Surely you're happy, Mr Feynman!

Michael Segal

In 1959 Richard Feynman called for researchers to improve the resolution of the electron microscope, and they have — but resolution is only part of the story.

"Is there no way to make the electron microscope more powerful?" was one of the questions that Richard Feynman asked in his famous 1959 talk 'There's plenty of room at the bottom'. Feynman devoted about a tenth of his lecture to electron microscopy, saying, among other things, that the electron microscopes of the day were "one hundred times too poor," and that their performance was far from being limited by electron diffraction. It should be possible, he said, to improve the resolution of electron microscopes by a factor of 100 by addressing shortcomings in the lenses used to focus the electron beam. This would allow scientists to answer a series of fundamental questions in chemistry and biology. As Feynman put it: "It is very easy to answer many of these fundamental biological questions: you just look at the thing!"

Seeing atoms with advanced electron microscopes would, Feynman added, be a necessary complement to the manipulation of atoms that was one of the talk's broader themes. Indeed, the microscopy section of 'plenty of room' is a microcosm of the entire lecture. As with the rest of the talk, Feynman's observations on electron microscopes are prescient but incomplete (see Thesis articles on pages 783 and 785 of this issue). His observations have become modern-day rallying cries for different areas of electron microscopy, but they seem to have had little direct impact on the field. And underlying much of what he said was a reductionist or physics-centric attitude that rankles some scientists outside the physics community[1,2].

Today, 50 years after Feynman's lecture, the impact of the electron microscope is ubiquitous. It has been used to discover carbon nanotubes, to observe single-atom lattice vacancies and superconducting vortices, and accounts for virtually all of the cellular imaging that has been done between 10 and 10,000 Å. Part of this success derives from better resolution, as Feynman expected: the most advanced electron microscopes can now achieve a resolution of just 0.5 Å, which is a factor of 20 better than the state-of-the-art in 1959. These increases in resolution have been achieved through a combination of improvements in the brightness of the field-emission guns that produce the electron beams, higher accelerating voltages, improved electron optics and better instrument stability.

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The TEAM at the Lawrence Berkeley National Laboratory has achieved a resolution of 0.5 Å. It operates at energies between 80 and 300 keV, and is a collaboration between the Berkeley, Argonne and Oak Ridge national laboratories, the University of Illinois in Urbana and two companies — FEI Company and CEOS GmbH in Germany.

But just as important have been parallel developments such as cryogenic imaging, which involves covering the sample in ice to help maintain its structure under vacuum conditions and electron-beam irradiation. Moreover, the electron microscope has had less of an impact than Feynman predicted, having been beaten to landmark results such as DNA sequencing by alternate approaches. Feynman was clearly right that the electron microscope would be a valuable instrument in many areas of science, but his focus on resolution, and his optimism about the use of the electron microscope for tasks as varied as chemical synthesis and DNA sequencing reflect the limits of his vision.

