Lawrence Livermore National Laboratory

Science & Technology Review

July/August 2018

Forensic Science BOOSTS Global Security

Also in this issue:

Speeding Up Computational Solutions

A Postdoctoral Slam Dunk

Upgrade Enhances Flash X Ray

About the Cover

For more than 25 years, Lawrence Livermore's Forensic Science Center (FSC) has supported its national and global security missions, providing essential research and development efforts to the forensic science community as well as operational support for local, state, federal, and international entities. As the article beginning on p. 4 describes, the center's range of work spans multiple disciplines and encompasses wide-ranging capabilities and expertise. The FSC's activities promote the modernization of traditional forensic science techniques and the development of improved methodologies for strengthening global security.

Cover design: Mary J. Gines

About *S&TR*

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

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Prepared by LLNL under contract
DE-AC52-07NA27344

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S&TR, a Director's Office publication, is produced by the Technical Information Department under the direction of the Office of Planning and Special Studies.

S&TR is available on the Web at str.llnl.gov

Printed in the United States of America

Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22161

UCRL-TR-52000-18-7/8
Distribution Category UC-99
July/August 2018

Science & Technology REVIEW

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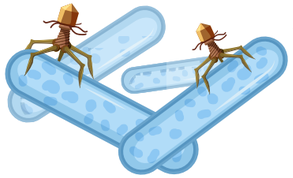

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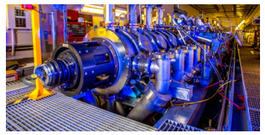

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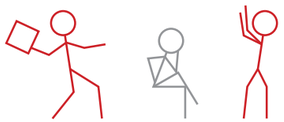

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Advancing Bioscience with "Brain-on-a-Chip"

Lawrence Livermore scientists and engineers have developed an in vitro "brain-on-a-chip" device for testing and predicting the long-term effects of biological and chemical agents, disease, or pharmaceuticals on the brain. The device, part of the Laboratory's iCHIP (in vitro chip-based human investigational platform) project, simulates the central nervous system by recording neural activity from multiple brain cell types deposited and grown onto microelectrode arrays. The results were published in the November 21, 2017, edition of *PLOS ONE*.

To re-create the structural anatomy of the brain, researchers divided the chip into four distinct areas—an inner region further split into three subregions and an outer region representing the brain's cortex. They deposited primary hippocampal and cortical cells onto the inner and outer regions' electrodes, and then monitored the "bursts" of electrical potential that cells emit when communicating and observed how the cells interacted over time. The researchers also successfully performed tests with a four-cell insert to prove more cell types could be used simultaneously. "While we cannot fully recapitulate a brain outside of the body, this work is an important step in terms of increasing complexity of these devices and moving in the right direction," said co-lead author and Laboratory engineer Dave Soscia.

Scientists say the platform is part of the Laboratory's broader vision for countering emerging and existing threats. It allows them to study the networks formed among various regions of the brain, and obtain timely, human-relevant data without animal or human testing. With the brain-on-a-chip platform, researchers could analyze how disease spreads through the organ, model epilepsy and other neurological disorders, or examine the effects of chemical or biological exposure over several months.

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Laboratory Licenses Nanolipoprotein Technology

Ann Arbor, Michigan–based EVOQ Therapeutics has licensed the Laboratory's nanolipoprotein (NLP) technology for cancer immunotherapy, which deploys the body's own immune system to fight cancer. Developed by Livermore biomedical researchers over the last decade with funds from the Laboratory Directed Research and Development Program, NLPs are water-soluble molecules that are 6 to 30 billionths of a meter in size and resemble high-density lipoproteins (HDL), known as "good cholesterol" in humans. EVOQ is developing a vaccine delivery platform that uses synthetic HDL.

Checkpoint inhibitors, which block normal proteins on cancer cells, are one immunotherapy approach that holds promise in the cancer treatment community. Although inhibitors have only been successful in 20 to 40 percent of cases, research using animal models has shown that when the synthetic HDL delivery is combined with the approach, complete tumor regression is exhibited in about 85 percent of colon carcinoma and melanoma cases. Ideally, synthetic peptides, small fragments of neoantigen proteins, would be incorporated into NLPs along with adjuvants (molecules that activate the patient's immune system). NLPs would then enter the body's lymph nodes, activating T-cells that circulate throughout the body and destroy the cancerous tumor cells.

