Biological Low-Voltage Scanning Electron Microscopy

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Edited by

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Preface

Ever since its advent in the 1950s, the scanning electron microscope hs offered an image of surface structures that was both uniquely detailed and easily interpretable. Although initially, the resolution of the images it produced did not match that of those made using the transmission electron microscope, this deficit no longer relevant on biological specimens where, for both instruments, image quality of is now limited fundamentally by the fragility of the specimen.

High-resolution, low-voltage scanning electron microscopy (LVSEM) is now a powerful tool to study biological structure. Particularly when coupled with novel specimen preparation techniques, it has allowed us to understand in three dimensions objects that previously could only be imaged from serial-sectioned material analyzed with transmission electron microscopy.

Because LVSEM provides images with clear topographic contrast from specimens coated with only an extremely thin metal coating, it can provide high-resolution images of macromolecular complexes and of structural interactions that are free from the confusion of structural overlap. What is more, it provides this analysis in the context of being able to view the large specimen areas needed to provide context.

These capabilities are particularly useful for applications in cellular biology. In addition, specific molecular components on surfaces and internal cell structures can be identified by using colloidal-gold labeling techniques. Particular internal structures can be viewed either by isolating them or by using novel fracturing and sectioning techniques that cause the internal components to occur on the outer surface of the specimen. There, the LVSEM can image them with a degree of topological precision that is often not possible with conventional TEM.

Given these tremendous capabilities, it seemed to us both surprising and unfortunate that that LVSEM was not used more often to study biological structure. In a time when interest is extending from the genome to the proteome and when we increasingly want to know not only the existence but also the localization and interactions of specific molecules and molecular complexes, it seemed to us that LVSEM was the ideal modality for answering a myriad of important questions in cellular biology and development. What seemed to be needed was a way to make potential users more aware of LVSEM's unique and powerful capabilities and also to provide the reader with both meaningful examples from a variety of applications and suitable protocols for preparing specimens.

We approached a number of leaders in the field with this idea and received a most enthusiastic response. The topics chosen were selected to be of interest to scientists, technicians, students, teachers, and to all who are interested in expanding their knowledge related to LVSEM. The specific topics covered in this book include high-resolution LVSEM applications to cellular biology and detailed specimen preparation techniques for molecular labeling and correlative microscopy, cryoSEM of biological samples, and new developments in LVFESEM instrumentation in x-ray microanalysis at low beam voltage.

Biological Low Voltage Scanning Electron Microscopy covers many aspects of specimen preparation and provides specific protocols for practical applications that are commonly not available in research papers. It also gives general as well as detailed insights into the theoretical aspects of LVSEM. The book is intended for a large audience as a reference book on the subject. By providing both theory and practical applications related to imaging biological structures with LVFESEM, we hope that it will fill a gap in the literature.

During the editing process of this book, two of our most treasured colleagues, who have advanced the field immensely, passed away. Both the late Dr. Hans Ris and the late Dr. Stanley Erlandsen were passionate about the usefulness of LVSEM to enhance their own research, and as such, they left a wealth of new knowledge, novel techniques, and ideas for new applications for the scientific community. Their contributions are of great value to future scientist, students, technical staff, and many other using LVSEM.

The editors are most grateful to all authors who have contributed their superb and unique expertise to this project and shared their insights with the present community interested in microscopy and those who will enter the field in the future.

We would like to thank Kathy Lyons, our ever-so-patient editor at Springer. In addition, one of the editors (JP) would also like to thank Bill Feeny, the Zoology Departmental artist, and Kandis Elliot, the Botany Department artist, for their help in preparing the figures.

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Chapter 1
The Early Development of the Scanning Electron Microscope

Dennis McMullan

It has been forty years since the scanning electron microscope (SEM) became a significant instrument in the scientific community. In 1965, the Cambridge Instrument Company in the United Kingdom marketed their Stereoscan 1 SEM, which was followed about 6 months later by JEOL of Japan with the JSM-1. Before 1965, there were about thirty years of intermittent SEM development in Germany, the United States, England, and Japan, although Japanese development was apparently not covered in the published literature. Development began in the 1930s in Germany, and then began towards the end of that decade in the United States.

During these early years, there were two different approaches: the first, which had some specific relevance to the low voltage scanning transmission microscope (LVSEM); and the later one that was linked to the transmission electron microscope (TEM) and led to the scanning transmission electron microscope (STEM) and the future form of the current SEM. But first, we must share a few words about the early history of scanning and its application in microscopy.

Invention of Scanning

In the 1840s, Alexander Bain, a Scottish clockmaker, invented the principle of dissecting an image by scanning, and he was granted a British patent (Bain 1843) for the first fax machine (McMullan 1990). At the transmitter, a stylus mounted on a pendulum contacts the surface of metal type forming the message, thus closing an electrical circuit. At the receiver, a similar stylus, also on a pendulum, records electrochemically on dampened paper. Following each swing of the pendulums, the type and the recording paper are lowered by one line. The means for starting the pendulums swinging simultaneously and synchronising them magnetically are described in the patent.

Scanning Optical Microscopy

The first proposal in print for applying scanning to microscopy was made in Dublin by Edward Synge (1928). The proposal was for a scanning optical microscope, and his goal was to overcome the Abbe limit on resolution by

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what is now called "near-field microscopy"—that is, the production of a very small light probe by collimation through an aperture smaller than the wavelength of the light.

Synge was a scientific dilettante who had original ideas in several scientific fields, but did not attempt to put them into practice (McMullan 1990). However, he considered some of the problems that would be encountered with a scanning microscope and he proposed the use of piezo-electric actuators (Synge 1932), which are now used with great success in the scanning tunneling microscope and other probe instruments—including, of course, the near-field optical microscope itself. He envisaged fast scanning of the sample so that a visible image could be displayed on a phosphor screen, and he also pointed out the possibility of contrast expansion to enhance the image from a low contrast sample—probably the first mention of image processing by electronic means (as distinct from photographic).

Charged Particle Beams

A proposal for using an electron beam in a scanning instrument was described in German patents by Hugo Stintzing of Giessen University (1929). These patents were concerned with the automatic detection, sizing, and counting of particles using a light beam, or—for those of below-light microscopic size—a beam of electrons. The focusing of electrons was (at that time) unknown to him, as to most others, and he proposed obtaining a small diameter probe using crossed slits. The samples would be either mechanically scanned in the case of a light beam, or one could use electric or magnetic fields to deflect an electron beam. Suitable detectors would be used to detect the transmitted beam that was attenuated by absorption or scattering. The output was to be recorded on a chart recorder so that the linear dimension of a particle could be given by the width of a deflection, and the thickness by the amplitude—the production of a two-dimensional image was not suggested. Stintzing did not apparently attempt the construction of this instrument and there are no drawings accompanying the patent specification. Thomas Mulvey (1962), however, published a block-schematic diagram of Stintzing's proposal much later.

The Transmission Electron Microscope

In the early 1930s, the main center for the development of the electron microscope was the Berlin Technische Hochschule in the laboratory of Professor Matthias where Max Knoll was a research assistant supervising students—including Ernst Ruska, whose subject area was electron optics. Arising from this work, Knoll and Ruska demonstrated the first transmission electron microscope with a magnification of x16.

