JESSE WINTER FOR *NATURE*

PUSHING THE LIMITS

Technological advances are triggering a revolution in electron microscopy.

BY RACHEL COURTLAND

Scientists can't study what they can't measure — as David Muller knows only too well. An applied physicist, Muller has been grappling for years with the limitations of the best imaging tools available as he seeks to probe materials at the atomic scale.

One particularly vexing quarry has been ultra-thin layers of the material molybdenum disulfide, which show promise for building thin, flexible electronics. Muller and his colleagues at Cornell University in Ithaca, New York, have spent years peering at MoS_2 samples under an electron microscope to discern their atomic structures. The problem was seeing the sulfur atoms clearly, Muller says. Raising the energy of the electron beam would sharpen the image, but knock atoms out of the MoS_2 sheet in the process. Anyone hoping to say something definitive about defects in the structure would have to guess. "It would take a lot of courage, and maybe half the time, you'd be right," he says.

This July, Muller's team reported a breakthrough. Using an ultra-sensitive detector that the researchers had created and a special method for reconstructing the data, they resolved features in MoS_2 down to 0.39 angstroms[1], two and a half times better than a conventional electron microscope would achieve. (1 Å is one-tenth of a nanometre, and a common measure of atomic bond lengths.) At once, formerly fuzzy sulfur atoms now showed up clearly — and so did 'holes' where they were absent. Ordinary electron microscopy is "like flying propeller planes", Muller says. "Now we have a jet."

Muller's images represent the latest of a burst of technological advances that are triggering a revolution in what researchers can probe using transmission electron microscopes (TEMs) — devices as tall as a room that send beams of electrons through samples to explore structures down to a size scale smaller than an atom. The machines promise to give scientists the ability to see details previously out of reach, from the structure of fragile next-generation electronics materials, to the innards of porous substances that can separate gases.

The excitement isn't just about high-resolution images. The new capabilities also let researchers explore invisible properties of materials as never before, including electric and magnetic fields as well as hard-to-detect vibrations inside crystals. And some researchers are converting the vacuum-filled

David Muller with his team's electron microscope.

interiors of electron microscopes into tiny laboratories, so that they can study how samples behave when they are exposed to liquids and gases or varying temperatures.

A large contributor to the improvements has been speedy detectors that are sensitive to electrons. Early incarnations of these detectors have already made an impact on biology, revealing details about the construction of proteins and other substances that would be time-consuming — if not impossible — to measure through conventional X-ray crystallography. But researchers say that many of the rewards of these fresh capabilities are only now within reach — particularly when it comes to the study of nanomaterials and other synthetic systems. For a long time, people were "figuring out what you can do at all", says Haimei Zheng, a materials scientist at Lawrence Berkeley National Laboratory in California. "I think that this field is now getting ready to address more significant questions."

NEW RESOLUTIONS

In some ways, the electron microscope hasn't changed much since its introduction in the 1930s. The modern TEM still shoots a beam of electrons through a sample. At the far end, a detector then registers the resulting image, or researchers can use information from scattered electrons to reconstruct the sample's structure. Because electrons can have wavelengths that are thousands of times shorter than those of visible light, they are able to resolve much finer details than can an ordinary optical microscope.

Although this basic design has stayed intact, the resolving power of TEMs has improved by a factor of more than 1,000. The last big leap got its start around 20 years ago, with the emergence of electromagnets that could correct for distortions in the electron beam. By the late 2000s, these long-awaited aberration correctors had enabled advanced TEMs to reach sub-angstrom resolution.

"For materials folks, aberration correctors were a big revolution," Muller says. "It not only let you see every kind of atom that you wanted to see, but it also let you work much quicker than you worked before." But to take full advantage of this jump in resolution, microscopists still had to deliver intense doses of electron beams to their samples — which meant that fragile materials, including anything biological, would be damaged.

