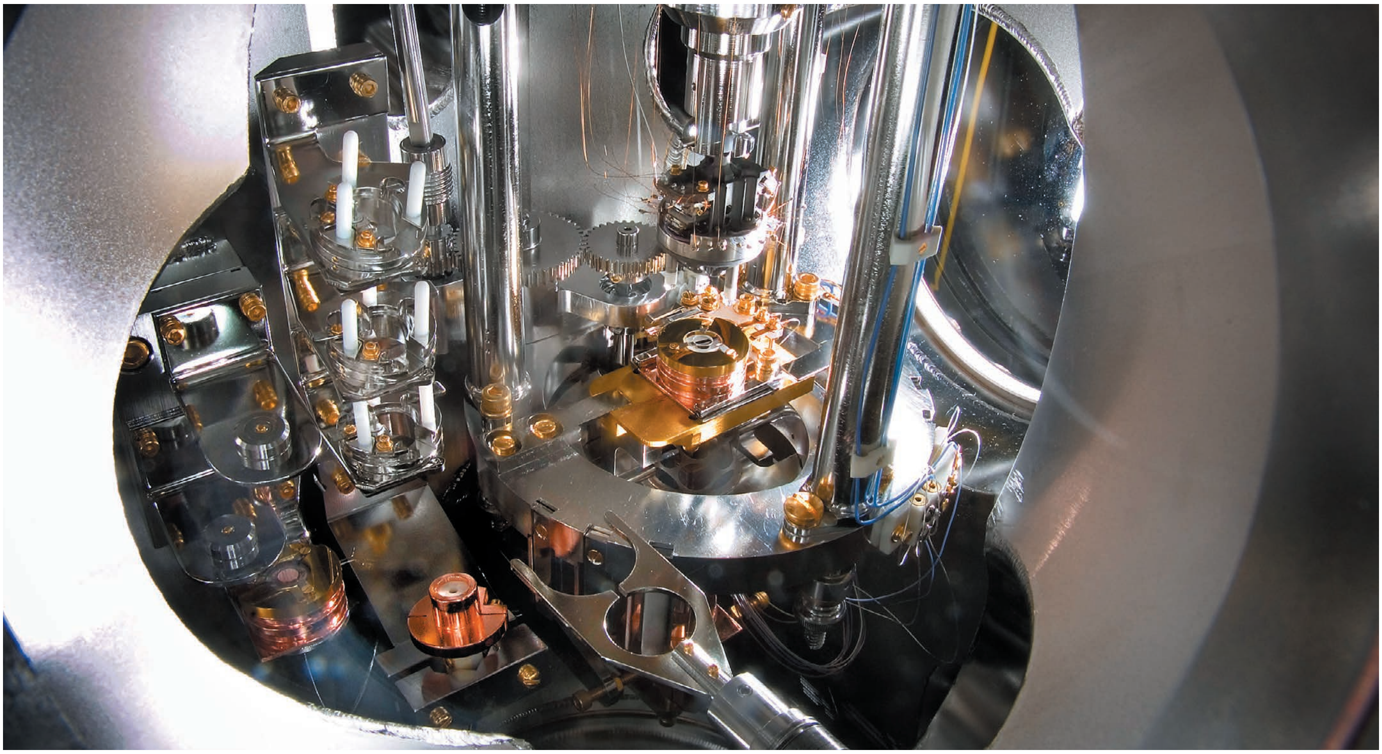
TECHNOLOGY FEATURE

THE ATOMIC-FORCE REVOLUTION

Atomic force microscopy is revealing molecular structures with startling clarity. Artificial intelligence and automation could expand its potential.

BROOKHAVEN NATIONAL LABORATORY



The atomic force microscope enables researchers to image the surfaces of materials at atomic resolution.

BY ANDY EXTANCE

While collecting images of the molecular structure of an antiseptic compound called 8-hydroxyquinoline, nanoscientist Xiaohui Qiu saw something that he didn't expect.

His team was using a method called atomic force microscopy, dragging an ultrasharp probe across the surface of a sample to reveal its topography at subnanometre resolution. The technique had previously revealed single molecules, complex mixtures and even chemical reactions in extraordinary detail. But Qiu's image of 8-hydroxyquinoline contained something peculiar: connecting silhouettes of the organic molecule's two rings were fine tendrils of contrast in regions where hydrogen bonds should form[1]. Nobody had ever captured a picture of these electronic interactions before. Qiu's image seemed destined to become iconic.

"I was seduced by the image," says Mervyn Miles, a physicist at the University of Bristol, UK, who visited Qiu's laboratory at the Chinese National Center for Nanoscience and Technology in Beijing in September 2013, at the same time as the images were published online in *Science*. He recalls feeling both amazement at the finding and uncertainty that atomic force microscopy could really capture such a level of molecular detail. "You go, 'Oh, I'll think about that later,'" Miles says. More than four years on, researchers' awe has turned to scepticism. And there lies the challenge of using atomic force microscopy to capture images at subnanometre resolution.