From the very beginning of electron microscopy, the imaging of solid samples was an important goal, particularly as the methods for producing thin samples were not developed until later. The first attempt was by Ruska (1933), with the sample

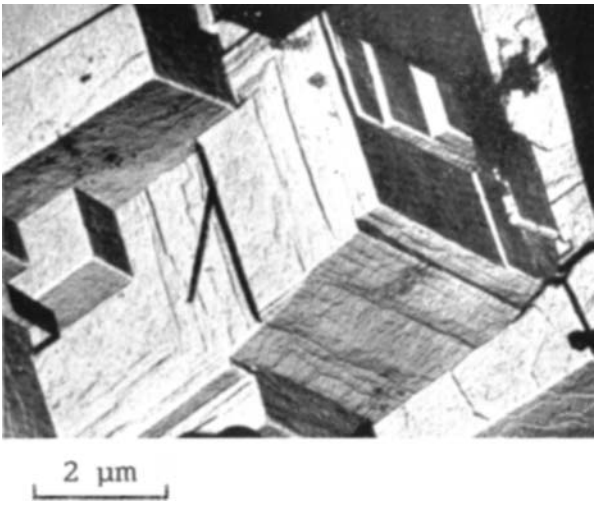

Fig. 1.1 Early TEM image of an oxide replica of etched aluminium (Mahl 1940)

surface normal to the viewing direction and illumination by an electron beam at grazing incidence to the surface. Ruska obtained images of copper and gold surfaces but at a magnification of only x10. A few years later, he made a second attempt (Ruska & Müller 1940) with the same geometry and with only marginally better results. von Borries (1940) was much more successful with his grazing incidence method in the transmission electron microscope (TEM), where the sample surface is at a few degrees both to the viewing direction and to the illuminating beam.

A breakthrough in the microscopic imaging of surface topography in the TEM was the 1940s introduction of replicas by H. Mahl (1940), and these set the standard for the next 25 years—although they were tedious to make and could be subject to serious artifacts. An early example is shown in Fig. 1.1.

Electron Beam Scanner

Knoll, the co-inventor of the TEM with Ruska, was the first to publish images from solid samples obtained by scanning an electron beam (Knoll 1935). In 1932, very soon after the building of the first TEM at the Berlin Technische Hochschule, he joined the Telefunken Company as the director of research to develop television (TV) camera tubes. There, he designed an electron beam scanner for studying the targets of these tubes. A schematic block diagram is shown in Fig. 1.2 (the sample was mounted at one end of a sealed-off glass tube, and an electron gun was located at the other). The accelerating potential was in the range of 500–4,000 V, and the beam was focused on the surface of the sample and scanned by deflection coils in a raster of 200 lines and 50 frames/s. The current collected by the sample (the difference of the incident and secondary emitted currents) was amplified by a thermionic tube

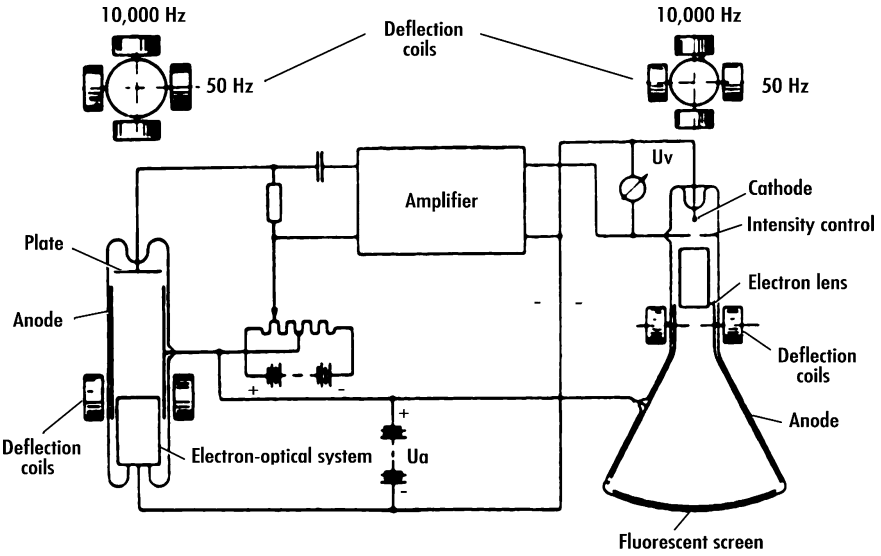

Fig. 1.2 Schematic diagram of Knoll's (1935) electron beam scanner; (labels translated by T. Mulvey)

amplifier and intensity-modulated cathode-ray tube that was scanned by deflection coils connected in a series with those on the electron-beam scanner. By changing the ratio of the scan amplitudes, the magnification could be varied. Knoll used mainly unity magnification, but he could increase it to about x10 before the resolution was limited by the diameter of the scanning probe.

This apparatus had virtually all the features of an SEM, but Knoll surprisingly (in view of his earlier work on the TEM) did not use additional electron lenses to reduce the size of the probe below 100 μm. The resolution he obtained, however, was entirely adequate for his purpose. The beam current was relatively high—on the order of microamps—and therefore thermionic tubes could be used to amplify the signal current in spite of the fast scan rate. He must have realized that reducing the size of the probe would be counter-productive because there were no suitable high-gain electron detectors in existence at that time.

Similar images were produced by others working on the development of TV cameras in the 1930s (e.g., von Ardenne 1985), but Knoll was the only one at the time who looked at samples other than camera tube targets (e.g. silicon iron in Fig. 1.3), and he also elucidated the contrast mechanisms of secondary electron coefficients and topography. The images were true secondary electron images because the electron gun and sample were enclosed in the highly evacuated and baked glass envelope, and there was therefore little or no contamination of the surface. It is only comparatively recently that ultra-high vacuum (UHV) SEMs have been available that can work in this imaging regime. Knoll continued using his electron beam scanner (which he named *der Elektronenabtaster*) for a number of purposes, including the study of oxide layers on metals (Knoll 1941).

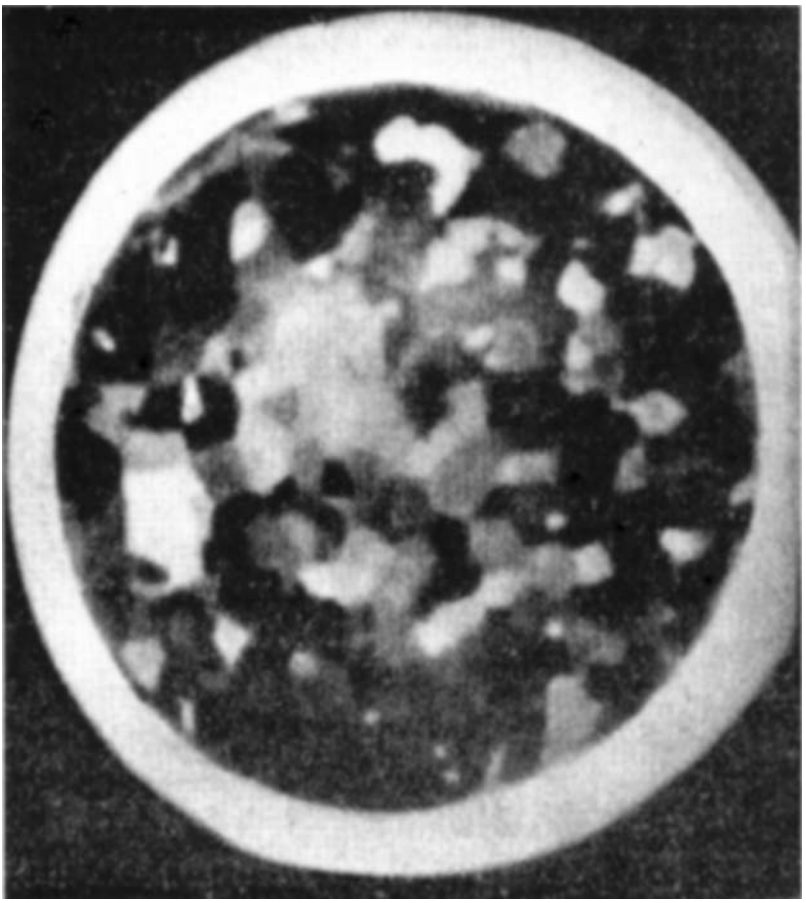

Fig. 1.3 Electron-beam scanner image of a silicon iron sheet showing electron channeling contrast; horizontal field width = 50 mm (Knoll 1935)

A few years later, Manfred von Ardenne, also in Berlin, built a very different instrument that was in fact a scanning transmission electron microscope (STEM) that he intended to also use for solid samples. His hope was not realized at the time, but his work established many of the principles used in all future SEMs. This is described further in this chapter, but first we will consider Vladimir Zworykin's RCA microscope—which actually came after von Ardenne's but was related much more closely to Knoll's pioneering work—and also to some aspects of later LVSEMs.

