

ADVANCED TECHNIQUES IN PHYSICAL FORENSIC SCIENCE

The techniques and tools of modern physics have long been profitably applied to forensic science.¹ Since the 1900s, forensic scientists have analyzed gunshot residues, examined fired munitions, determined the refractive indices of glass—to name a few of their diverse activities.

As portrayed in television news or crime novels, forensic science supports law enforcement agencies in their criminal investigations. The Federal Bureau of Investigation, Scotland Yard and the International Criminal Police Organization (Interpol), for example, all have large forensic laboratories.² Less well known to the public is that certain Federal and military forensic laboratories, whose core missions embrace national security and intelligence, also conduct forensic research. Free from the overwhelming caseloads that beset mainstream law enforcement personnel, specialists at such nontraditional forensic labs can focus on doing their research aimed at developing increasingly sophisticated instrumentation with which to conduct investigations.

Livermore's Forensic Science Center

In 1991, Lawrence Livermore National Laboratory (LLNL) consolidated its various independent forensic activities and established the Forensic Science Center (FSC). Charged with researching, developing and applying modern forensic science techniques, the FSC drew together experts in the general technical areas of physical, chemical, biological and nuclear forensic science. And after President George Bush announced a moratorium on underground nuclear testing in October 1992, several other US Department of Energy (DOE) national laboratories launched their own forensic support efforts as part of a strategy to diversify their activities and revenue sources.

The activities of LLNL's FSC fall into three areas of application: nonproliferation of weapons of mass destruction (WMD), counterterrorism and law enforcement support. The center conducts R&D on advanced techniques for collecting and analyzing samples in the field, on portable analytic instrumentation and on the application of state-of-the-art technologies to diverse sets of samples. The FSC also analyzes samples submitted by agencies outside DOE. These samples are scrutinized not only for evidence of the more conventional forensic analytes—such as drugs, poisons and explosive residues—but also for the diagnostic signatures of nuclear, biological and chemical WMD and other uncommon species.

Past investigations have included the World Trade

Ultrasensitive mass spectrometry is now being applied in forensic investigations.

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Center bombing, UNABOM evidence analyses, the smuggling of illicit materials and forensic characterization of interdicted biotoxins.

In this article, we discuss advanced physical techniques developed and implemented by the FSC for modern forensic analyses. As well as describing the underlying physics, we illustrate

the techniques with case studies. Although we focus on two techniques in particular—ion store/time-of-flight mass spectrometry and secondary ion mass spectrometry—we also emphasize that the real strength of modern forensic investigations of completely unknown specimens lies in adopting a multidisciplinary but integrated approach to sample analysis.

Trapping ions and mass spectrometry

Trace analyses of airborne compounds often provide important—and, on occasion, the only—clues in forensic investigations. Conventionally, a two-step process is followed. First, a sample is collected in a container, which may be a polyethylene bag, glass or metal canister or a solid sorbent trap. Then, the container is transported to a laboratory for subsequent analyses.³

Although these types of collection container are easy to handle and operate, their use is problematic when continuous monitoring is required. On the way to the lab, samples can be lost through irreversible adsorption or decomposition. Serious analytic errors can result.⁴

Real-time, on-site direct analysis with portable equipment eliminates many of the limitations of field collection, and several new instrumental concepts are being developed to apply it. The best combination of sensitivity and specificity over the broadest range of compounds is offered by mass spectrometry, of which the most promising implementation exploits a variant of ion trap technology developed by Nobel Prize winners Wolfgang Paul and Hans Dehmelt.⁵ The ion trap's principal advantage is that diagnostic target molecules become significantly easier to detect as they accumulate within the trap.

At the FSC, we have recently developed the ion trap as a pulsed extraction source for time-of-flight mass spectrometry (TOF-MS).⁶ By combining the storage capability of the ion trap with the speed and efficiency of TOF-MS, we can continuously and sensitively measure trace quantities of gases and volatile compounds in ambient air.

