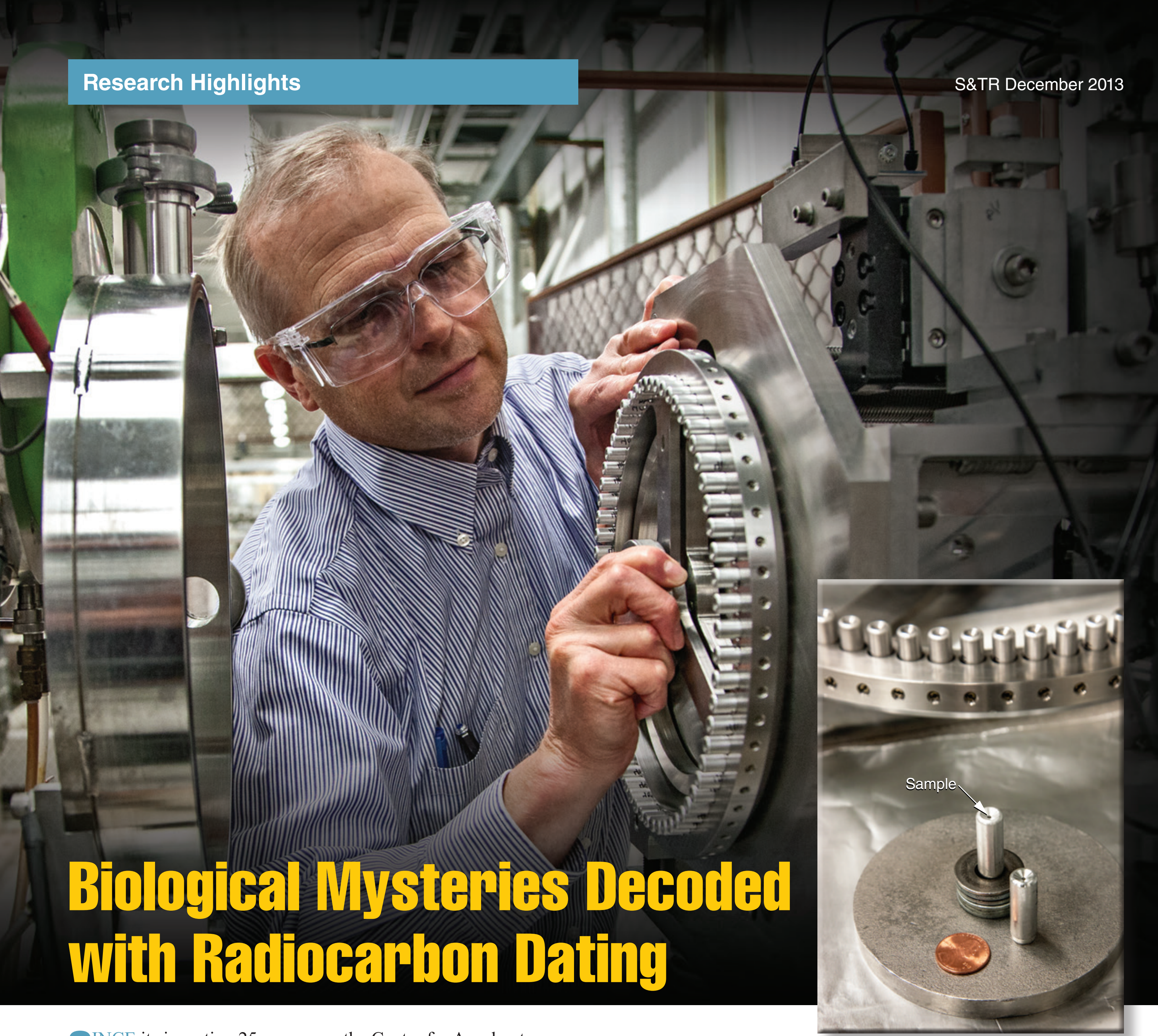
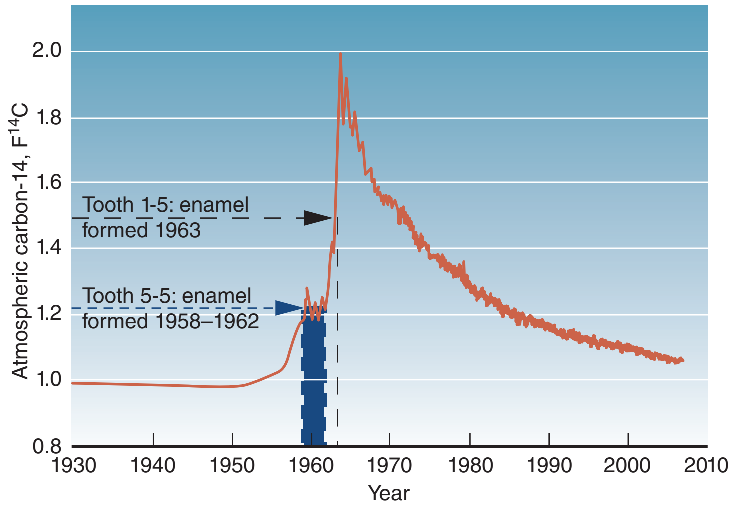


