortunately, several lines of evidence from independent sources can be used to validate our assay.

Researchers at Livermore Laboratory and in Canada and Japan have used the hamster technique to collect baseline information establishing the normal

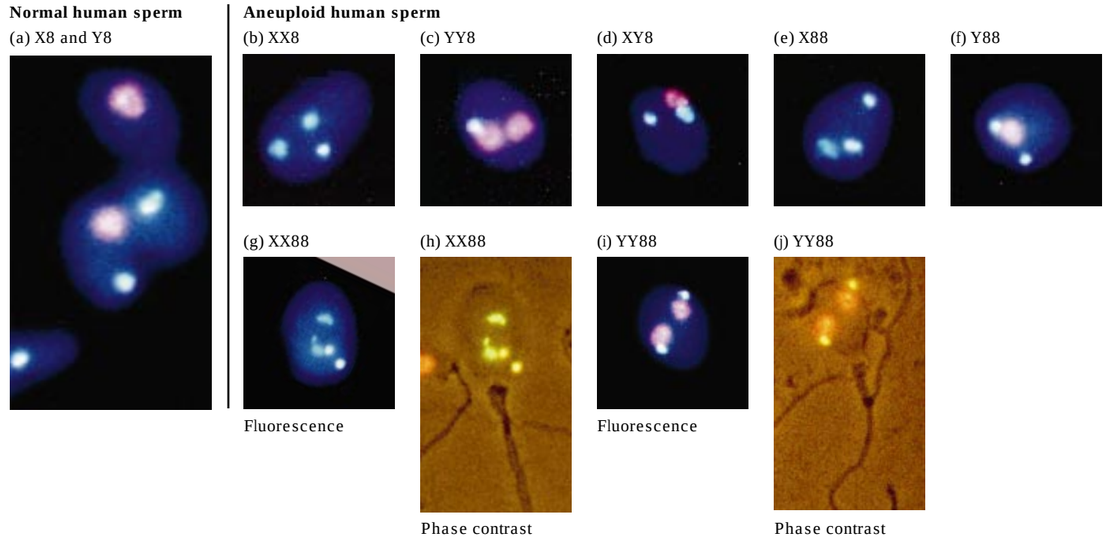


Figure 5. Our three-probe FISH procedure applies a mixture of probes specific for chromosomes X, Y, and 8, each tagged with a different fluorescent dye. Here, human chromosomes X fluoresce green, Y fluoresce red, and 8 fluoresce yellow. (a) Normal sperm carry either X8 or Y8 and are marked by only two different colors and two domains. (b–f) Abnormal sperm, such as XX8, YY8, XY8, X88, or Y88, have three domains but of varying colors. (g–j) Abnormal sperm, such as XX88 or YY88, have two colors but four domains. (h) and (j) show the sperm tail using phase-contrast imaging.

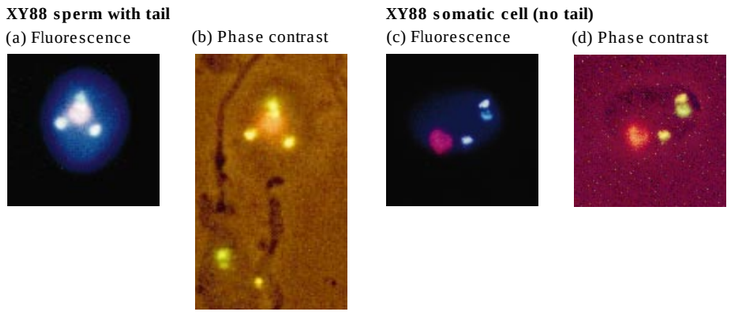


Figure 6. Phase-contrast microscopy, together with fluorescence microscopy, allows us to differentiate between (a and b) XY88 sperm, which have tails, and (c and d) XY88 somatic cells normally present in semen, which do not have tails.

burden of chromosome damage in human sperm. These studies suggest that the baseline frequency of aneuploid sperm in young, healthy males is 3 per 10,000 chromosomes. This is the reference value we used in assessing the new FISH assay. We also have hamster data on the frequency of abnormal chromosomes after administering doses of some mutagenic drugs. Finally, hospitals publish the results of population-based surveys that provide additional statistics on the frequency of XYY babies, XXY babies, and other genetic anomalies at birth.

Baseline FISH Research

Using the one-, two-, and three-probe FISH assays in human sperm, we have assessed chromosomes X, Y, 1, and 8 for evidence of aneuploidy in hundreds of thousands of cells from healthy men. The frequencies of aneuploid sperm can vary among the different chromosome types and among individual male donors. Furthermore, most healthy men give consistent results over time (up to four years).

Our assays show that human sperm contain the abnormal chromosome pairs 8-8, XX, YY, and XY at frequencies of roughly one per 2,000 sperm analyzed, averaging across donors. This frequency is quite similar to the value of 0.6 per 2,000 sperm obtained by the hamster technique. We found that the abnormal chromosome pairs 1-1 had the highest frequency of all, about 3 per 2,000 sperm. No sperm of the many thousands we tested from different healthy donors contains more than two of the same chromosome type (for example, we do not find the triplets 888, 111, or YYY). The most common sex chromosomal abnormality we found using the three-probe assay was XY8, with an average frequency of 9.5 per 10,000 human sperm scored.[6] Table 2 summarizes the frequencies of abnormal sperm types we found in various studies of young, healthy, human males using FISH.

Table 2. Frequencies of human sperm, per 10,000 cells analyzed, with abnormal numbers of chromosomes determined by the FISH method. This summary table represents data from 14 donors and more than 220,000 scored cells. (Remember: normal sperm carry a single X or a single Y and a single copy of each numbered chromosome. Any other combination constitutes a chromosomal abnormality.)

Type of FISH assay	Type of chromosomal abnormality in sperm	Frequency (pooled data for donors studied)
One probe	1-1	14.0
Two probe	YY	5.7
Two probe	XX	3.9
Two probe	XY	6.2
Three probe	XX8	3.1
Three probe	YY8	3.1
Three probe	XY8	9.5
Three probe	88X	3.0
Three probe	88Y	3.6
Three probe	XX88	2.2
Three probe	YY88	1.7
Three probe	XY88	10.6*

*This frequency may be elevated because it may include a small number of somatic cells of the type XY88 normally found in semen.

Bridging Biomarkers

We recently developed corollary methods for detecting aneuploidy in the sperm of mice and rats using two- and three-probe FISH. We used a multicolor FISH procedure to evaluate chromosomes X and 8 in more than 80,000 sperm from healthy, young adult mice. About 3 sperm per 10,000 cells evaluated showed XX or 88 aneuploidies (Figure 7).[7] The frequencies we found for these particular numerical errors in two strains of mice were indistinguishable from those for sperm from healthy men using similar procedures and scoring methods. This work serves to demonstrate what we call "bridging biomarkers" between humans and animals for detecting sperm aneuploidy.

Bridging biomarkers, in essence, allow us to use the same type of measurable variation as we assess similarities and differences among species. With this type of information, we can compare the mean error rate of specific chromosomal defects in human versus rodent sperm, especially the sperm of mice. Our data show that the mean error rate is about the same in otherwise healthy male mice and humans. The bridging biomarkers of sperm aneuploidy also allow us to compare human and laboratory species for effects of physiological changes (e.g., diet, age), effects of exposure to toxicants, and effects of genetic differences. The studies of age effects are summarized to illustrate the utility of bridging biomarkers.

Age Effects in Mice and Men

We divided the 14 men in our three-probe study into two groups with average ages of 47 versus 29 years. Compared to the younger men, older men had higher fractions of abnormal sperm with either two copies of the X chromosome (XX8) or two copies of the Y chromosome (YY8). However, older men did not have higher frequencies of the other possible aneuploid conditions, such as XY8, X88, XY88, and so forth.[6]

Our findings on age effects are preliminary and should be interpreted with caution. A more detailed study using a larger population of men is needed. We have recently received funding from the National Institutes of Health to carry out such a study. The new collaboration will be one of the first human tests potentially linking chromosomally abnormal sperm to age and other life-style factors of the father.

Our preliminary findings of age effects in men are strikingly similar to our recent results on aneuploid sperm in aged mice. Aged mice (mice normally live for about two and a half years) had higher levels of sex-chromosome aneuploidy in sperm than did young mice. Mice of advanced age (older than about two years) had about twice as many aneuploidies of the types XX8, YY8, 88X, and 88Y than did younger mice (slightly older than two months). As with human males, we found the largest age-related increases in the XX8 and YY8 aneuploidies (Figure 8).

If our findings on aging continue to hold up with further research, they may point to an intriguing possibility. According to several lines of evidence on the production of sperm and egg cells with genetic errors, age effects in human females are predominant in the first stage of meiosis (the first of a two-stage process in forming an egg or sperm). Our preliminary data on both mice and human males suggest that age effects in males are predominant at the second stage of meiosis. Thus, males and females may differ in terms of the exact stage at which some genetic errors, such as aneuploidy, arise.

Effects of Smoking

Cigarette smoking is one of the most pervasive examples of the self-administration of toxic compounds. Research over several decades at Livermore and elsewhere has shown that cigarette smoking can cause defects in sperm quality. However, no information has been available on its mutagenic potential in sperm.