During an analysis run, air is introduced directly into a Paul-type ion trap through a small orifice on a vacuum chamber inlet. Volatile species are simultaneously ionized by electron impact and accumulated in the trap cavity, while positive ions are confined to an orbit around the center of the trap by an electric field. Once the trap is filled, the ions are pushed out into the TOF-MS unit by a short (8 ns) voltage pulse applied to the entrance and exit endcaps. (See box 1 on page 34.) Since the voltage pulse imparts the same amount of kinetic energy on ions

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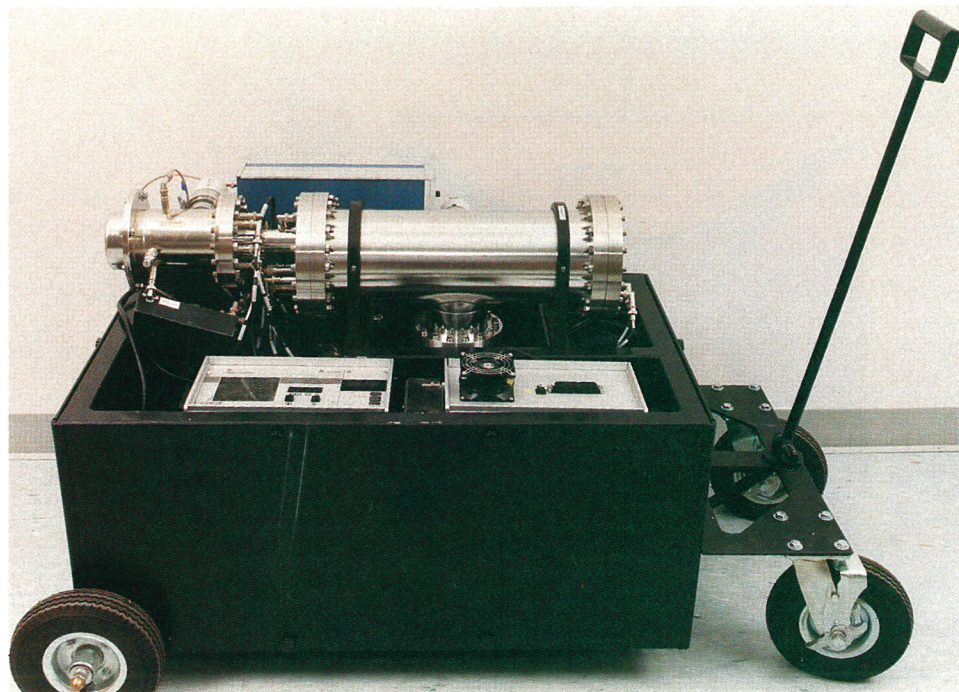


FIGURE 1.
FIELD-TRANSPORTABLE
ion store/time-of-flight
mass spectrometry
(IS/TOF-MS) instrument.
On the top of the unit,
commercial Finnigan
ITMS™ electrodes are
contained in the smaller
chamber (left), which is
interfaced to a customized,
50 cm Jordan reflectron
flight tube (right).
Instrument vacuum is
maintained by a 180 L/s
turbomolecular/diaphragm
pumping system.
Electronics were fabricated
at LLNL.

of equal charge, measuring how long the ions take to fly from the trap to the detector yields their masses.

Exactly how the ion trap is controlled is critical for optimizing the sensitivity and resolution of the IS/TOF-MS unit (IS = ion store) as a whole. When operated properly, the trap produces an ion packet of narrow velocity and spatial distribution, which is ideal for TOF-MS analysis.⁷ Another advantage: The trap requires only a small radio frequency (RF) potential to store ions, which makes it possible to operate it at relatively high background air pressures (10^{-4} torr and higher). This advantage not only makes direct sampling of air possible, but it also improves storage and ionization. For when the pressure is high enough—that is, when the mean free path and ion orbit are of the same order—collisions between the ions and the atoms of the background gas enhance the ions' storage. These dampening interactions also help to keep the ions in the center of the trap and away from the trap's outer areas, where trajectories are less stable.

Collisions within the trap cavity also enhance the ionization efficiency of most organic analytes (whose ionization potentials are about 10 eV) through charge exchange with the abundant air species of oxygen (ionization potential of 12.1 eV) and nitrogen (15.6 eV).

The undesirable accumulation of ionized background molecules relative to the target ions is controlled by inducing unstable orbits in the background ions. In particular, by increasing the RF amplitude on the ring electrode, instability in the trap is selectively produced in ions whose ratio of mass to ionic charge, m/z , is below diatomic oxygen's 32 atomic mass units. After the trap is saturated, the ion cloud is extracted for TOF-MS analysis by pulsing both end caps at the proper RF phase of the ring electrode, which is simultaneously clamped to ground. The resultant spatial and energy distribution of ions is then focused in the TOF-MS unit. We routinely obtain an instrument resolution of $m/\Delta m = 1200$ (FWHM) and mass accuracy of approximately 0.01%.