NLPs were initially developed between 2005 and 2008 by a team of Livermore scientists led by Paul Hoeprich. In addition to the use of NLPs for transporting cancer vaccines, Lawrence Livermore scientists continue exploring other applications for the technology. These research areas include developing vaccines for influenza and chlamydia, formulating drug molecules to enhance NLP efficacy, and shuttling therapeutics across the blood–brain barrier.

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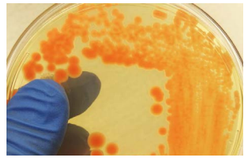
Researchers Create Tunable, Green Detergents

Surfactants, also known as detergents, are used extensively in the cosmetics, oil, food, agriculture, healthcare, and pharmaceutical industries. However, the majority of surfactants are petrochemicals, which can have a negative impact on the environment and increase the nation's dependence on foreign and domestic oil. With the demand for biosurfactants—those produced from microorganisms—on the rise, scientists at Lawrence Livermore have begun studying a new "tunable" kind that is environmentally friendly and can have broad industrial utility. The research appeared in the January 2, 2018, edition of *PLOS ONE*.

The new Laboratory surfactant is derived from the red yeast *Rhodotorula* (see image below) and is composed of a linear carbohydrate connected to a fatty acid. "The primary challenge is that microorganisms produce biosurfactants as a complex mixture of closely related surfactants, not a pure, single type of compound," says Matt Lyman, a Livermore biologist and lead author of the paper. "If we can harness the surfactant diversity found in nature and provide it in a pure form that is 'tunable' for industry needs, then it becomes a powerful technology beyond what exists today for commercial biosurfactants." The research was conducted through a collaboration between the Laboratory's Biosciences and Biotechnology Division and the Forensic Science Center.



The goal of the Livermore team is to use *Rhodotorula* as a "microbial factory" to produce a base surfactant compound at minimal cost. The team will then perform routine chemical modifications to enable it to slide up and down the hydrophilic–lipophilic balance (HLB) scale, which determines whether a surfactant has more of an affinity toward water or oil. Such a system would provide a "made-to-order" biosurfactant with a specific HLB most useful to customers and their applications.

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Commentary by Bruce E. Warner

Constant Vigilance in Forensic Science

EVERY day, Lawrence Livermore advances science and technology in service to national security. Our specialized centers and institutes pursue this mission with novel research projects, first-class facilities, and multidisciplinary staff. As the feature article beginning on p. 4 describes, the Laboratory's Forensic Science Center (FSC) is a notable example of our longstanding leadership in a rapidly evolving field.

Founded in the early 1990s, the FSC brings many strengths to modern forensic science. Much of the center's success comes from commitment to both operational and research activities. Analyzing samples for the criminal justice system, the intelligence community, and international entities drives research and development in forensic techniques and instrumentation. Such scientific improvements, in turn, enable FSC staff to process increasingly complicated samples for multiple sponsors. Recent proteomics breakthroughs are direct outgrowths of this balanced approach.

These remarkable achievements are made possible through FSC scientists' technical excellence and the center's advancement of analytical methods for chemical, biological, radiological, nuclear, and explosives samples. The FSC applies data-driven forensic analyses to real-world cases, routinely supporting evidentiary efforts for the U.S. Department of Justice and its Federal Bureau of Investigation.

Indeed, the FSC brings many best practices to conventional forensic science, such as using nondestructive techniques that preserve materials for further evaluation. Demonstrating its commitment to producing data of the highest quality, center operations comply with the International Organization for Standardization's competency and calibration requirements. In addition, the center consistently receives top marks on proficiency tests that require detection of minute quantities of chemicals in highly complex samples. These practices enable FSC scientists to study the composition of a sample and understand its origin through chemical synthesis and attribution projects.