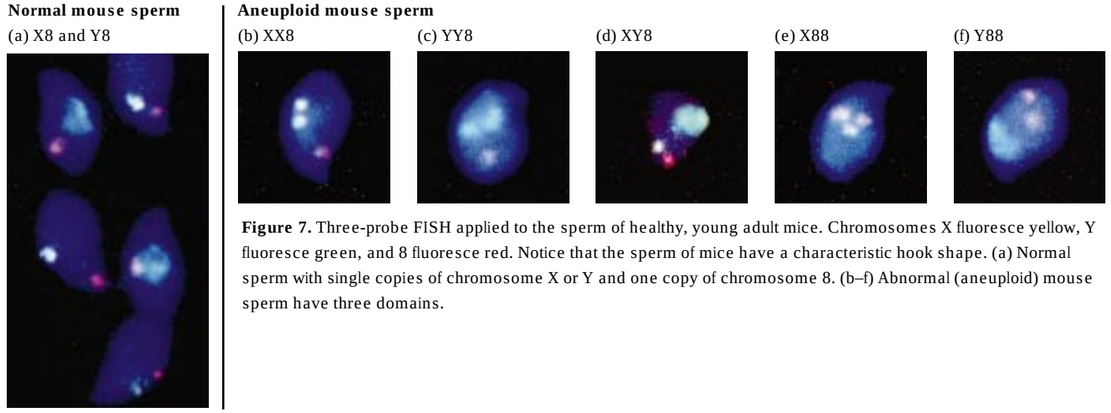


Figure 7. Three-probe FISH applied to the sperm of healthy, young adult mice. Chromosomes X fluoresce yellow, Y fluoresce green, and 8 fluoresce red. Notice that the sperm of mice have a characteristic hook shape. (a) Normal sperm with single copies of chromosome X or Y and one copy of chromosome 8. (b–f) Abnormal (aneuploid) mouse sperm have three domains.

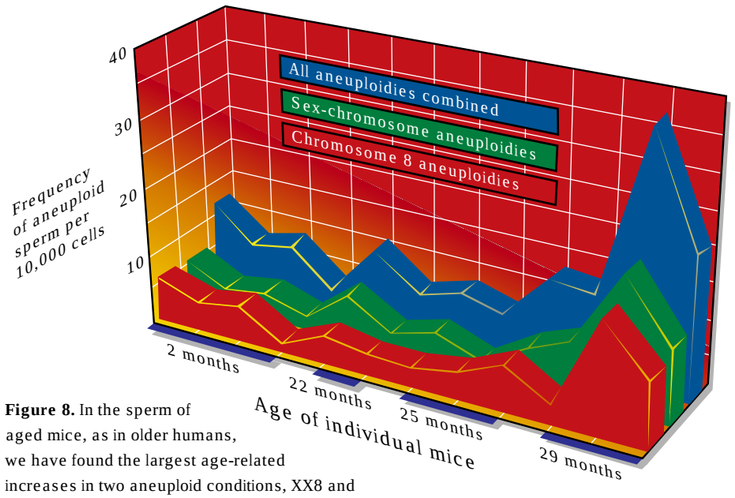


Figure 8. In the sperm of aged mice, as in older humans, we have found the largest age-related increases in two aneuploid conditions, XX8 and YY8. These curves show that the frequencies of aneuploid chromosomes in the sperm of mice increase with increasing age, especially after two years. This figure includes only those instances in which cells have gained a chromosome (ref. 7).

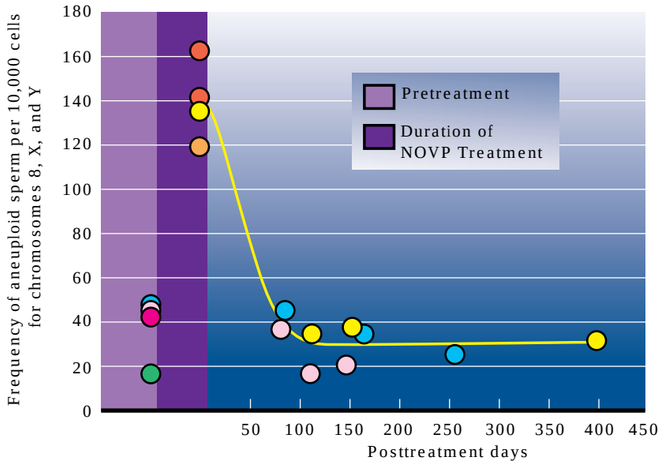


Figure 9. Frequency of aneuploid sperm in young men with Hodgkin's disease undergoing NOVP chemotherapy. The baseline levels of abnormal sperm increase twofold to fivefold following treatment with NOVP and then return to pretreatment levels approximately three months after chemotherapy. Each color represents samples obtained from a separate donor (ref. 6).

We recently studied 15 smokers and 15 nonsmokers from the Czech Republic and found that smokers produce approximately twice the number of aneuploid sperm as nonsmokers. Cigarette smoking is a life-style that often includes alcohol consumption and possible stress factors. Thus, further research is needed to determine whether the effects we found are indeed due to tobacco products or to other aspects of a smoker's life-style.

Effects of Chemotherapy

We have applied the three-probe FISH method to sperm cells of cancer patients before, during, and after treatment with the combination chemotherapy NOVP. Our basic question was whether the aneuploidies induced in sperm might persist following treatment, raising the possibility that genetic damage could be passed on to future offspring. We elected to study NOVP treatment because it contains drugs known to produce aneuploidy in model systems.

Figure 9 shows our results for young male patients with Hodgkin's disease (a kind of lymphatic cancer). When compared with healthy controls, these patients had elevated frequencies of aneuploid chromosomes X, Y, and 8 even before treatment (twofold to sixfold increases over normal levels). Just after NOVP treatment, the frequencies of numerical abnormalities increased twofold to fivefold compared to pretreatment levels. Following chemotherapy, aneuploid sperm returned to pretreatment levels within two to six months—clear evidence that at least one type of chemotherapy has transient effects on aneuploidy in human sperm. Further studies are needed to determine whether other drugs induce aneuploidy in human sperm and whether the effects are also transient.

New Research on Embryos

In very recent work, we have begun to assess damage in embryos, using the mouse as the model species. Figure 10 summarizes the various developmental stages of the mouse embryo at which we can now apply the FISH method to assess numerical and other errors in chromosomes. For example, we can study the embryos immediately after fertilization (just before first cleavage of the embryo when it divides into two cells), at the two-cell stage, or about four days later just before the embryo implants into the uterine wall and when it consists of 30 to 50 cells. This work has required the development of special FISH probes and techniques that are suitable for embryos.

One of the advantages of studying one-cell mouse embryos (called "zygotes") is that the chromosomes from the paternal and maternal mice

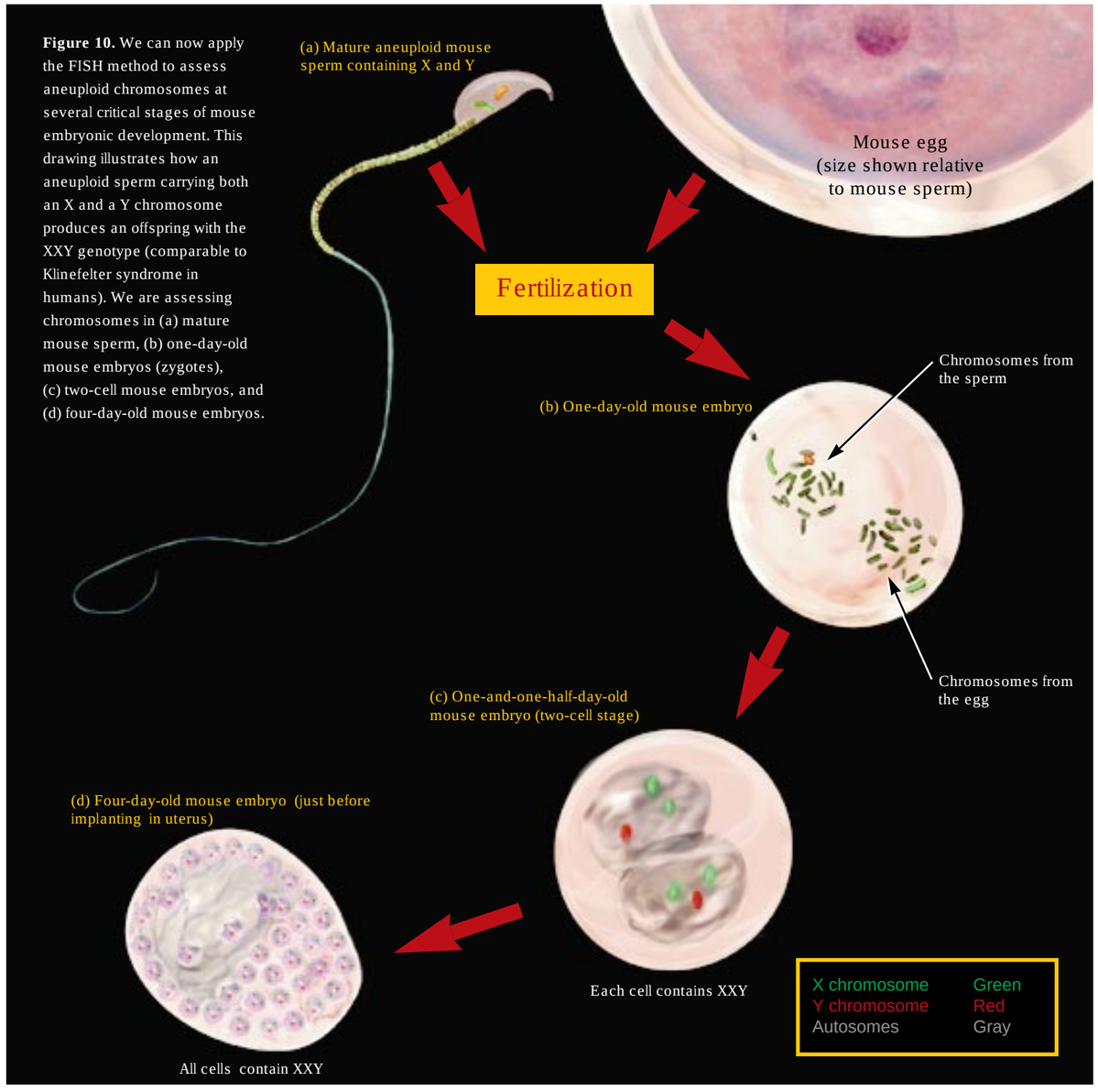


Figure 10. We can now apply the FISH method to assess aneuploid chromosomes at several critical stages of mouse embryonic development. This drawing illustrates how an aneuploid sperm carrying both an X and a Y chromosome produces an offspring with the XXY genotype (comparable to Klinefelter syndrome in humans). We are assessing chromosomes in (a) mature mouse sperm, (b) one-day-old mouse embryos (zygotes), (c) two-cell mouse embryos, and (d) four-day-old mouse embryos.

remain separated until the first cellular division (Figures 11a through 11c). This means that we can determine the contribution of each parent independently. Another advantage is that we can easily validate our results on mice because investigators have published data on the frequencies of aneuploid mouse chromosomes at the time of first cleavage.