When in operation, the TOF-MS accumulates ions in the trap for 200 ms, after which they are extracted and analyzed within 100 μ s to yield a duty cycle near 100%.

IS/TOF-MS limits of detection for the most volatile organic species are in the low parts-per-billion range, which is somewhat better than sniffer dogs (which can't, of course, detect odorless compounds).

Direct sampling with real-time analysis greatly minimizes surface interactions and is optimum for monitoring sticky or surface-reactive chemical compounds. However, for polar compounds (which contain a covalent bond whose shared electrons are closer to one bonded atom than the other), our detection limits are presently 100 times worse than for nonpolar species. We are working to improve instrumental response for this class of compounds.

A field-transportable IS/TOF-MS unit is shown in figure 1. Although such units were developed primarily for conducting surveillance from an airborne platform, a ground-based forensic application of the technology—the identification of near-fatal toxic fumes—is discussed in box 2 on page 35.

Sputtering ions and mass spectrometry

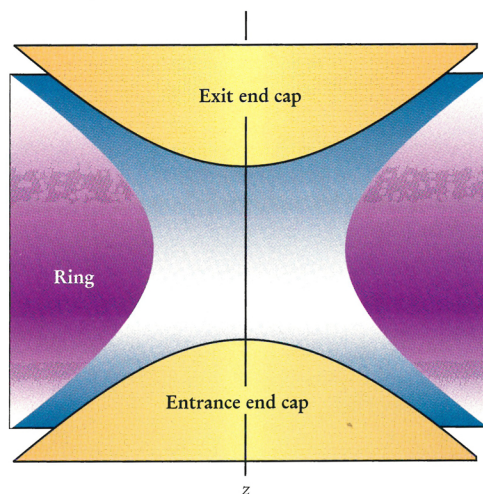
In secondary ion mass spectrometry (SIMS), a beam of primary ions ($^{16}\text{O}^-$ or $^{133}\text{Cs}^+$) is directed at a sample to sputter off secondary ions from the sample's surface. These secondary ions of various m/z ratios (and, hence, various initial velocities) pass through a stigmatic-focusing mass spectrometer, which focuses them to a single point according to their m/z ratios. Because the primary beam can be so finely controlled, SIMS—and, at present, only SIMS—makes it possible to perform ultrasensitive elemental and isotopic analysis in combination with submicrometer spatial resolution.^{8,9}

Since detection limits for all elements (with the exception of the hard-to-ionize noble gases) are around a few parts per billion, SIMS constitutes an extremely sensitive indicator of sample chemistry. Statistically meaningful results can be obtained from a specimen containing as few as 10^4 atoms of the element in question. This high sensitivity, coupled with the ability to interrogate virtually any solid sample and measure secondary ions over a dynamic range greater than 10^{10} , gives SIMS an unsurpassed capability to determine the compositions of samples

Box 1. The Quadrupole Ion Trap as an Analytical Tool

A quadrupolar ion trap consists of three electrodes with rotational symmetry about the z axis and with the surface of the center (ring) electrode forming a hyperboloid of one sheet. Two end caps are oriented above and below the ring electrode to form a hyperboloid of two sheets. (See the accompanying figure.) An electrodynamic quadrupolar field is created by applying radio frequency power to the ring electrode, along with a DC voltage between the end caps. Within this operational configuration, an ion experiences a strong, three-dimensional focusing field that continually drives it toward the center of the trap.

An ion trap is most commonly used as a mass analyzer, which typically involves mass-selective axial ejection to generate mass spectra. In many systems, this process is achieved by



applying a low-frequency AC voltage to the end-cap electrodes while the RF potential on the ring electrode is ramped. As the ions approach their stability limit with increasing RF, the AC voltage promotes ejection along the z axis by exciting ion secular motion.

