# 1

# A Brief Historical Overview

## 1.1 SCANNING ELECTRON MICROSCOPY

### 1.1.1 The Beginnings

The birth of scanning electron microscopy (SEM), in the 1930s and 40s, represented a major breakthrough in the study of the microstructure, composition and properties of bulk materials. SEM combines high-resolution imaging with a large depth of field, thanks to the short wavelengths of electrons and their ability to be focused using electrostatic and electromagnetic lenses. In addition, the strong interaction of electrons with matter produces a wide variety of useful ‘signals’ that reveal all kinds of secrets about matter at the microscopic and even nanoscopic level.[1]

The earliest demonstration is attributed to Knoll, who obtained the first scanned electron images of the surface of a solid (Knoll, 1935). In 1938, von Ardenne established the underlying principles of SEM, including the formation of the electron probe and its deflection, the positioning of the detector and ways of amplifying the very small signal current (von Ardenne, 1938a, 1938b). Then Zworykin and his team at RCA Research Laboratories built an SEM which had several important original features (Zworykin *et al.*, 1942). The resolution was about 50 nm which, compared to the performance of the already established transmission electron microscope (TEM), was unfortunately not sufficient to convince people of SEM’s usefulness at that time.

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[1] Convention dictates that features measuring less than 100 nm are termed ‘nano’.

*Principles and Practice of Variable Pressure/Environmental Scanning Electron Microscopy (VP-ESEM)*
D. J. Stokes

The cause of the SEM was then taken up in 1948 by Oatley at Cambridge University where, over a number of years, he and his research students built five SEMs of increasingly improved performance. The first of these showed how SEM could reveal the three-dimensional nature of surfaces (McMullen, 1952; 1953), and the students that followed made various important contributions to the development of SEM and its applications, leading to an instrument with 10 nm resolution by the 1960s. Some examples of the literature at that time include: Smith and Oatley (1955); Smith (1956); Oatley and Everhart (1957); Wells (1957); Everhart and Thornley (1960); Broers (1965) and Pease and Nixon (1965). The culmination of this work was the production of the first commercially available SEM in 1965: the Cambridge Instruments Stereoscan (see Figure 1.1).

This marked the start of a new era. The resolution of SEM was not as good as that of TEM, but the difficulties of preparing thin samples for TEM were avoided. In addition, it became appreciated that the ability to observe the surfaces of bulk specimens, to visualise the topography of the features and to obtain quantitative information was highly valuable in its own right.

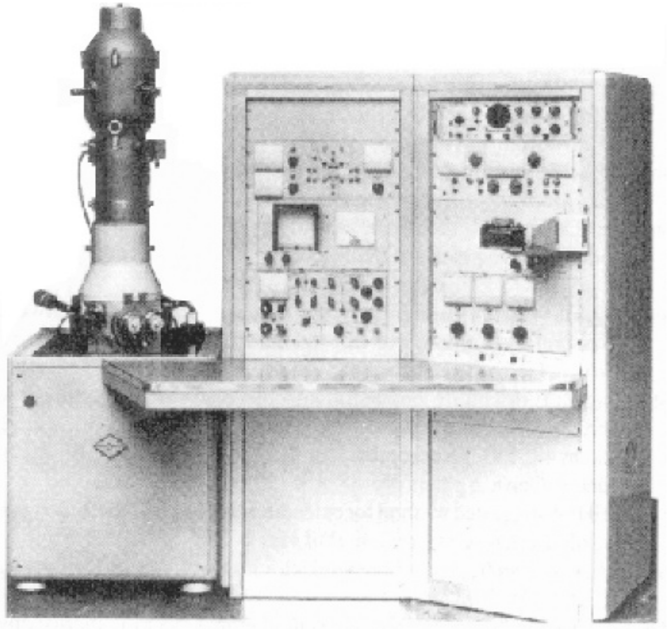

**Figure 1.1** The first commercially available scanning electron microscope, the Cambridge Instruments Stereoscan Mk1, 1965

### 1.1.2 The Need for Added Capabilities

For applications involving metallic materials, SEM imaging and analysis is a comparatively straightforward matter, subject to a proper interpretation of the results and an understanding of the factors that can affect these (such as cleanliness and roughness of surfaces, oxide formation, etc). However, numerous methods are needed when dealing with most other types of material, due to practical operational limits of the instrument and the physics of electron beam–specimen interactions.

