A MATERIAL WORLD

By RICHARD LIPKIN

A deep look at the ties that bond

Imagine this scenario. A large, loosely fastened bedspring hangs in a pitch-black room. In the darkness, with only a bucket of baseballs at your feet, you stand facing that invisible bedspring.

Your job is to figure out what the bedspring looks like — or, more precisely, how it is physically constructed.

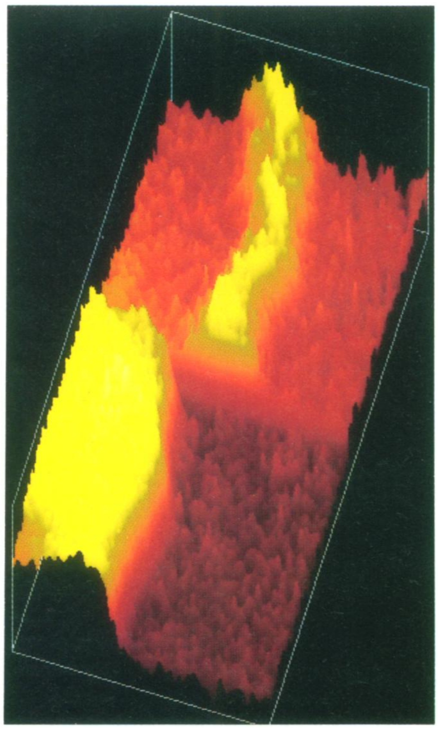
So you begin throwing baseballs at the bedspring. You notice that most baseballs go straight through. Some bounce back. Others rebound at strange angles. A few even get stuck. Continuing, you also notice that each time a ball hits a single coil, you hear a distinctive ringing. The sound's pitch, created by the coil's unique vibration, varies depending on how fast you throw the ball and where the ball strikes. Soon, you discover that those vibrations relate to the amount of energy the ball imparts to a given coil, as well as that coil's size and shape.

Now, you devise a plan. You build a machine that throws balls at the bedspring with an exact speed and direction, then tracks the angles of their rebounds, the forces they carry, and the energies they have imparted to the bedspring. After throwing a few million balls, you take all the data and feed them into a computer, which figures out roughly what the bedspring looks like, how it's built, and the nature of the material from which it is made.

While not literally true, this analogy captures a sense of the process used by some physical chemists to determine the structure of certain materials, right down to the level of individual atoms. Think of the balls as electrons or other high-energy particles and the bedspring as a well-ordered material.

Indeed, advances in analytical microscopy during the past decade have enabled researchers to probe the depths of matter with surprising precision. The ability to see individual atoms is slowly coming into reach. Today, scientists can detect in specific regions of certain materials exactly what atoms are present, where they are located, and how they bond together — doing so with previously unattainable accuracy.

Analytical electron microscopy, which combines more than one detection technique, is among the most innovative methods for delving into matter's nooks and crannies. One impressive pairing brings together scanning transmission electron microscopy (STEM) and electron energy loss spectroscopy (EELS).



Muller *et al.*/NATURE

A computer-enhanced STEM-EELS image of the interface between a diamond film and a silicon surface. The top yellow region shows amorphous carbon atoms just above the interface. The lower orange region reveals a diamond film.

When it comes to detecting objects on the atomic scale, STEM and EELS offer advantages and disadvantages — sensing some things well, others poorly. Yet when joined, the two methods can yield enough information to piece together a reasonably detailed picture of just a few atoms in a specific region of a crystal. In recent months, scientists have achieved unprecedented atomic resolutions this way.

The technique involves passing a thin stream of high-energy electrons through wafer-thin, 100-nanometer slices of matter. STEM collects information revealing the basic structure and spatial arrangement of the atoms, which are stacked up in columns. EELS then detects how atoms in the slices have deflected those electrons and how much energy each electron has yielded to the atoms in its path. With those data, EELS can identify the elements present based on each atom's unique spectrum.

From the combined STEM and EELS information, researchers can determine the identities of individual atoms, their exact locations, and the nature of the bonds between them.

