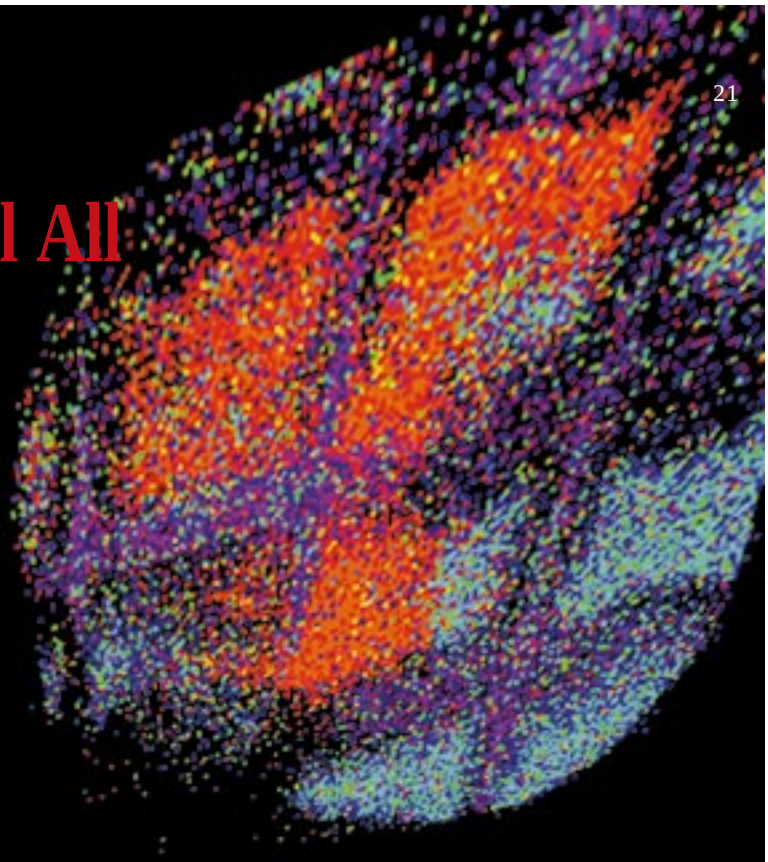
When Collisions Reveal All



AS researchers focus on ever-smaller dimensions to engineer advanced materials, they increasingly demand new tools to scrutinize these materials. The need is particularly acute for semiconductor chip makers as they continue to shrink the size of chips and their internal features. Lawrence Livermore researchers also want a better way to image and characterize the all-important surfaces of critical materials.

Now a team from Livermore's Physics and Space Technology, Chemistry and Materials Science, and Engineering directorates has developed a diagnostic instrument called a time-of-flight secondary ion mass spectrometry (SIMS) emission microscope. For the first time, the instrument simultaneously provides extremely sensitive surface analysis, high-resolution imaging, and chemical determination of surface constituents. Recent tests on a variety of materials show that the new microscope may well prove valuable in solving vexing surface analysis problems in fields as diverse as precision optics and amino acid sequencing.

SIMS is a widespread technique in which a stream of energetic, primary ions bombards the surface of a material under investigation. Upon impact, these ions generate positively and negatively charged secondary ions, which are gathered by electrically charged lenses, imaged, and identified. (Neutral atoms and molecules are also given off but are not detected.)

NASA scientists used the first SIMS instrument in the 1960s to analyze moon rocks. Today, SIMS is widely used for analyzing trace elements and contaminants in solid materials, especially semiconductors and thin films.

Traditional SIMS instruments employ a stream of single-charged primary ions (for example, xenon +1) to bombard a sample. With this technique, about a thousand bombardments are needed to produce one secondary ion, a slow process during which a spectrum of surface constituents is gradually built up.

Greater "Pop"

The new Livermore instrument uses not single-charged, but multiple-charged ions (for example, gold +69), which produce a thousandfold increase in secondary ions. "Highly charged ions make our instrument unique," says materials scientist Alex Hamza. "The higher the charge, the greater the 'pop,' the more ions that come off." More ions mean more—and faster—information about the composition of the surface layer, including any contaminants.