Biological Mysteries Decoded with Radiocarbon Dating

Bruce Buchholz loads a wheel of samples into the spectrometer at the Laboratory's Center for Accelerator Mass Spectrometry (CAMS) to determine the materials' concentration of carbon-14. The inset shows a closeup of a sample holder.

SINCE its inception 25 years ago, the Center for Accelerator Mass Spectrometry (CAMS) at Lawrence Livermore has supported scientific research for a diverse range of disciplines. The precise measurement capabilities at CAMS allow researchers to identify the isotopic composition of a given sample. Continued efforts to improve the center's sample preparation techniques and detection methods have ensured that CAMS can help address important scientific challenges in fields ranging from archaeology and geophysics to pharmacology and nonproliferation.

One important research endeavor involves determining the precise age of biological material generated in the past 60 years by measuring the ratio of radiocarbon (or carbon-14) to the carbon-12 and carbon-13 in samples. Scientific forensics using radiocarbon bomb-pulse dating is possible because of the isotopic signature created by aboveground nuclear testing between 1955 and 1963, which nearly doubled the amount of carbon-14 in the atmosphere. When the aboveground test-ban treaty took effect in 1963, atmospheric levels of radiocarbon began to decline as carbon-14 migrated into the oceans and biosphere. Living organisms naturally



Researchers at CAMS helped solve a missing-person's cold case by analyzing teeth from a skull fragment found in Canada in 1968. The orange line shows the ratio of atmospheric carbon-14 to carbon over time (measured in a concentration unit called $F^{14}C$). Results indicated that the baby tooth (5-5) formed between 1958 and 1962, whereas the premolar (1-5) formed in 1963 or later.

incorporate carbon into their tissues as the element moves through the food chain. As a result, the concentration of carbon-14 leaves an indelible time stamp on every biological molecule when it comes into being.

To extract carbon for measurement, researchers at CAMS turn a sample into carbon dioxide through either combustion or a chemical process and then reduce the carbon dioxide to graphite—a form of carbon—on an iron catalyst. "The graphite is what we measure," says Livermore scientist Bruce Buchholz, who helped pioneer this technique at CAMS. "A full-size sample is about the size of a grain of salt, weighing between 100 micrograms to 1 milligram, although we often measure amounts as small as 20 micrograms."

Any sample that contains enough carbon to measure—dental enamel, proteins, or DNA, for example—can be dated using the highly accurate spectrometer at CAMS. (See the box on p. 18.) Recent projects have applied bomb-pulse dating to help resolve three biologically based mysteries involving a missing-person's cold case, neuron growth in the human brain, and proteins in the lens of the human eye.

Tooth Leads to Truth on Cold Case

Cold cases are seldom solved at the speed shown in a TV crime drama. However, recent breakthroughs have resulted from the combined use of modern forensic technology and analysis methods. One such case involved a skull fragment found in 1968 on the banks of a Canadian river. In 1969, an anthropological assessment of a tooth in the fragment placed the age of the deceased at 7 to 9 years. No local children within this age range had been reported missing, so the evidence was placed in storage.

