The frontiers of microscopy

There have been remarkable developments in microscope technology in recent years, driven in part by the nanotechnology revolution and the need to investigate ever smaller and more complex objects with higher resolution. We now not only need to know where the atoms are and what they are, but also how they interact with one another at the atomic scale. Microscopy is a large and growing area, and here we focus our discussion on two main areas that have advanced greatly in recent years: scanning probe microscopy and electron microscopy.

P.A. Midgley[a] and C. Durkan[b]

[a]Department of Materials and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK, [b]Department of Nanoscience, University of Cambridge, 11 JJ Thomson Avenue, Cambridge CB3 0FF, UK

Frontiers of scanning probe microscopy

Since the advent of scanning probe microscopy (SPM) over a quarter of a century ago, several scientific fields, including materials science, have been transformed. Initial work with the scanning tunneling microscope (STM), whereby real-space imaging at the atomic scale was used to verify the hitherto predicted 7×7 reconstruction of the Si(111) surface, immediately catapulted the STM into mainstream science. So much so, that it led to the award of the Nobel Prize in 1986 to G. Binnig and H. Rohrer for their invention of the STM, along with the pioneer of electron microscopy, E. Ruska. Since those early days, aided by the development of the atomic force microscope (AFM) a few years after the STM, we have learned much about the structure and function of matter at the nanometer scale and below. As the operation of the STM itself is dependent on quantum mechanics, it has proven to be invaluable for fundamental research into surface and electron physics. Initial work involved probing the nature of the tunnel barrier between the STM tip and a metal or semiconductor surface, and was quickly followed by investigations of the surfaces themselves, culminating in the now well-known beautiful visualizations of electron standing waves in Eigler's 'quantum corral' structures[1].

These structures had themselves been fabricated using the STM tip to manipulate individual Fe atoms on a Cu surface, demonstrating the smallest circle ever made, with a diameter of a few nanometers. It is this combined ability to manipulate and image at these unprecedented scales that has made SPM so universally applied today as a complementary tool to optical and electron microscopy. The STM has long moved on from bare surfaces to investigations of adsorbed atoms and molecules, with recent work concentrating on molecules on thin insulating films in an attempt to decouple the molecules from a conductor. This enables us to examine the molecules closer to their pristine, or gaseous, state[2]. The ability to perform STM through thin insulating films is, however, beyond many commercially available instruments because of the low current levels (sub-picoamp) involved, so is presently undertaken with custom-made microscopes. The first atomic resolution STM images of silicon were recorded using a tunnel current between the STM tip and the surface of around 10 nA; this

ISSN:1369 7021 © Elsevier Ltd 2008

can now be achieved with currents of less than 100 fA, an increase in sensitivity of five orders of magnitude. This is approaching the shot-noise limit, which, for the bandwidth needed for STM, is around 10 fA.

This increase in sensitivity comes with the additional advantage that the distance between the STM tip and the sample being studied can be several Ångstroms larger than previously. This has opened up the possibility of increasing the scan speed from a few minutes per image to video rates. In fact, video-rate AFM was first demonstrated more than a decade ago[3], and major advances are still being made in this field today. Other advances in STM have come about through the ability to investigate various properties of the surface/system under investigation; for example, vibrational spectroscopy on single molecules has been demonstrated at low temperatures[4], as has single-spin manipulation of molecules and atoms[5]. Whilst STM offers the ultimate in resolution, it suffers from the drawbacks that the sample under investigation must be conductive and that, at the atomic scale, there is no way to distinguish routinely between materials. This lack of material characterization capability was one of the reasons driving the development of the AFM. Although the resolution of AFM can be better than that of STM under very special conditions, the vast majority of AFM work is done under ambient conditions with a lateral resolution of around 1-2 nm. This loss of resolution is more than compensated for with by the advantage of being able to spatially map many different properties of samples, e.g. magnetic fields (magnetic force microscopy, MFM), electric fields (electric force microscopy, EFM), conductivity (C-AFM) and surface potential or work-function (Kelvin probe force microscopy, KPFM), as well as mechanical properties such as piezoresponse, adhesion, stiffness, friction and, more recently, optical properties via nano-Raman spectroscopy (see Fig. 1). Chemical forces may also be measured and mapped using chemically functionalized tips. All these measurements can be made using a standard AFM, the main differences being the type of sensor probe, or tip, that is used, and the specific detection scheme (contact mode, non-contact mode, tapping mode, force modulation, frequency-shift detection, etc.). One of the difficulties with scanning probe microscopy is that, now there are so many different implementations and mapping techniques, there is an ever-increasing list of acronyms to remember!