Hamza says studies at Livermore show that during the first few femtoseconds (quadrillionths of a second) of impact, the highly charged ions deposit a huge amount of potential energy into a surface area several nanometers (billionths of a meter) square. In contrast, single-charged ions deposit large amounts of kinetic, not potential, energy. This kinetic energy transfer is not localized at the surface but is distributed more deeply into the sample.

Although the exact mechanism of highly charged ion energy transfer isn't fully elucidated, Hamza says it is probable that electrons from nearby surface atoms are attracted to the strongly positive primary ion. The resulting electron transfer removes the "glue" that once held the nearby atoms in place, allowing them to fly off. As they leave the surface, they are attracted to the electrostatic lens of the microscope and accelerated to a detector located about a half meter from the sample. Finally, an image of the surface magnified at from 40 to 400 times is created (Figure 1).

The chemical determination of the secondary ions is performed through time-of-flight techniques in which the time a secondary ion takes to arrive at the detector is directly related to the mass. Histograms of the arrival times are built up to form mass spectra of the secondary ions emitted from the sample (Figure 2). With a collision rate of about a thousand per second, the Livermore instrument takes roughly 15 minutes (corresponding to about a million events) to build up a useful image such as that in Figure 1.

Because of the number of secondary ions produced per collision and the small area being investigated, the microscope

is particularly useful in determining the location of secondary ions through coincidence counting. By seeing what molecules come off together from the impact of primary ions, the instrument can reveal impurities in the location of interest. This feature is particularly important as chips shrink and can be contaminated by fewer impure atoms or molecules, which, nevertheless, must be detected.

Focusing on Sensitivity, Resolution

The new Livermore instrument can detect 10 parts per million, a sensitivity equal to that found in a typical SIMS instrument. Resolution of feature sizes has been demonstrated at 6 micrometers. The development team is confident it can achieve resolutions down to 10 nanometers within a year through better lens design and improved detector resolution.

The instrument uses beams of highly charged ions generated by the electron-beam ion trap (EBIT), developed by a Lawrence Livermore research team a few years ago. With this device, the charge, energy, and mass of the primary beam can be varied independently. Electrostatic lenses and apertures control the intensity and width of the primary ion beam.

Conceived, designed, and fabricated by Livermore physicist Alan Barnes with the assistance of mechanical engineer Ed Magee, the SIMS emission microscope features a novel "acorn-shaped" objective lens used to image the secondary ions while a sensitive detector determines the up–down position and time of arrival of the secondary ions at the microscope image plane. Contrast in the image can be based on the intensity of the electrons detected or the presence of particular secondary ions.

Because of this technology's potential importance to the semiconductor industry, the team has used it to analyze the deposition of tungsten on patterned silicon wafers, a common step in computer chip manufacturing. Figure 1 is an image collected from a wire-mesh-covered sample of a silicon wafer patterned with silicon dioxide and tungsten. The colors in the image indicate the type of secondary ion observed, as measured by time of flight: red indicates a tungsten-related region and blue a silicon dioxide–dominated region.

By selecting events from the blue and red regions, researchers can provide a spatially resolved analysis of the surface composition. Figure 2a shows the time-of-flight spectrum of the blue region, while Figure 2b is the spectrum from the red region. Both spectra reveal the outstanding sensitivity of the instrument.

According to Hamza, an impressive array of instruments is available to image materials with very high resolution—examples include transmission electron microscopy, scanning electron microscopy, and scanning tunneling microscopy. An equally impressive array of instruments and techniques is available to determine material composition (Auger electron spectroscopy, photoelectron spectroscopy, and SIMS). However, no other technique combines high-resolution images, high sensitivity to trace elements, and the chemical structure of the secondary ions, all in one package.