The RCA Scanning Electron Microscope

Zworykin, who was director of research at the RCA Laboratories in Camden, N.J., initiated a development program for SEM (Zworykin et al. 1942) in 1938 that continued until about 1942. He was a pioneer of electron-scanned TV camera tubes

dating back to the 1920s, and had also developed the first optical microscope with video output (Zworykin 1934). The development of the SEM was done in parallel with that of a TEM, and by the same staff—in particular, J. Hillier, E.G. Ramberg, A.W.Vance and R.L. Snyder as well as Zworykin himself.

Although Zworykin had every microscope paper from Germany translated as soon as it was received (Reisner 1989), he was apparently not influenced by von Ardenne's work on the STEM/SEM. Instead, he started by repeating Knoll's beam scanner experiments (in effect) using a *monoscope*. The monoscope was a pattern-generating, cathode-ray tube that had been invented in Knoll's department at Telefunken (1935) and further developed by RCA for television use (Burnett 1938). His team then built an SEM based on the monoscope, but with two magnetic lenses to produce a very small focused probe, and a demountable vacuum system so that the sample could be changed (Zworykin et al. 1942). The scan rate was the US TV standard—441 lines and 30 frames/second—and the signal was amplified by a thermionic tube video amplifier. For a signal-to-noise ratio of 10, the signal current had to be 3×10^{-8} A, which could only be reached if the probe diameter was about 1 μm.

He then tried to obtain a high current in a smaller probe by using a field emission gun with a single-crystal tungsten point, presumably based on experience with the point projection microscope that had been built in the RCA Laboratories by G.A. Morton and E.G. Ramberg (1939). To reach a sufficiently high vacuum, Zworykin had to return to having the gun and the sample in a glass envelope that had been baked and sealed off. A single magnetic lens was used, and fleeting images were obtained at x8,000 magnification, with scanning at TV rate and a thermionic tube amplifier. Stable images could no doubt have been achieved, but at that time a practical microscope would not have resulted because demountable UHV techniques did not then exist.

To overcome the noise problem, Zworykin therefore decided to build an SEM with an efficient electron detector and a slower scan. The detector was the combination of phosphor and photomultiplier that T.E. Everhart and R.F.M. Thornley (1960) used nearly twenty years later in an improved form. To bring the secondary electrons to it, he designed an electrostatic immersion lens that retarded the beam electrons and accelerated the secondaries. Figure 1.4 shows the final electron optical arrangement. Electrostatic lenses were used to produce a demagnified image of the source on the sample that was held at +800 V relative to the grounded gun cathode. The electron beam leaving the gun was accelerated to 10 keV in the intervening electron optics. The secondary electrons returning from the sample were similarly accelerated, and diverged as they passed through the 4th electrostatic lens and hit the phosphor screen with an energy of 9.2 keV.

In the first instrument, the scanning was done by electro-mechanically moving the sample relative to the beam using loudspeaker voice-coils and (later) hydraulic actuators—it was only in the final version that magnetic scanning of the beam was employed. The scan time was fixed at 10 min by the facsimile recorder that was used for image recording, and also controlled the microscope scans. There was no provision for a faster scan or the production of a visible image on a TV monitor—this seems strange remembering Zworykin's TV background, but it may have been because the signal bandwidth was seriously limited by the decay time of

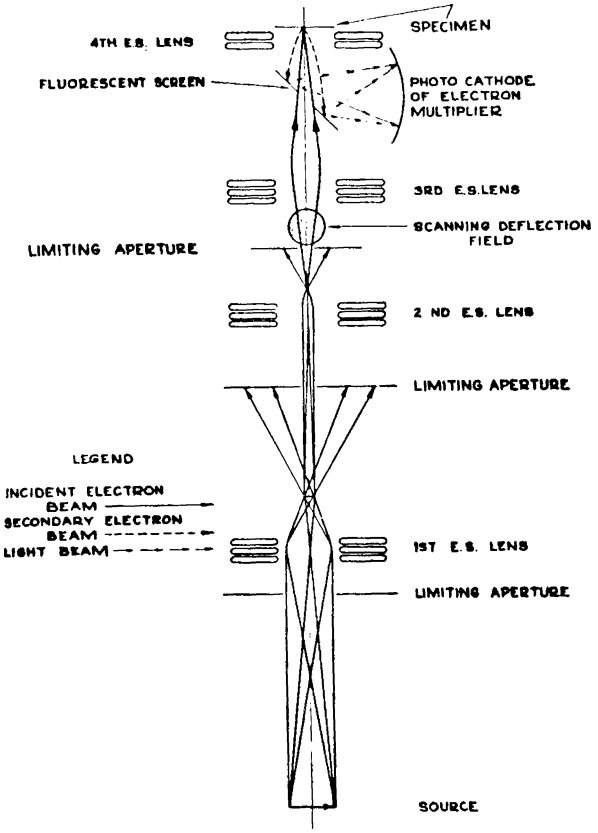

Fig. 1.4 The electron optics of the SEM built by Zworykin et al. (1942)

the phosphor, which was a problem important in later work at Cambridge (McMullan 1952). The optimum focus setting was found by maximizing the high frequency components in the video waveform observed on an oscilloscope, a method that was originally proposed by von Ardenne (1938b).

Although the intention was to produce contrast by differences in the secondary emission ratio of the surface constituents, and the incident beam energy of 800 eV was chosen with this in mind, contamination of the surface in the rather poor vacuum prevented meaningful compositional contrast from being obtained. Surprisingly, Zworykin did not anticipate this, although he was an experienced vacuum physicist. Only two years earlier, secondary emission measurements and the effects of contamination had been published by Bruining and de Boer (1938). Actually, all of Zworykin's published micrographs were of etched or abraded samples, and contrast was topographic (Zworykin et al. 1942)—for example, etched brass (see Fig. 1.5). The quality of the recorded images was rather disappointing, and together with the lack of a visible image, must have been a factor in RCA's decision to discontinue the project. The main reason, however, was undoubtedly the excellent results that were, as mentioned earlier, being obtained with replicas viewed in the