Using FISH probes for the Y chromosome, our early results from more than 200 mouse zygotes show that about 9% of the embryos have numerical errors, and two of those cases were of paternal origin (see Figure 11b). We will investigate the effects on the embryo of exposing mouse sperm to chemical agents known to cause genetic defects.

Our new studies on four-day mouse embryos (Figures 11d and e) are a collaboration with the University of California at Berkeley. Very little work has been done on such embryos, so our research will be among the first. We are applying FISH and other biological imaging methods to understand how the development and survival of implanted embryos are affected by mutagen exposure of the father's sperm before mating.

Acrylamide is the model mutagen for our four-day embryo project because it is known to induce heritable defects. The damage we are seeing in embryos includes aneuploidy, mosaics (a combination of some normal cells and some chromosomally altered cells), chromosome breakage, and polyploidy (the occurrence of chromosomes that are three or more times the haploid number). We expect that the methods will also be useful for future studies on the outcomes of human *in vitro* fertilization.

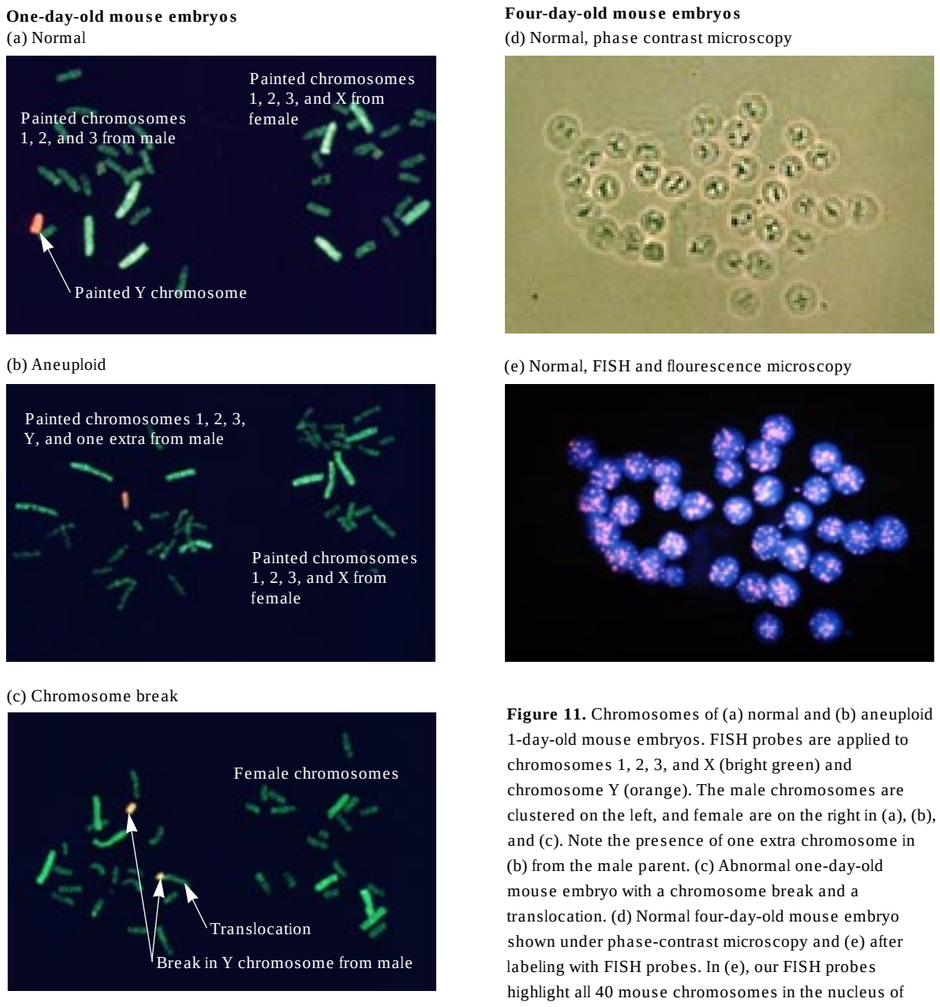


Figure 11. Chromosomes of (a) normal and (b) aneuploid 1-day-old mouse embryos. FISH probes are applied to chromosomes 1, 2, 3, and X (bright green) and chromosome Y (orange). The male chromosomes are clustered on the left, and female are on the right in (a), (b), and (c). Note the presence of one extra chromosome in (b) from the male parent. (c) Abnormal one-day-old mouse embryo with a chromosome break and a translocation. (d) Normal four-day-old mouse embryo shown under phase-contrast microscopy and (e) after labeling with FISH probes. In (e), our FISH probes highlight all 40 mouse chromosomes in the nucleus of each cell of the embryo.

New Research on Klinefelter Syndrome

We are beginning to look at blood samples from humans who carry the genetic abnormality associated with Klinefelter syndrome (47, XXY). Such individuals tend to be slower than normal in physical and behavioral development, they eventually grow taller on average, and they are all sterile. The work is a collaboration involving LLNL and five other institutions.

About half of 47, XXY cases receive the extra X chromosome from the father (such aneuploid sperm would carry both the X and Y chromosomes). Male children with this syndrome and their fathers provide us with a unique opportunity to learn about the relation between sperm aneuploidy and aneuploidy at birth. We will study 40 families with children whose diagnosis of Klinefelter syndrome has been genetically confirmed. We want to know if fathers who are responsible for the syndrome in their child produce inherently elevated levels of aneuploid sperm, especially XY sperm. To increase the speed of scoring defective chromosomes and the use of objective criteria in the FISH assay, we are also developing new automation and image-analysis techniques.

Looking Ahead

For decades, genetics researchers, concerned physicians, and many parents have struggled to come to terms with the causes and conditions that may underlie abnormal reproductive outcomes. With highly efficient FISH probes, we are beginning to understand some of the ways chromosomal abnormalities can arise in sperm and how those defects may lead to defects in the embryo.

Our expectation is that the new procedures will lead to a far greater understanding of the relations among certain genetic defects in human sperm, the effects of age, environmental exposures, and life-style factors, and the probability of fathering a chromosomally defective child. On the horizon are improved FISH assays for more chromosomes, new assays that can detect chromosome breakage in sperm, and automation and objective image processing. Such advances will help to make our methods more accessible to the rest of the research community.

Key Words: aneuploidy; chromosomal abnormality; DNA probes; fluorescence *in situ* hybridization (FISH); Klinefelter syndrome; sperm—human, rodent; sex chromosomes.

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About the Scientist

ANDY WYROBEK joined the Biomedical Sciences Division of the Laboratory in 1975. He is currently the principal investigator of the sperm and embryo research team within the Biology and Biotechnology Research Program at the Laboratory. He received his B.S. in physics from the University of Notre Dame in 1970 and his Ph.D. in medical biophysics from the Ontario Cancer Institute at the University of Toronto in 1975. In more than 80 publications, Andy Wyrobek has explored male-mediated developmental toxicology, human male reproductive hazards, and mammalian testing systems for detecting the genetic effects of environmental, occupational, and therapeutic agents in sperm and embryos. His special interests are understanding the mechanisms leading to birth defects and identifying the environmental and genetic risk factors for abnormal pregnancies.

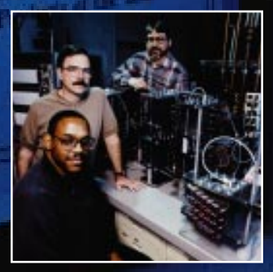
R&D 100 Awards Recognize Five Laboratory Inventions

THE Laboratory has done it again. In receiving five R&D 100 awards, the "Oscars" of applied research, the Laboratory has demonstrated its stature among the premier research institutions in the world.

Every year, scientists from corporations, government laboratories, private research institutes, and universities all over the world submit entry materials to *R&D Magazine*, vying for an award. Editors of the magazine and a panel of experts judge the entries, looking for the most technologically significant products and processes, ones that promise to change people's lives for the better.

Many past winners have become part of our everyday lives—Polacolor film, the halogen lamp, anti-lock brakes, the automated teller machine, the fax machine, the nicotine patch, and color computer printers. The Laboratory has won 55 R&D 100 awards since the competition began in 1963, including the process for the diamond turning of optics (1978), the three-dimensional chemical x-ray microscope (1988), and the hard x-ray lens (1991).

There is no telling what can happen to an R&D 100 award winner. In 1993, the Laboratory won an award for the world's fastest solid-state digitizer, a product that grew out of research related to the Nova laser. Today that digitizer has been put to use in micropower imaging radar (MIR), a $10 to $15 mini-radar system that can do a job that used to require equipment costing up to $40,000. MIR can be used on automobiles to warn



of collisions, can detect buried land mines, and can help rescuers searching for people trapped alive in destroyed or collapsed buildings. The range of MIR applications is so broad that the Laboratory is selling licenses to apply MIR technology in 14 different areas.

This year's awards were given to six teams of Laboratory scientists who made major progress in the fields of aerogels, lasers, mass spectrometry, and electron beam processing. One is a shared award for two aerogel processes:

- A new injection molding process for aerogels was developed that is similar to the molding process used to manufacture some plastics. This method of mass-producing aerogels is 30 times faster than any other production system, reduces liquid wastes by 40%, and uses 10 times less energy.
- Capacitive deionization with carbon aerogel electrodes provides an economical and efficient method for removing salt and impurities from water. It generates no wastes and is more energy-efficient than competing technologies. It can be used for waste treatment, water purification and softening, and desalination.

Two awards are related to the development of new types of lasers:

- A simple and inexpensive lens device scales up the output power of diode-pumped, solid-state lasers to 250 times that of similar systems. With this increased output energy, these lasers can be used in surgery, to treat infertility, in laser radar, and perhaps eventually in inertial confinement fusion.
- A flashlamp-pumped, solid-state laser with a unique amplifier system is the world's brightest solid-state, average-power laser. Divergence has been reduced almost to its physical limits, and the beam has an extremely narrow bandwidth. The laser is being used to generate x rays for the production of integrated circuits and in long-range laser radar systems.