"Remember that we're using these techniques to investigate incredibly small bits of matter," says physical chemist Dale E. Newbury of the National Institute of Standards and Technology in Gaithersburg, Md. "The electron-beam diameter of our system is only 1 nanometer. If we shoot that beam through a 50-nanometer film, we're talking about exciting an extraordinarily small amount of matter — a single column of atoms with a mass of 10^{-19} grams. That's really small."

First conceived in the 1930s, EELS remained largely neglected, owing to technical inefficiencies, until the mid-1970s. Better electron-beam detectors and improved ways of collecting and interpreting spectral data have renewed interest in EELS as a basic research tool.

EELS offers another advantage. Electrons from the microscope's beam "travel more or less in the same direction coming out of the specimen as they did going in. They don't scatter very much," Newbury says. "The beam continues straight down the column of the instrument. So, with a relatively modest detector, you can get a significant fraction of the available signal, a feature that gives EELS a great advantage over X-ray methods."

Recently, Newbury and Richard D. Leapman, a physicist at the National Institutes of Health in Bethesda, Md., reported "unprecedented sensitivity" in detecting trace elements — in the parts-per-million range — in materials derived from both living and nonliving sources.

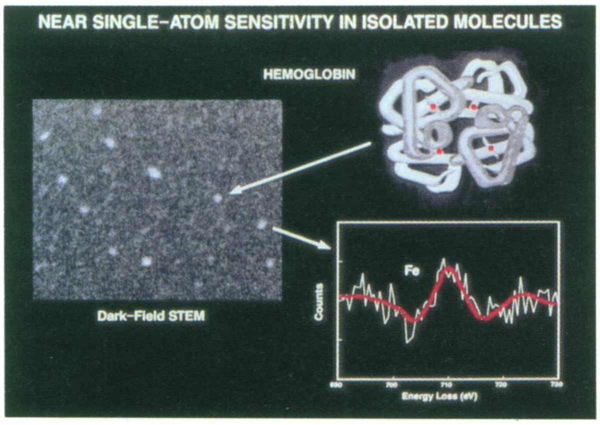
Using STEM and EELS together — STEM for basic atomic structure, EELS for identifying elements — the chemists could distinguish concentrations of trace elements below 10 parts per million in regions of a specimen only 10 nanometers wide, "which translates to near single-atom sensitivity."

"What's really significant here," says Newbury, "is that we've shown for the first time that not only can we measure very small amounts of matter, but that we can measure extremely dilute trace elements. Normally, they wouldn't even show up. We measured samples with about 1 million atoms in total and could see 50 of one type, 50 of another. Not only can we see a tiny mass, but we can detect a tiny fraction of that tiny mass. We've never been able to do that before."

Typically when chemists look for trace elements in a lump of matter, they can tell that it contains a few atoms of a particular type mixed in among the material's millions of other particles. But where are those elements? No one knows.

"Now we can ask, how are those trace elements distributed? Are they uniformly distributed, or are they in a little clump somewhere?" says Newbury. "That can have great significance in terms of how a material behaves, whether it's in a semiconductor or a cell."

In Leapman's lab at NIH, he and Newbury have employed STEM and EELS to find as few as 320 copper atoms in a single hemocyanin molecule, 4 iron atoms in a hemoglobin molecule, and 200 phosphorus atoms in a strand of virus RNA. They've also found specific phosphorylation sites of cell proteins, identified immunolabeled antigens within cells, and determined the water content of cell organelles — all useful facts for deciphering basic cell mechanisms.



A STEM-EELS image of isolated hemoglobin molecules on a thin carbon film. The technique could isolate four iron atoms (shown in red) in a single molecule, as depicted in the accompanying drawing.

Leapman *et al.*

Of late, Leapman and his colleagues have been peering into freeze-dried sections of mouse brain cells, mostly from the cerebellar cortex. In the dendrites of these cells they find scant numbers of calcium ions — a fact critical for understanding the cells' signal-sending messenger molecules. So small is the calcium concentration that it remains virtually undetectable by other methods.