AFM is also a technique that lends itself particularly well to operation in various environments, ranging from ambient to ultra-high vacuum to liquid, which is why AFMs are used in many different disciplines from materials science to biology, chemistry and physics, and why STM tends to be used mostly for physical and chemical studies of surfaces. This enormous breadth of application of AFM makes for some tantalizing possibilities: if video-rate AFM can be accomplished readily and easily without loss of image resolution or quality, then it may soon replace the optical microscope as the tool for routine surface characterization.

Whilst SPMs are undoubtedly getting faster, more sensitive and more reliable, there can be little doubt that the real strength of this burgeoning field is the enormous flexibility of the microscopes. However, much still remains to be done – interpreting SPM images is a fine art due to the myriad forces and interactions between the tip and the sample, and to the inherent uncertainty in the exact nature of the tip. Nonetheless, these are exciting times for SPM, with new challenges and questions arising all the time about nanoscale mechanics and dynamics.

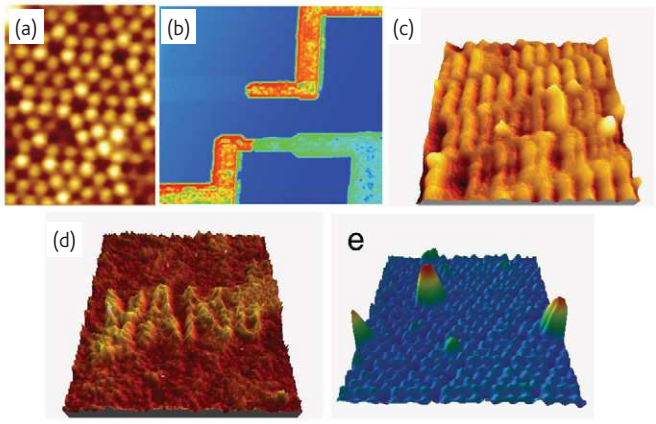

Fig. 1 A montage of images illustrating the varied modalities of SPM: (a) STM image, atomic resolution on Si(111); (b) potential map taken using AFM on two Au nanowires, the top one of which has broken, showing discontinuity in voltage – used for fault finding in circuits, 2 mm × 2 mm; (c) MFM image of data tracks on hard disk, 3 mm × 3 mm; (d) AFM image of ferroelectric pattern using piezoresponse force microscopy, 2.5 mm × 2.5 mm; and (e) STM image of C60 molecules on Si(111) surface.

Frontiers of electron microscopy

For many years, and especially in the 1960s and 1970s, there was a tremendous push to improve the performance of electron microscopes in order to resolve the atomic structures of metals and semiconductors. It had long been recognized that aberrations in electromagnetic lenses degraded the image quality but that resolution could be improved by increasing the beam voltage, hence reducing the electron wavelength. Million-electron-volt instruments were (and still are being) built but, although atomic structure was visible in such instruments, the radiation damage and the cost of the instruments made this approach of limited value. In the last decade or so, attention has returned to improving the lens design.

Scherzer[6] had shown how the aberrations in the round lenses used in most electron microscopes could be minimized by the use of multipole lenses. Rose's theoretical work[7] led to aberration-corrected lenses being built successfully by Haider and co-workers [8] in Germany (now at CEOS), and similar success was had by Krivanek et al.[9] at Cambridge and in the USA (now at Nion). This new technology has been incorporated remarkably quickly into commercial instruments.

