

Preface

In these two volumes, we have asked many of the leaders in the field of modern microscopy to summarize the latest approaches to the imaging of atoms or molecular structures, and, more especially, the way in which this aids our understanding of atomic processes and interactions in the organic and inorganic worlds.

Man's curiosity to examine the nanoworld is as at least as old as the Greeks. Aristophanes, in a fourth-century BC play, refers to a burning glass; the Roman rhetorician Seneca describes hollow spheres of glass filled with water being used as magnifiers, while Marco Polo in the thirteenth century remarks on the Chinese habit of wearing spectacles. Throughout this time it would have been common knowledge that a drop of water over a particle on glass will provide a magnified image, while a droplet within a small hole does even better as a biconvex lens. By the sixteenth century magnifying glasses were common in Europe, but it was Anthony van Leeuwenhoek (1632–1723) who first succeeded in grinding lenses accurately enough to produce a better image with his single-lens instrument than with the primitive compound microscopes then available. His 112 papers, published in *Philosophical Transactions of the Royal Society*, brought the microworld to the general scientific community for the first time, covering everything from sperm to the internal structure of the flea. Robert Hooke (1635–1703) developed the compound microscope, publishing his results in careful drawings of what he saw in his *Micrographia* (1665). The copy of this book in the University of Bristol library shows remarkable sketches of faceted crystallites, below which he has drawn piles of cannon balls, whose faces make corresponding angles. This strongly suggests that Hooke believed that matter consists of atoms and had made this discovery long before its official rediscovery by the first modern chemists, notably Dalton in 1803. (Greeks such as Leucippus (450 BC) had long before convinced themselves that a stone, cut repeatedly, would eventually lead to “a smallest fragment” or fundamental particle; Democritus once said that “nothing exists except atoms and empty space. All else is opinion” (!))

This atomic hypothesis itself has a fascinating history, and is intimately connected with the history of microscopy. It was Brown's observation in 1827 of the motion of pollen in water by optical micro-

scopy which laid the basis for the modern theory of matter based on atoms. As late as 1900 many chemists and physicists did not believe in atoms, despite the many independent estimates that could be made of their size. These were summarized by Kelvin and Tait in an appendix to their *Treatise on Natural Philosophy*, together with an erroneous and rather superficial estimate of the age of the earth, to be used against Darwin. (This text was the standard English language physics text of the late nineteenth century, despite its failure to cover much of Maxwell's work.) Einstein's 1905 theory of Brownian motion, and Perrin's (1909) more accurate repetition of Brown's experiment, using microscope observations to estimate Avogadro's number, finally settled the matter regarding the existence of atoms. Einstein does not reference Brown's paper, but indicates that he had been told about it. But as Archie Howie has commented, it is interesting to speculate how different the history of science would be if Maxwell had read the Brown paper and applied his early statistical mechanics to it. By the time of Perrin's paper, Bohr, Thomson, Rutherford and others were well committed to atomic and even subatomic physics.

In biology, the optical microscope remained an indispensable tool from van Leeuwenhoek's time with many incremental improvements, able to identify bacteria and their role in disease, but not viruses, which were first seen with the transmission electron microscope (TEM) in 1938. With Zernike's phase contrast theory in the thirties a major step forward was taken, but the really dramatic and spectacular modern advances had to await the widespread use of the TEM, the invention of the laser and the CCD detector, the introduction of the scanning mode, computer control and data acquisition, and the production of fluorescent proteins.

The importance of this early history should not be underestimated—in the words of Feynman "If in some cataclysm, all scientific knowledge were to be destroyed and only one sentence passed on to the next generation of creatures, what statement would contain the most information in the fewest words? I believe it is the atomic hypothesis—that all things are made of atoms."

Images of individual atoms were first provided by Muller's field-ion microscope in the early 1950s, soon to be followed by Albert Crewe's Scanning Transmission Electron Microscope (STEM) images of heavy atoms on thin-film surfaces in 1970. With its subångström resolution, the modern transmission electron microscopes (TEM) can now routinely image atomic columns in thin crystals. For favorable surface structures, the scanning tunneling microscope has provided us with images of individual surface atoms since its invention in 1982, and resulted in a rich spin-off of related techniques.

