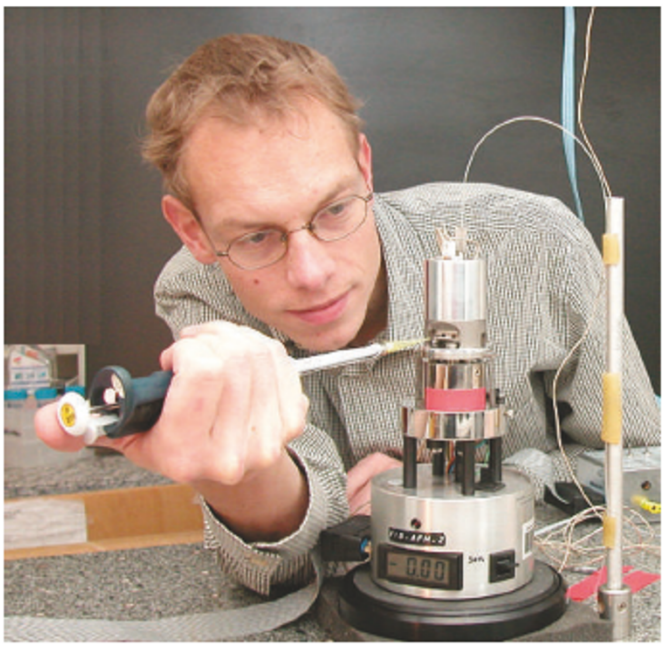
FOCUS ON THE LIVING

Atomic force microscopes have revolutionized the study of materials, but probing watery biological systems has proved more difficult. Jenny Hogan asks whether a fix is at hand.

Bart Hoogenboom's windowless room is a cramped space, almost filled by the sturdy table at its centre. Cables dangle from piles of electrical devices that climb to the ceiling. In the midst of the tangle of equipment is a stack of three small metal cylinders. Hidden inside the top one is a sliver of silicon, its tip quivering up and down 200,000 times each second.

"It is an experimental physicist's dream and a biologist's nightmare," says Hoogenboom. Happily, Hoogenboom is a physicist, a postdoc at the University of Basel in Switzerland, and for him the roomful of gadgetry to tinker with is a treat. But the outcome of his tinkering could enrich the lives of biologists



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Physicist Bart Hoogenboom's love of gadgetry could pay off for biologists.

The cylinders on the table are the working parts of an atomic force microscope, or AFM. Invented 20 years ago[1], AFMs are based on a sharp tip at the end of a flexible beam, or cantilever; the topography of a surface is detected by the bending of the cantilever as the tip scans the specimen. Under the right conditions, AFMs can produce images that are accurate down to the last atom.

The AFM has revolutionized the way material scientists study surfaces. But in general, it has been harder to apply AFMs to the study of delicate biological molecules and the soft tissues in which they are found. The team Hoogenboom works in, and others in labs around the world, are planning to change that, by finding out how to use AFMs in ways that don't damage the samples.

Biologists already have pretty good methods for studying things down to molecular or even atomic scales, but these involve taking the objects of study out of their normal biological context. Electron microscopy requires samples to be fixed and exposed to a vacuum; X-ray crystallography requires the relevant proteins to be forced into crystalline arrays. "The advantage of the AFM is clear," says Peter Hinterdorfer, a biophysicist at the University of Linz in Austria, who has used the technique to study how antibodies bind to their targets: "you can image in physiological conditions."

Some of the most impressive biological AFM images, including the image shown opposite, are the work of Andreas Engel, the head of Hoogenboom's team. But even this beautiful picture highlights the limitations of the technique. The golden rings are rotors that form part of an energy-conversion machine found in cell membranes, called an ATPase. Each protein ring has a diameter of around 5.4 nm — and although it is possible to see the molecules that make up each ring, you can't make out the amino acids of the protein, never mind see the thousands of atoms from which the rings are built.