Unfortunately, the efficiency of this process is poor because only half the ion cloud is directed toward a detector behind the exit end cap. Furthermore, ion ejection of this type is susceptible to space-charge interferences, especially of the ion secular motion, and can result in decreased resolution and shifts in apparent mass. By contrast, using the ion trap solely as a storage device in the IS/TOF-

MS unit circumvents these limitations by eliminating any axial ejection step.

as small as a few picograms.

Two different SIMS techniques are used for forensic applications at Livermore: ion microscope and ion microprobe. (See box 3 on page 36 for more details.) Both use a high mass resolution, magnetic-sector mass spectrometer with ion-counting detection. In each approach, mass-resolved images can be acquired as the primary beam erodes the specimen surface. The resultant three-dimensional analysis contains a complete record of the sample's chemical and isotopic variations, with simultaneous lateral and depth resolution. This singular capability is particularly effective in analyzing the chemically heterogeneous materials often encountered as forensic specimens.

By virtue of its spatial resolution, sensitivity, dynamic range and versatility, SIMS has been an invaluable asset in FSC investigations. Despite these advantages, however, SIMS instrumentation is relatively uncommon in forensic laboratories.

In practice, the principal challenge in applying SIMS to forensic problems is to relate the composition of the secondary ion flux to the composition of the sample. Whether a sputtered atom is ionized depends strongly on many factors, including the properties intrinsic to the sputtered particle (its ionization potential, for example), the characteristics of the sample (its bulk composition, for example) and the properties of the primary beam (the ion species present, for example). Inher-

ent variation in ionization probability spans more than five orders of magnitude for different elements, and since there is no comprehensive theory of sputtered ion formation available at present, SIMS data can be accurately quantified only by comparing them to primary standards of similar major element composition, measured under the same analytic conditions.

Ranging from uranium metal to newsprint, the wide diversity of specimens interrogated for forensic purposes requires, therefore, a particularly extensive set of standards. Our LLNL library, for example, includes more than 50 glass and mineral standards certified for 30–40 trace elements, as well as a set of 200 ion-implanted standards, each containing 15 validated species. By comparing secondary ion intensities from a sample to those for one or more of these standards, trace element abundances customarily can be determined to an accuracy of 5–10% and isotope ratios to $\pm 0.1\%$.

Quantitating secondary ions is also hampered by the copious production of ionized atomic clusters (molecular ions) and multiply charged ions during sputtering. Since

FIGURE 2. DEBRIS FROM A FATAL "cold fusion" cell explosion that occurred in a Menlo Park, California, electrochemistry laboratory on 2 January 1992. Shown are the main stainless steel electrolysis cell and its detached base, a ripped Teflon insert and pieces of brass heat-exchanger fins.



Box 2. Investigation of Suspect Toxic Fumes Emission

In the winter of 1993, a plant worker collapsed at the Department of Energy's Savannah River Site and nearly died after inhaling the fumes that were produced as he cut an iron pipe with an oxyacetylene torch. The pipe, which was an old hydrogen sulfide gas flare from a heavy water production plant, had been abandoned with one end open to the environment for about 20 years.

Samples taken before the cutting began revealed no residual hydrogen sulfide, nor were the victim's symptoms indicative of exposure to high concentrations of either H_2S or sulfur dioxide. The pipe cutting procedure was routine, and no accidents like it had ever been reported during many years of similar plant deconstruction operations in either the US or Canada. However, the interior of the pipe did contain corrosion and ash deposits that smoldered after torch cutting. The potential involvement of carbonyl sulfide (COS), a lesser-known toxic gas, became the target of the investigation undertaken by the Forensic Science Center.

During 1995–96, the pipe cutting procedure was reenacted at the Livermore FSC. Leftover deposits from inside the Savannah River pipe were used in the reenactment, and volatile species emanating from a hot pipe were measured. Because the properties of the emitted gas plume changed as the cut progressed, we performed the analysis with an ion store/time-of-flight mass spectrometry (IS/TOF-MS) unit, which monitored volatile analytes at the open end of the pipe, 6 m away from the cut, while "grab" samples for later analysis were collected at various times from ports on the side of the pipe, 3 m and 5.8 m from the cut. The grab samples were then extensively analyzed in FSC laboratories by using quantitative gas chromatography–mass spectrometry (GC–MS).