Organizations at home and abroad value the FSC's capabilities. As an example, the U.S. Environmental Protection Agency relies on the FSC as the reference laboratory for the Environmental Response Laboratory Network, providing analysis methods, standards, and training. Moreover, the FSC is one of only two U.S. laboratories certified by the Organisation for the Prohibition of Chemical Weapons (OPCW) for analysis of environmental and biomedical samples and is one of only 22 laboratories worldwide that supports OPCW treaty verification and alleged-use investigations. In addition, FSC staff, through the Department of State's Chemical Security Program, train other countries' first responders to properly identify and investigate chemical threats.

Together FSC's strengths—a comprehensive portfolio, high-quality research and development, rigorous methodologies, practical experience, and accreditations—tell a compelling, but incomplete, story. Even the best commercial laboratories lack two additional factors contributing to the center's success. First, since the FSC is a federally funded research and development center—motivated by mission, not profit—sponsors are assured of objective results. Second, the center has access to capabilities beyond its own walls. Whether the need is expertise or equipment, the FSC can draw upon the skill and proficiency of Laboratory colleagues who are experts in a range of scientific and engineering disciplines. With this in-house support, FSC staff can collaborate with the U.S. Department of Homeland Security, the National Nuclear Security Administration, and other partners in an even broader scope of work.

As world events evolve in complexity, the nation's problems have become increasingly challenging. The FSC's most important assets are the outstanding people who work together as a team to solve problems. Livermore's national security mission depends on constant vigilance, and the FSC's dedication and technical excellence is reflective of the entire Laboratory workforce. Our ability to anticipate and adapt to changing needs makes us an asset to our sponsors, who are motivated to send their most difficult problems here.

■ Bruce E. Warner is principal associate director for Global Security.

S&TR July/August 2018

The Case for Modern Forensic Science

Armed with wide-ranging expertise and analytical capabilities, scientists at Livermore's Forensic Science Center are prepared for anything that comes through its doors.

Chemist Brian Mayer cleans a source component of a mass spectrometry system. The Forensic Science Center (FSC) relies on a suite of complementary instrumentation to meet proficiency requirements for multiple agencies. (Photo by George Kitrinos.)

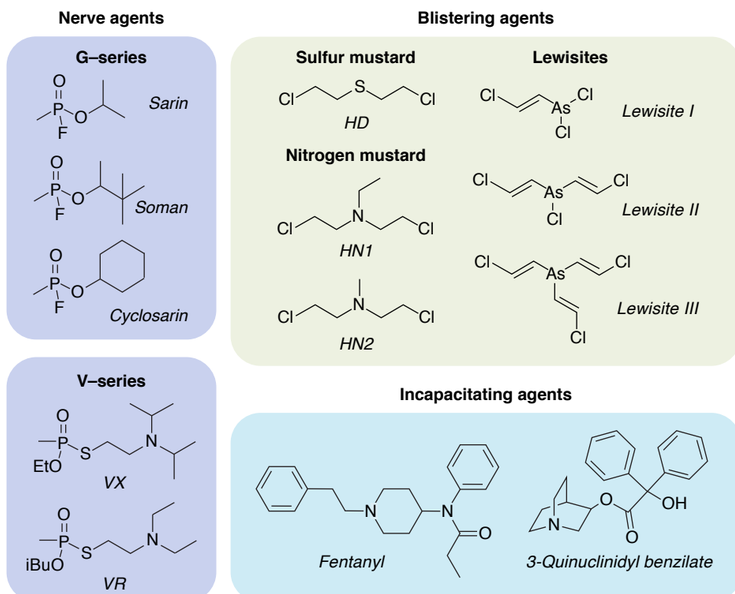
SUCCESS in a high-stakes field does not come easily. Last fall, Lawrence Livermore's Forensic Science Center (FSC) completed another grueling 15-day testing period for the Organisation for the Prohibition of Chemical Weapons (OPCW), the international body that oversees compliance with the Chemical Weapons Convention treaty. With the ability to detect evidence of chemical warfare agents (CWAs) both as intact chemicals and through their degradation products, the FSC has earned its standing among OPCW-certified laboratories in the United States as one of only two that analyzes environmental samples and one of three that determines human exposure to CWAs. (See *S&TR*, June 2013, pp. 13–15.)