The method's sensitivity to the electron density that surrounds atoms and exists between them, including the bonds that hold them together as molecules, enables researchers to probe chemistry's fundamental secrets. "If I have just a single molecule of something, I can — in principle, at least — record the structure of that," says Peter Liljeroth, a physicist at Aalto University in Espoo, Finland. "There is no other experimental technique currently able to do this."

But this sensitivity can also produce artefacts in images, which means that scientists must take extreme care to show that what they see represents a real structure. And Liljeroth says that the subnanometre techniques he uses currently are "too complicated and too time consuming" to be adopted widely. He and other researchers are therefore hopeful that automation and machine-learning algorithms will help to simplify the technique. "This, I ▶

see like an engineering problem," Liljeroth says. "This can be done."

TECHNOLOGY TO WATCH

An atomic force microscope is like an atomic-scale record player (see 'Anatomy of an atomic force microscope'). It requires a probe that comprises a cantilever (the record player's arm) and a tip (the record player's stylus). Both cantilever and tip are usually made from silicon, and the tip is coated with diamond or a metal. Tracking the probe as it moves over the surface of a sample (the record) maps the forces that the tip experiences. Because the tip and sample can both attract and repel each other, with the attraction potentially locking the tip in place, scientists typically make the probe vibrate. As the probe approaches the sample's surface, the force that the tip experiences changes the frequency at which the probe vibrates, which researchers measure by shining a laser on the cantilever.

Although atomic force microscopy can now image structures at atomic resolution, researchers had to overcome several hurdles to make this feasible in practice. For example, experiments must be carried out in an ultrahigh-vacuum chamber, where working with lasers can be difficult. In the early 1990s, Franz Gießibl, while at Park Scientific Instruments (now Park Systems) in Sunnyvale, California, modified silicon cantilevers so that vibrational changes altered their electrical properties. This enabled him to convert changes in frequency of the probe's vibration into electronic signals, making the use of lasers redundant.

Another problem was that silicon is relatively floppy. The vibrations of a silicon cantilever can span around 20 nanometres, a distance far greater than the diameters of the atoms that researchers want to observe. Needing a stiffer material, in 1994, Gießibl, who by then was living in Munich, Germany, turned to a surprising solution: the quartz tuning forks with which modern clocks keep time. Using a borrowed atomic force microscope that he set up in his flat, Gießibl found that the tuning forks from Swatch wristwatches were "almost ideal in stiffness", he recalls, because they vibrate on the subnanometre scale. Quartz, a piezoelectric material, can turn changes in frequency into electronic signals in a similar way to Gießibl's modified cantilever. In 1996, Gießibl began to produce the qPlus sensor, a cantilever substitute that incorporates a quartz tuning fork. Now at the University of Regensburg in Germany, he licenses the associated patents to six companies that manufacture similar sensors with custom-made tuning forks.

One further advance was required to make imaging at atomic resolution routine. Because the shape and makeup of probe tips varies, it is difficult to precisely measure what they are doing. Tips can also move samples during scanning, leading to blurred images. To resolve these problems, scientists at IBM Research in Zürich, Switzerland, borrowed from previous work on scanning tunnelling microscopy. Similar to atomic force microscopy, the method uses a probe to scan a surface, passing an electrical current through the sample to measure changes in conductivity rather than force. In the 1990s, researchers at Cornell University in New York affixed a single molecule of carbon monoxide to the scanning tunnelling microscope's probe to create an atomically sharp tip. In 2009, the team at IBM applied the same approach to atomic force microscopy, producing striking images of pentacene, a chain of five interlinked six-membered carbon rings[2]. The bonds between atoms appeared as clearly as if drawn in ink.

> *"This is a learning field and we're getting better at it."*

Carbon-monoxide tips are "very special", says IBM's Leo Gross, lead author of the 2009 paper. The molecule comprises just one carbon atom and one oxygen atom, with the carbon flush against the tip and the oxygen positioned below like an atomic-scale stylus. As the tip approaches the sample, the atoms being studied repel the oxygen atom, causing the carbon monoxide to tilt to the side.

The resulting images show "very sharp bonds and you can see a lot of detail," Gross says. If there is a ridge of electron density — like that associated with a chemical bond — tilting helps the atomic force microscope to obtain a clear contrast with the background. Indeed, that is precisely what Qiu saw for 8-hydroxyquinoline, with the structure's carbon–carbon and carbon–nitrogen covalent bonds depicted in sharp relief. Yet tilting also creates "apparent bonds that you see sometimes where there are none", Gross warns — for instance, between molecules. And in 2013, Liljeroth and coworkers suspected that Qiu's team had imaged such a phenomenon.