Probes of condensed and biological matter must possess a long lifetime if they are to be used as free-particle beams. For the most part this has limited investigators to the use of light, X-rays, neutrons and electrons. The major techniques can then often be classified as imaging, diffraction, and spectroscopy. These may be used in both the transmission and reflection geometries, giving bulk and surface information respectively. Chapter 8 (Bauer) reviews both the low-energy electron

microscope (LEEM) and spin-polarized LEEM methods which, using reflected electrons, have recently revolutionized surface science and thin-film magnetism. Here the high cross-section allows movies to be made of surface processes at submicrometer resolution, while Auger electron spectroscopy is conveniently incorporated. Chapter 9 (Feng and Scholl) deals with the closely related photoelectron microscopy, where a LEEM instrument is used to image the photoelectrons excited by a synchrotron beam. Here the superb energy selectivity of optical excitation can be used to great advantage. Chapter 3 (Reichelt) describes advances in scanning electron microscope (SEM) research, where the lower-energy secondary electrons provide images with large depth of focus for the most versatile of all electron-optical instruments. The numerous modes of operation include X-ray analysis, cathodoluminescence, low-voltage modes for insulators and the controlled-atmosphere environmental SEM (ESEM). Turning now to the transmission geometry, we review the latest work in atomic-resolution TEM in Chapter 1 (Kirkland and Hutchison), the technique which has transformed our understanding of defect processes in the bulk of solids such as oxides, and the STEM mode in Chapter 2 (Nellist). STEM provides an additional powerful analytical capability, which, like the STM, can provide spectroscopy with atomic-scale spatial resolution. An entire chapter (Chapter 4, Botton) is then devoted to analytical TEM (AEM), with a detailed analysis of the physics and performance of its two main detectors, for characteristic X-ray emission and energy-loss spectroscopy. The remarkable recent achievements of *in-situ* TEM are surveyed in Chapter 6 (Ross), including transmission imaging of liquid cell electrolysis, observations of the earliest stages of crystal and nanotube growth, phase transitions and catalysts, superconductors, magnetic and ferroelectric domains and plastic deformation in thin films, all at nanometer resolution or better. Again, the large scattering cross-section of electron probes provides plenty of signal even from individual atoms, so that movies can be made. Chapter 5 (King, Armstrong, Bostanjoglo and Reed) summarizes the dramatic recent revival of time-resolved electron microscope imaging, which uses laser-pulses to excite processes in a sample. The excited state may then be imaged by passing the delayed pulse to the photocathode of the TEM in this “pump-probe” mode. Single-shot transmission electron diffraction patterns have now been obtained using electron pulses as short as a picosecond. Most of these techniques are undergoing a quiet revolution as electron-optical aberration corrector devices are being fitted to microscopes. The dramatic discovery, that, after 60 years of effort, aberration correction is now a reality, was made about ten years ago, and we review the relevant electron-optical theoretical background in Chapter 10 (Hawkes). Finally, in biology, potentially the largest scientific payoff of all is occurring in the field of cryo-electron microscopy, where single-particle images of macromolecules embedded in thin films of ice are imaged, and a three-dimensional reconstruction is made. While the structure of the ribosome and purple membrane protein (among many others) have already been determined in this way, the grand challenge of locating every protein and molecular machine in a single cell remains

to be completed. We summarize this exciting field in Chapter 7 (Plitzko and Baumeister).