Understanding how those ions move in and out of cells requires detecting a change of about 10 percent — perhaps four atoms. That these atoms can be detected at all, says Leapman, gives a big edge to biologists piecing together the picture of ion transport within cortex cells, all part of a larger effort to understand how brain cells function.

"Essentially, we can look at a small region of any cell where there might be some interest in an element, such as calcium in postsynaptic terminals of brain cells," Leapman adds. "But applying EELS to biological materials isn't easy. Since the energy levels are high, the specimens can get damaged by the electron beam. So we have to quickly freeze the cells to about 20 [kelvins], to keep all of the ions in their normal positions, then cut sections about 100 nanometers thick."

Seeking details on such subtle matters as the way calcium moves within a cell, researchers cannot go far with conventional optical microscopes. "In biology, there's this hazy area between very-high-resolution techniques with X rays on the one hand, and lower-resolution optical microscopy on the other," says Leapman. "You have big molecules that aren't amenable to high-resolution analysis but are too small for optical methods. In this gray area, I think STEM and EELS give us the best data."

Much of EELS' improved sensitivity derives from new parallel-array detectors.

"Parallel detection was a critical breakthrough," says Newbury. "We used to measure a spectrum one increment at a time, which was very slow. But the parallel detectors let us measure 1,000 increments simultaneously."

Using charge-coupled devices (CCDs), researchers can convert many parts of a spectrum directly to digital data, which are easily managed by computers. Better computer algorithms for collecting and analyzing raw spectral data have done much to sharpen and hasten the EELS process. "All together, these various improvements permit us to extract very minuscule signal changes from the spectral data," Newbury says.

On the cutting edge of materials research, several scientific groups have honed EELS and STEM, devising creative ways to study molecular structure and bonding. For instance, Philip E. Batson at the IBM T.J. Watson Research Center in Yorktown Heights, N.Y., employs these two methods to study silicon atoms.

Scrutinizing a sliver of silicon oxide less than 50 nanometers thick, Batson has extracted key details about the molecular interface created when atoms of silicon bond to atoms of the oxide. Such clarity can help scientists divine what gives crystals their unique properties. His results "show convincingly" that EELS data can reveal critical bonding and electronic features, Batson said in the Dec. 23/30, 1993 NATURE.

Along similar lines, David A. Muller, a physicist at Cornell University, is struggling to learn how diamond films form on silicon, a process that has many potential industrial applications. To attain such an understanding, however, scientists need to know more precisely how diamonds nucleate, or form seed crystals. In the same issue of NATURE, Muller explains how STEM and EELS reveal subtleties of carbon bonding at the interface between diamond and silicon.

"We're trying to make out the fine features of carbon atoms, so in essence we've made a bonding map of carbon," Muller says. "Now not only can we say that a particular atom is carbon, but we can show how it's bonded to other atoms around it. This information will help us understand in detail, for example, why graphite is soft and diamond is hard, even though they're both made of carbon."

"There are many theories about how diamond grows, but the process is not well understood," Muller adds. "By looking at how carbon atoms behave right on a boundary, we may get some clues."

Batson's and Muller's reports follow another by Nigel D. Browning, a physicist at Oak Ridge (Tenn.) National Laboratory. In the Nov. 11, 1993 NATURE, Browning tells how STEM and EELS permitted him to see single-atom columns at the interface between cobalt silicide and silicon. With a very fine electron beam probe, he first distinguished and excited specific columns of atoms, then generated a "compositional map," proving that "atomic resolution microanalysis from a single column [of atoms] is possible in principle."

"The beauty of this technique," says Browning, "is that, right there, at the interface, you can see exactly what's happening."

What difference could that make?

"Let's say you want to make a high-temperature superconductor, for instance," Browning explains. "And let's say that you want to know what's going on at the interface between the superconductor and a normal material or between two superconductors. Or suppose you need to look at a defect. The only reliable way to characterize what's happening at that interface, in terms of electronic structure, is to use EELS and STEM together." □