Indeed, almost a year to the day on which Qiu's paper was published online, Liljeroth's team presented atomic force microscopy images that contained lines connecting atoms that do not interact through hydrogen bonding[3]. They suggested that such lines might be artefacts induced when neighbouring atoms nudge the carbon-monoxide tip, creating contrast where little or no electron density exists.

Other researchers interviewed by *Nature* say that hydrogen bonds are likely to make little contribution to what Qiu's team observed. Gießibl, for example, thinks that the lines are "probably not hydrogen bonds". However, "that's not trivial" to conclude, he adds. "I had seen the paper when it came out and it sounded legitimate to me."

Qiu says that what the images actually show is still up for debate. Both hydrogen bonding and nudges from nearby atoms might be involved, and when his team's paper was published, the effect of tip tilting with carbon-monoxide tips hadn't been studied fully. "Nobody, so far, found a real benchmark that is clear enough to distinguish the two," he says.

EXORCISING PHANTOMS

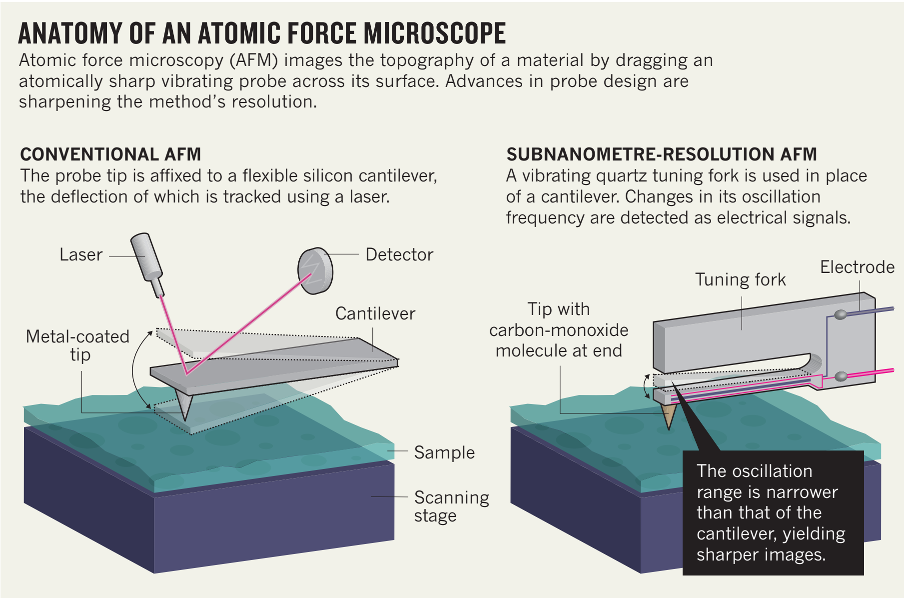
Such uncertainty is not a fatal flaw, Gross says. "This is a learning field and we're getting better at it." But how can researchers ensure that they aren't misinterpreting what they see? One option is to anticipate results in advance of image collection. Pavel Jelinek from the Czech Academy of Sciences in Prague and co-workers have developed computational methods that reliably predict what scientists using carbon-monoxide tips should observe for many molecules[4]. Being able to make such a comparison is important because molecular images can be so compelling, explains Liljeroth, who used the simulations in his 2014 paper[3]. "If you see it, it must be real," he says. "That's the gut reaction." But thanks, in part, to the computer simulations, scientists can now better anticipate the method's idiosyncrasies.

High-speed image collection

Recording conventional atomic force microscopy images can take more than a minute. But some microscopes can record images in less than a second, even at nanometre resolution.

To achieve such speeds, researchers such as Toshio Ando at Kanazawa University, Japan, use extremely small cantilevers (around 7 micrometres long), which resonate at very high frequencies. Standard cantilevers can exceed 100 micrometres in length. Using this approach, Ando's team has been able to watch the motor protein myosin V 'walk' along a strand of actin at the rate of up to 20 images per second[6].

At the University of Bristol, UK, Mervyn Miles's team uses a quartz tuning fork, similar to that found in the cantilever substitute qPlus sensor, as a vibrating microscope stage for mounting samples on during scans[7]. The microscope's probe initially rests on the sample, which is covered by water. As the stage vibrates more rapidly, the tip lifts off and 'surfs' over the water, explains team member Rob Harniman. This enables the researchers to scan samples at more than 100 images per second and up to around 1,300. "We've watched antimicrobial peptides making a hole in a membrane," Miles says. A.E.