OPCW's proficiency tests challenge the world's best forensic laboratories in technical skills, analytical methodologies, safety protocols, chain-of-custody procedures, and more. Test samples can be made of any material spiked with unknown chemical compounds at varying concentrations. A single mistake—failure to identify spiking compounds, false-positive results, or misreported data—can strip a laboratory of its accreditation. For the eighth consecutive test, the FSC came out on top with an "A" grade.

"These proficiency tests push the limits of our team, technologies, and technical expertise. The system we have developed over the years to enable success across these testing areas has become foundational to much of the work we execute at the FSC," says the center's director Brad Hart. According to Glenn Fox, former FSC director and now associate director for the Laboratory's Physical and Life Sciences Directorate, "The vision for the FSC was ahead of its time. The Laboratory saw

a need for, and invested in, its unique capabilities."

As the scientific community questions the reliability of traditional forensic science in law enforcement investigations, the FSC provides leadership in advancing conventional methodologies through its accuracy in chemical, biological, radiological, nuclear, and explosives (CBRNE) forensic analyses and its research and development efforts in analytical techniques. Bruce Warner, Livermore's principal associate director for the Global Security Principal Directorate, states, "The Forensic Science Center has been providing a balanced operational and research portfolio for 27 years. Government sponsors rely on the center's deep technical capabilities for sample analysis and origin determination and for development of new techniques to address emerging threats."



Categories of chemical warfare agents (CWAs) include nerve, blistering, and incapacitating agents. The FSC's chemical forensic work includes characterizing the constantly evolving range of CWAs.

Inflection Point

In 2016, the U.S. President's Council of Advisors on Science and Technology (PCAST) released a report recommending better scientific standards for forensic methods, specifically those used to compare different forms of evidence such as firearm ballistics, bitemarks, and hair, among others. The document drew on numerous papers, criminal cases, and reports from other federal agencies to capture the scope of the current techniques' shortcomings. PCAST concluded that methods reliant on "significant human judgment" are too subjective and, therefore, not scientifically defensible in court.

The PCAST report built on an earlier study from the National Research Council regarding the state of forensic science, and the cumulative scrutiny leads to what Hart calls an inflection point in transforming this branch of the discipline. He explains, "Inadequate techniques undermine the criminal justice system and increase resistance to their use in the broader national security community. We see opportunities to improve objectivity in this area because of our focus on advancing capabilities that generate high-quality, defensible data."

The FSC is already making strides in traditional forensic analysis with projects that expand the possibilities for human identification. Hart states, "Lawrence Livermore has a unique opportunity to influence this field. Few places can offer what we provide—extensive basic science research that informs our development of technologies and capabilities for application to real-world scenarios."

Strict Science and Safety

One of the FSC's key functions is advancing science in the public interest, and the center's staff thrive on the challenge of growing its forensic capabilities. "We like to push technology in new directions. For example, we consider how next-generation versions of instruments, such as mass spectrometers, will affect what we can detect in the environment," says Carolyn Koester, the center's deputy director for operations. FSC colleague and chemist Brian Mayer adds, "The center joins sophisticated equipment with multidisciplinary expertise in the same physical laboratory space. This synergy is critical to our continual modernization." (See the box on p. 10.)

Although most work done at the FSC is performed by in-house staff, the depth and breadth of Livermore's expertise across the complex enables investigators from other areas to help conduct diverse examinations. For example, a specialist from another area of the Laboratory may participate in a specific project, or another facility may provide access to complementary equipment. Chemist Pat Grant says, "We can 'borrow' scientists from other Laboratory programs for an integrated evaluation, thanks to the scientific and technical diversity onsite."