> "Being able to see small molecules binding to proteins would vibrate the whole community."
> — Daniel Müller

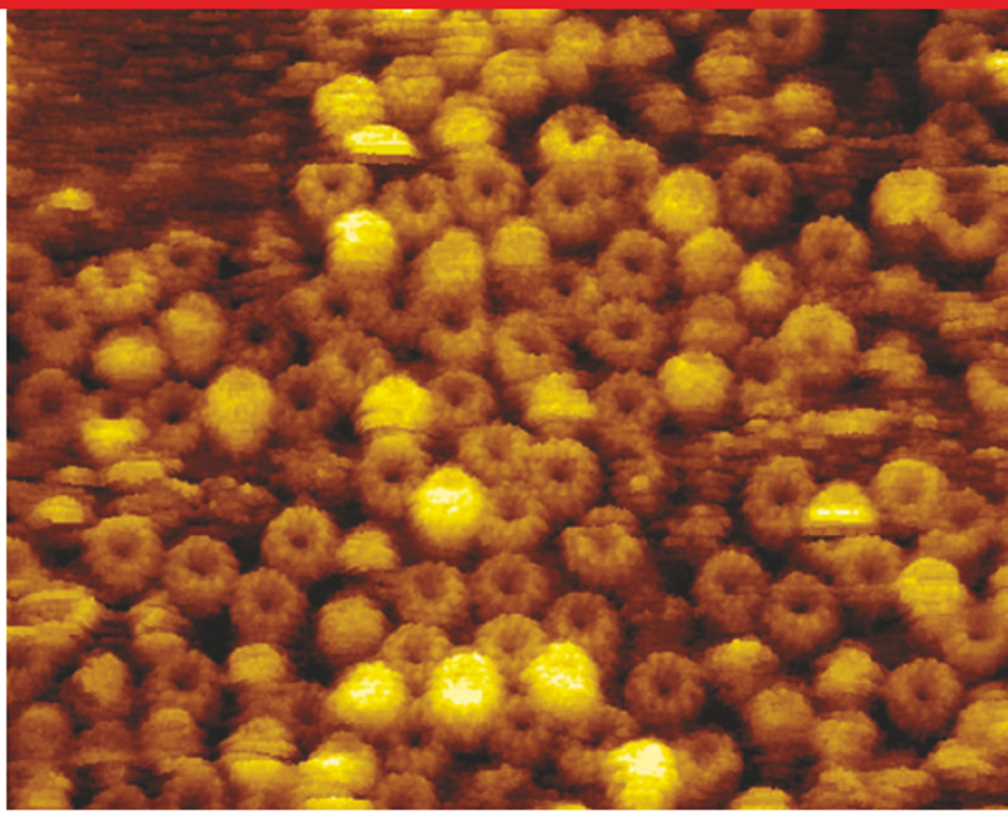
Close encounters

This is poor resolution compared with that possible for hard surfaces, but is good for biological images taken with AFMs in 'contact mode'. AFMs can work in a bewildering variety of ways, but contact mode is the most straightforward. The sample is brought into contact with the tip and moved back and forth and from side to side. But it is not the most precise way of doing things. The AFM tip tends to damage or dislodge the things it is scanning, which degrades the image resolution and limits the types of sample the technique can be used to study.

The obvious solution is to make the tip behave more gently when scanning the samples, and it is to this end that various researchers have turned to the 'frequency modulation' or FM mode. In FM mode, the tip hovers just above the surface under study. A pulse of energy is used to make the cantilever tremble, and the topography of the surface below affects the frequency at which it does so. So long as the forces that the tip senses as it hovers above the surface can be inferred from changes to the cantilever's frequency, the tip doesn't actually have to touch the sample at all.

AFMs used in FM mode (FM-AFM) can achieve stunning results — even resolving features below the scale of an individual atom, thought to be the signature of atomic orbitals, on a hard, flat silicon surface[2]. But that sort of resolution requires the system to be used in a vacuum. For a long time, getting the FM mode to work in a messier, wetter environment seemed out of the question. Having water around the cantilever was expected to deaden the vibrations. "Most experts would not have expected FM-AFM to work in water," says Stuart Lindsay of Arizona State University in Tempe, whose group develops AFMs and other scanning-probe instruments for biological applications. "I would have been included in that class. Otherwise, I would have tried it first."

The first evidence that FM-AFM might work in water came from Takeshi Fukuma of Kyoto University's electrical-engineering department in Japan. When Fukuma was a postdoc in Hirofumi Yamada's lab, which does research into molecular electronics, he sought to reduce the noise in the AFM's output by optimizing the



Atomic force microscopy has revealed the detailed structure of energy-converting ATPase rotor rings[6].