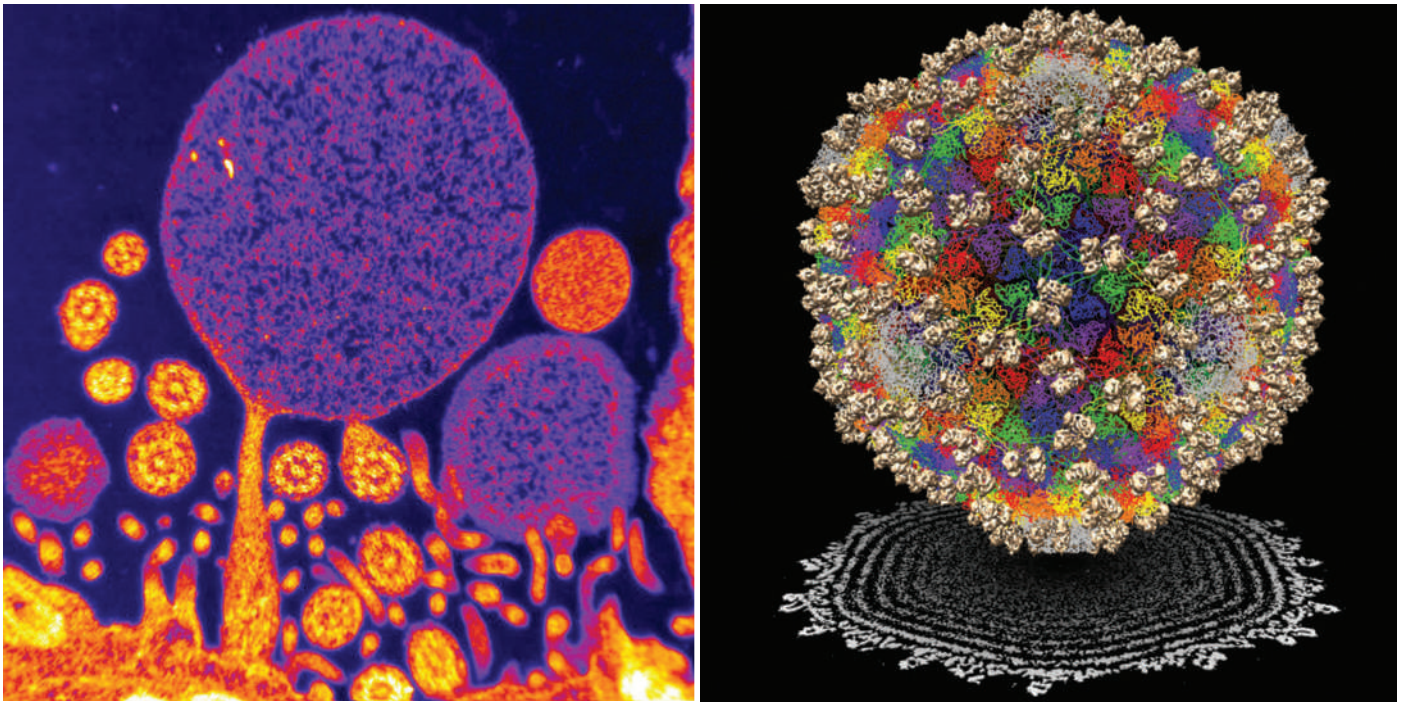
No harm done

A primary reason for these limits is the fact that shooting energetic electrons at samples tends to destroy them. Whether these electrons pass through the sample in transmission mode, or interact with its surface in scanning mode, they can cause thermal damage to the sample, ionize it, transfer momentum to it directly (known as 'knock-on' damage), charge it sufficiently to steer the beam away, break bonds, move atoms, release gases and turn proteins to ashes[3]. These issues are exacerbated when the resolution falls below 1 Å, and such beam damage may well set the ultimate resolution limit to biological imaging.

"Radiation damage prevents you from ever getting an atomic structure by electron microscopy of one molecule of an organic material," says Richard Henderson, director of the Medical Research Council Laboratory of Molecular Biology at Cambridge University. In X-ray crystallography, on the other hand, beam-induced damage is distributed among trillions of molecules, which is one reason why this technique has been able to image biological structures that electron microscopes have not.

Electron beams can also damage non-biological materials. Elements lighter than calcium are susceptible to knock-on damage for beam energies above 100 keV, which means that the resolution cannot be improved by simply increasing the beam energy. This consideration can affect both the type of samples that are imaged and the supports they are placed on. For example, graphene makes an attractive sample support because its perfect periodicity allows its contribution to the image to be removed by Fourier filtering — but not if it is destroyed by the beam.

Beam damage, which Feynman did not discuss in his talk, makes something that he did allude to — aberration correction — all the more important. This is because when



Left: a high-angle annular dark-field scanning transmission electron microscope (STEM) image shows details of the cilia that sweep dirt and mucus out of the lung. The image, which measures 3 μm across, was taken with an aberration-corrected STEM at the SuperSTEM facility in the UK by Mhairi Gass (University of Liverpool), Alexandra Porter (Imperial College) and Trevor Douglas (Montana State University). Although the electron beam has a diameter of just 1.3 Å, the actual resolution of the image is limited by the detector pixel size of ~3 nm. The sample was taken from a mouse. Right: an atomic model of the epsilon 15 virus derived by Wen Jiang, Wah Chiu and co-workers at Purdue University, Baylor College of Medicine and the Massachusetts Institute of Technology[5] from 20,000 individual images that were collected at liquid helium temperatures and a beam energy of 300 keV. The model has a resolution of 4.5 Å. Epsilon 15 is a virus that attacks the salmonella bacteria.

beam energies are lowered to reduce damage, aberration correction can partially compensate for the accompanying drop in resolution. Aberrations cause the optical properties of an uncorrected electron lens to be similar to "the bottom of a beer bottle", in the words of David Muller, an electron microscopist at Cornell University[3]. The problem of aberration in electron optics was first identified by Otto Scherzer in 1936, and he suggested how to overcome it in 1947 (something not mentioned in Feynman's lecture), but the first aberration-corrected electron microscope was not built until 1998.