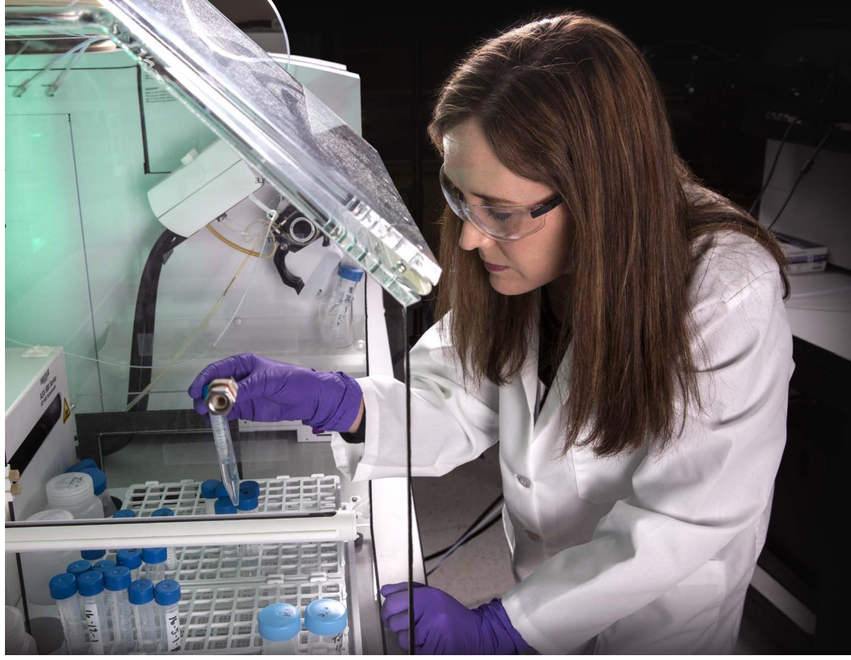
Safety is ingrained in all FSC activities. A strict acceptance procedure ensures proper triage, routing, and screening of materials while minimizing human exposure and sample contamination. Biological materials are handled in a dedicated biosafety area, and the cradle-to-grave custody process includes decontamination and proper disposal of all samples after analysis. "Inappropriate handling could result in severe consequences," notes Koester. "Until we confirm what the specimen is, we treat all samples with the highest precautions," says FSC deputy director Audrey Williams.

When needed, the center runs 24 hours a day, 7 days a week, and staff are dedicated to optimum and efficient turnaround. "We address real-time problems and think comprehensively across the forensic landscape—an attribute that our external partners value," explains Hart, citing the FSC's long-standing chemical attribution signatures program with the U.S. Department of Homeland Security. Also, as a partner laboratory with the Federal Bureau of Investigation (FBI), the Laboratory's FSC analyzes special nuclear materials and various chemical threats. The center's relationship with the FBI became codified after the September 11, 2001, terrorist attacks. Grant is the principal investigator for the center's FBI casework, which includes analysis of weapons-grade materials and other questioned samples. He notes, "When the FBI needs chemical or nuclear forensic capabilities for uncommon investigations, they often come to us."

The C in CBRNE

Chemical threats account for a significant portion of the FSC's investigations, so research and development efforts include synthesizing small amounts—as allowed under the Chemical Weapons Convention treaty—of chemical warfare agents to understand possible production methods clandestine actors may use. For example, FSC scientists have studied multiple pathways for making sulfur mustard, also known as mustard gas. "We look for data that make a given synthesis route or the final product unique," explains Williams. This work informs investigations by comparing a sponsor's questioned sample to idealized materials. Furthermore, comparing samples obtained by different sponsors may reveal whether they are related.

The FSC's chemical forensic expertise extends to chemical attribution. By building profiles or "signatures" of different CWAs, FSC staff can determine important information about a sample. A chemical signature is defined as a collection of impurities, including reaction byproducts, degradation products, and unreacted starting chemicals and reagents and their relative concentrations, detected in a final synthetic product. These chemical signatures are used to provide clues to how the sample was synthesized.



Forensic chemist and FSC deputy director Audrey Williams loads prepared samples into the FSC's inductively coupled plasma–mass spectrometry system, which is commonly used to analyze a sample's trace metal components or contaminants. Beforehand, samples undergo acid digestion to remove organic materials while generating and retaining metal ions. (Photo by George Kitrinos.)