An individual IS/TOF-MS mass spectrum from these experiments identified SO_2 , COS and other volatile, sulfur-containing compounds. A complete mass-spectral data file was written to disk every 2–4 seconds, and all outgassed species were examined on-line. (See the accompanying figure.) By analyzing changes from spectrum to spectrum, new volatile chemicals could be immediately detected whenever they were emitted. Temporal profiles of various questioned toxins were recorded and displayed.

As can be seen in the accompanying figure, the most abundant compound produced was SO_2 , which was tracked by its SO^+ fragment ion at mass-to-charge ratio m/z of 48 atomic mass units. The concentration of this species rapidly increased after the cut was started, then fell gradually, with occasional surges, to background level. An ion of $m/z = 64$ amu exhibited a similar rise but with an earlier and sharper decay. Although $m/z = 64$ corresponds to that of an SO_2^+ parent ion, its intensity relative to the $m/z = 48$ fragment was much larger than that observed in standard SO_2 spectra. Consequently, the majority of the intensity at $m/z = 64$ was interpreted as arising from S_2^+ , which has a similar m/z . COS

($m/z = 60$ amu) was produced only marginally, while carbon disulfide was measured at the instrumental limit of detection. By comparison with mass spectra of primary analytical standards, the maximum concentration of evolved SO_2 during these experiments was determined to be approximately 27 000 parts per million (ppm), while for poisonous species it was 70 ppm for COS and 6 ppm for CS_2 .

To kill a lab rat with COS requires that the rat inhale a concentration of at least 600 ppm over four hours. An empirical measurement of an order of magnitude lower concentration in the incident simulation, combined with the victim's exposure time of much less than four hours, did not support a contention that COS had been a likely contributor to the Savannah River Site accident.

The FSC investigation, though inconclusive, proved that IS/TOF-MS

instrumentation could indeed monitor tiny concentrations of lethal toxins in real time. But it also reinforced the idea that laboratory simulations often cannot suitably reproduce important variables in real-world incidents—either because they are not known or appreciated, or because critical factors in the interplay of complex systems are impossible to adequately duplicate.

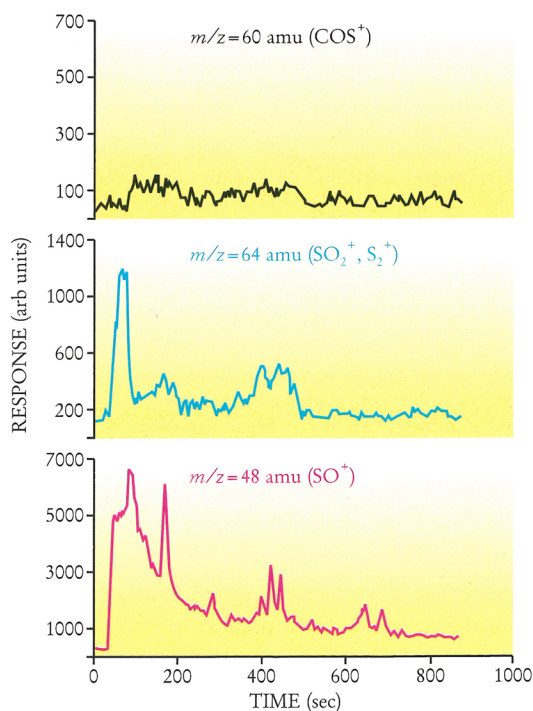
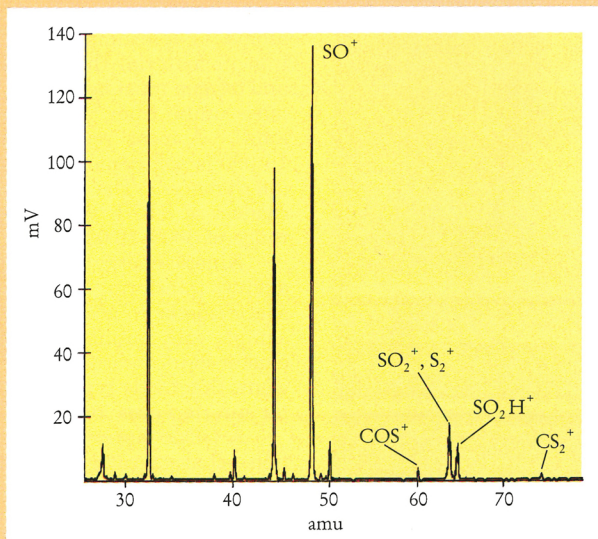




FIGURE 3. SIDE VIEW of the main “cold fusion” vessel that exploded accidentally in a Menlo Park, California, electrochemistry laboratory on 2 January 1992. Note the bulge below the metal threads. An increase in internal pressure prior to the explosion deformed the 0.2 cm thick, stainless steel cell from an initial configuration of a right-circular cylinder.

the m/z ratios of these interfering ions can be very close to those of the analytes of interest, high-mass-resolution or voltage-offset techniques must be implemented.