The electron microscope has had less of an impact than Feynman predicted.

Although microscopes like the 3.5 MeV machine at Osaka University were built without aberration correction[4], more recent projects combine aberration-corrected electron optics with much lower energies. The R005 microscope in Japan and the Transmission Electron Abberation-corrected Microscope (TEAM) in the US (see photo on page 786) operate between 80 and 300 keV and use aberration correction to achieve a 0.5 Å resolution. The super scanning transmission electron microscope (SuperSTEM) facility in the UK hosts two 100 keV electron microscopes fitted with aberrations correctors made by the US company Nion, and both microscopes have achieved subångström resolution.

Building aberration-corrected electron microscopes is usually beyond the capabilities of a single laboratory: R005, for instance, is a collaboration between the Tokyo Institute of Technology, CREST and JEOL Ltd, and the Sub-Angstrom Low Voltage Electron (SALVE) microscope in Germany, which will operate in the 20–80 keV range, is being built by the University of Ulm, Carl Zeiss Ltd and Corrected Electron Optical Systems (CEOS) GmbH. Ute Kaiser of the University of Ulm, project manager of SALVE, remembers the improvements that aberration correction brought to an earlier 80 keV microscope, an FEI Titan, in Ulm. "It was really a new world after switching on the aberration correction," she says. "Seeing single carbon atoms from nanotubes and graphene stable under the 80 keV electron beam was a very big surprise."

Resolving the future

Although electron microscopists have not yet met Feynman's challenge to reach a resolution of 0.1 Å, they have certainly tried. But how important is it to keep trying? For biologists, the answer may be "not very". Although a materials scientist might be interested in picometre displacements of a single oxygen vacancy, which can have profound effects on bulk material properties, subångström resolutions are both less interesting and much more difficult for objects such as cells, proteins, DNA and ribosomes. "The electron microscope is basically perfect now for anything you'd ever want to do in biology, although some components could still be improved," says Henderson, "and I don't think there is any interest in biology in anything below 1 Å." A resolution of 3.5 Å is the threshold for biologists because this is roughly the length scale at which chemical bonding information can be discerned: "3.5 Å is the dividing line between blobs and chemistry," according to Henderson.

Developments such as aberration correction, which have allowed materials scientists to achieve subångström resolutions, may therefore be of limited importance to biologists: "It is not clear whether correctors

as such will advance biological imaging that much," says Wolfgang Baumeister, head of molecular structural biology at the Max Planck Institute of Biochemistry. "We have so many limitations further upstream, that these are not really the big issues we have."

Although biologists are interested in improving the resolution of their images, their efforts are less focused on improving the electron microscope itself. Instead, biologists are focusing on the imaging of intact samples in their native environment, and on reducing artefacts from staining, slicing, freezing and beam damage. Whole-cell tomography is being developed to construct three-dimensional images from multiple individual projections, as are staining techniques appropriate for the electron microscope, new ways of stabilizing instruments, more efficient detectors that allow lower, less-damaging beam energies, and phase plates that will produce increased contrast and signal-to-noise ratios. And as the structures of the components of cells are solved, biologists have started to study the location, movement and interactions of these components. Such research has benefited from the development of hybrid microscopy: in this approach different length scales are imaged by different techniques — including electron, X-ray, NMR and optical microscopy — and the results are then combined to give a single multi-resolution picture.

The history of biological imaging also reveals the limited importance of resolution in electron microscopy. Feynman listed a dozen fundamental questions in biology — such as "What is the sequence of bases in the DNA?" and "How are proteins synthesized?" — that he thought could be addressed by improved electron microscopes. "If you go through Feynman's questions one by one, they've all been completely solved in fantastic detail," says Henderson, "but although there have been contributions from electron microscopy they weren't the decisive ones." Baumeister describes efforts in the 1970s to use electron microscopes for DNA sequencing as mostly "fruitless".