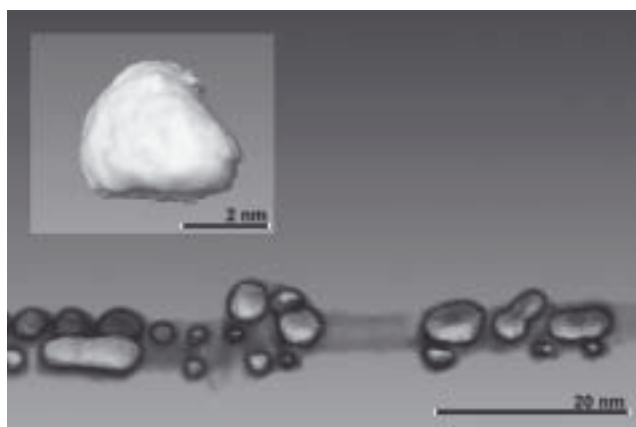
Electrons, with the largest cross-section and a source brighter than current generation synchrotrons, provide the strongest signal and hence the best resolution. They do this in a manner that can conveniently be combined with spectroscopy, and we now have aberration-corrected lenses for them. But multiple scattering and inelastic background scattering often complicate interpretation. X-ray imaging of nanostructures, even at synchrotrons, involves much longer data acquisition times but the absence of background and multiple scattering effects greatly improves quantification of data, and thicker samples can be examined. (It is easy to show that the small magnitude of the fine-structure constant will almost certainly never permit imaging of individual atoms using X-rays. We should also recall that in protein crystallography, about 98% of the X-ray beam hits the beam-stop and does not interact with the sample. Of the remaining 2%, 84% is annihilated in production of photoelectrons, and 8% in Compton scattering, while only the remainder produces Bragg diffraction. For light elements the inelastic cross-section for kilovolt electrons is about three times the elastic.) Success with X-rays has thus come mainly through the use of crystallographic redundancy to reduce radiation damage in protein crystallography. However, soft X-ray imaging with zone-plate lenses provides about 30 nm resolution in the “water window” with the advantages of thick samples and an aqueous environment. Applications have also been found in environmental science, materials science and magnetic materials. In addition, the equivalent of the STEM has been developed for soft X-rays: the scanning transmission x-ray microscope (STXM), which uses a zone-plate to focus X-rays onto a sample that can be translated by piezo motors. This arrangement can then provide spatially-resolved X-ray absorption spectroscopy. This work is reviewed in Chapter 13 (Howells, Jacobsen and Warwick).

Both X-ray and electron-beam imaging methods are limited in biology by the radiation damage they create, unlike microscopy with visible light, which also allows observations in the natural state. Optical microscopy is undergoing a revolution, with the development of super-resolution, two-photon, fluorescent labeling and scanning confocal methods. These methods are reviewed in Chapters 11 and 12. Chapter 11 (Diaspro, Schneider, Bianchini, Caorsi, Mazza, Pesce, Testa, Vicidomini, and Usai) discusses two-photon confocal microscopy, in which the spot-scanning mode is adopted, and a symmetrical lens beyond the sample collects light predominantly from the excitation region, thereby eliminating most of the “out-of-focus” background produced in the normal full-field “optical sectioning” mode. Three-dimensional image reconstruction is then possible. Two-photon microscopy combines this with a fluorescence process in which two low-energy incident photons are required to excite a detectable photon emitted at the sum of their energies. This has several advantages, by reducing radiation damage and background, and allowing observation of thicker samples. The method can also be used to initiate photochemical reactions for study. Chapter 12 (Hell and Schönle) describes the

latest super-resolution schemes for optical microscopy, which have now brought the lateral resolution to about 28 nm and, by the symmetrical lens arrangement (4π confocal), increased resolution measured along the optic axis by a factor of up to seven. The lateral resolution can be improved by modulating the illumination field or by using the stimulated emission depletion microscopy mode (STED), in which saturated excitation of a fluorophore produces nonlinear effects allowing the diffraction barrier to resolution to be broken.

For the scanning near-field probes new possibilities arise. Although restricted to the surface (the site of most chemical activity) and requiring in some cases complex image interpretation, damage is reduced, while the subångström resolution normal to the surface is unparalleled. The method is also conveniently combined with spectroscopy. Early work was challenged by problems of reproducibility and tip artifacts, but Chapters 14–17 in this book show the truly remarkable recent progress in surface science, materials science and biology. Chapter 14 (Nikiforov and Bonnell) describes the various modes of atomic force microscopy which can be used to extract atomic-scale information from the surfaces of modern materials, including oxides and semiconductors. Work-functions can be mapped out (a Kelvin probe with good spatial resolution) and a variety of useful signals obtained by modulation spectroscopy methods. In this way maps of magnetic force, local dopant density, resistivity, contact potential and topography may be obtained. Chapter 15 (Sutter) describes applications of the scanning tunneling microscope (STM) in materials science, including inelastic tunneling, surface structure analysis in surface science, the information on electronic structure which may be extracted, atomic manipulation, quantum size and sub-surface effects, and high temperature imaging. Weierstall, in Chapter 16, reviews STM research at low temperatures, including a thorough analysis of instrumental design considerations and applications. These include measurements of local density-of-states oscillations, energy dispersion measurements, electron confinement, lifetime measurements, the Stark and Kondo effects, atomic manipulation, local inelastic tunneling spectroscopy, photon emission, superconductivity and spin-polarized tunneling microscopy. Finally, in Chapter 16, Amrein reviews the special problems that arise when the atomic force microscope (AFM) is applied to the imaging of biomolecules; much practical information on instrumentation and sample preparation is provided, and many striking examples of cell and macromolecule images are shown.