Biologists were quick to leap on another innovation. For many years, the best electronic method for taking TEM images began with radiation-sensitive scintillators, which were used to convert incoming electrons into photons that could then be detected. But the process was indirect and inefficient and led to a lot of blurring.

That changed in the early 2010s, when 'direct-electron detectors' became widely available. Such devices could directly and efficiently register electrons, generating cleaner images from fewer incoming particles.

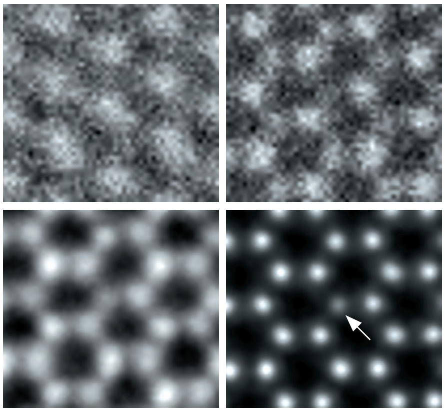
Biologists paired these detectors with frozen samples to create a TEM technique called cryo-electron microscopy (cryo-EM), which has illuminated the structures of a wide range of biomolecules. Last year, three pioneers of the approach won the Nobel Prize in Chemistry for their work.

For many materials scientists, Muller says, these detectors held less appeal. For one thing, they couldn't tolerate many electrons per pixel, which prevented researchers from using the kind of high-intensity beam they would need to observe objects at the tiniest scales. The devices were especially ill-suited for scanning transmission electron microscopy (STEM), in which electrons are focused into a smaller, brighter beam that can then be moved across a sample. The problem was that the cryo-EM detectors were not designed to capture both the flood of electrons that pass undiverted through the sample and the small fraction that get deflected from their original path, which is crucial in STEM.

> "WE ARE MAKING STEPS IN UNDERSTANDING THE FUNDAMENTALS OF SCIENCE."

A decade ago, Muller and his colleagues began working on a detector that could nab all those electrons. Unlike those used for cryo-EM, which can have millions of pixels, the team's eventual device, called the electron microscope pixel-array detector (EMPAD), boasts fewer than 20,000 pixels. But the EMPAD is built on a half-millimetre-thick slab of silicon, so it can capture all the energy of electrons that hit it and thereby discern individual particles as well as the main beam. Muller likens the detector's million-to-one dynamic range to a back-lit picture on a sunny day. "This is a detector that would be able to get an image of all the sunspots on the Sun and the image of my friend's face in the shadow at the same time," he says.

It was this advance that allowed Muller's team to clearly image the MoS_2 slivers this year, with the aid of a computational method to process multiple scattering patterns, called ptychography[1]. But the ability to capture all the electrons scattered by a sample gives researchers much more information to work with. Electric and magnetic fields, for example, alter how electrons are scattered. In 2016, Muller and his colleagues[2] showed that they could use data collected by the EMPAD to map out the magnetic field at various points in the sample — a feat difficult to accomplish through other methods. One subject that Muller is excited to study now is skyrmions — nanometre-scale swirls of magnetism that could potentially be used to store data.



REF. 1

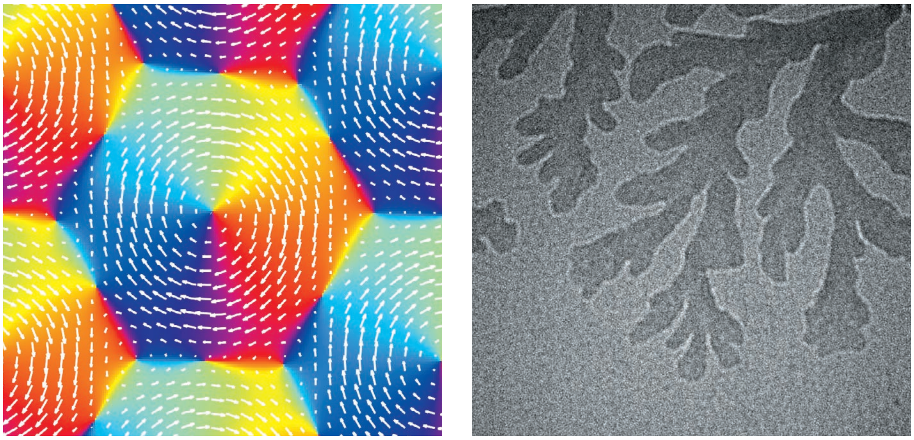
The 0.39-Å MoS_2 image (bottom right) shows a sulfur vacancy unclear in lower-resolution images.