SOURCE: ANDY EXTANCE/FRANZ GIESSIBL/CLAIRE WELSH/*NATURE*

Liljeroth's team has used carbon-monoxide tips to study electronic components embedded in graphene nanoribbons. Yet current approaches cannot simulate non-flat structures well enough to help in their interpretation. Together with his Aalto colleague Adam Foster, Liljeroth is developing machine-learning algorithms and artificial-intelligence programs to predict images of objects of any size, configuration or orientation. The pair have assembled a network of collaborators, and are seeking funding to support the effort.

Ultimately, this approach could lead to fully automated data interpretation, Liljeroth says. But Philip Moriarty, a physicist at the University of Nottingham, UK, suggests that this is unlikely to work in all cases, because even experienced researchers disagree on what atomic force microscopy data show. Moriarty cites the results of a recognition test in which his team was asked to classify images into categories — such as whether they showed atoms assembling individually, in rows or as pairs. The highest scoring participant succeeded only about 70% of the time. "If humans can't recognize one image from another, we've got a bit of a problem," Moriarty warns, because researchers' judgement provides the benchmark by which algorithms are trained. Yet by participating in Liljeroth's network, Moriarty hopes to explore the possibility that, with access to appropriate image data, artificial-intelligence-based classification systems could outperform people.

But there's little image data available on which to train such systems, Gross says. Only about 100 known molecules have been resolved to atomic resolution using carbon-monoxide-tip atomic force microscopy, he estimates. Although automated classification should be tried "at some point", Gross thinks that it's too early, at present.

Simulations such as Jelinek's could provide a suitable training set, Liljeroth suggests. "The question is whether these synthetic images are close enough to experiments."

SOUL-DESTROYING REPETITION

If nothing else, automation could help to mitigate the arduous practical challenges faced by researchers who use atomic force microscopy (see 'High-speed image collection'). Filipe Junqueira, a PhD student in Moriaty's lab, is studying how to produce arrays of thin columns of gallium arsenide. Each column, known as a nanowire, has a diameter of 10 nanometres or greater and is grown inside a stainless steel ultrahigh-vacuum chamber. To image the nanowires, Junqueira must overcome practical obstacles such as experimental noise and sample manipulation using a metal arm known as a wobble stick. His measurements contain interference that might be related to construction work being carried out several hundred metres away, even though the atomic force microscope he uses is housed in a basement and is supported by a table that can dampen vibration.

Other labs have taken more drastic steps to minimize the effects of noise. At Vienna University of Technology, Ulrike Diebold's team suspends its microscope from 36 vibration-damping elastic cords. When combined with an automated system that keeps the system level, this enables carbon-monoxide-tip atomic force microscopy that provides "beautiful images", according to team member Martin Setvin.

And there are further challenges. To mount a carbon-monoxide molecule on a tip, researchers must push the microscope's probe up to a surface coated with carbon monoxide, and then pass an electric current through the probe. It can take hours to get the process right, Setvin says. "If you lose the carbon-monoxide molecule, you have lost a day of work."

For a PhD student with little experience of scanning-probe techniques, it can take "a couple of months to start getting very nice atomic force microscopy images", Liljeroth says. Although some level of expertise will always be necessary, he hopes that machine-learning algorithms and automation will help to reduce the time that is needed for tip preparation.

Moriarty echoes this sentiment, as preparing a good tip can require a lot of trial and error. The only way of knowing whether a researcher has attached the correct molecule or atom is to record a clear image. The researcher must then repeat the image-collection process with fresh tips, to gather enough observations to be confident in his or her interpretation. Moriarty admits that scientists can find this a "soul-destroying" practice. "Automating that process is the way to go," he says.

Moriarty and others have already taken a step in this direction by developing an automated process for using tips to move hydrogen atoms[5].

Despite slow progress, Moriarty finds cause for optimism in astronomy, a field in which machine learning is helping researchers to judge when data might be meaningful. The Laser Interferometer Gravitational-Wave Observatory (LIGO) collaboration, for instance, successfully applied computational algorithms to distinguish possible gravitational waves from artefacts.

But limitations remain, Moriarty admits, because even LIGO needs scientists to confirm manually whether potential detections are real. Tip preparation at the push of a button would require a system to be able to accurately judge the quality of the images that it gathers, he says. "The only way to automate is to train the blasted machine to recognize when it's got a good image," Moriarty says. "If a machine could do that, those astronomers would be out of a job." ■

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CORRECTIONS

The Toolbox article 'The future of scientific figures' (*Nature* **554,** 133–135; 2018) implied that Benjamin Delory developed the persistence barcode method. In fact, he and his colleagues developed an analysis pipeline that relied on and adapted an existing method.

The Technology Feature 'Deep learning for biology' (*Nature* **554,** 555–557; 2018) erroneously affiliated Mark DePristo at Verily Life Sciences. He is, in fact, at Google. Also, the DeepVariant tool was developed jointly by Verily and Google.