The final two awards were given for developments in fields as diverse as mass spectrometry and electron beam processing:

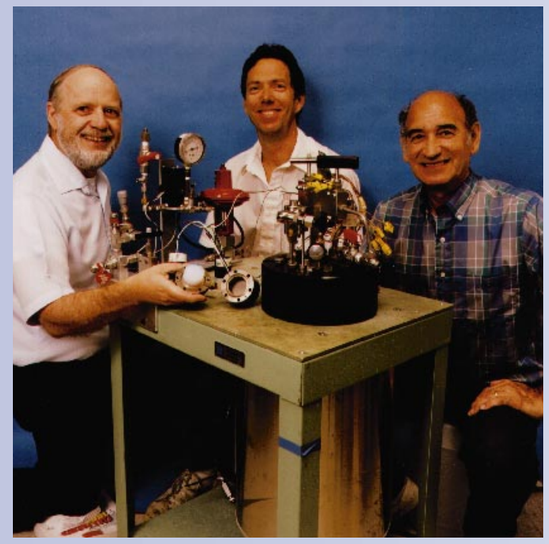
- The first hand-portable mass spectrometer, based on the principle of ion-cyclotron motion, combines the ion source and mass analyzer/detector with an integral vacuum system. In spite of its small size and simplicity, the system can achieve sufficient resolution to detect trace compounds or contaminants in air samples.
- A small, inexpensive electron beam gun can be used to process paints, inks, computer floppy disks, medical supplies, and other materials. The beam exits the gun through a new, thin-film window and delivers higher beam energies much more efficiently than large, expensive, conventional electron beam systems.

The group of articles that follows highlights each of these R&D 100 award-winning inventions and introduces you to the men and women of Lawrence Livermore National Laboratory who made them possible.

A Shared Award in Aerogel Process Technology

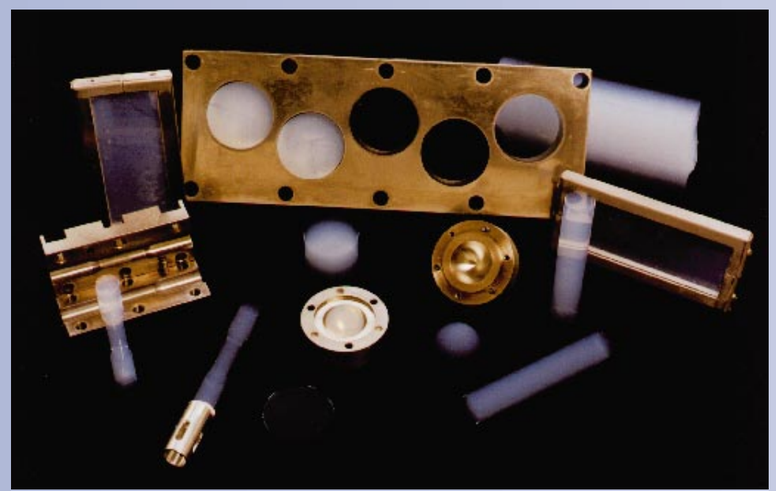
AEROGELS are among the lightest solids known, with some varieties consisting of 99.8% air. Many aerogels are nearly transparent and are called "frozen smoke" for their ghostly appearance. At the same time, of all known solids, aerogels have the highest internal surface areas per gram of material, thanks to their complicated, cross-linked internal molecular structure. An aerogel the size of a grape has the surface area of about two basketball courts. The internal microstructures give aerogels exceptional strength—some aerogels can support 1600 times their own weight.

First made in 1931, silica aerogel, composed mainly of silicon dioxide (sand), is probably the best known type of aerogel. Another type is organic aerogel, made up mainly of carbon and hydrogen atoms. At Lawrence Livermore National Laboratory, one of the world's leading centers of both silica and organic aerogel research and development, scientists attracted international attention early in this decade when they created a silica aerogel some 10 times less dense than the previous lightest version.



Pictured with the injection molding apparatus for rapid production of silica aerogels are, from left to right, Larry Hrubesh, John Poco, and Paul Coronado.

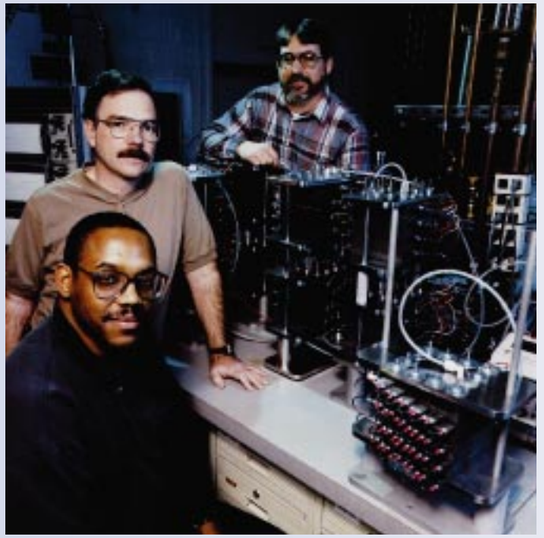
Aerogels exhibit many remarkable properties, such as the best electrical, thermal, and sound insulation of any known solid. As a result, few materials offer as many potential applications as aerogels. These versatile materials could be used in electronics, optics, thermal pane window inserts, solar panels, novelties, toys, jewelry, helmets, insulation tiles, refrigerators, freezers, and space applications and as catalysts for chemical reactions. Two recent advancements at the Laboratory in inorganic and organic aerogel process technology have improved the position of aerogels as a commercially viable technological innovation.



Getting Aerogels into Shape

A major stumbling block to the entry of silica aerogel products into the marketplace in a significant way is their high production cost, due mainly to a long and tedious manufacturing process. In response, a team of Laboratory researchers developed a process for producing shaped aerogel parts that is more than 30 times faster than conventional methods.

The injection molding process for net-shaped aerogels uses various molds (samples shown) to rapidly produce silica aerogels in a variety of shapes.



Pictured from left to right are Greg Mack, Joe Farmer, and Dave Fix, who developed the capacitive deionization process using carbon aerogel electrodes.

"We believe this process holds the key to making aerogels readily available for commercialization in many applications and prospective markets," says team leader and physicist Larry Hrubesh.

Hrubesh notes that silica aerogels are traditionally prepared by mixing a silicon-alkoxide—$Si(OC_xH_y)_4$—compound with alcohol, water, and a small amount of ammonium hydroxide. When mixed, these ingredients form a gel containing a network of silicon dioxide particles. If the gel is carefully dried, an intact aerogel remains. The challenge is to heat the gel to remove the liquid without destroying the fragile silicon dioxide microstructure.

The problem facing manufacturers is that during drying, the liquid molecules exert a force called surface tension that pulls the gel in on itself and crushes the microstructure. One means of circumventing this problem is to carefully heat the liquid-filled gel under pressure until the liquid converts to a supercritical state. In this state, material behaves like neither a pure liquid nor a gas and no longer exerts surface tension. The fluid can then be directly removed from the gel without cracking it. However, this process, used by aerogel manufacturers, takes many hours to complete and is very costly.

The Livermore team's method eliminates the stress in the gel during conversion of the liquid to the supercritical state, dramatically speeding the drying process. The new process is very similar to injection molding, a common process used to manufacture certain types of plastics. First, the precursor chemicals are injected directly into a two-piece, sealed mold. While the mold is rapidly heated, the liquids react to form the gel. The gel's shape is defined by the mold walls, which keep it from straining under the influence of the rising hydrostatic pressure of the liquid within the gel.

After as little as 20 minutes, the temperature and pressure within the mold produce the supercritical fluids, which are rapidly purged from the confined gel without cracking it. The internal pressure in the mold is rapidly lowered by releasing it through an opening. After the mold is cooled for a few minutes, it is opened and the finished aerogel part is removed. The entire process, from start to finish, takes minutes instead of the hours (or even days) required by conventional processes, and the finished aerogels require no further machining or polishing.

"Our process is the only one that can mass-produce aerogels of precise sizes and shapes while maintaining high surface tolerances," says Hrubesh. "This is possible because the mold totally defines the size, shape, and surface quality of the aerogel object. Other processes require at least one free surface, which can distort during supercritical drying."

The Livermore team estimates that the cost for aerogels made using the new process is less than $3 per liter, as opposed to the $25-per-liter price of aerogels available today using conventional processes. Furthermore, the new process reduces liquid waste by 40% over other methods because the liquid purged from the gel can be reused to make more aerogels. Finally, the process uses about 10 times less energy than other techniques because it does not require the pumping of fluids and because it heats the molds for only a short time.

The Livermore aerogel processing breakthrough was aided by the analytical work of Paul Coronado and John Poco, who fabricated double-wall containers with thermocouples inside to monitor the pressure and temperature of the gelling process. Using these containers led to a much greater understanding of aerogel chemistry and the discovery that changing small amounts of starting ingredients has a significant effect on the gelation and drying processes.

The research team is continuing to refine the injection molding process. Taking advantage of the new technology, team members are also making small double-paned windows containing a sealed layer of silica aerogel. Studies have shown that double-paned aerogel windows could have an insulating efficiency value of R-19, equivalent to the insulation value of a house wall backed with a 8.75-cm-thick roll of fiberglass.

Aerogels for Purifying

One of the most promising new applications for aerogels is in a cost-effective water purification process developed at the

Laboratory. The aerogel-based process can have a variety of uses ranging from extracting harmful contaminants from industrial waste water to desalinizing sea water. Known as carbon aerogel capacitive deionization (CDI), the patented process will probably consume less energy per unit of water purified than conventional technologies, does not require costly membranes or pumps, operates at ambient temperature, and is resistant to chemical attack.

The carbon aerogel CDI process works by sending solutions with various positively and negatively charged ions through an electrochemical cell consisting of numerous electrodes containing organic aerogels. Laboratory researchers Rick Pekala and John Poco fabricated the double-sided electrodes by gluing two sheets of a carbon aerogel composite to both sides of a titanium plate that serves as both an electrical current collector and a structural support.