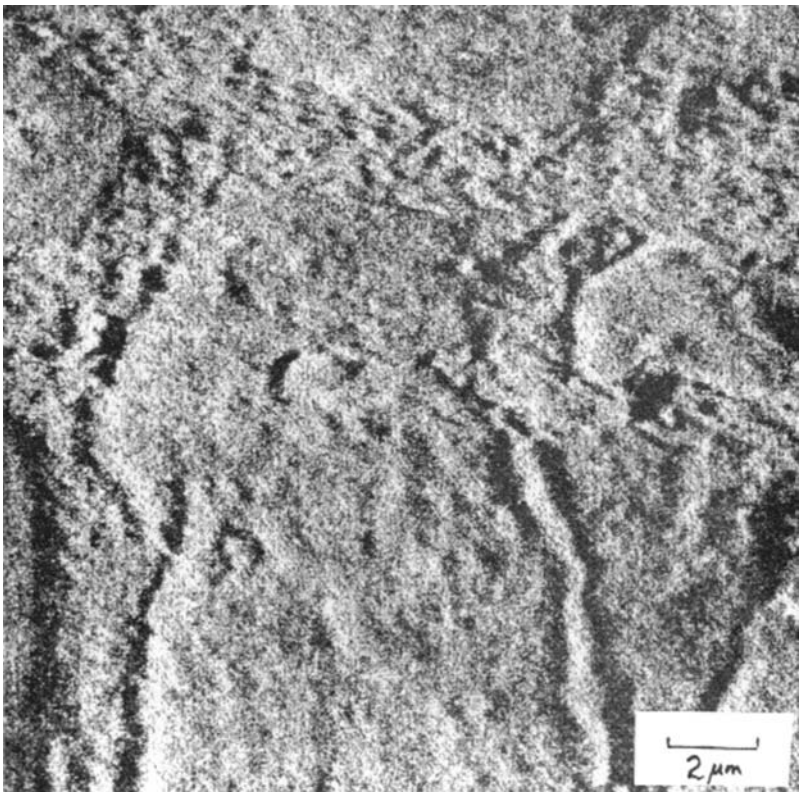

Fig. 1.5 Micrograph of etched brass produced by the SEM of Zworykin et al. (1942)

TEM (Mahl 1940). In any event, all available technical effort had to be directed to the highly successful RCA EMB TEM, which was just then coming into production (Reisner 1989).

Von Ardenne's Scanning Electron Microscope

While RCA was developing its SEM, Manfred von Ardenne (a private consultant who had his own laboratory) was developing the first scanning electron microscope with a submicron probe. In 1936, he was contracted by Siemens & Halske AG to investigate the possibility of using a scanned electron probe to avoid the effects of objective lens chromatic aberration with thick samples in TEM. In the course of this work, he laid the foundations of electron probe microscopy by making and publishing (von Ardenne 1938a,b) a detailed analysis of the design and performance of probe-forming electron optics using magnetic lenses. The analysis covered the limitations on probe diameter due to lens aberrations and the calculation of the current in the probe. He also showed how detectors should be placed for bright-field

and dark-field STEM and for imaging a solid sample in a SEM, and considered the effects of beam and amplifier noise on imaging.

To fulfill the Siemens contract, von Ardenne built the first scanning transmission electron microscope (STEM) and demonstrated the formation of probes down to 4 nm in diameter. But in the short time available, he was limited to employing existing technology, and because there was no suitable low-noise electronic detector, he used photographic film—consequently there was no immediately visible image. A schematic of the microscope column is shown in Fig. 1.6. A demagnified image of the crossover of the electron gun was focused on the sample with two magnetic lenses, and X-Y deflection coils were mounted just above the second of these. Immediately below the sample, was a drum around which was wrapped the photographic film. The image was recorded by rotating the drum and simultaneously moving it laterally by means of a screw while the currents in the deflection coils were controlled by potentiometers mechanically coupled to the drum mechanism. The intensity of the beam was very low (about 10^{-13} A) and it was necessary to record the image over a period of about 20 minutes. Because the image was not visible until the film had been developed, focusing could only be accomplished indirectly by using the stationary probe to produce a shadow image of a small area of the sample on a single-crystal Zinc sulfide screen that was observed through an optical microscope and prism system. The recordings were inferior to those from the TEM that was being constructed by Ruska and von Borries at Siemens, and the hoped-for advantages of STEM with thick samples were not realized.

Von Ardenne spent a short time trying to use the instrument in the SEM mode on bulk samples, but could only obtain low resolution images because of the detector problem. The sample current was amplified by thermionic tubes and a large probe current was needed. He did not publish any images.

In total, von Ardenne worked for less than two years on scanning electron microscopy before concentrating on the development of his universal TEM (von Ardenne 1985). Then, with the start of World War II, he began work on a cyclotron and isotope separators for nuclear energy projects. If he had been able to continue, there is little doubt that he would have built an efficient SEM within a year or two: this is evidenced by a patent (von Ardenne 1937) that included a proposal for double-deflection scanning, two papers (von Ardenne 1938a,b), and a book (von Ardenne 1940). Two of the chapters in the book were on scanning microscopy and were based on the 1938 papers, but included additional material relating to imaging the surfaces of solid samples. Most importantly, he proposed a detector using an electron multiplier with beryllium copper dynodes (see Fig. 1.7) that could be opened to the atmosphere and worked with efficiently under poor vacuum conditions. Measurements of the secondary emitting ratio of beryllium copper and its stability when exposed to the atmosphere were otherwise only first reported in 1942 by I. Matthes (1942) of the AEG Research Institute in Berlin, but von Ardenne was probably aware of this research a year or two before.

In his book, von Ardenne also discussed the interaction between the beam electrons and the sample, and suggested that back-scattering would cause a loss of resolution, illustrating this with a diagram that has quite a modern look

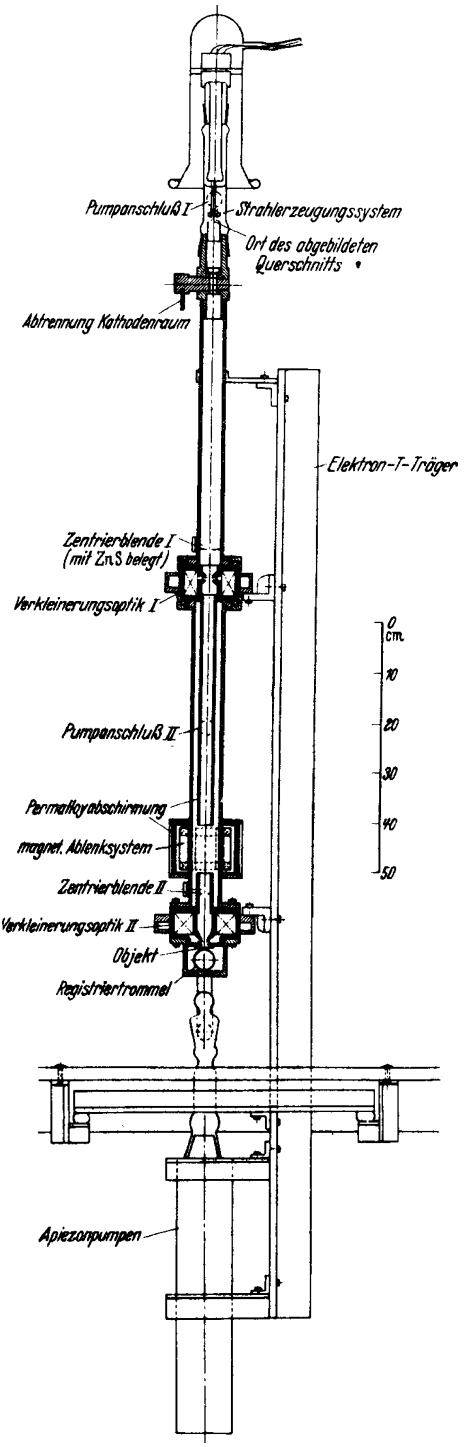

Fig. 1.6 Cross-section of the column of von Ardenne's (1938b) STEM

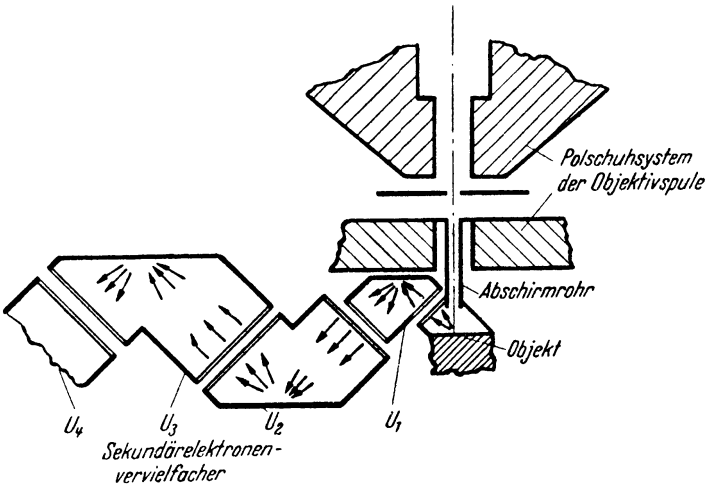

Fig. 1.7 Electron multiplier with beryllium copper dynodes proposed by von Ardenne (1940) as a secondary electron detector for SEM. The drawing shows the first three stages of the multiplier and its position relative to the objective lens and sample