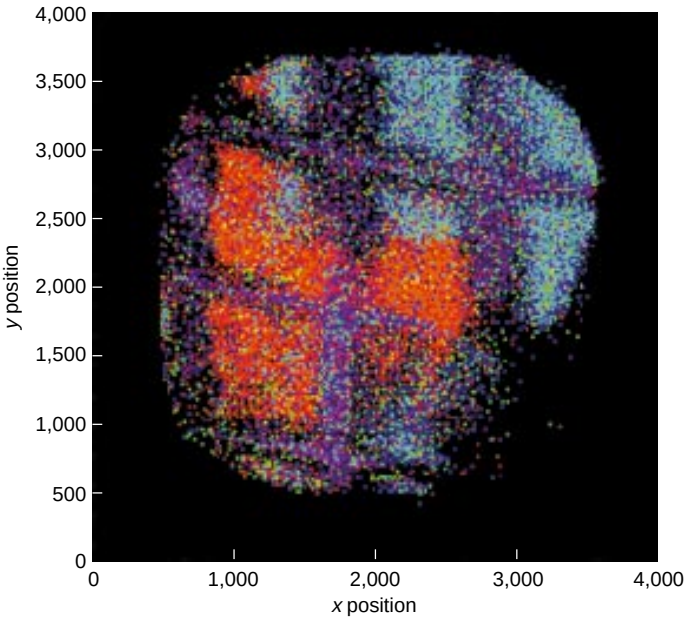


Figure 1. Image of a 70-line-per-inch copper grid over a silicon wafer. The colors indicate the mass of the secondary ions measured. Blue and green indicate silicon dioxide features of the material, while red and orange indicate tungsten features. Purple is chlorine and carbon contamination. The lines are 360 micrometers apart, and the smallest observed feature is 6.4 micrometers wide.

An Eye on the Future

The first Livermore instrument was built to demonstrate the concepts necessary to construct more powerful versions. Plans for the next two years include improved resolution, data collection, and primary beam focusing. To image smaller areas, the team will experiment with using ion streams chilled to low temperatures.

Hamza reports significant interest from Semitech, a national semiconductor industry forum. Semitech officials have suggested that semiconductor companies could send samples to a central location housing several of the Livermore microscopes.

As for other applications, the research team sees significant potential wherever chemical structure must be determined at high resolution. A natural fit is stockpile surveillance activities such as investigating corrosion in metals and inspecting high explosives to determine their reliability. In fact, the research team has already used the new microscope to examine the distribution of high-explosive molecules in their binding material—a factor affecting reliability.

Another important application is the investigation of possible links between glass failure and polishing residue in optical components used in powerful lasers such as Lawrence Livermore's forthcoming National Ignition Facility. One intriguing application is analyzing biological materials. By using a highly charged ion stream to break molecular bonds, the microscope could be used to determine the sequence of amino acids forming proteins and thereby become a powerful tool used in molecular biology as well as forensics.

If planned refinements succeed, the instrument could well become a mainstay in research laboratories everywhere.