Molecular ions differ in mass from atomic ions due

to the differing nuclear binding energies of the cluster atoms. For example, a SIMS search for trace levels of iron in material that contains abundant silicon, calcium and oxygen must be able to distinguish ^{56}Fe (55.935 atomic mass units) from $^{28}\text{Si}_2$ (55.954 amu) and $^{40}\text{Ca}^{16}\text{O}$ (55.958 amu). A mass resolving power ($m/\Delta m$ at FW0.1M) of 10 000 is sufficient to eliminate about 70% of all molecular interferences. The microcharacterization examinations we conduct at LLNL typically require that $m/\Delta m$ be in the range of 3000–10 000.

Kinetic-energy filtering is an effective discriminator against interfering species. It entails adjusting the mass spectrometer to accept only those ions that exit from the sample with initial energies in excess of 60–80 eV. Since cluster-ion formation at such high energies is very inefficient, this tactic typically reduces the intensity of molecular interferences 100–1000 times.

A SIMS application in a specific forensic investigation—characterizing counterfeit currency—is discussed in box 4 on page 37.

Interdisciplinary forensic techniques

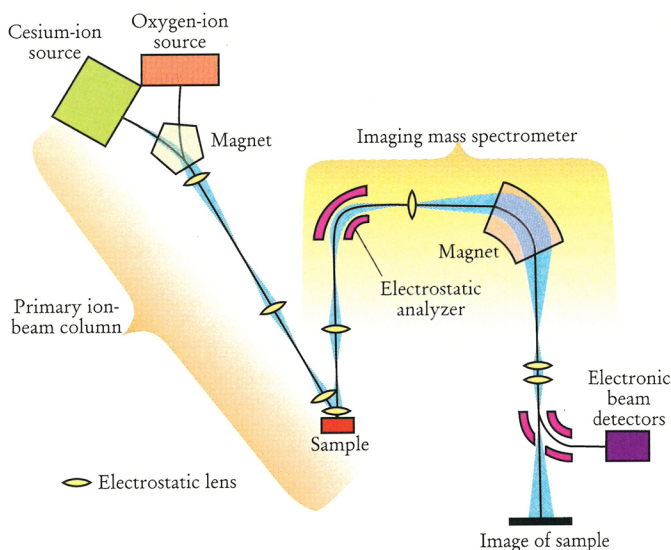
It would be a mistake to conclude that advanced physics instrumentation alone is sufficient to perform effective forensic analysis. Rather, conducting successful investigations is grounded fundamentally in a strong multidisciplinary approach to sample analysis. A forensic speci-

Box 3. Secondary Ion Mass Spectrometry

Secondary ion mass spectrometry (SIMS) uses a focused beam of 10 keV $^{16}\text{O}^-$ or $^{133}\text{Cs}^+$ primary ions to sputter the surface of a sample. Material within a few atomic layers of the surface (with a depth resolution of around 5 nm) is ejected with typical sputter yields of one to several atoms per incident ion. A small proportion—less than 1%—of the ejecta is ionized, electrostatically collected and analyzed according to mass-to-charge ratio m/z in a double-focusing mass spectrometer.

The ion microscope approach to specimen analysis uses a defocused primary ion beam with a diameter typically greater than 100 μm . Stigmatic ion optics form a magnified, real image of the sample surface in mass-separated secondary ions on a microchannel plate-fluorescent screen detector. This image can be digitized with a charge-coupled-device (CCD) camera and subsequently processed by computer. The result is a quantitative mapping of the trace-element and isotopic distributions over the entire field of view with a spatial resolution of about 1 μm .