(see Fig. 1.8). He argued that the incident beam electrons produce secondary electrons at or near the surface from an area approximately equal to the beam diameter, and give a high-resolution image (*nutzbare Strahlung*). The beam electrons penetrate the sample, and a proportion of them is backscattered and reach the surface where they produce further secondaries. These two signals are now generally referred to as SE-I and SE-II, respectively (Drescher et al. 1970, Peters 1982). The backscattered electrons are emitted from an area of diameter comparable to the penetration depth, and the secondaries they produce (*schädliche Strahlung*) may impair the resolution (he did not, however, consider the case of a sample with small inclusions below the surface). He concluded that good resolution might be obtained either with a very low-energy beam (1 keV), or with one having a high energy (50 keV). In the first case, the backscattered electrons would emerge from an area of the surface a little larger than the incident beam, and the resolution would be unaffected. On the other hand, the secondary electrons produced by the backscatter of a 50-keV beam would affect a much larger area, and would be evenly distributed so that their main effect would be to increase the background (reduce the contrast) rather than affect the resolution.

Von Ardenne's scanning microscope was destroyed in an air raid on Berlin in 1944, and after the war he did not resume his work in electron microscopy but researched in other fields—first in Russia and then in Dresden (in 1955), which was then in East Germany. Additional information about von Ardenne's scientific work is available in his autobiography (von Ardenne 1972) and by McMullan (1988).

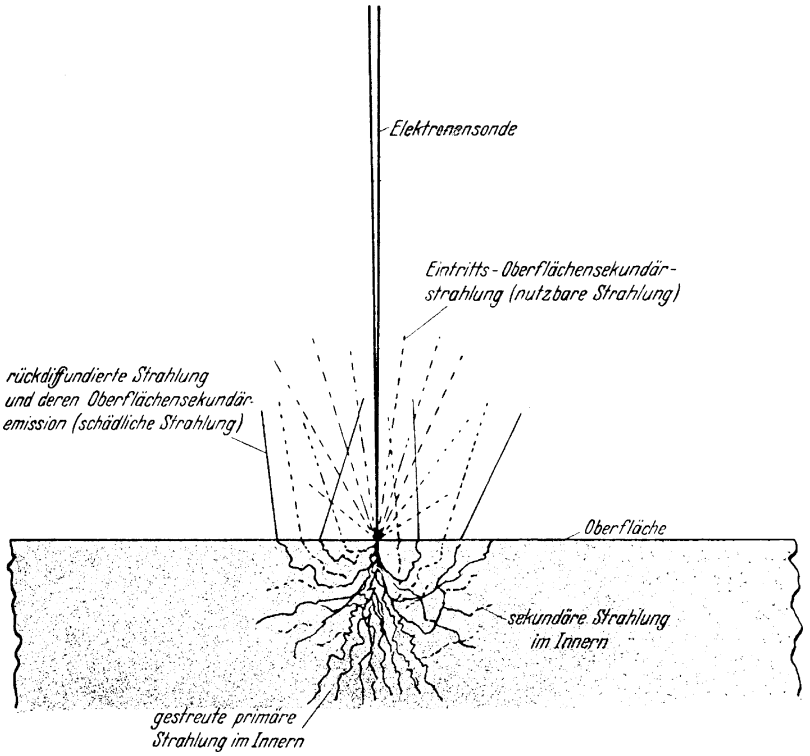

Fig. 1.8 Diagram illustrating von Ardenne's (1940) discussion of secondary electron imaging of a surface

The Cambridge Scanning Electron Microscopes

Apart from a theoretical analysis of resolving power by a French author (Brachet 1946), no other substantial work on SEMs was reported until 1948, although recent research has revealed that some rather primitive experiments were done by A. Léauté (1946) at L'Ecole Polytechnique in Paris during World War II (Hawkes & McMullan 2004). In the 1940s, and for many years after, the feeling among most electron microscopists was that the SEM was not worth further consideration in view of the apparent failure at RCA—if such an experienced team could be that unsuccessful, it seemed very unlikely that anyone else could produce an effective instrument. A notable exception to this general opinion was that of Denis Gabor (1945).

It was then that Charles Oatley at the Department of Engineering of the University of Cambridge decided to take another look at the SEM, although he related that "several experts expressed the view that this [the construction of an SEM] would be a complete waste of time" (Oatley et al. 1985). He explained at some length the reasons that brought him to this decision, but the main technological justifications were that "Zworykin and his collaborators had shown that the scanning principle

was basically sound and could give useful resolution in the examination of solid surfaces" and "improvements in electronic techniques and components had resulted from work during the war" (Oatley, 1982). He also felt that the RCA detector had a low efficiency and only a small proportion of the secondaries were reaching it, with the result that the images were noisy in spite of the long recording time. Independently of von Ardenne, he proposed to use an electron multiplier with beryllium-copper electrodes (Allen 1947), having been promised one by A.S. Baxter at the Cavendish Laboratory, who was making multipliers of this type (Baxter 1949). The full story of Oatley's achievement is presented in a recent publication (Breton et al. 2004).

I was selected by Oatley to build an SEM as a Ph.D. project—it was a challenging task because electron microscopy was a completely new subject for everyone in the laboratory, although I had had some experience in the radar and television industries, including the development and manufacture of cathode-ray tubes. I first completed a 40 keV electrostatically focused TEM that had been started by another PhD student, K.F. Sander. He abandoned it at an early stage and changed the subject of his research project to electron trajectory plotting (Sander 1951). I converted it to a STEM, and then to an SEM, by the addition of scan coils, an electron multiplier detector, and a long persistence cathode-ray tube monitor (McMullan 1952).

It was not apparent how Zworykin's results might be improved upon. A higher-incident beam energy was expected to be beneficial, but it was not clear how image contrast would be formed. As mentioned earlier, Bruining and de Boer (1938) had shown that the secondary emission from a surface is critically dependent on the vacuum conditions, and it was plain that the achievable vacuum would not be good enough for there to be meaningful secondary-electron compositional contrast from a polished sample.

Images of surfaces were obtained at grazing incidence and viewing direction (2 deg) in the TEM by Bodo von Borries (1940) and others, and it seemed probable that similar images could be produced in the SEM. I therefore mounted a sample of etched aluminium at a rather larger angle (30 deg; because the backscattered electrons did not have to be focused) and was rewarded by the now commonplace three-dimensional appearance that is the hallmark of SEM images and a consequence of their large depth of focus. One of the first images—of etched aluminium—is shown in Fig. 1.9: (a) the direct view image (about 0.9-sec frame period), and (b) a 5-min recording. The beam energy was 16 keV and the resolution about 50 nm, limited by astigmatism in the objective lens and insufficient magnetic shielding.

A block diagram of the SEM is shown in Fig. 1.10 (McMullan 1953). There was a relatively fast-scan, long-persistence cathode-ray tube display (405 lines, 1.8 fields/sec interlaced and a 5-min frame scan for photographic recording). Other features included a nonlinear amplifier for gamma control, and beam blanking for DC restoration. Double-deflection scanning coils were added later.