The benefits of aberration correction are many, both for transmission electron microscopes (TEMs) and for scanning transmission electron microscopes (STEMs). In the TEM, contrast in

high-resolution lattice images arises typically through phase contrast brought about by defocusing the image. The Scherzer defocus sets an optimum interpretable resolution limit. For higher-frequency information (better resolution) the contrast oscillates rapidly and interpreting image contrast directly is difficult. However, such high-resolution information can be 'decoded' using techniques such as through-focal series or tilt series restoration.

By minimizing lens aberration, especially spherical aberration, the interpretable limit can now be extended and, perhaps just as importantly, the problems of image delocalization are greatly reduced. By defocusing the image, the contrast is 'delocalized' over a wide field of view, leading to 'ghost' images and making the interpretation of images, for example at interfaces, difficult or impossible. Microscopes with aberration correctors (so-called Cs correctors) minimize this problem. Indeed, correctors even allow the user to choose to have a spherical aberration of the opposite sign, leading to remarkable new imaging modes such as 'negative Cs imaging' (NCSI), developed in Jülich, wherein atom contrast is reversed and light atoms appear to have stronger contrast than in other imaging modes[10].

The performance of the microscope is now limited by other aberrations and instabilities. Chromatic aberration, brought about by forming images using electrons with a spread of energies, can be minimized by improving the performance of the objective lens with a so-called Cc corrector. To achieve 0.5 Å resolution (the aim of the TEAM[11] project in the USA), the TEM needs both Cs and Cc correctors. Instabilities (mechanical and electronic) have been addressed by the instrument manufacturer, and new designs of microscopes have recently been introduced, such as the Zeiss 'frame', in which the microscope is suspended, or the FEI 'boxed' Titan, in which a stiff massive column is shielded in a metal box (the latter has to be remotely operated).

In the STEM, aberration correction is also of great benefit. The aberration corrector is now on the probe-forming lens (before the specimen) such that a very finely focused spot (with sub-Angstrom diameter) can be formed. However, not only is the spot very small, the current density in that electron probe is many times higher than conventional STEM instruments. This allows STEM images of very high resolution to be acquired quickly: for example, Batson et al.[12] (IBM) were able to record with remarkable clarity TV-rate movies of Au atoms 'dancing' on carbon support films. As well as high-resolution imaging, the great benefit of any STEM instrument is the ability to record other signals, such as electron energy loss spectroscopy (EELS) and X-ray microanalysis, simultaneously to enable a one-to-one correspondence between structural and chemical signals. A recent and impressive demonstration of that process was performed by the Muller group at Cornell[13], who were able to map the chemical composition at atomic resolution near an oxide boundary (see Fig. 2).

The ability to collect spectral information using the STEM is enhanced greatly by the development of a monochromator. Although