The ion microprobe approach employs a finely focused primary ion beam, typically with a diameter of 1–10 μm , to sputter a small region of a sample. Secondary ion images can be produced by rastering the primary beam over the specimen and using the secondary ion intensity (normally measured with an electron-multiplier, ion-counting detector) to modulate a synchro-



nously rastered video monitor. Lateral resolution is determined by the primary beam diameter and can approach 0.2 μm .

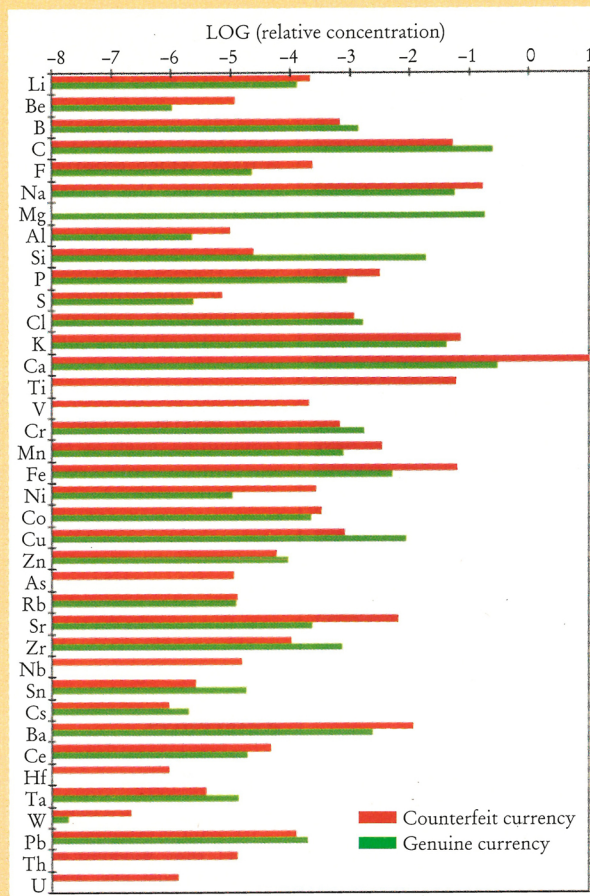
Although the microscope mode offers greater sensitivity, superior depth resolution and diminished sample charging give the microprobe approach greater utility for most forensic applications.

Box 4. Counterfeit Currency

For the past several years, counterfeit US currency of exceptionally high quality has been intercepted by law enforcement authorities. Identifying the sources of illicit currency is a primary mission of the US Secret Service, and the Forensic Science Center has supported the agency's work by applying secondary ion mass spectrometry (SIMS) to the measurement of the trace element "fingerprints" of paper and inks in counterfeit banknotes.

Trace analytes in the paper are concentrated in particles of rutile (a mineral form of titanium dioxide) that are characteristically a few micrometers across. Their small size precludes sensitive and specific elemental analysis by conventional techniques, but SIMS can be used to delineate striking chemical differences between genuine and counterfeit banknotes.

Rutile is used as a filler and coloring agent in currency paper. Natural rutiles contain iron, vanadium, niobium and tantalum, whose concentrations—along with those of other minor constituents—serve to identify the mine that produced the rutile. The synthetic rutile used in US currency, by contrast, is chemically very pure, and its size distribution sharply peaks below $1\ \mu\text{m}$. SIMS analyses in one FSC investigation of counterfeit notes measured anomalously large rutile particles that were extremely enriched in tungsten. These specimens contained up to 0.5%



by weight of tungsten (a proportion that varied by 100 times or more from grain to grain) and were also unusually enriched in sodium, aluminum, silicon and calcium. This composition was quite distinct from those of natural rutiles and indicated that the counterfeit contained synthetic rutile produced in a relatively unsophisticated manufacturing process.

Another feature of contemporary counterfeiting is the use of intaglio printing, which imprints bills by means of an etched plate to impart a distinctive, embossed quality. Intaglio ink was found to be unexpectedly rich in trace elements. The accompanying figure shows a detailed SIMS comparison of the relative abundances of 38 elements in green intaglio ink layers, only a few micrometers thick, in genuine and counterfeit banknotes. Several striking differences in chemical composition were evident. This particular counterfeit ink contained concentrations of titanium, vanadium, arsenic, niobium, hafnium, thorium and uranium at least 100 times higher than in genuine inks, while the inverse was true for the analytes of magnesium and silicon. Such characteristic differences in composition constitute a diagnostic signature, and it is a potential evidentiary link to establish the source of the counterfeit currency.

men is often interrogated with many complementary technologies—especially if it is completely unknown, or if such efforts are appropriate in an important case.