The most important differences between this instrument and Zworykin's were the much higher incident beam energy (15-20 keV), and the contrast produced mainly by scattered electrons from the tilted sample. The mechanism of contrast formation was investigated and shown to be topographic. No attempt was made to collect

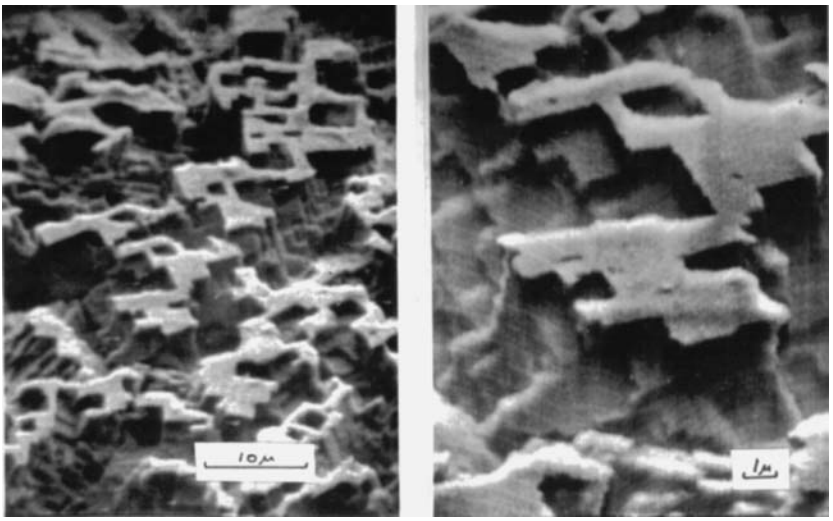

Fig. 1.9 One of the early images (etched aluminium surface) produced with SEM1. Angle of incidence of 16 keV electrons 25°: (a) visible image, 0.95 frames/s; beam current 1.5×10^{-10}A. (b) 5 min recording; 10^{-13}A. (McMullan 1952, 1953)

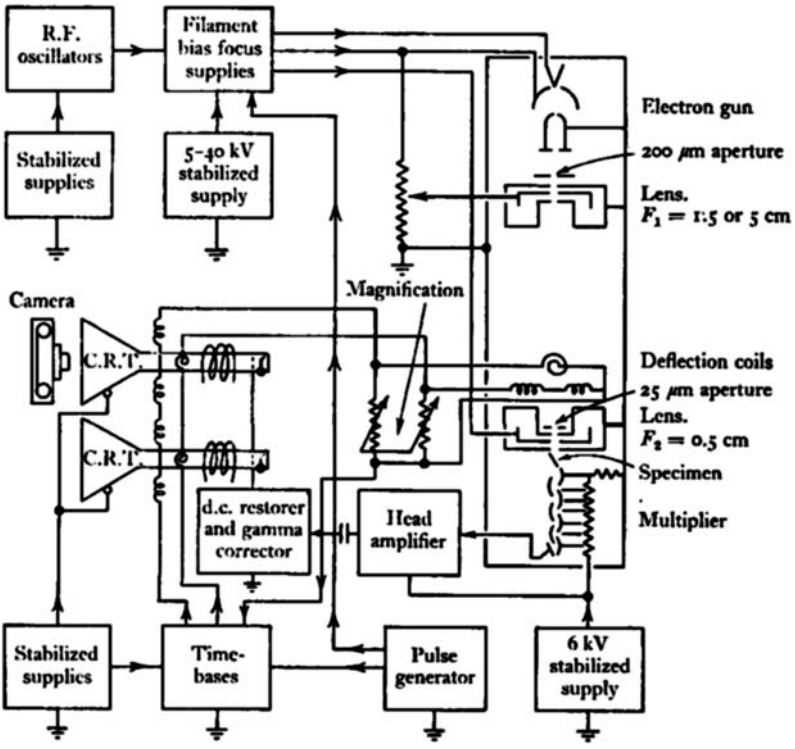

Fig. 1.10 Block schematic of SEM1. (McMullan 1952, 1953)

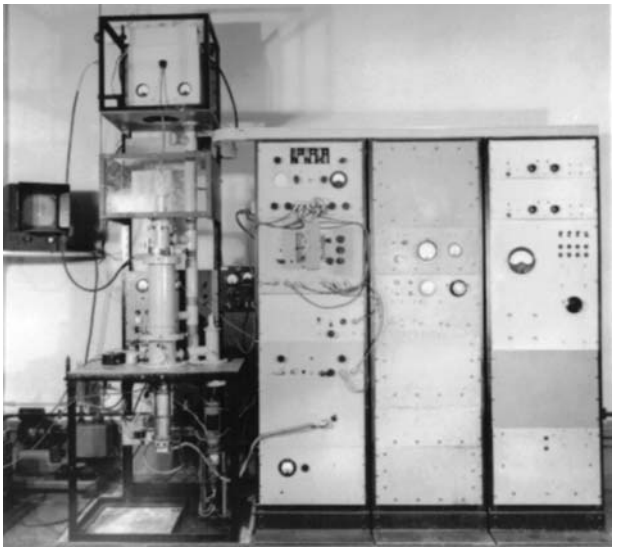

Fig. 1.11 Photograph of SEM1 taken in 1953 when K.C.A Smith took over

low-energy secondaries. In fact, I thought that they would be detrimental because of the inevitable contamination on the surface of the sample. I overlooked the increase in signal that is obtained from the low energy secondaries.

I realized that there was another advantage in using a high-energy scanning beam: this was that in principle atomic number contrast was possible using backscattered electrons. An experimental curve of emission ratio (for 20 keV primaries) versus atomic number had recently been published by Palluel (1947), but an attempt at obtaining atomic number contrast failed. Some years later, Oliver Wells (1957) was more successful. The obvious disadvantage of high-beam energies was that the resolution was limited by penetration of the primary electrons. I suggested low-loss electron imaging to minimize this, but was not able to implement it (this was also done many years later by Wells, who published in 1971). One other contrast mechanism that I tried was cathodoluminescence, and I was able to demonstrate that phosphors with too long a decay constant to be used for producing images at a 0.9-sec frame time with a Zworykin-type detector were completely satisfactory when excited at a high-current density by a focused probe (Smith & Oatley 1955). Figure 1.11 is a photograph of the microscope (now named SEM1) taken in 1953 shortly before K.C.A. Smith assumed responsibility for it and turned it into an SEM that could produce images comparable with some of those from modern microscopes.

Further Development of the Microscope

Smith introduced many improvements to SEM1, including a stigmator and a tilting sample stage, and he increased the efficiency of the detection system by moving the electron multiplier nearer the sample so that low-energy secondary electrons were

collected, thus increasing the signal current. He showed that metalized insulating samples could be imaged, and he examined a wide variety of samples including germanium point-contact rectifiers and biological specimens. He also built an environmental cell for wet specimens (anticipating the environmental scanning electron microscope, ESEM); this had thin windows to admit the focused beam and allow the scattered electrons to reach the multiplier. Although the results were rather disappointing, it led Oatley to suggest replacing the second window with a short-decay-time plastic scintillator and photomultiplier, and dispensing with the bulky electron multiplier (Smith 1956). This, in turn, resulted in the development of the Everhart and Thornley (1960) detector.

Further SEMs were built in the engineering department at Cambridge: SEM2 (Wells 1957); SEM3 (Smith 1960); SEM4 (Stewart 1962); and SEM5 (Pease & Nixon 1965). All were used on a wide variety of samples and for the development of new techniques. Other important instrumental advances made by Oatley's group during the remainder of the 1950s through to 1965 included: atomic number contrast (Wells 1957); stereomicroscopy (Wells 1960); voltage contrast (Oatley & Everhart 1957); low-voltage (1–2 keV) SEM (Thornley 1960a); high temperature (1200 °C) imaging of thermionic cathodes in a SEM (Ahmed 1962); high-resolution (10 nm) SEM (Pease & Nixon 1965); etching of surfaces in a SEM by ion bombardment (Stewart 1962); ion etching and microfabrication in SEM (Broers 1965); and microelectronics in SEM (Chang 1966). Most of this work is described in papers by Oatley (1982) and Oatley et al. (1985).