To begin with, a fundamental requirement of SEM is the need for high-vacuum conditions throughout the column, typically around $10^{-3}$–$10^{-5}$ Pa ($10^{-5}$–$10^{-7}$ torr), sometimes better, depending on the electron source, in order to minimise primary electron scattering and hence maintain a focused beam. An immediate consequence of the high vacuum requirement is that specimens must be vacuum-friendly: no volatile components may be present in the specimen, since this would compromise the vacuum as well as putting the electron source at risk of contamination. Of course, many biological specimens, foams, emulsions, food systems and so on contain water and/or oils – substances that evaporate in the absence of their corresponding vapour. Hence, before imaging can take place, such samples require preparation in order to remove potentially volatile substances, and many procedures have been developed. These include chemical fixing, dehydration in a graded alcohol series, freeze-drying and critical point drying. The methods can be very sophisticated and/or time consuming. An added factor is that the sample preparation technique itself can often change the structural or chemical nature of the specimen to be examined, leading to the imaging of unwanted artefacts.

Moreover, high-vacuum electron microscopy of specimens in the liquid state is, of course, impossible, unless cryogenic procedures are employed to render the specimen solid. It should be emphasised, however, that the methods associated with cryo-preparation are extremely effective for high-resolution observation of frozen-hydrated material and, similarly, the other techniques mentioned certainly have their place. As always, it is a matter of choosing techniques that are appropriate to the system under study.

Another consideration in SEM is that the bombardment of samples by relatively high-energy electrons quickly results in a build up of negative charge unless the sample is electrically conductive, in which case the charge can be dissipated via a grounded specimen holder. Thus, metallic samples, being electrically conductive and containing no volatile components, can be imaged with ease in SEM. Less conductive samples dissipate negative charge much less efficiently and therefore charge builds up. The electric fields in and around the sample quickly become distorted, leading to a deterioration in image quality, often so serious that the sample cannot be imaged at all. Figure 1.2 illustrates the well-known 'mirror effect'.

In the case shown in Figure 1.2, an insulating specimen has first been imaged using a primary electron beam energy $E_0$ equal to 20 keV, followed by imaging at $E_0 = 3$ keV. For $E_0 = 20$ keV, an excess of electrons is implanted, setting up a strong negative potential below the surface. The 3 keV primary electrons, being much lower in energy, are influenced by the negative potential inside the specimen to such an extent that they are turned back in the opposite direction without entering the solid, striking the polepiece and other fixtures in the chamber and generating electron signals that are collected at the detector. Hence, the specimen surface acts as a mirror.

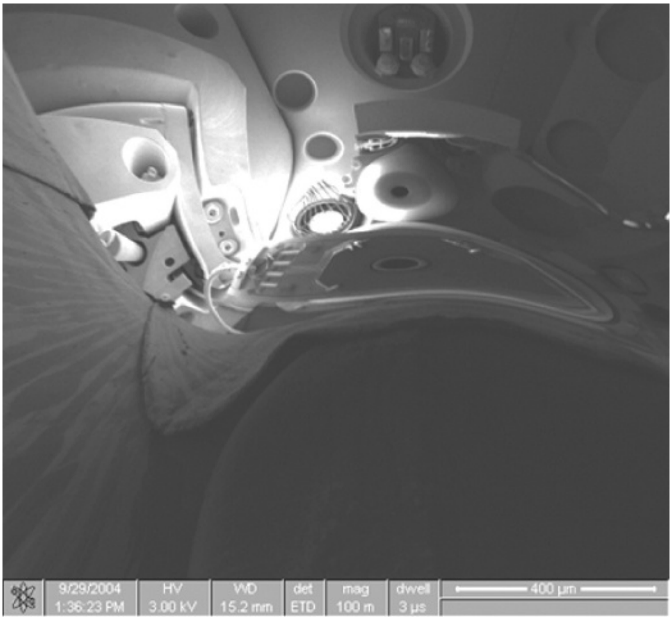

**Figure 1.2** The 'mirror effect'. Instead of landing on the specimen and forming an image of the specimen surface, primary electrons are repelled by the electric field arising from electrons implanted in the specimen, and turn back to strike the lens and other parts of the microscope. This generates signals that form an image of the inside of the chamber (distorted in this case)