The carbon aerogels have exceptionally high surface areas (400 to 1100 m^2/g) for a total of about 3 million cm^2 per electrode. (A stack of 192 pairs of carbon aerogel electrodes, used in the most recent series of experiments, has a total surface area of about 1 billion cm^2, the equivalent of 25 acres.)

"The electrically conductive, monolithic sheets of carbon aerogel have exceptionally high specific surface area that can be exploited by CDI," notes Joe Farmer, project leader.

After application of a voltage between two adjacent carbon aerogel electrodes, cations (positively charged ions) and anions (negatively charged ions) are drawn toward the electrode's cathode and anode, respectively. These ions (in the case of sea water, mainly sodium and chlorine) are electrostatically removed from the water and held at the surfaces of the electrodes, leaving purified water. The trapped ions are then released into a separate stream of rinse water comprising 1% or less of the volume of the product water.

Farmer says carbon aerogel CDI may offer significant advantages over competing methods (such as reverse osmosis and ion exchange), which typically consume large amounts of energy, involve costly and often troublesome membranes or high-pressure pumps, and often generate large quantities of corrosive wastes that must then be specially treated. For example, ion exchange columns, commonly used to remove heavy metals and radioisotopes from waste water, require about 100 kg of acid to regenerate 1 kg of cation exchange resin. In contrast, carbon aerogel CDI regenerates its cells by electrically discharging them; the cells are then rinsed with a small volume of water.

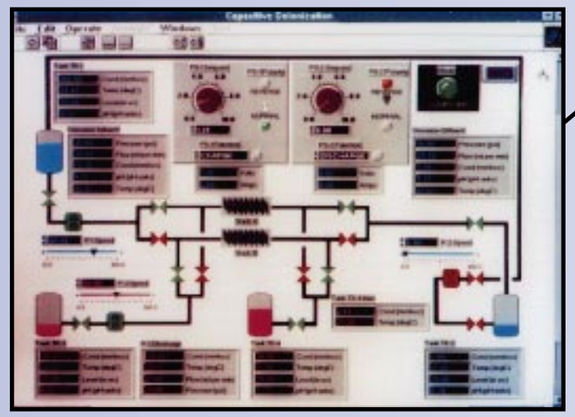
One of the most interesting potential applications for carbon aerogel CDI is desalinization of brackish water (containing typically 800 to 3200 ppm salt) for residential, commercial, and agricultural purposes. Preliminary studies show that the process may require less energy than other competing technologies because it does not employ complex membranes and does not require flow-through porous media. At some point in the future, it also may be possible to treat sea water (35,000 ppm salt), an important application for many parched California coast communities.

The Livermore research team experimented with various salt concentrations and operating voltages and showed that the system is capable of removing 95% of salt before the carbon aerogel electrodes become saturated. After several months of operation at 1.2 volts per cell (judged the most effective setting), the electrodes lost only 6 to 8% of their capacity.

Perhaps the most important application involves treating liquids containing radioisotopes. Farmer notes that both the U.S. Department of Energy and the former Soviet Union have large inventories of solutions contaminated with radioactive materials. Unlike ion exchange, carbon aerogel CDI can treat these radioactive wastes without generating secondary wastes.

Other potential applications for carbon aerogel CDI include the purification of boiler water for fossil and nuclear power plants, treatment of agricultural waste water containing pesticides and other toxic compounds, purification of water for semiconductor processing and other manufacturing processes, and treatment of waste water from electroplating operations.

Currently, LLNL is discussing plans with the U.S. Army to apply carbon aerogel CDI as part of an effort to destroy old stocks of mustard gas and for developing compact desalination units that can be stored for long periods without deteriorating. It is also holding talks with the U.S. Air Force about using carbon aerogel CDI to remove contaminants from rinse water generated from plating operations. Any commercialization of the technology will be done by private industry through appropriate agreements with the Laboratory.



Farmer says that more research and development is needed before carbon aerogel CDI technology can be incorporated into any large-scale plant. Future efforts will include component aging tests, more precise energy analyses, and experiments with high concentrations of various salts as well as acidic and basic electrolytes. Finally, cost-effective, high-volume production processes are needed for carbon aerogel electrodes to achieve their practical benefits.

For further information

Injection Molding Process for Net-Shaped Aerogels
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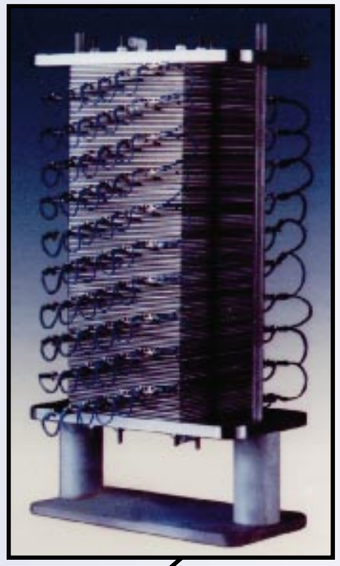
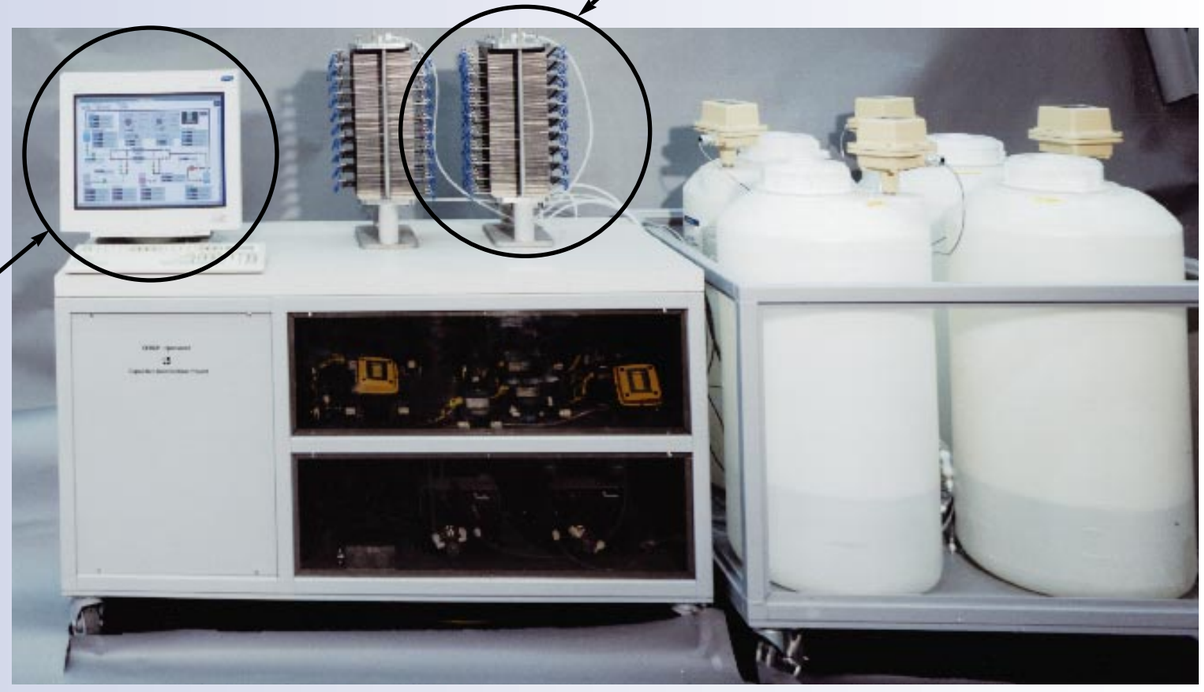
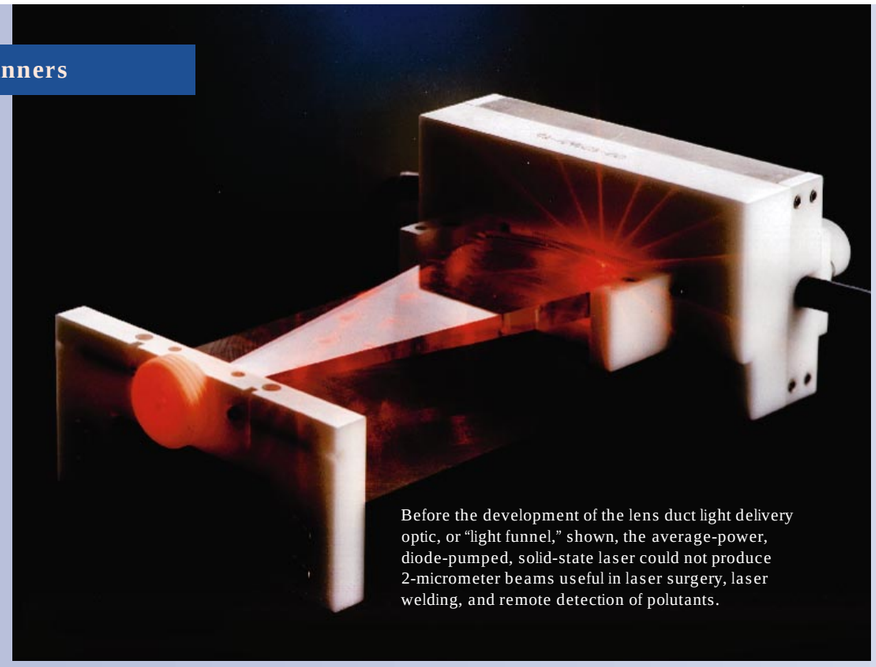




All control of the fully automated, continuous-flow carbon aerogel capacitive deionization system shown is done with a "click of a mouse" on the control screen. At the left is a stack of carbon aerogel electrodes, the heart of the system.

A Light Funnel for Diode-Pumped, Solid-State Lasers



Before the development of the lens duct light delivery optic, or "light funnel," shown, the average-power, diode-pumped, solid-state laser could not produce 2-micrometer beams useful in laser surgery, laser welding, and remote detection of polutants.

LASER beams do not just happen. Atoms must be pumped with energy to "excite" them. As excitation continues, the energy in the atoms increases, and the atoms give off energy in the form of intense light. The light can be visible light or invisible light like infrared or ultraviolet light. Depending on the type of laser, the excited atoms may be in a gas or a solid material, and the pumping mechanism may take a variety of forms.