Materials Research

In 1955, Smith used SEM1 for the first three applications of a scanning electron microscope in materials research:

1. Smith was visited by F.P. Bowden, head of the Surface Physics Laboratory in the Department of Physical Chemistry at Cambridge University, and J. McAuslen of Imperial Chemical Industries, who brought a sample of silver azide crystals. The thermal decomposition of these was being investigated in their laboratory using a TEM in the reflection mode, but had failed due to the premature ignition of the crystals under the intense illumination that was needed. In the SEM, with the crystals mounted on a small hot-plate, the decomposition could be readily controlled and observed without difficulty (Bowden & McAuslan 1956; McAuslen & Smith 1956; see Fig. 1.12.
2. J.H. Mitchell, controller of research at Ericsson Telephones, Ltd. approached Oatley about their work on the etching of germanium surfaces and the emergence of edge dislocations. They wished to establish whether there were pits or raised areas of sublight-microscopic size. J.W. Allen brought specimens to Cambridge and the micrograph in Fig. 1.13 shows a feature produced by the etchant CP4 (a mixture of acids with a small amount of bromine). (Allen & Smith 1956).
3. The third event occurred when Dr D. Atack—then on sabbatical leave from the Pulp and Paper Research Institute of Canada (PPRIC) where he was director of

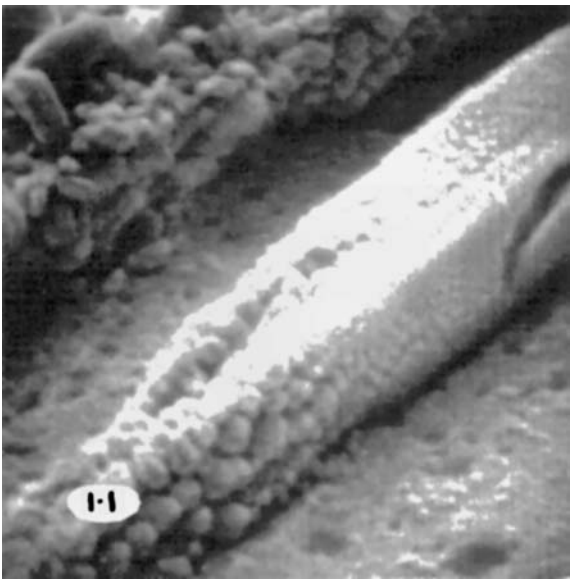

Fig. 1.12 Partial decomposition of a small needle of silver azide. (Smith 1956)

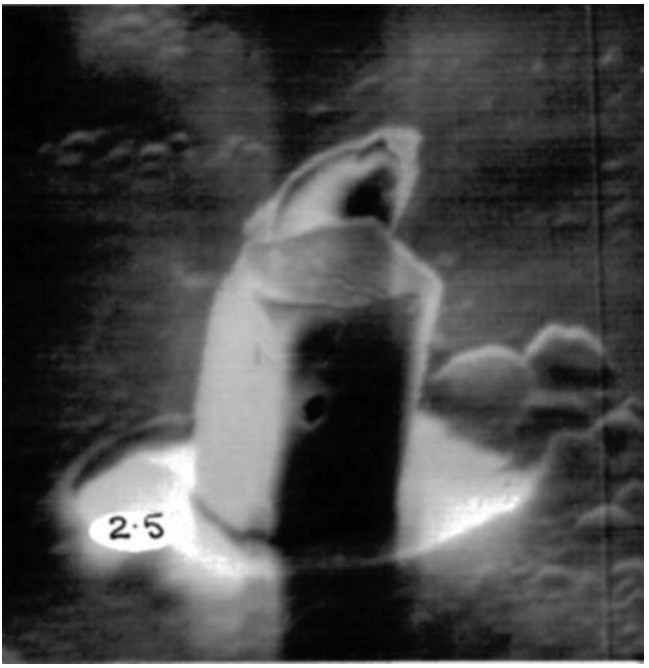

Fig. 1.13 Crystallographic feature on a germanium surface etched with CP4. (Allen & Smith, 1956)

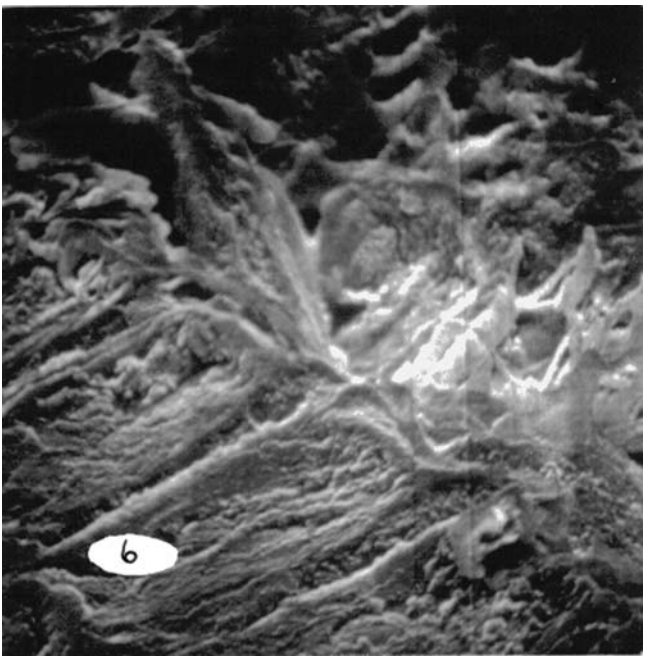

Fig. 1.14 Application of the SEM to the study of wood fibers: a surface of newsprint. (Smith 1956)

the Applied Physics Division—came and asked if he could try some of his pulp and paper samples in the SEM. In this case, a TEM had also been tried with unsatisfactory results (Page 1958). The experiments were highly successful, as shown in Fig. 1.14 (Smith 1956) and led to PPRIC purchasing a SEM (see next section).

The First Low Voltage Scanning Electron Microscope (LVSEM) Imaging

In the present context, the work of R.F.M. Thornley on LVSEM in Oatley's laboratory (Thornley 1960a) needs to be described in more detail. He investigated some of its possibilities, and by meticulous testing and modification was able to greatly improve the performance of the instrument he had been allocated (SEM2), which had been built and used by Wells (1957). This included eliminating the many sources of interference (mainly 50 Hz) he had identified and making alterations to various components—some quite minor—so that eventually he was able to obtain a probe diameter of 200 nm at 1 keV. This is, of course, ridiculously large by today's standards, but for then it was a considerable achievement. His modified SEM2 was the first LVSEM (Thornley 1960a) of the modern type (strictly speaking, Zworykin's (1942) was the first, but the low voltage was irrelevant to the rather poor images it was able to produce).

To quote from a paper Thornley presented at the European Regional Conference on Electron Microscopy in Delft in 1960 (Thornley 1960b):

> "Previous work at Cambridge has used electron accelerating voltages greater than 6 kV, limiting the use of the instrument to the examination of conducting surfaces in order to avoid the formation of charging artefacts. Insulators can be covered with a thin metallic film to overcome this restriction, but deformation while under observation is difficult because the conducting film cracks away from the substrate as shown by Wells (1957). If the beam voltage is reduced until the secondary emission coefficient of the specimen is equal to or greater than unity, the surface potential will be automatically stabilised at that of its surroundings, in this case, at earth potential. Under these conditions, the effective secondary emission coefficient of an insulator is unity, regardless of the angle of incidence of the primary beam and the picture contrast is controlled only by collector modulation. . . . ."