We include two chapters on unconventional “lensless” imaging methods—Chapter 18 (Dunin-Borkowski, Kasama, McCartney and Smith) deals with electron holography and Chapter 19 with diffractive imaging. Gabor’s original 1948 proposal for holography was intended to improve the resolution of electron microscopes, and only recently have his plans been realized. Meanwhile, electron holography using Möllenstedt’s biprism and the Lorentz mode has proved an extremely powerful method of imaging the magnetic and electrostatic fields within matter. Dramatic examples have included TEM movies of superconducting vortices as temperature and applied fields are varied, and ferroelectric and magnetic domain images, all within thin self-supporting films. Chapter 19 (Spence) describes the recent develop-



A projection from a three-dimensional image of a carbon nanotube with gold clusters attached. This was reconstructed by taking a series of projected STEM-ADF images at different tilt angles. A faceted gold cluster is shown in the inset. Electron tomography makes it possible to study the three-dimensional nanotube-metal contact geometry which determines the electrical contact resistance to the nanotubes (courtesy of J. Cha, M. Weyland, and D. Muller, 2006).

ment of new iterative solutions to the non-crystallographic phase problem, which now allows diffraction-limited images to be reconstructed from the far-field scattered intensity distribution. This has produced lensless atomic-resolution images of carbon nanotubes (reconstructed from electron microdiffraction patterns) and phase contrast images from both neutron and soft X-ray Fraunhofer diffraction patterns of isolated, non-periodic objects. In this work, lenses are replaced by computers, so that images may now be formed with any radiation for which no lens exists, free of aberrations. Our volumes end with a chapter by van Aert, den Dekker, van Dyck and van den Bos on the definition of resolution in all its forms.

Coverage has been limited to high-resolution methods, with the result that some important new microscopies have been omitted (such as magnetic resonance imaging (MRI), projection X-ray tomography, acoustic imaging etc.). Field-ion microscopy and near-field optical microscopy are also absent. Conversely, although there is no chapter on tomography in materials science, we must mention the rapid progress of these techniques, which has culminated in a remarkable near-atomic reconstruction by J. Cha, M. Weyland and D. Muller of a carbon nanotube to which gold clusters are attached (see figure). For further information on this branch of tomography, see Midgley and Weyland (2003), Midgley (2005), Weyland et al. (2006) and Kawase et al. (2006).

The ingenuity and creativity of the microscopy community as recorded in these pages are remarkable, as is the spectacular nature of the images presented. Neither shows any signs of abating. As in the past, we fully expect major advances in science to continue to result from breakthroughs in the development of new microscopies.

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References

- Kawase, N., Kato, M., Nishioka, H. and Jinnai, H. (2006). Transmission electron microtomography without the “missing wedge” for quantitative structural analysis. *Ultramicroscopy* (2006) forthcoming.
- Midgley, P.A. (2005). Tomography using the transmission electron microscope. In *Handbook of Microscopy for Nanotechnology* (Yao, N. and Wang, Z.L., Eds) 601–627 (Kluwer, Boston).
- Midgley, P.A. and Weyland, M. (2003). 3D electron microscopy in the physical sciences: the development of Z-contrast and EFTEM tomography. *Ultramicroscopy* **96**, 413–431.
- Weyland, M., Yates, T.J.V., Dunin-Borkowski, R.E., Laffont, L. and Midgley, P.A. (2006). Nanoscale analysis of three-dimensional structures by electron tomography. *Scripta Mater.* **55**, 29–33.

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