Flashlamps have been used for excitation since the birth of lasers in the early 1960s. But flashlamps need to be replaced frequently, and they do not deliver energy very efficiently. So, about 15 years ago, scientists began to investigate the diode, a semiconductor device that converts electric power directly into light, as a pumping device for some solid-state lasers. The diode pump source began to be widely used about five years ago. Yet, diode-pumped lasers had drawbacks, too. While the diodes could produce laser light in high-intensity pulses, their overall power output was low (less than 1 kW/cm^2), which reduced their usefulness.

A team of Laboratory scientists led by Ray Beach hit upon a remarkably simple solution to the low power output problem. "We imagined a 'light funnel,'" says Beach, "wherein the output from the diodes is captured and manipulated to concentrate the pump light." This light funnel is a small lens for concentrating, or "conditioning," the output radiation of the diodes.

With higher power outputs, other advances became possible. Previously, applications requiring high-average power were limited to a 1-micrometer (µm)-wavelength beam when using a diode-pumped, solid-state laser. But with this new lens technology, a broader range of solid materials can be used that produce a variety of wavelengths, increasing the applications of these lasers. A 2-µm beam is even more useful. In surgery, for instance, a 1-µm beam causes collateral damage to tissue around surgical area. A 2-µm beam, on the other hand, cuts and cauterizes the surgical area while causing much less collateral damage to surrounding tissue. A 2-µm beam also has a variety of remote sensing applications—it can be mounted on an airplane to detect wind shear or placed in a suspected area to detect the byproducts of chemical or biological warfare.

With a light funnel lens, the output power of diode-pumped, solid-state lasers is increased by a factor of 250, and their unit cost is reduced by a factor of 100. "Our product offers a simple, inexpensive, and commercially attractive technique for scaling up the output power of laser systems," says Beach. "And this ability to scale up the laser's output power drastically reduces its price."

The Lens Conditions the Light

The three components of the Laboratory team's all-solid-state system are a laser diode pump array, the light funnel or lens duct, and a laser crystal. The lens duct (shown above) lies at the heart of this new design. It condenses and homogenizes the diverging light output from the diode array, coupling the diode light to the laser material at the lens duct's exit face.

The pie-shaped lens duct can be fabricated of inexpensive glass or plastic. Its longer input face and shorter output face are coated with an antireflective material for more efficient transmission of light. The other four sides are uncoated and prevent light from escaping by reflective wave guiding, which is produced by internal reflection of the light beam. While this

optical device is particularly applicable to coupling diode array pump light to solid-state laser materials, it can be applied to any situation that requires focusing a beam of light to produce a smaller beam of greater intensity. This lens duct can be used to scale up both pulsed and average-power laser systems. The team has developed a pulsed, 1-µm, diode-pumped, solid-state laser with an output pulse energy of 100,000 microjoules (µJ). The nearest competitor produces just 400 µJ of output energy. This 250-fold gain in output pulse energy also costs much less to produce: $1 per microjoule compared to that competitor's $100 per microjoule. Other systems on the market produce even less output pulse energy at a higher cost per microjoule.
An average-power, 1-µm laser also performs markedly better than those of the competition. The Laboratory's system produces 13 W of output power, which is two and a half times what the nearest competitor can produce and at a much lower cost.

A 2-µm unit developed by the Laboratory team produces 25 W of average output power. A diode-pumped, solid-state laser producing this much power at the 2-µm wavelength was not possible before the development of the lens, and as a consequence, no comparable commercial system exists.

Applications from Medicine to Manufacturing

Applications for this new class of lasers abound in medicine, radar, manufacturing, and materials processing. The average-power, 2-µm system was developed as a surgical laser. The $78-million-per-year medical laser market is presently dominated by flashlamp-pumped lasers, which could be replaced by the far more reliable and efficient all-solid-state, diode-pumped versions.

The Laboratory team's pulsed, 1-µm unit is suitable for underwater illumination and sensing, and it is also being investigated for photon-assisted ignition in a new generation of jet turbine engines. In addition, it can be used for a lidar (laser radar) system in which laser beams are reflected and scattered off clouds, smog layers, and atmospheric discontinuities, and the return signals measured. All of these applications require a laser that is not only compact and reliable but produces significantly more pulse energy than is currently available.

The Laboratory is developing a 2-µm system for use in treating infertility in humans and livestock. This laser drills tiny holes in the surfaces of human and animal eggs to increase their probability of being fertilized by sperm.

A high-average-power (100-W), solid-state laser is being evaluated as a replacement for the copper vapor lasers presently used by the U.S. Enrichment Corporation Laser Isotope Separation Project. Advantages of this laser over the copper vapor laser include higher efficiency, longer lifetime, and lower lifecycle costs.

The Livermore team is planning a kilowatt-class prototype for use in material processing applications such as metal cutting, welding, and hole cutting. For the past several years, the material processing community has embraced flashlamp-pumped lasers. But a drawback of those systems is that they are large and expensive to install and operate.

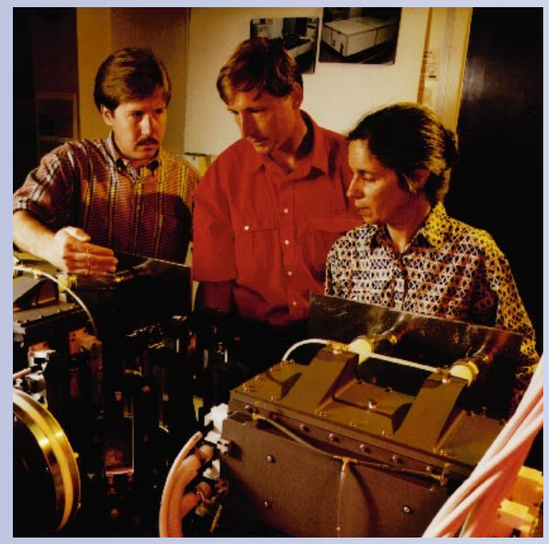
Looking even further into the future, megawatt-class lasers could be used for laser-driven, inertial confinement fusion power plants. In fact, Laboratory scientists developed one of their 1-µm systems as a subscale prototype for this application. Until very recently, scientists thought that solid-state lasers, even under ideal conditions, did not provide adequate efficiency and robustness for a commercial power plant setting. But by extending the current laser fusion technology base to include this lens duct technology and other advancements in solid-state lasers, a laser fusion driver concept appears feasible for the first time.

All-solid-state laser with diode-irradiance conditioning and the team that developed it. Back row from left to right: Steve Payne, Chris Marshall, John Lang, Chuck Petty, Scott Mitchell. Bottom row, left to right: Steve Mills, Ray Beach, Mark Emanuel, Bill Benett.

The diode-pumped, solid-state laser was developed under Cooperative Research and Development Agreements (CRADAs) with the Beckman Laser Institute and Medical Clinic at the University of California at Irvine, and Wellman Laboratories of Photomedicine, Boston, Massachusetts.

For further information contact Raymond Beach (510) 423-8986 (beach2@llnl.gov).

One of the World's Brightest Lasers



High-average-power solid-state laser developers (from left) Brent Dane, Lloyd Hackel, and Mary Norton with an advanced, two-amplifier prototype of their invention in the laboratory. Using recently improved amplifier glass developed with Schott Technologies, this laser should exceed the average power of the award-winning system by a factor of 4.

A powerful beam of green light shoots up into the night sky, beyond the Earth's atmosphere. The beam starts out pencil thin, and its divergence has been reduced almost to the limits that physical laws allow—it diverges to just a few meters in diameter after traveling hundreds of kilometers. The beam is precisely aimed to illuminate satellites, allowing researchers to identify and track them. Because of atmospheric distortions, the researchers cannot directly record clear images. But using the special capabilities of the laser generating this beam and a process known as high-resolution speckle imaging, they can collect a time-resolved "speckle" pattern and numerically reconstruct an undistorted image.

Another powerful beam of green laser light focuses on a jet of gas in a small vacuum chamber. With each pulse of light, a small ball of highly energized gas forms. This plasma converts the laser light into x rays that pass through an image-containing reticle and onto a silicon wafer coated with photoresist. The image on the reticle contains the ultra-fine circuit features of a very advanced computer chip. The x rays create shadows of the circuit features on the wafer and print them in the photoresist. The x rays generated by the laser represent a new commercial production technique for the next generation of integrated electronic circuits.

A single laser system developed by a team at the Laboratory enables both of these new applications. The laser produces high-energy pulses more frequently than any other laser system—30-joule (J) pulses six times a second (i.e., 6 hertz). This results in a peak power of over 2 gigawatts (GW) for each pulse and an average power of up to 180 W. This average power is 10 times greater than that of any commercially available solid-state laser in this pulse energy range. Even more importantly, the 2-GW peak power, which is equivalent to the peak output of a nuclear power plant, can be focused to a spot only a few micrometers in diameter, thanks to the low divergence of the beam. As Brent Dane, team leader for the Laboratory's effort on this laser, notes, "The combination of high average power and very low divergence has produced a world's record for average brightness for a solid-state laser."

While producing high-energy pulses, the laser can also operate at a broad range of pulse durations, from 10 to 1,000 nanoseconds (1 ns = 1 billionth of a second, or 1×10^{-9} s). Solid-state laser systems have typically operated in pulse durations of either 10 to 30 ns or more than 100,000 ns. In the past, achieving pulse durations between 30 ns and 100,000 ns with a solid-state laser has been very difficult and has resulted in pulse energies of only a few millijoules.

A Unique Amplifier

A new type of laser amplifier consisting of mirrors, rotators, polarizers, special laser glass, and other optical devices is behind all of these "firsts." All materials used in the amplifier have high damage thresholds to withstand the extremely high energies.

The laser starts at an oscillator that produces a low-power, high-quality beam of invisible light with a narrow bandwidth. This beam then passes many times through the amplifier, its energy increased from millijoules to several tens of joules. A full transit from the oscillator to the amplifier's exit takes about 40 ns.