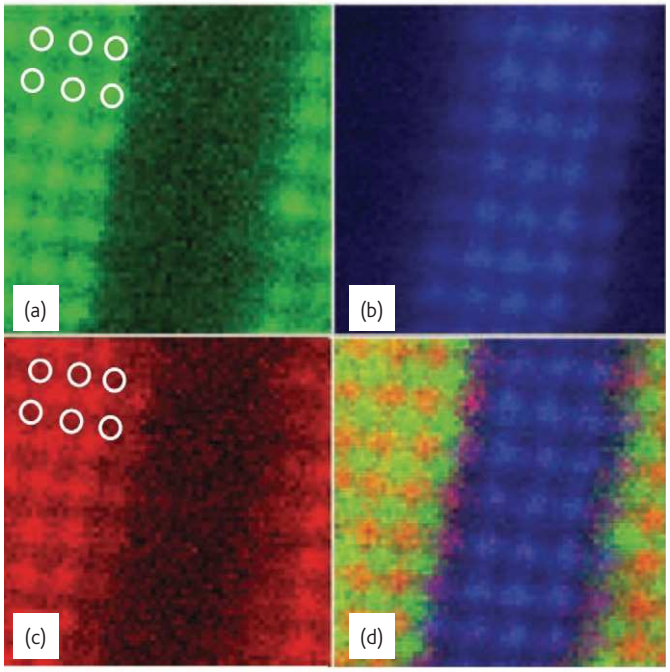

Fig. 2. Images of a $La_{0.7}Sr_{0.3}MnO_3/SrTiO_3$ multilayer, formed using the (A) La M edge; (B) Ti L edge; and (C) Mn L edge. (D) An RGB false colour image combining the three spectroscopic images. The field of view is 3.1 nm. (Reprinted with permission from13. © AAAS.)

the beam current is inevitably reduced, close to 0.1eV energy resolution is now possible in monochromated instruments. This enables spectral details to be seen in the low loss region of the EELS spectrum, and in the fine structure at ionization edges, that were not possible before. In the low loss region it is possible to measure accurately band gaps of semiconductors and to reveal the low energy excitations that are so important in some metallic nanostructures[14]. The EELS fine structure can yield important information about the local chemical environment and valency. This fine spectral detail, coupled with atomic resolution imaging, makes the new generation of STEM instruments remarkably powerful.

An additional benefit of aberration correctors, especially for TEMs, is the possibility of achieving atomic resolution with (corrected) objective lenses with large pole-piece gaps. This has led to the emergence of new in situ instruments that enable a variety of experiments to be undertaken at high resolution and often in movie mode. The large gap allows novel holders to be inserted, including: in situ STM and AFM holders, to enable simultaneous TEM and SPM; nanoindenters, which reveal in real-time the microstructural changes brought about through mechanical deformation; electrical stages, to apply current and voltages, especially in combination with techniques such as electron holography, to reveal local electromagnetic potentials[15]; and environmental stages that allow high (often reactive) gases within the microscope and at elevated temperatures. Dedicated aberration-corrected environmental instruments, or 'E-TEMs', have emerged

recently that are of particular interest to those studying nanoscale growth (of, for example, nanotubes and nanorods) and heterogeneous catalysis[16].

The possibility of studying the dynamics of a reaction can now be taken to the limit using electron microscopes fitted with laser-pulsed photocathodes. Building on the work of Zewail in Caltech and Bostanjoglo in Berlin, a number of groups worldwide have begun to develop electron microscopes that can record images and diffraction patterns with ultrafast electron beams. Femtosecond lasers can be used to excite a specimen and, with a suitable time delay, to excite the photocathode to produce a pulsed electron beam in a 'pump-probe' experiment. Two different modes of operation are emerging: one with multiple femtosecond pulses building up the image pulse by pulse[17] and the other a one-shot method whereby a single nanosecond pulse is used to record a single image[18]. A resolution approaching 1 nm is now possible with this method and, with further redesigns of the microscope gun and column, it may be possible to record atomic resolution images, perhaps with a sub-nanosecond acquisition.

To enable such images to have a sufficient signal-to-noise ratio, ultrafast experiments often use beams with extremely high electron currents. Unless damage (by electron or laser) is deliberate, specimens must be suitably robust to withstand these. The concerns about beam damage have led to microscopes being operated at ever-lower voltages (in contrast to the early push for higher voltages), especially for carbonaceous materials such as carbon nanotubes. Aberration-corrected microscopy at 80 kV is now available routinely, and even lower voltages have been tried on such instruments. Of course, as the voltage falls one starts to approach the realm of the SEM, and it would be remiss not to mention just briefly here that modern field emission gun-based SEMs are themselves remarkable instruments which can offer sub-nanometer resolution. Indeed, the distinction between STEM and SEM technology continues to blur: STEM detectors on SEMs are available to examine thin samples, and it is likely that we will see commercial aberration-corrected SEMs in the near future (indeed, Haider's prototype corrector was based on an SEM).