> ". . . . [Fig. 1.15], of a fractured ceramic surface, shows that, with a suitable choice of collector position, contrast similar to that expected from oblique viewing at high voltage can be obtained at low voltages, in this case, 1.5 kV. Provided the stability requirements can be met, the ultimate resolution, for voltages above 500 V, is limited by the same factors as in the high voltage case to between 50 and 100 Å as shown by Everhart (1958). It has been found that the reduction in gun brightness at low voltages is largely compensated by the increase in secondary emission, so no change in recording time has been necessary. The micrograph shown was recorded over 2 min, using an instrument originally designed for 25 kV operation, but fitted with a modified gun, permitting operation down to 300 volts. Contrast due to surface films is enhanced at low voltages because differences in secondary emission coefficients are more pronounced and penetration effects are reduced, the range of a 500-V electron in aluminium being roughly 30Å. . . . . "

Further work on ceramics was reported in a paper with L. Cartz of Morganite Research and Development Ltd. (Thornley & Cartz 1962):

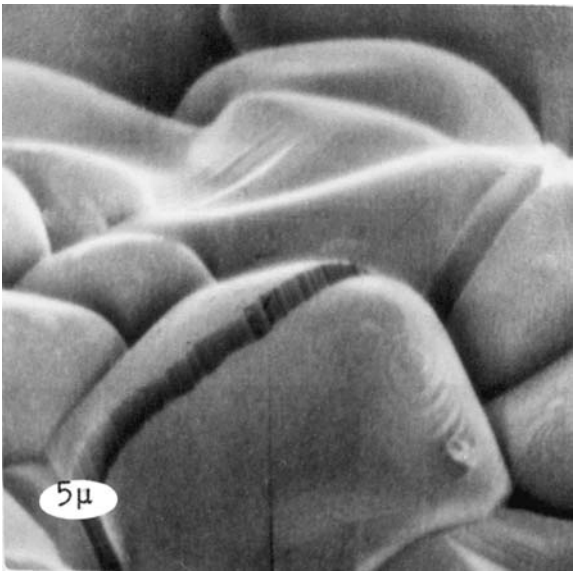

Fig. 1.15 Imaging the surface of a sintering fault in an alumina ceramic with 1.5-keV electrons. (Thornley 1960)

> "A direct electron-optical method of observing an insulator surface is described and applied to a series of alumina ceramics. The surface of the object requires no previous treatment of any kind, and a resolving power of 2,000 Å has been obtained with a depth of focus of about 50 μm. Different phases and components can be distinguished. Fractured surfaces, a fault region, and polished surfaces of various alumina ceramics are examined."

Commercial Production of SEMs

Following the encouraging results obtained using SEM1 to study wood fibers (Atack & Smith 1956), L.R. Thiesmeyer, director of the Canadian Pulp and Research Institute, ordered a fully engineered microscope (SEM3) from the Cambridge University Engineering Department. This was used for many years in their Montreal Laboratories and was the earliest industrial application of an SEM on a daily basis.

Smith developed SEM3 and completed it in 1958: it was the first magnetically focused SEM (Smith 1959). The lower section of the column below the table consisted of a modified Metropolitan Vickers (later AEI)-type EM4 TEM (Page 1954) and contained the electron gun, condenser lens, transmission sample stage, objective lens, and double pole piece projector lens. For scanning operations, the transmission objective and projector were used together in various combinations and powers according to the spot diameter required to provide the first stage of spot demagnification. Immediately above the table, there was a section of the column containing the scanning coils and the objective lens that was of the pin-hole type (Liebmann 1955), with three adjustable apertures. There was a tilting sample stage and the Everhart-Thornley type of secondary electron detector.

Thiesmeyer and Atack were among the very few who (at that time) saw the great potential of SEM. Although Oatley's group had produced and published high-quality micrographs from many different samples, there was still considerable resistance to SEM among microscopists. Over several years, Oatley expended much effort in trying to persuade electron microscope manufacturers to market an SEM (Jervis 1971, 1972; Oatley 1982; Breton et al. 2004) but he was only finally successful in 1962 when the Cambridge Instrument Company decided to go ahead with the production of an SEM based on the instruments developed by Oatley's group. This decision was influenced by A.D.G. Stewart, one of Oatley's students who agreed to join the company and later played a leading part in the development of the *Stereoscan*, as the new SEM was named (Stewart & Snelling 1965; Stewart 1985).

The prototype Stereoscan (see Fig. 1.16) went to the Dupont Chemical Corporation in the United States in 1964, and in the following year the first two production models were sold to P.R. Thornton at the University of North Wales and to J. Sikorski at Leeds University in the United Kingdom, the third to G. Pfefferkorn at Münster University in Germany, and the fourth to the Central Electricity Laboratories in Leatherhead, United Kingdom. In the words of Professor Sir Charles Oatley (1982), "By this time the Company had launched a publicity campaign and orders began to roll in. An additional batch of twelve microscopes was put in hand; and then a further forty . . . . . the scanning microscope had come of age."

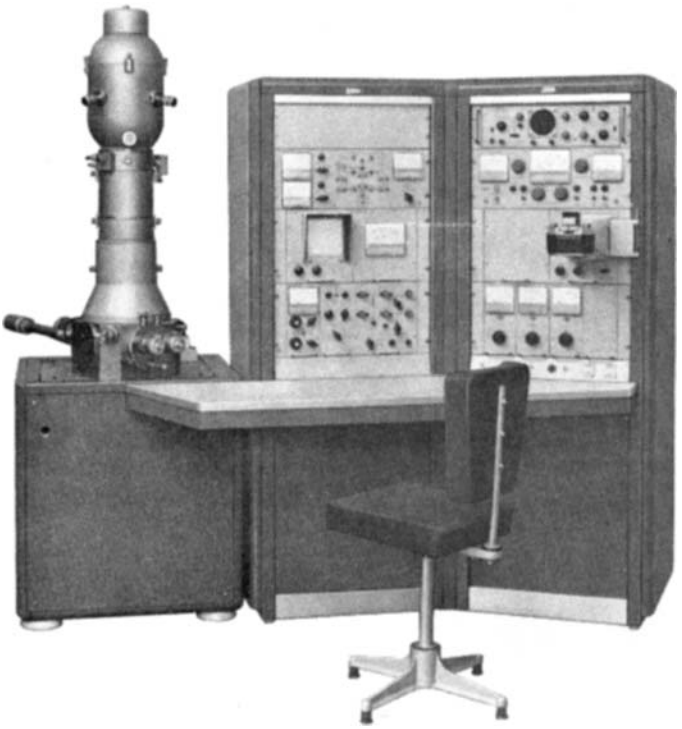

Fig. 1.16 The Cambridge Instrument Company's Stereoscan Mk 1 prototype (Stewart & Snelling 1965)

The first commercial competitor was the Japanese firm, JEOL, who marketed their JSM-1 SEM about six months later and were soon followed by others (McMullan 2004).

Other SEMs up to 1965 and Beyond

SEM developments in other laboratories prior to 1965, as evidenced in scientific publications, included the following:

- An SEM was built in France by Bernard & Davoine (1957) at the National Institute of Applied Science in Lyon. It had a probe size of the order of 1 μm and was used over a period of years mainly for cathodoluminescence studies.
- AEI in the United Kingdom—the major TEM manufacturer at that time—developed an SEM but did not proceed after the first instrument, sold in 1959, turned out to be unsatisfactory (Jervis 1971, 1972).
- Wells et al. (1965) built an advanced SEM for semiconductor studies and microfabrication for the Westinghouse Laboratories in Pittsburgh, Pennsylvania, and demonstrated EBIC imaging.