The amplifier incorporates a solid-state slab gain medium made of neodymium-doped glass (Nd:glass) with xenon flashlamps mounted around it. The flashlamps pulse rapidly, pumping light energy into the glass slab. The beam extracts this energy as it passes through the slab, generating an output pulse that has thousands of times more peak power than the flashlamp source.

The slab, designed and constructed at the Laboratory, has one thinner dimension to allow the heat to conduct out. The laser light "zig-zags" through the slab gain medium, internally reflecting off the side walls of the slab and averaging much of the thermal distortion within the slab. Hot spots in the slab, which cause optical distortion and could damage the gain medium, are virtually eliminated.

Most laser systems degrade during this amplification stage because of the high thermal loading placed on the gain medium. In this system, however, the team uses a device called a phase conjugator, which the beam enters midway through its transit in the amplifier. The stimulated Brillouin scattering (SBS) phase conjugator is a special mirror that reverses the beam's phase and corrects distortions. Although the flashlamp pump and the slab have been designed to reduce distortion, heat builds up during the multiple pulses of the flashlamps and distorts the laser beam wavefront. The phase conjugator reverses the sign of the beam distortions and returns the beam back through the amplifier for an equal number of passes, almost completely canceling the phase error and producing near-perfect beam quality and very stable beam pointing.

The phase conjugator is simply a glass cell with a quartz window. A lens focuses the input light into a liquid, carbon tetrachloride, that fills the cell. Through a mechanism called the electrostrictive effect, a density grating builds in the cell and reflects the beam with a reversed phase. No moving parts or electrical circuits are required. The technique reverses the wavefront with very high resolution without doing a single computer calculation or moving a single actuator.

Glass cells filled with carbon tetrachloride are not what we generally think of as mirrors. They are, in fact, a type of nonlinear mirror. SBS phase conjugators have been discussed in scientific literature for many years and experimented with in laboratories. But this is the first instance of one put to practical use in a working, high-power laser system.

Electrooptics technician James Wintemute completes final alignment of a packaged laser system. Several of these systems have been installed and are in use, one of them at Kennedy Space Center in Florida, where it will be used to illuminate rocket launches.

Another feature of this system is its ability to double the frequency of the output beam so that it changes from infrared light to visible, green light. Green light diverges less than infrared light, an important attribute for long-range uses where a beam with low divergence is needed.

To obtain even higher average power output from the laser, a new laser glass with much higher mechanical strength has been developed that could double the average power of a single amplifier. Furthermore, the use of SBS phase conjugation allows multiple, sequentially firing amplifier heads to be combined in a single laser beam train. A two-head laboratory prototype is currently in operation, and a version of the laser using four amplifier heads is under construction. The average power of the latter is expected to easily exceed 1,000 W.

Lighting Launches and Removing Graffiti

High-resolution speckle imaging and x-ray lithography are just two applications for this new laser.

The frequency-doubled green light output of this system, with its narrow bandwidth and accurate long-range pointing stability, is ideal for advanced laser radar uses. For the U.S. Navy, the team developed a high-energy, green-output laser with a 500-ns pulse duration and delivered it to the Navy at Cape Canaveral, where it will be used to illuminate rocket launches from the Kennedy Space Center. During independent testing prior to shipment, the laser fired over one million shots with no maintenance or realignment required.

A version of the laser, operating at around 100 joules per pulse, could provide the pump source for a tabletop-size x-ray laser, whose coherent output would be used to produce very-high-resolution, three-dimensional images.

The team has also discovered that high-energy, short-pulse lasers are excellent for quickly removing paint from surfaces with an intense acoustic wave that does not harm the surface beneath the paint. This is an environmentally sound method of removing paint from aircraft and ships, lead-based paint from public and private buildings, and painted graffiti from a variety of surfaces. "Graffiti removal is a hot topic," notes Dane, "and is being explored further by other scientists at the Laboratory."

For further information contact Brent Dane (510) 424-5905 (dane1@llnl.gov).

A Miniature Mass Spectrometer

Because nothing small enough was available commercially to fit their needs, the Livermore team designed a miniature electronics package (shown in the background image) to excite and detect ions inside the miniature mass spectrometer's ion trap.

CHEMISTS and forensic scientists have been using mass spectrometers for decades to analyze a variety of sample materials. In the early 1980s, atomic physicists began to use a highly sensitive type of mass spectrometer called an ion trap to study individual particles. Some research ion traps are sufficiently sensitive to measure one ion at a time with a mass accuracy of one in one quintillion, or 1 in 1,000,000,000,000,000,000 (1:10^{18}).

Dan Dietrich, a senior researcher at the Laboratory, and his colleague, Bob Keville, had been using these ion traps for several years, and began to wonder whether the theory behind them could be applied to a smaller commercial mass spectrometer. Small fixed or portable mass spectrometers would be immensely useful in a variety of situations—as a remote detection device for monitoring air quality or for sniffing out drugs or compounds related to nuclear or chemical weapons.

The central detection device in research mass spectrometers is very small, which is what Dietrich and Keville found attractive. But the accompanying equipment fills a room. So, they developed the first truly hand-portable mass spectrometer, which fits into a small briefcase. It weighs just 12.3 kg (33 lb) and operates off a battery. Its accuracy is 1 in 1,000 (1:10^3) and will ultimately be 1:10^4, which is a long way from the accuracy of the large research units but sufficient for the uses planned for it.

Although this miniature mass spectrometer is still under development, it could have greater sensitivity and efficiency than conventional laboratory-based, single-pass mass spectrometers. In a single-pass unit, there are an ion source where the sampling material is ionized, an analyzer where the ions are separated in space according to their charge, mass, and velocity, and a detector that measures the number of ions analyzed. Some loss of ions as they move from region to region within this type of unit is inevitable.

A New Twist on Penning Ion Traps

Ion traps are very different from single-pass technology. The Laboratory's new unit is based on the principles of the Penning ion trap. Ions are created inside the trap, and analysis and detection are done there as well. Both features reduce ion losses and allow the ions to be sampled many times.

A traditional Penning ion trap used by chemists in mass spectrometry is a rectangular box that sits inside a powerful electromagnet. A gas is introduced into the box and ionized by an electron beam. Two opposing ends of the box receive a positive electrical charge; the four sides, forming a square tube along the magnetic field direction, receive a less positive charge. The positively charged ions are repelled by the positively charged ends; thus, the electrical voltage prevents the ions from leaving the box along the direction of the magnetic field. If the positive ions try to leave the box across the direction of the magnetic field, i.e., toward any of the less positive sides, they are deflected by the magnetic field into cyclical orbits. This motion is called cyclotron motion. The frequency of this cyclical motion is determined only by the strength of the magnetic field and the ratio of the ions' electrical charge to their mass. By rapidly reversing the sign of the voltage on opposing pairs of these less positive sides, the turning ions can be pulled into orbits optimized for detection. Because the values of the magnetic field and the ions' electrical charge are known, the mass of a "trapped" ion can be determined from the frequency of its orbit. This method of determining an ion's molecular weight and thus its identity is known as ion cyclotron resonance and is the operating principle of the Penning ion trap.

High resolution ion traps developed for use by atomic physicists use the basic principles of the rectangular trap but are open-ended cylinders that fit inside powerful superconducting magnets. In a cylindrical ion trap, the homogeneous magnetic field keeps the ions in a very narrow orbit in the middle of the cylinder. The combination of huge magnets and ultra-high vacuums, which can keep individual ions in orbit inside the trap for weeks, is what allows these ion traps to be so extraordinarily accurate.

While the ion trap devised by the Laboratory team is about the same size as the trap in the research units, the magnetic field is provided by a small permanent magnet, greatly reducing the overall size and power requirements of the unit. The design of the trap, the electron-beam, the vacuum system, and the inlet valve are also new. The goal is an ion residence time of 100 milliseconds, which provides sufficient resolution to detect trace samples of contaminants or other compounds in air.

The permanent magnet is designed to optimize magnetic field. It is cylindrical, slightly less than 7.6 cm across and 5.7 cm high, with a center bore of slightly less than 2.5 cm. A thin-walled vacuum chamber containing the ion trap fits inside the hole. A small ion pump, along with a miniature cryogenic pump, maintains the tube's vacuum pressure. A minature Piezo electric inlet valve admits gas to be sampled into the tube. There is also a pulsed electron source beam that ionizes the gas in the trap.

Ions are confined in a volume defined by electrodes that are plated on the inner diameter of the vacuum tube. Changing voltages on the electrodes excites the ions into their cyclotron orbit within the trap. Between the excitation electrodes are the detection electrodes that sense the ions' orbital frequency and thus the mass and identity of the ions in the trap.

Dan Dietrich (left) and Bob Keville with their miniature mass spectrometer.

Several innovations in our ion trap keep power consumption very low—to about half a watt. (That figure does not include the 20 W needed by the laptop computer that accompanies the device.) First, the permanent magnet is the most obvious power saver. Second, smaller is better. The smaller the trap is—that is, the closer the particles are to the detection electrodes—the greater the trap's sensitivity and resolution are and the lower the electrical power consumption. Next, the unique design of the inlet valve is important in reducing energy consumption. In most mass spectrometers, gas is continually bled in, and thus, large pumps are needed to maintain the vacuum in the spectrometer. This new miniature Piezo electric inlet valve lets in gas in small pulses, reducing vacuum pumping requirements. Finally, the vacuum pump itself is also a new power-saving design.

Uses in the Field

Research mass spectrometers used in laboratories are complex, bench-top instruments requiring a highly trained operator. Our inexpensive and portable mass spectrometer, in contrast, will find numerous applications outside of the laboratory.

These units can act as air quality monitors in a closed or confined space, such as a factory where chemical weapons manufacture is suspected. Someday, with additional front-end filters or sensors, they could be used to indicate the presence of air-borne disease agents. In a home, office, or factory, they could be incorporated into feedback control loops and alarm systems to warn of hazardous conditions. A home sensor could monitor freon and radon as well as carbon monoxide, carbon dioxide, methane, propane, and other hydrocarbons.

Law enforcement agencies could replace breathalyzers and drug- and explosives-sniffing dogs with these sensors. Testing drivers for alcohol use or sniffing out drugs could be performed remotely, which would mean increased safety for police officers and other officials.