Muller's team is not the only one to create detectors with a large dynamic range. Quantum Detectors in Oxford, UK, is one of three companies that are building electron-microscopy detectors based on Medipix, a class of chip developed at CERN, Europe's biggest particle-physics laboratory, near Geneva, Switzerland. "I think they've taken the big manufacturers by surprise," says Damien McGrouther, a microscopist at the University of Glasgow, UK, which is working with the company. Muller, meanwhile, has licensed his technology to Thermo Fisher Scientific — a large research-supplies company headquartered in Waltham, Massachusetts.

DELICATE IMAGING

Direct-electron detectors also allow the number of electrons in a beam to be reduced — and therefore used to illuminate a range of radiation-sensitive materials. These include, for instance, metal–organic frameworks (MOFs), porous crystalline materials that researchers are exploring for many uses, such as extracting moisture from desert air and separating natural gas from other hydrocarbons. These targets can be even more sensitive to electron dose than proteins are, says Ming Pan, a physicist who works in business development at Gatan, an electron-microscopy company in Pleasanton, California. In 2017, he was part of a team that imaged a MOF at atomic resolution using one of Gatan's detectors on a TEM[3].

The sensitivity and speed of direct-electron detectors, which can be faster than 1,000 frames per second, has also captured the attention of researchers working on moving electron microscopy beyond static structures. Thanks to microfabrication techniques, it is now possible to make sample holders that can do more than simply sit inside the high vacuum of an electron microscope. Researchers can control temperature, apply tension and compression, expose samples to gases and even confine liquid solutions to see how materials change in phase, structure or chemistry.



Swirls of magnetism imaged in an iron–germanium film, where colour and arrows show field direction (left). Seaweed-like iron oxide nanodendrites grow on the membrane of a liquid cell in a TEM (right).

LEFT: ZHEN CHEN AND DAVID MULLER; RIGHT: MATTHEW R. HAUWILER, WEN-I LIANG AND HAIMEI ZHENG

Many of these ideas aren't new, says Frances Ross, a materials scientist at the Massachusetts Institute of Technology in Cambridge. Combing through old papers, she was inspired to find discussions from the 1940s about how to look at water between two thin windows. "The ideas were out there," she says. "But they didn't have the materials, the fabrication techniques, to make it happen in a practical way."

Ross is widely credited with moving liquid cells into the practical realm. As a researcher at IBM in the early 2000s, she and her colleagues created a holder with a silicon nitride pane that was thin enough to allow electrons to pass through relatively unimpeded[4]. Since then, researchers have explored other materials for use in liquid cells, such as graphene[5].

At the Lawrence Berkeley laboratory, Zheng is leading a multimillion-dollar US Department of Energy programme dedicated to developing the technique further. She and others have trained a variation of a detector designed for cryo-EM on liquid samples. Among other targets, they are interested in the interface between battery electrodes and electrolytes — a crucial area in which problems such as the formation of metallic filaments called dendrites can shorten a battery's lifetime, and even cause it to explode. Such studies, she says, could help in devising ways to improve performance and investigate new battery compositions. When researchers want to test materials, they often construct small batteries called coin cells to see how the ensemble performs. But, Zheng says, that cell is "almost like a black box. They don't know what is going on inside." With liquid cells, she says, researchers have a window on to the sorts of nanoscale behaviour that ultimately determine the performance of batteries, including how the dendrites grow.