All images are to some extent two-dimensional (2-D) projections of a 3-D structure, and it is worth mentioning that 3-D imaging, or tomography, continues to grow as a key tool in this area. Many modern nanoscale devices are 3-D by design and function, and so 3-D imaging is essential to fully characterize the structure and properties[19]. Atomic resolution tomography using the TEM or STEM is not far away and will act as a genuinely complementary technique to the atom probe microscope, which, with modern designs (such as the LEAP[20]) and the possibility of laser pulsing the tip, has emerged as a far more versatile technique than was the case even 10 years ago.

Before concluding this article, we should mention just briefly the progress made in electron cryomicroscopy for structural biology. Over the past few years, the automation of data collection, better low-temperature microscopy techniques and new image processing methods have all contributed to improvements in the resolution of biological structures, one recent example[21] illustrating near-atomic-resolution 3-D density maps of a rotavirus. Phase contrast imaging required in cryomicroscopy should be enhanced with the introduction of phase plates, akin to those used in optical imaging, and the requirement to produce phase contrast by defocusing (and thus necessarily delocalizing the image information) will no longer be necessary. More exotic methods are also being proposed to study individual frozen molecules with femtosecond pulses of electrons and X-rays[22]. Given these advances, it may yet be possible to achieve true atomic resolution maps of large asymmetric macromolecules by electron microscopy[23].

The technical developments seen in electron microscope design have been remarkably swift in the past few years. We are now quickly reaching the point where the information available in the microscope image is no longer limited by the deficiencies of the microscope but by the sample. Focused ion beam technology has allowed thin membranes to be cut from almost any material, but damage (e.g. amorphization, implantation) is still a problem. New methods will have to be developed to ensure that each microscope sample is as damage-free as possible and to allow the microscopist to get the best from these remarkable new instruments. mt

References

1. Crommie, M. F., et al., *Science* (1993) **262**, 218
2. Repp, J., et al., *Phys. Rev. Lett.* (2001) **86**, 252
3. Manalis, S. R., et al., *Appl. Phys. Lett.* (1996) **68**, 871
4. Stipe, B. C., et al., *Science* (1998) **280**, 1732
5. Heinrich, A. J., et al., *Science* (2004) **306**, 466
6. Scherzer, O., Z. *Physik* (1936) **101**, 593
7. Rose, H., *Optik* (1990) **85**, 19
8. Haider, M., et al., *Nature* (1998) **392**, 768
9. Krivanek, O. L., et al., EMAG '97. Inst. Phys. Conf. Ser. (1997) **153**, 35
10. Jia, C. L., and Urban, K., *Science* (2004) **303**, 2001
11. http://ncem.lbl.gov/TEAM-project/
12. Batson, P. E., et al., *Nature* (2002) **418**, 617
13. Muller, D. A., et al., *Science* (2008) **319**, 1073
14. Nelayah, J., et al., *Nature Physics* (2007) **3**, 348
15. Twitchett-Harrison, A. C., et al., *Nano Lett.* (2007) **7,** 2020
16. Helveg, S., et al., *Nature* (2004) **427**, 426
17. Lobastov, V. A., et al., *Nano Lett.* (2007) **7**, 2552
18 Armstrong, M. R., et al., *Ultramicroscopy* (2007) **107**, 356
19. Midgley, P. A., and Weyland, M., *Ultramicroscopy* (2003) **96**, 413
20. Thompson, K., et al., *Science* (2007) **317**, 1370
21. Zhang, X., et al., Proc. Natl Acad. Sci. USA (2008) **105**, 1867
22. Spence J.C.H. and Hawkes P.W *Ultramicroscopy,* (2008) **108**, 1502
23. Glaeser, R. M. Proc. *Natl Acad. Sci.* USA (2008) **105,** 1779