In 2005, the remains were shipped to the Ancient DNA Laboratory at Simon Frazier University in Canada for analysis with newer techniques, including radiocarbon dating. "The measurements we make at CAMS don't necessarily solve a case, but they can help authorities narrow the possibilities," says Buchholz.

For the cold case, the Livermore team analyzed the enamel of two teeth from the skull fragment: a fully formed deciduous molar (a baby tooth) and a partially formed premolar (an adult tooth). Buchholz notes that once tooth enamel has developed, it does not incorporate new carbon, making it a remarkably accurate indicator of a person's age. Because the teeth from the fragment would have formed at different times in the child's growing cycle, the amount of carbon-14 in each tooth would indicate whether the deceased was born before or after the peak of aboveground nuclear testing. Enamel from the baby tooth corresponded to a birth year between 1958 and 1962. The premolar enamel formed around 1963, and because this tooth was incomplete, researchers concluded that the child died in 1963 or later.

The team's collaborators also analyzed the sample using newer anthropological techniques, for example, determining the amount of skeletal ossification and the head circumference. Those results yielded an age at death of 4.4 years ± 1. The combined measurements suggested the child was born between 1958 and 1962 and died between 1963 and 1968. More detailed DNA analysis led authorities to a local child who had been missing since early 1965 and was presumed to have drowned, allowing them to close the case.

"This is just one example of how we assist law enforcement," says Buchholz. "In the U.S. alone, there are thousands of unidentified remains, and those John and Jane Doe cases might benefit from analysis using such a combination of techniques."

Neurons Keep on Growing

Buchholz also worked with a CAMS team on an international collaboration to study neuron growth in the human brain. For most of the 20th century, scientists thought that neurogenesis stops shortly after birth. In the 1990s, however, studies of rodents showed that neuron growth continues in the olfactory bulb region associated with smell. Although this growth was not found in nonhuman primates, researchers wondered if the human brain generated neurons in appreciable numbers throughout life, and if so, what areas of the brain were involved.

CAMS researchers measured the concentration of carbon-14 in genomic DNA of neurons from the hippocampus to help collaborators in Europe develop a model for exploring how and how often different types of hippocampal cells regenerate. The team then took cells from the hippocampus of human cadavers, isolated the nuclei from neurons and nonneuronal cells, and extracted the DNA for analysis. Results showed that, in nonneuronal cells, the turnover rate declines with age. That is, as

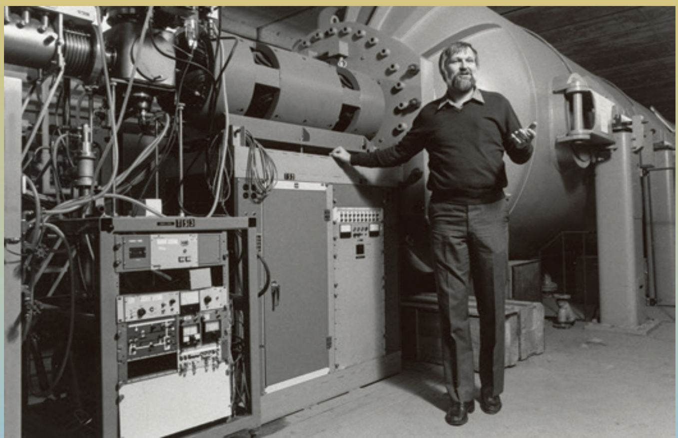
Celebrating 25 Years of Teamwork and Collaboration

It makes perfect sense that, 25 years ago, the Center for Accelerator Mass Spectrometry (CAMS) began as a joint effort. From the beginning, CAMS has been about collaboration, bringing excellent people and excellent science together in an environment that encourages teamwork. In 1985, the University of California Regents joined Lawrence Livermore and Sandia national laboratories as equal partners to fund the Multi-User Tandem Laboratory, with Livermore physicist Jay Davis as the facility's first director.