—Arnie Heller

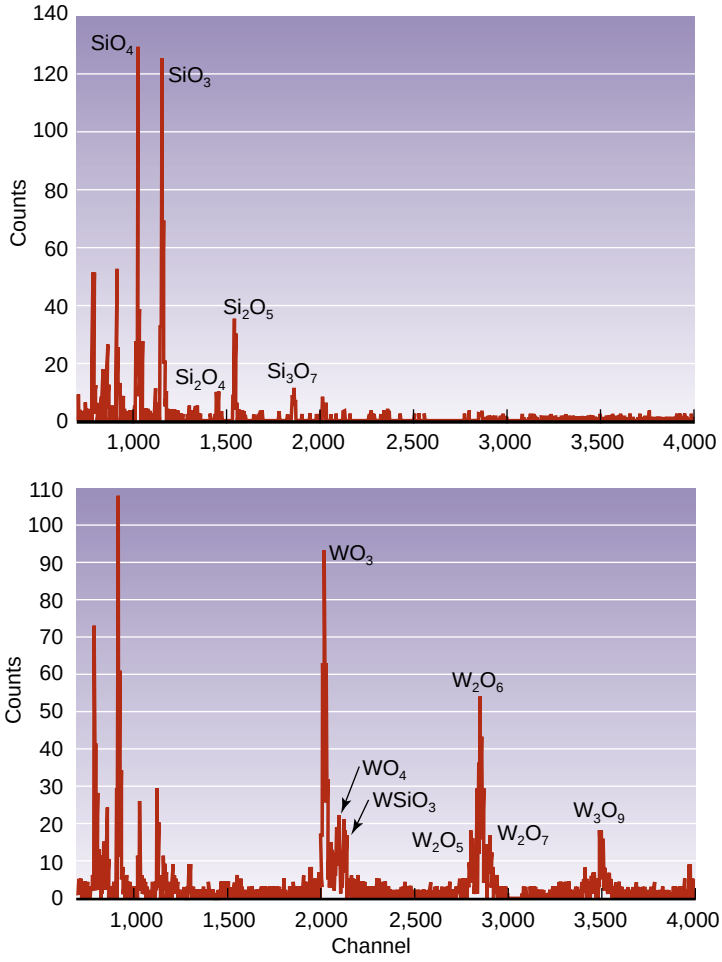


Figure 2. (a) The time-of-flight spectrum of the blue region in Figure 1 dominated by silicon compounds (SiO_x). (b) The time-of-flight spectrum from the red region in Figure 1 dominated by tungsten compounds (WO_x).

Key Words: highly charged ions, SIMS (secondary ion mass spectrometry), time-of-flight secondary ion mass spectrometry (SIMS) emission microscope.

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Collaboration Opens Door to Understanding Genetic Kidney Disorder

THE search for genes—just as for gold—may be long and arduous, but the rewards are great. Genes and the proteins they produce hold the keys that unlock the mysteries of genetic diseases and allow the development of gene and drug therapies. The worldwide Human Genome Project has the ultimate goals of finding all the genes in the DNA sequence, developing tools for using this information to study human biology and medicine, and improving human health.

The task of locating a particular gene in the human genome can be more daunting than searching for a vein of precious ore underground. For perspective, the human body has 100 trillion cells, each of which contains 23 pairs of chromosomes. Each chromosome carries a complete set of DNA. If the DNA of one cell, which contains about three billion nucleic acid units or "base pairs," were formed into a single continuous strand, it would stretch six feet long. (The four chemical "bases"—adenine, thymine, guanine, and cytosine—bind together to form base pairs that are the building blocks of DNA.) About 3 percent of this DNA forms working genes. The task facing gene hunters is to search through the "genetic junk" of the human genome to find that one string of DNA that comprises the gene in question.

It's a task that makes digging for gold in a mountain of dirt and rock look easy.

The Search for a Kidney Disease Gene

For many years, researchers from the Karolinska Institute in Stockholm, Sweden, and the University of Oulu in Finland had been seeking the gene for congenital nephrotic syndrome, an inherited kidney disease that causes massive amounts of proteins to be excreted by the kidneys. The disorder, which occurs primarily in families of Finnish origin, develops shortly after birth and usually causes death within a year. The only alternative for this progressive disease is a kidney transplant.

By 1993, the researchers, led by medical chemistry professor Karl Tryggvason from Karolinska Institute's Department of Medical Biochemistry and Biophysics, had narrowed their search to chromosome 19. Because Lawrence Livermore is well known for its mapping and sequencing of chromosome 19, Tryggvason contacted Livermore biomedical scientist Anne Olsen for assistance.

"Other laboratories and institutions are sequencing pieces of 19, but we are the only one addressing the entire chromosome," Olsen explained. (For more information about the Laboratory's work in DNA sequencing, see *S&TR*, November 1996, pp. 24–26, and July/August 1997, pp. 18–20.)

When the European researchers contacted Livermore, they knew where the gene resided on the genetic linkage map, but not on the physical map of chromosome 19. (See the box on p. 25.) In 1993, the physical map of chromosome 19 was less developed than it is today. Olsen and other biomedical scientists worked for more than a year to complete a physical map of the genetic region in question, providing the European team with well-characterized DNA fragments or clones. The collaborators used those cloned pieces of chromosome 19 to further narrow down the site of the fatal gene, tracing it to an area containing 150,000 base pairs.