For example, in conjunction with SIMS analysis of trace-element signatures as discussed in box 4, counterfeit currency has also been analyzed at LLNL by using thin-layer chromatography and gas chromatography-mass spectrometry for organic ink components, x-ray fluorescence for nondestructive elemental screening, thermal ionization mass spectrometry for lead isotope ratios, polymerase chain reaction amplification for DNA constituents and accelerator mass spectrometry for carbon-14 dating.

The FSC has recently begun to provide technical field support to Federal law enforcement for threat assessment, nuclear forensics and illicit material countersmuggling. However, our first nuclear forensic investigation in the public domain occurred after the fatal explosion of a "cold fusion" electrochemical cell on 2 January 1992.

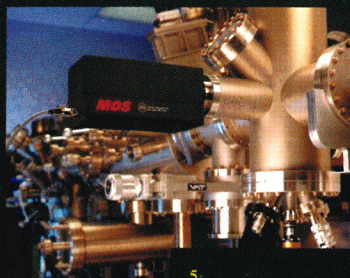
Following the accident, which took place in an electrochemistry laboratory at SRI International in Menlo Park, California, the Occupational Safety and Health Administration asked us to perform general forensic analysis of selected debris (figures 2 and 3) to determine any potential causes of the blast. Gamma-ray spectrometry

was conducted to search for activation products that might have been produced by a possible pulse of neutrons associated with an hypothesized cold fusion excursion.¹⁰ More-comprehensive forensic analyses were also performed, involving approximately 65 LLNL staff members from the disciplines of chemistry, physics, nuclear science, materials science and engineering.¹¹ The results were quite unexpected and indicated that explosive oxidation of residual lubricating oil could have initiated or contributed to the total energy inventory of the incident.¹²

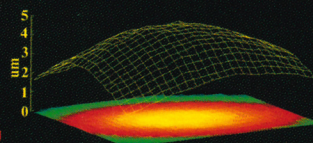
Other diverse forensic research efforts undertaken at the FSC have included the development of capillary electrophoresis with radioactivity and photothermal deflection detection modalities (analytical science),¹³ the nondestructive interrogation of latent registration numbers on vehicle licenses (laser science)¹⁴ and a detailed characterization of pepper spray formulations (chemistry).¹⁵

Perhaps the most publicized and controversial of FSC investigations to date has been the analysis of the Riverside General Hospital (California) "mystery fumes" incident.¹⁶ When emergency room personnel were seriously incapacitated while treating a dying cancer patient, extensive analyses of the episode by numerous health and safety organizations yielded no satisfactory scientific ex-

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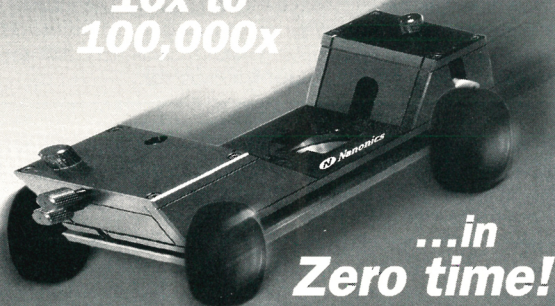
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planation. However, through a combination of chemical and toxicologic reasoning, the FSC proposed a detailed and specific explanation. The patient was suspected of having ingested a significant quantity of dimethyl sulfoxide (DMSO). In a process perhaps accelerated by the patient's final emergency therapy and by the unusual enzymes and free radicals present in the dying patient, the DMSO oxidized to produce the very toxic dimethyl sulfate, which entered the atmosphere in the emergency room when the patient's body fluids were withdrawn or lost.¹⁷

Rarely do FSC scientists perform analyses or interpret their results in isolation. A project coordinator is responsible for integrating all forensic information in a given investigation, and the resulting scenario must be consistent with all observations. Interpretation of forensic data generally ensues from conversations among analysts from different disciplines, who deliberate and correlate results from diverse sample interrogations. Advanced physical techniques, such as those discussed in this article, add to the multidisciplinary arsenal available to modern forensic scientists and often provide the crucial knowledge that leads to a successful investigation.

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