The Multi-User Tandem Laboratory initially focused on using accelerator mass spectrometry (AMS) to diagnose fission products of atomic tests and to conduct research in materials science, nuclear astrophysics, nuclear spectrometry, and neutron physics. Academic collaboration was encouraged from the very first, and in 1988, Lawrence Livermore established the Center for Accelerator Mass Spectrometry to coordinate the increasing number of experiments with academic users.

Today, CAMS is the world's most versatile and productive AMS facility. The center operates around the clock, performing up to 25,000 measurements per year. The research made possible by CAMS covers areas as diverse as archaeology, atmospheric chemistry, biomedicine, carbon-cycle dynamics, earth system processes, cell biology, alternative fuels, forensic dating, and forensic reconstruction of radiation doses.

AMS is an exceptionally sensitive technique for measuring concentrations of isotopes in small samples, typically less than 1 milligram, and the relative abundance of isotopes at low levels. For example, it can identify one carbon-14 isotope among a quadrillion other carbon atoms. In the CAMS spectrometer, negative ions made in an ion source are accelerated in a field of hundreds of thousands of volts. The accelerated ions smash through a thin carbon foil or gas that destroys molecular species. After passing through a high-energy mass spectrometer and various filters, the ions slow to a stop in a solid-state or gas-ionization detector. The system identifies individual ions by the rate at which they slow down.



In 1988, Lawrence Livermore established the Center for Accelerator Mass Spectrometry to diagnose fission products from nuclear tests and study climate and geologic records. Shown here is Laboratory physicist Jay Davis, who served as the center's first director.

"It's amazing to see the variety of problems brought in for analysis," says CAMS director Graham Bench. By measuring the carbon-14 isotopes in various samples, CAMS researchers have helped solve cold cases and plumb the mysteries of the human brain and eye (this highlight), tested the efficacy of cancer drugs (*S&TR*, September/October 2008, pp. 12–18), and established the age of a potential Mayan codex. Challenges come from near and far. "We have strong ties to research communities worldwide in academia, private industry, and government agencies," says Bench. "We also continue to support the Laboratory's programs. For instance, we have become a leader in biological AMS research, and we have generated isotopes to calibrate sample recovery instruments for the National Ignition Facility." (See *S&TR*, December 2012, pp. 18–20; September 2011, pp. 4–10.)

In addition, CAMS offers research opportunities for graduate students and postdoctoral fellows. According to Bench, in the past 15 years, the center has generated data for more than 300 graduate-level theses and annually supports the work required for 20 PhDs—a number that even a first-flight academic department would envy. Bench notes that several Lawrence scholars have crossed the center's threshold. "Throughout its history, CAMS has helped prepare and train the next generation of scientists," he says. Some of these scientists launch long-term careers at the Laboratory, such as geochemist Tom Guilderson. In 2011, Guilderson won the Department of Energy's E. O. Lawrence Award for groundbreaking radiocarbon measurements of corals, helping researchers to better understand the ocean's paleohistory and how oceanic processes affect the global carbon cycle. Other early-career researchers from CAMS have become senior scientists at facilities worldwide, such as Susan Trumbore, the current director of Biogeochemical Processes at the Max Planck Institute for Biogeochemistry in Germany.

"We provide an environment for extremely bright people to be creative, take initiative, and truly collaborate," says Bench. "CAMS has been successful because of this culture. It's the kind of place where people will stop what they are doing on their own projects to help others. We have a group focus, a community of true team players. This innovative culture makes CAMS and Lawrence Livermore a great place to work and to deliver breakthrough discoveries on important scientific challenges."

humans grow older, their brains create fewer nonneuronal cells. The measurements in neuronal genomic DNA told a different story. The oldest subjects studied (who had died at age 90 or older) had higher carbon-14 levels than would have been present in the atmosphere before 1955, indicating neuron growth continued in the hippocampus at least into the subjects' fifth decade. These markers and others showed no dramatic decline in hippocampal neurogenesis with age.

The team is still exploring the exact role of hippocampal neurons, in particular, because neuron growth is important for healthy aging. New neurons are required for efficient pattern separation and allow the brain to process and store similar experiences as distinct memories. Older cells are necessary for pattern completion, helping people to associate similar memories with each other. Studies also indicate that reduced neurogenesis plays a role in psychiatric diseases such as depression, but questions remain about the processes involved. Knowing that neuron growth continues could even lead to therapies for regenerating brain tissue lost to trauma or disease.