According to Williams, the FSC approaches each research question and chemical sample as a puzzle to be solved. "Our research, development, and operational missions strengthen each other because we have built a collective knowledge base," she says. For instance, the center's CWA detection strategies for OPCW proficiency tests underpin everyday work identifying chemical signatures and characterizing chemical threats. Williams continues, "We provide expert-level evaluation of our measurements, not just the raw data. We go beyond simply reporting that certain chemicals are present in a sample to pull all the clues together in context. Essentially, X and Y together may suggest Z is occurring."

The B in CBRNE

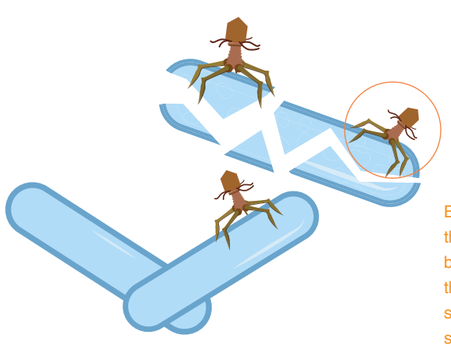
The FSC's success in biological forensic analysis springs from a robust cycle of research followed by applied proof-of-concept studies; partnerships with Livermore's Biosciences and

Biotechnology Division as well as other laboratories; and collaborative scale-up, testing, and deployment phases. Brian Souza, senior biologist and group leader for Biosecurity and Bioforensics, describes this process as unique. “Our biologists think outside the box. In addition, we have access to select agents, including dangerous pathogens,” he says. Souza’s team works closely with the Department of Defense, its Defense Threat Reduction Agency, and other agencies to advance development of biological countermeasures using Livermore’s capabilities in bioinformatics and computational biology.

Biological countermeasures neutralize or reverse physiological responses to naturally occurring and engineered pathogens. To fight antimicrobial resistance, FSC scientists are exploring the potential of viruses that attack bacteria—known variously as phages, bacteriophages, or microbial viruses. Souza’s team and collaborators are developing new methods for characterizing phage genomes. “We need to understand how viruses are organized genetically. Computer modeling of phage proteins streamlines research and development prior to experimentation,” he notes.

Phage genomes are not as predictably structured as bacterial genomes, and popular DNA-annotation tools fall short of identifying phage genes. Using Livermore’s high-performance computing resources, the team has designed a one–two punch for conducting the required analysis. First, PHANOTATE, a novel computational algorithm, identifies genes by maximizing their translatable parts—called open reading frames—within the phage’s DNA. Next, the Python-based Phage Annotation Toolkit and Evaluator (PhATE) program uses PHANOTATE’s results to predict protein structure and function encoded in the open reading frames. The combination of PHANOTATE and PhATE enables researchers to determine and model genes that when translated into proteins are used during infection and destruction of their host cell. Understanding these mechanisms of action may help researchers discover better and more effective protein-based countermeasures and antimicrobials.

Beyond national security and public health applications, research into viral DNA markers can guide further forensic endeavors. Souza explains, “Informatics tools enable us to assemble DNA sequences faster, so the methods we develop for biological studies can be shared to benefit human forensic work that uses DNA.” In addition, a microbe’s epigenetic DNA changes (those not related to DNA sequencing) and its genetic markers based on environmental exposure can inform studies of similar mechanisms in human proteins. Souza adds, “Our center-based approach to forensic science is crucial. We have several boutique capabilities that, along with investments and skilled partners, allow us to move quickly in this space.”



Biological countermeasures “go viral” with the use of phages (inset)—viruses that attack bacteria. A phage’s head contains DNA while the rest of the body is made of protein-based structures. Phages attach to receptors on the surface of a bacterium, penetrate the cell, and replicate their DNA, ultimately destroying the cell.