D. J. MÜLLER & A. ENGEL, BIOZENTRUM, UNIV. BASEL

way the cantilever's deflection was being measured. He thought this might compensate for the water damping down the oscillations.

In most commercial AFMs, a laser beam is bounced off the top surface of the cantilever to work out the tip's position. Fukuma and his coworkers set to improving each element of this sensor system, boosting the laser power and introducing a technique used in CD-ROM and DVD drives to stabilize the laser performance. The combined effect of the changes was to reduce the noise in the system to near its theoretical limit[3]. Without even trying to minimize the noise from other sources, such as the buffeting of the cantilever by water, the team got striking results. "I did just a few experiments to get atomic resolution," says Fukuma. In July last year, the research team reported their findings[4]. The paper's centrepiece is an image of mica taken in pure water, showing the atoms which are just half a nanometre wide.

Scaling down

The developments in Yamada's lab are attracting attention. "This is certainly an innovation we're interested in and something we're watching," says Craig Prater, director of technology development for Veeco Metrology in Santa Barbara, California; Veeco is one of the world's leading suppliers of AFMs. Yamada says that Seiko Instruments and Jeol, two other companies that build AFMs, have also expressed interest in his work.

But the advance is not yet on the radar of the researchers whom it might benefit. "Biologists will pay attention as soon as the technique is used to image biological samples," says Daniel Müller, who collaborated with Engel on the ATPase work, and is now at the University of Technology in Dresden, Germany.

Yamada has recently presented images of biological samples, such as the membrane protein bacteriorhodopsin, at conferences. And looking for a closer collaboration with biologists, Yamada's student Fukuma has now moved to work with an interdisciplinary group at Trinity College Dublin in Ireland headed by Suzi Jarvis. Within three months of arriving in Jarvis's lab, Fukuma had optimized its AFM, as he had done in Kyoto, making many of the electrical components himself.

Fukuma's results have spurred Hoogenboom's group to ever greater efforts. His team has now achieved true atomic resolution of mica in water too. It worked independently of the Kyoto group on optimizing the measurement of cantilever deflection, completely replacing the optical beam deflection system used in standard AFMs with a component designed by the team[5] — hence all the clutter.

The Basel team has also produced images of bacteriorhodopsin (a paper on this is under review at *Applied Physics Letters*). The FM-AFM images of bacteriorhodopsin seem slightly fuzzy compared with the best made in contact mode. But that doesn't worry Hoogenboom.

"We have ten years' experience doing contact mode; we haven't yet optimized the FM technique," says Hoogenboom. "If you drive a car for the first time, you don't know how fast you can take a corner."

FM-AFM is unlikely to achieve atomic resolution for biological samples, however. AFM is in general a rather slow technique, and during the minutes that a scan takes, the molecules under study will wriggle. Also, the sides of the tip will interact with any portion of the sample that rises up near it. Several groups are working on reducing these effects, for example by making the tip sharper or speeding up the process of scanning.

T. FUKUMA/TRINITY COLLEGE DUBLIN

Fix finder: Takeshi Fukuma boosted the efficiency of an atomic force microscope.

But even a small improvement in resolution over the contact-mode images could help, according to Müller, who worked on the ATPase rotors. Just being able to see small molecules binding to proteins "would vibrate the whole community", he says. This could provide, for example, a quick way to see how drugs bind to their target receptors — providing a new tool in drug discovery.

But until people start to use FM-AFM, no-one is really sure what the implications will be. "There are some interesting results coming out now," says Jason Cleveland of Asylum Research, an AFM supplier that is collaborating with Jarvis's group to explore how to introduce FM-AFM capability to their instruments. "But it's right at the beginning of the snowball, and you don't really know how big it will be."

Back in Basel, Hoogenboom recalls a discussion at a conference where biologists explained how they wanted an AFM to work. Their request was for a black box where you press a button and it gets you nice images. The FM-AFM technique is more complex than that, but the instrument needn't be a nightmare, assures Hoogenboom. "A bit more development and it will almost look as simple as your CD player." ■

Jenny Hogan is a reporter for *Nature*.

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