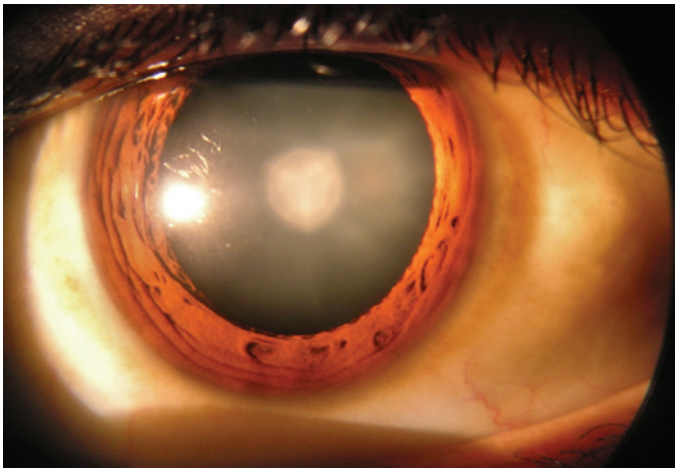
An Eye on Renewal

Another CAMS collaboration studied proteins in the lens of the human eye to better understand how cataracts form and how best to treat them. Cataracts are caused by a clouding in the eye's crystalline lens and, if left untreated, can lead to blindness. According to the Centers for Disease Control and Prevention, about 20.5 million Americans age 40 or older have cataracts. By 2020, that number is expected to increase to 30.1 million.

At the core of the eye lens are highly specialized, long-lived cells made of crystalline, transparent proteins. The structural and functional integrity of lenticular proteins helps keep the cells transparent, allowing for proper vision. "How these lenses cope with a lifetime of stress and aging, without any capacity for protein turnover and repair, is not completely understood," says Buchholz. In the mid-2000s, CAMS researchers collaborated with Paul FitzGerald, a professor at the University of California at Davis, to determine whether proteins in the core are made from cells of differing ages. "If they are, we'd know there is a process for protein turnover and renewal," says Buchholz.

Using samples from cadavers, researchers removed the cell layers of adult human eye lenses to reach the core. They then separated the core proteins into water-soluble and water-insoluble fractions, which were analyzed at CAMS to determine the average age for each sample. The water-insoluble samples—which contained the membrane proteins—had ratios of carbon-14 to carbon consistent with the age of the cells, whereas the water-soluble crystalline protein samples contained carbon that was younger.

"Our study provided the first direct evidence of carbon turnover in this type of protein in adult humans," says Buchholz.



Cataracts are a national health problem, growing in proportion with the aging population. CAMS research on proteins in the eye lens could lead to better treatments for this debilitating condition.

The findings suggest that the lens nucleus is a dynamic system, maintaining health and resisting injury through unknown protein transport mechanisms and possibly protein repair. "If scientists can understand protein transport, they may eventually find ways to delay the onset of cataracts or even prevent or heal this debilitating condition," says Buchholz.

The Curve of the Future

As time passes, the bomb-pulse curve continues to flatten. However, carbon-14 has a half-life of nearly 6,000 years, so traces of it will linger. Livermore researchers are working to improve the analysis capabilities at CAMS so that smaller and smaller traces of the isotope can be measured. The CAMS team is contributing to other biological research as well, including a study on the formation of aneurysms.

Using the bomb pulse from aboveground nuclear tests to date biologically based materials has grown from a novel technique into an integral part of many scientific endeavors. This innovative spin-off from the Laboratory's primary national security mission is helping to advance law-enforcement efforts and medical research. Twenty-five years in and still counting, CAMS continues to offer its unique capabilities to help solve important scientific mysteries.

—Ann Parker

Key Words: bomb-pulse radiocarbon dating, carbon-14, cataracts, Center for Accelerator Mass Spectrometry (CAMS), cold case, hippocampus, neurogenesis, scientific forensics.

For further information contact Bruce Buchholz (925) 422-1739 (buchholz2@llnl.gov).