The R, N, and E

Before the FSC was formally established, Livermore radiochemists measured nuclear and radioactive materials resulting from nuclear explosive testing. The 1990s saw a worldwide increase in material seizures, and since then, FSC scientists have been at the forefront of nuclear forensics and attribution, helping to deter trafficking of illicit materials. (See *S&TR*, October/November 2014, pp. 12–18.) Grant remarks, “We have learned much more from comprehensive analyses of real-world interdictions and samples than from scripted exercises or fabricated test specimens.” This accumulated experience has produced a primary reference source for the nuclear forensic field. Grant, along with FSC associates Ken Moody and the late Ian Hutcheon, authored the seminal textbook, *Nuclear Forensic Analysis*, now in its second edition.

Different aspects of the center’s CBRNE capabilities often dovetail with surprising results. In the FSC’s first high-profile case, a controversial nuclear energy experiment at a California research laboratory caused a fatal explosion. Debris analysis revealed only natural background radioactivity, but further investigation uncovered another potential explosion initiator unrelated to the original hypothesis. The case underscores the importance of comprehensive investigation—an imperative for objective forensic analysis—and reinforces the need for access to a variety of skills and techniques.

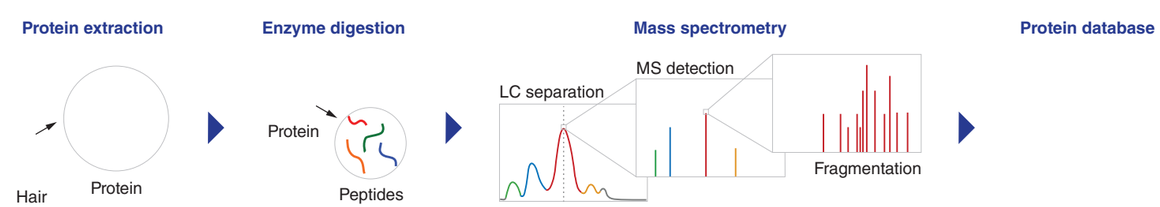
When conducting an investigation, FSC scientists must consider both principal and collateral aspects of the work at hand. For explosives, such analysis means identifying impurities and additives within explosive compounds, any residues, and the composition of the primary container. For interdicted radiological or nuclear materials, a complete evaluation may also include microscopic analysis of packaging materials and fibers, biological traces left by the traffickers, and other substances such as dusts or pollens, which could provide route and storage information.

This careful approach means FSC scientists can uncover a hoax just as well as revealing a real threat. In one notable investigation, two containers advertised as smuggled nuclear materials were acquired. Despite being labeled as containing uranium and plutonium, neither object held radioactive or weapons-grade materials. FSC scientists, along with colleagues from Livermore’s Nondestructive Characterization Institute, evaluated all aspects of the containers. They began with a series of nondestructive tests, including gamma-ray spectrometry and x-ray and neutron radiography, to interrogate the specimens’ interiors for safety assessment. Ultimately, subsequent forensic analysis determined that the contents were not dangerous.

(left) Shown here is one of two specimens obtained from a nuclear smuggling enterprise that turned out to be a scam. (right) Researchers evaluated all aspects of the container using multiple imaging techniques, such as neutron radiography. After “breaking and entering” the specimen, the team identified an assortment of packaging and filler materials, including plant matter, pharmaceutical compounds, synthetic fibers, and magnetic objects. (Photo by Pat Grant; radiograph courtesy of Harry Martz, Bill Brown, Randall Thompson, and John Rodriguez.)

Promising Proteomic Identification

Enhancing objectivity in traditional forensic science requires maximizing the potential of tiny pieces of evidence, such as a skin cell or bone fragment. Proteins are a viable source of human identification because they can express DNA mutations and are found in many types of tissue. According to chemist Deon Anex, proteomics can augment DNA analysis in degraded samples such as human remains. He says, “Proteins are more robust in the environment than DNA.” In a recent collaboration, FSC scientists developed a technique for biological identification that uses proteins found in human hair. (See *S&TR*, July/August 2015, pp. 15–17.) Anex and Hart are now leading an effort to expand this work to other human tissues.



Proteomic analysis of hair protein begins with an extraction process that dissolves the hair and isolates the peptide chains. Liquid chromatography–mass spectrometry (LC–MS) is used to separate the peptides and analyze their amino acid sequences.