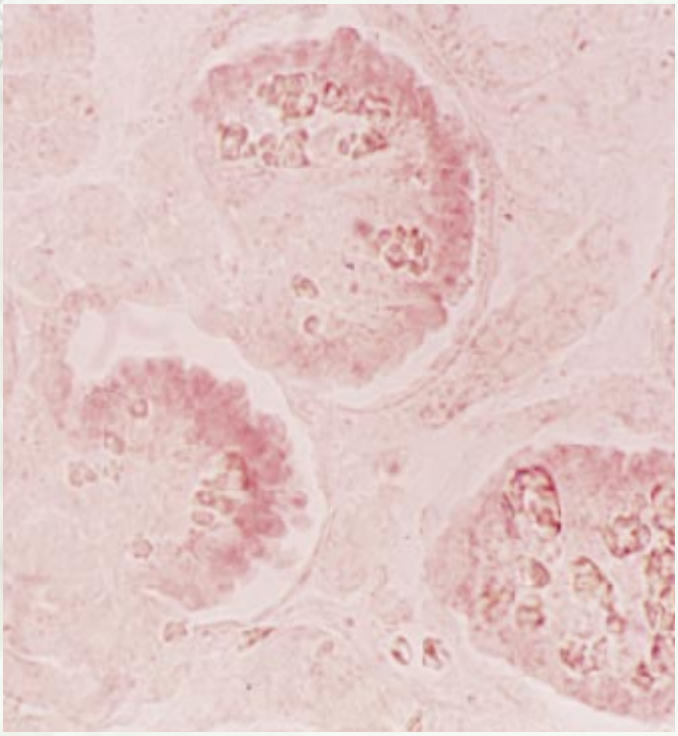
Narrowing Down the Search

At this point, the teams had gone as far as they could go with physical mapping, and it was time to sequence the individual base pairs to determine their exact order on the chromosome. Jane Lamerdin and Paula McCready led another Lawrence Livermore team that sequenced the bases using the Laboratory's high-throughput sequencing machines. The Finnish collaborators used the clones provided by Livermore for biological analysis and located 11 likely genes in the candidate region.

Of those genes, they finally narrowed it down to one. That particular gene was mutated in the families carrying the disease, and the protein associated with the gene was well-expressed in the kidneys.

"Even though our part is done, the story is just beginning," said Olsen. "Since the main symptom of this disease—protein excreted in the urine—appears in other conditions, this work may offer insights into other kidney ailments as well."

The breakthrough was announced in March 1998 when a paper on the research appeared in the journal *Molecular Cell*



Expression of the congenital nephrotic syndrome kidney gene (Nephrin) in the cellular material from a blood vessel in the kidney of a human embryo. Nephrin was discovered by Karl Tryggvason and his team of Finnish and Swedish researchers with the assistance of Livermore genetic scientists Anne Olsen, Jane Lamerdin, and Paula McCready. (Image courtesy of Karl Tryggvason of the Division of Matrix Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden, and the Biocenter and Department of Biochemistry, University of Oulu, Oulu, Finland.)