Industries with critical process control functions could monitor for sensitive manufacturing byproducts such as chlorofluorocarbons, hydrazine, helium, nitrous oxide, nitrous dioxide, and sulfuric acid. Even small industrial accidents could be avoided with these sensors in place.

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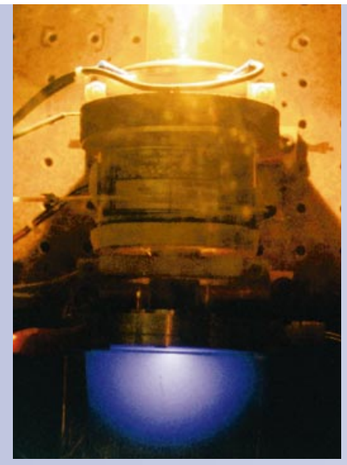
More Efficient, Less Expensive Electron Beam Processing

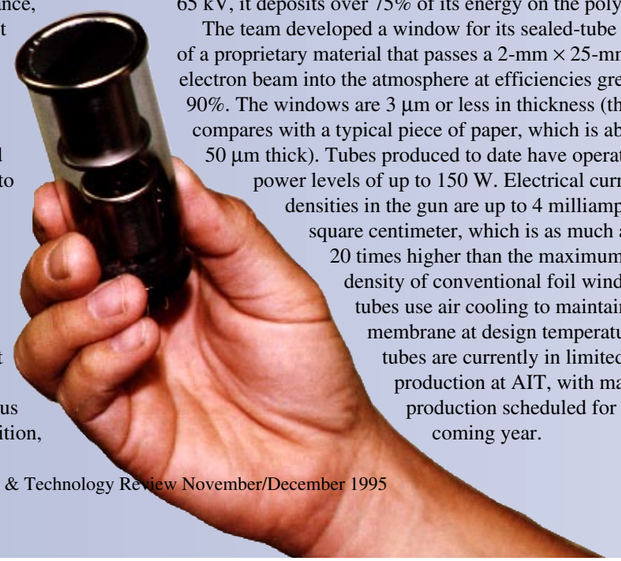


The sealed-tub electron beam gun can generate 150 W of electron beam power.

ENVIRONMENTAL concerns affect almost every industry. Thermal methods of curing inks, adhesives, and coatings produce volatile organic compounds (VOCs), which are considered hazardous materials. So polymers that can be cured using ultraviolet light and electron beams, which do not produce VOCs, have been developed. Although electron beam processing has many advantages over ultraviolet processing, the high cost and complexity of commercial electron beam equipment has limited its use to less than 1% of the multibillion-dollar radiation-curable materials market, which is now growing at more than 20% per year.

Thousands of ultraviolet-curable products and applications are developed every year, but concerns are being raised because their formulations contain highly toxic photoinitiators, which can cause numerous health problems. On the other hand, electron-beam-curable materials are much less toxic, cure independent of color, and use 3 to 7 times less energy than ultraviolet curing equipment. But conventional electron beam equipment could never compete with ultraviolet due to its large size and astronomical cost.

That could change if industry puts to use a new product developed by scientists at the Laboratory and American International Technologies Inc. (AIT) of Torrance, California. Together they developed a low-cost electron beam gun in a vacuum tube as a replacement for these expensive systems. A system using sealed tubes costs ten times less than conventional electron beam systems of similar power, is smaller and easier to use, and increases worker safety by reducing exposure to x rays and high electrical voltages.



Booth Myers, technical leader of the Laboratory portion of the team, says, “Despite the high cost of existing systems, electron beam processing is a growing field, driven by industry needs to avoid the use of VOCs in ink, adhesive, and paint curing. Our lower cost electron beam gun is not only competitive for existing applications, but will open up numerous new uses for electron beam processing. In addition, the ability to mass produce these tubes makes it possible to compete with ultraviolet systems in both size and cost.”

The New Window

The large vacuum vessels used in conventional electron beam systems have titanium or aluminum foil windows through which the beam exits into the atmosphere, where the processing takes place. Typical current densities for metal foils are 200 microamperes per square centimeter at 150 to 300 kilovolts (kV). The minimum voltage of 150 kV is significant because that is the energy required just to push the electrons through the metal foil window. So for coatings less than 40 micrometers (μm) thick (a typical thickness for ink, paint, or adhesive coatings), these conventional systems are very inefficient because more energy is deposited into the metal foil window than into the coating being cured. For example, a 300-kV accelerator deposits less than 5% of its beam energy on a 40-μm-thick polymer. Because most of the emerging electron beam processing applications are for thin films, this inefficiency is a significant problem. The sealed-tube electron beam gun developed by the LLNL/AIT team offers vastly increased electrical efficiency—operating at 65 kV, it deposits over 75% of its energy on the polymer.

The team developed a window for its sealed-tube gun made of a proprietary material that passes a 2-mm × 25-mm, 60-kV electron beam into the atmosphere at efficiencies greater than 90%. The windows are 3 μm or less in thickness (this compares with a typical piece of paper, which is about 50 μm thick). Tubes produced to date have operated at power levels of up to 150 W. Electrical current densities in the gun are up to 4 milliamperes per square centimeter, which is as much as 20 times higher than the maximum current density of conventional foil windows. The tubes use air cooling to maintain the membrane at design temperature. The tubes are currently in limited production at AIT, with mass production scheduled for the coming year.

Pictured from left to right are Hao-Lin Chen, Booth Myers, Glenn Meyer, James Davin, and Dino Ciarlo. At the bottom are American International Technologies Inc. team members George Wakalopulos, President of AIT, and Peter Bond.

Smaller, Safer, Less Expensive

Depending on the power and features selected, the initial cost of a conventional system ranges from $300,000 to $2 million. A comparable sealed-tube unit and supporting equipment will cost $25,000 to $100,000, depending on the power. Annual operating costs for existing systems are also high, while the new units will operate at one-tenth that cost. The new systems are less than one-tenth the size of a comparable conventional system and require very little maintenance, which should yield additional cost savings. Existing systems are large, fixed, custom-designed installations, uneconomical for small-scale, lower power applications. The new tubes can be used alone or in groups, and a wide variety of processes and applications can be addressed in a variety of configurations.

Operation at lower voltages, which is possible because of the new thin-film window, greatly reduces x-ray generation and improves worker safety. It is also more efficient for processing thin material layers such as inks, adhesives, and paints and for surface sterilization, because the reduced penetration depth of lower energy electrons is better matched to the thickness of the material being processed.

Conventional systems require periodic replacement of the foil windows after about 2,000 hours of use. This process demands extensive disassembly of the unit. The new sealed tubes can be unplugged after 2,000 hours of use and replaced with new ones, reducing unit downtime from days to minutes. In fact, laboratory tests and calculations show that lifetimes of 7,000 hours are feasible, which would reduce operating costs even further.

Expanding the Range of Uses

Currently, the primary commercial application for electron beam processing is curing polymers without using VOCs. Because electron beam polymerization does not produce VOCs and does not use the toxic photoinitiators used in ultraviolet light curing, it satisfies many of the existing and anticipated environmental restrictions on manufacturing processes.

Electron beam processing is also used to sterilize medical supplies and food packaging materials that cannot tolerate sterilization by high temperature or chemicals. Electron beam sterilization of food has not gained widespread acceptance in the U.S., although its use is increasing in other countries.

The availability of a low-cost, self-contained source of high-energy electrons expands enormously the range of uses for electron beam radiation. Some examples include:

- Fast, on-line chemical and elemental analysis. Electron beams induce visible, ultraviolet, and x-ray fluorescence whose spectrum identifies the elements in the irradiated sample. “Since this process is effectively instantaneous, it will likely find many applications both for on-line industrial monitoring as well as laboratory analysis,” notes Myers.
- Injection of electrons into liquid sprays. The addition of electrons greatly improves spray effectiveness by reducing the average droplet size formed at the nozzle. Applications include pesticide spraying, turbine fuel injection, and internal combustion engine fuel injection.
- Destruction of toxic industrial compounds or cleanup of stack or automotive exhaust gases. Because electron beams are known to decompose both organic and inorganic compounds, they could be used as an economical method of waste treatment without incineration. The government might find this application useful for site cleanup and restoration programs. Another application of this new technology, to disassemble retired nuclear weapons, is under evaluation.
- Generation of ozone. Ozone is gaining popularity as a purifier for water supply systems, either instead of or in addition to chlorine. Ozone generation with electron beams is known to be approximately twice as energy efficient as conventional means using electrical discharge devices.

The electron beam gun described in this article was developed under a Cooperative Research and Development Agreement (CRADA) with American International Technologies Inc., Torrance, California. For further information about commercial applications, contact AIT at (310) 328-3484.

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The Genetic Contribution of Sperm: Healthy Baby or Not?

We have developed a powerful new tool using chromosome-specific DNA probes to study the causes of chromosomal abnormalities in sperm and their effects on embryonic development. The technique, fluorescence *in situ* hybridization (FISH), involves binding fluorescent labels to repetitive sequences of DNA in specific chromosomes. Its use in humans can provide valuable information on the sensitivity of sperm to environmental, occupational, and drug exposures as well as to other risk factors, including age and genetic variation. Such factors can result in chromosomal abnormalities that may be passed on to the embryo and fetus. Our recent studies show that a small fraction of human sperm from healthy males contains genetic defects, specifically abnormal numbers of chromosomes (aneuploidies). The proportion of such defective sperm appears to increase with age and smoking. These findings are supported by preliminary results on the frequency of aneuploid sperm of mice, which appear to be good models for studying induced aneuploidy. Treatment of men with Hodgkin's disease using the chemotherapeutic drug NOVP induces a transient elevation in levels of aneuploid sperm. We are developing animal models to better understand how defective sperm affect the survival and development of the early embryo. We are also beginning to study fathers of children born with chromosomal defects, such as Klinefelter syndrome, to ascertain whether these men produce more aneuploid sperm than do fathers of healthy children.

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Diode-Pumped Laser	❑	❑	❑	❑
Brightest Laser	❑	❑	❑	❑
Mini Mass Spectrometer	❑	❑	❑	❑
Electron Beam Gun	❑	❑	❑	❑

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