Temporal resolution is also a major focus of research.

Outside of biology, however, the race for better resolution in electron microscopes goes on. Physicists and chemists interested in inorganic samples, for example, are now pushing towards a resolution of 0.1 Å, which would allow them to observe the spatial distributions of atomic wavefunctions, and to also meet Feynman's original target for resolution. "I believe the most fundamental issue is still the resolution," says Akira Tonomura, a pioneer of electron holography at Hitachi. "I am not satisfied with the present situation."

Temporal, rather than spatial, resolution is also a major focus of research. High-speed detectors are allowing researchers to move beyond taking static images and into the realm of diffusion dynamics, molecular excitation, phase transformations and chemical reactions. Whereas the typical detector can capture 30 frames per second, the TEAM collaboration has developed a detector running at 400 frames per second. Other groups are pushing into the picosecond time regime by capturing billions of images of a single repeated process, although such techniques cannot be applied to diffusion and other processes that are not perfectly repeatable.

The ability to study dynamic processes might eventually allow scientists to fulfil another of Feynman's predictions — the use of the electron microscope in chemical synthesis. "I expect that electron microscopes will go beyond observing what have we made, to take a greater role in synthesis by allowing *in situ* observation of reactions at atomic resolution," says Ulrich Dahmen, scientific director of the TEAM project.

The Feynman effect

So what effect did Feynman's speculations have on the subsequent development of electron microscopy? "Most people in the field haven't heard about Feynman's theses," says Baumeister. "He was undoubtedly a visionary and his statements are great fun to read, but they were perhaps too generic to have had a lot of impact." The talk had similarly little impact on Joachim Frank, a professor of biology at Columbia University: "I wasn't aware of his speech for a long time, and I have not heard it quoted."

For physical scientists working on the resolution frontier, however, the microscopy section of 'plenty of room' has proved to be more influential. "In the transmission electron microscope community it is accepted as a very important speech and people know of it," says Kaiser. "In fact my favourite title for a talk on SALVE is 'Microscopy at the bottom.'" Max Haider, founder of CEOS, a company that develops aberration-corrected electron optics, agrees: "At present it is a very important statement that is now cited much more often than it was in the past."

The TEAM collaboration, for example, regularly quoted from the talk when selling the project to the US Department of Energy. "The Feynman talk, which made the connection between nanomaterials and microscopy, hit just the right button," says Dahmen. "It was very important actually in that context." Indeed, the TEAM website frames the entire collaboration in terms of Feynman's speech, describing their microscope as meeting "the Feynman challenge".

The cunning of biologists

Some scientists have criticized Feynman for being too reductionist and too physics-centric in 'plenty of room'[1,2]. Is this true for the microscopy section of the talk? Clearly, Feynman could not have forseen that new techniques such as the polymerase chain reaction would trump electron microscopy for DNA sequencing. "Like many physicists he did not foresee the cunning of biologists to use the components of living systems to answer important questions," says David DeRosier, a biologist at Brandeis University. At the same time, Feynman's prediction that simply observing the physical building blocks of a biological, chemical or material system would be a powerful tool, has been validated. "The reductionist approach has worked very well," says Henderson. "Unless you can dismantle something, reconstitute it, and put it back together, you can't prove how it works."

Indeed, the structural biology that microscopy elucidates may be particularly suited to a meeting of physics, chemistry and biology. "I hope that one day structure will be used to establish boundary conditions to solve the equations of physics and chemistry that underlie biological function, as foreseen by Michael Polanyi," says Robert Glaeser, a molecular biologist at the University of California, Berkeley. And although Feynman did not by any means introduce an atomic-level perspective into structural biology (the first atomic-resolution protein images were completed in the same year as his talk), he understood that it was an area to which physics would contribute.

What would Feynman think if he were alive today? It is possible that he would care less about the impact that his talk had, and more about the simple fact that there has been so much progress in electron microscopy. As one researcher commented during a recent review of the TEAM project, referring to the title of Feynman's autobiography, "Surely you're happy, Mr Feynman!" ❐

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