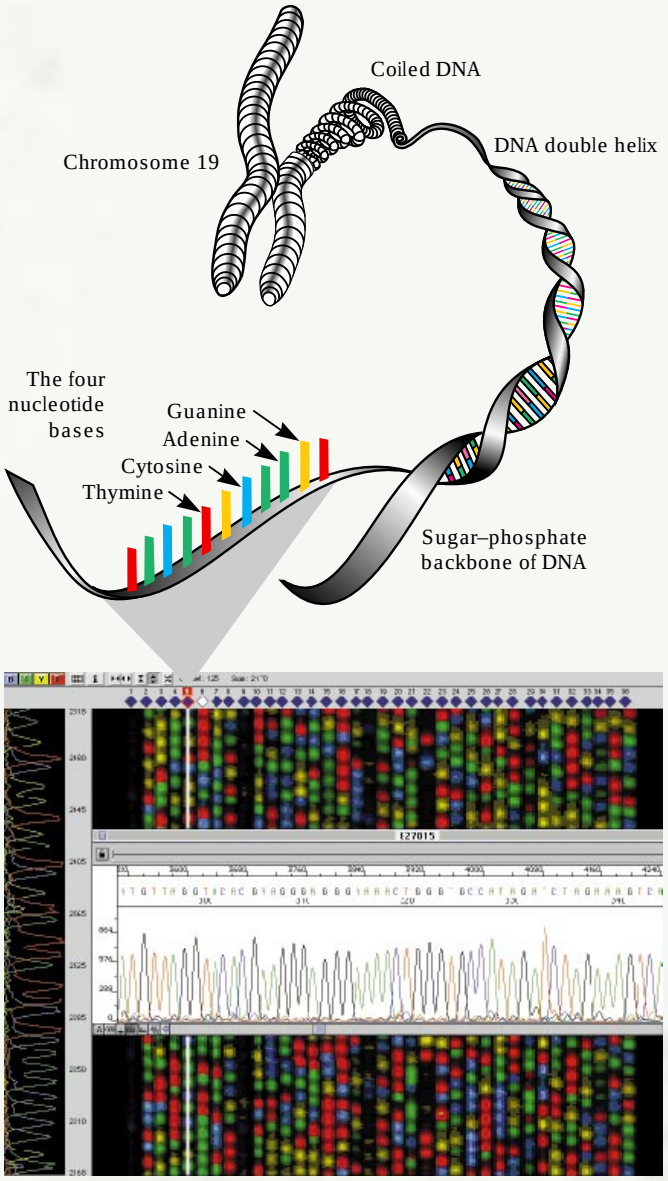
A Primer on Maps, Markers, and Sequencing

A genome map describes the order of genes or other markers and the spacing between them on each chromosome. Human genome maps are constructed on several different scales or levels of resolution. At the coarsest resolution are genetic linkage maps, constructed by observing how frequently two markers are inherited together. These maps depict the relative chromosomal locations of DNA markers (genes and other identifiable DNA sequences) by their patterns of inheritance. Two markers near each other on the same chromosome tend to be passed together from parent to child. During the normal production of sperm and egg cells, DNA strands occasionally break and rejoin in different places on the same chromosome or on the other copy of the same chromosome. This process— called meiotic recombination— can result in the separation of two markers originally on the same chromosome. The closer the markers are to each other, the more tightly linked they are, making it less likely a recombination event will separate them. Recombination frequency thus provides an estimate of the distance between two markers.

The value of the genetic map is that an inherited disease can be located on the map by following the inheritance of a DNA marker, even though the responsible gene is not yet identified.

Physical maps, in contrast, provide a finer resolution of the absolute location of a gene. A physical map lays out the order of all the base pairs on a chromosome. The ultimate physical map of the human genome is the complete DNA sequence, or the determination of all base pairs on each chromosome.

For more information about basic genetics as well as mapping and sequencing techniques, see the U.S. Department of Energy's "Primer on Molecular Genetics" on the World Wide Web at *http://www.ornl.gov/TechResources/Human Genome/publicat/primer/intro.html*.



At the lower left is a portion of a sequencing gel produced recently at Lawrence Livermore using the latest mapping and sequencing technology. Rapid advances in mapping and sequencing techniques and technologies are enabling researchers to find specific genes faster than they could just a few years ago when Livermore researchers collaborated with Swedish and Finnish colleagues in their search for the kidney disorder gene Nephrin.

(March 1998, pp. 575–582). This announcement came hot on the heels of another genetic discovery involving a rare hereditary susceptibility to a variety of cancers—the Peutz–Jeghers syndrome. Pinpointing the location of the Peutz–Jeghers gene took only one year.

"The difference in time indicates how far we've come with mapping and sequencing techniques and technologies over the past few years," Olsen said. "When Karl Tryggvason first contacted us about the kidney disease gene, we didn't have a highly developed physical map for that region. Three years later, when we were asked to collaborate on the search for the Peutz–Jeghers gene, our map was much better developed, and that search went more quickly. Better, more detailed maps mean the search for genes will only accelerate in the future."

—Ann Parker

Key Words: chromosome 19, congenital nephrotic syndrome, DNA clones, DNA mapping and sequencing, Human Genome Project, kidney disease, Peutz–Jeghers syndrome.

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