Others have trained the electron microscope on more-fundamental systems. At Eindhoven University of Technology in the Netherlands, Nico Sommerdijk and his colleagues have explored the formation of fluid-filled structures that resemble the vesicles in cells. In work yet to be published, the researchers have imaged a two-sided polymer as it self-assembles in liquid to form an artificial vesicle. And with a team led by Jim de Yoreo at the Pacific Northwest National Laboratory in Richland, Washington, Sommerdijk has studied how a polymer can bind to calcium, a process that could provide insight into how marine creatures grow the iridescent material known as nacre or mother-of-pearl. "It's not the invention of penicillin," Sommerdijk says, "but we are making steps in the fundamentals of understanding science."

Liquid-cell research has challenges. One of the biggest, says de Yoreo, is that electrons can wreak havoc when they hit water or an organic solvent, creating charged radicals that can destroy samples, shift pH or generate reducing agents that cause unintended reactions. It is also difficult to measure quantities such as pH and temperature inside the microscope.

But others are heartened by the latest research on the effect of electron beams. Patricia Abellan, a materials scientist at SuperSTEM, a research centre and user facility for advanced microscopy in Daresbury, UK, says she has seen "a revolution in the understanding of the interaction of the electron beam with matter", particularly in liquid systems. The change has been spurred in large part by collaborations with researchers who focus on studying materials affected by nuclear radiation. In the past few years, Abellan and others have explored how additives can control the growth of particles and alter pH, and how solvents other than water, such as toluene, might limit the effect of electron beams on samples in liquid[6].

BETTER BEAMS

Advances in electron microscopy have also come from improving the electron beams themselves. Devices called monochromators have allowed researchers to narrow the range of energies for electrons that reach the sample. Researchers are starting to use that tighter spread of energy, along with spectrometers and other instruments, to reach beyond the basic structure and composition of materials and map more-sophisticated properties at ever-finer resolutions. One such target is phonons — vibrations in the atomic lattice of materials. Mapping these vibrations at atomic resolution "would provide a lot of information on key processes behind most modern technology", Abellan says, such as how materials conduct electricity and heat.

Some researchers are turning the electron beam's potential to interfere with materials into a tool in its own right. Earlier this year, physicist Toma Susi at the University of Vienna and his colleagues used a STEM electron beam to move a silicon atom from site to site inside a hexagonal graphene lattice[7]. A similar sort of manipulation has been done for years on materials with weaker bonds in atomic-force and scanning tunnelling microscopes, Susi says, but in these cases, the results aren't stable. If the atoms aren't kept very cold, thermal energy erases the new structures. Electron microscopes are capable of higher-energy work. "Once something is manipulated," he says, "it really stays." Researchers hope that this ability may be useful for pushing atoms around inside 3D structures to, for example, create small devices for quantum computing[8].

At the University of Antwerp in Belgium, Johan Verbeeck is looking to make electrons into a more-sophisticated probe, by passing them through plates that can alter their phase. By embedding extra information in an electron before it passes through a sample, researchers might be able to find out more about the sample's properties. "The quest is to get more information from the same electron," says Verbeeck.

Sommerdijk points to work by Nigel Browning at the University of Liverpool, UK, who has been exploring how to control a STEM beam to minimize damage. Instead of doing a comprehensive scan, a microscope could hit a subset of points in the sample. Done right, such sparse sampling could still generate a large amount of useful data. "I think it's beautiful," says Sommerdijk, adding that it could be particularly useful in liquid studies.

Muller has his eyes on other ideas; he'd like to see, for example, whether detailed materials studies can be extended from room temperature down to cryogenic temperatures — a prospect that needs more mechanical stability than electron microscopes are currently capable of. But the field is moving fast, he says. "I don't think anyone is standing still. Everyone's thinking about what do you want to build next." ■

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