Assuming that the specimen is not already charged, a low-energy electron beam (arbitrarily a few tens to a couple of thousand electronvolts, eV) can be used so that the number of electrons emitted from the specimen is equal to the number of incident electrons,[2] thus maintaining a charge balance. However, this can be at the expense of image resolution[3] and it can be difficult to find the right criteria for charge balance when the specimen consists of materials with differing electrical properties.

The story so far, then, is that insulators and, very often, the types of samples that have undergone the preparatory stages mentioned earlier, must be subjected to further treatment in the form of a metallic coating. Commonly, insulating samples are sputter-coated with a conductive material such as gold, platinum, palladium, chromium, etc. Again, the introduction of artefacts is a possibility, along with the risk of obscuring fine structural details under the coating. Coated samples give only topographic contrast, due to the short escape depths of electrons from metals, and therefore valuable compositional contrast from the underlying specimen may be lost.

Another consequence arising from the imaging criteria discussed above is that it can be difficult to carry out dynamic experiments, such as mechanical testing, on insulating samples. Even if the sample is given a conductive coating, fracturing of the surface will expose fresh insulating material and lead to charge build up. That said, there are examples of successful results obtained with high-vacuum SEM using low beam energies and/or a backscattered electron (BSE) detector. Electrons forming the latter signal have relatively high energies and are therefore less sensitive to the electric fields that develop as a result of charge build up, compared to low-energy signal carriers such as secondary electrons.

More convincingly, direct, real-time SEM observations of reactions involving gases or liquids are clearly not possible in high vacuum. Such studies are conventionally carried out by observing separate samples, suitably prepared (i.e. fixed, dried, frozen, coated, etc.) at each different stage in the development of the process under study. Clearly, it would be useful if observations could be carried out dynamically, *in situ*, without the preparation steps and vacuum constraints.[4]

This brief introduction has outlined a few of the constraints that conventional high-vacuum SEM places on accessing information from

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[2] Electrons arriving from the primary electron beam.

[3] This is primarily because low-energy electrons are more easily affected by imperfections in the electron optics, leading to a less tightly focused beam. In modern SEM, though, low-energy resolution is much improved.

[4] The capability to do just this is one of the unique differentiators of VP-ESEM, enabling time-resolved dynamic observations on a single specimen.

certain types of specimen or for performing specific experiments. The desire to go beyond the imaging of extensively prepared, static specimens opened up the way for the development of a new type of SEM, as we shall see in the next section.

## 1.2 THE DEVELOPMENT OF IMAGING IN A GAS ENVIRONMENT

### 1.2.1 Overcoming the Limits of Conventional SEM

Since about the 1950s, workers have been experimenting with differentially pumped, aperture-limited ‘environmental chambers’ for TEM, while others have worked with sealed containers with thin film, electron-transparent windows (see, for example, Swift and Brown, 1970; Parsons, 1975). Then, in 1970, Lane demonstrated the use of an aperture-limited chamber for SEM, described in a relatively obscure, but detailed, conference paper (Lane, 1970). Lane discusses the design of an ‘environmental control stage’ as well as the scattering cross-sections and mean free paths of electrons in various gases, including hydrogen, oxygen, nitrogen and the noble gases, and demonstrates stable imaging of liquid water.

Meanwhile, Robinson, Moncrieff and others in the 1970s worked to develop an SEM that was capable of maintaining a relatively high pressure while affording controlled imaging, by adapting the SEM specimen chamber itself (Robinson, 1975). Stable imaging of water was also shown by Robinson at the International Congress on Electron Microscopy (ICEM 8) in Canberra, Australia (Robinson, 1974). He was working with a modified JEOL JSM 2 SEM, containing a 100 μm pressure-limiting aperture (PLA) to separate the vacuum at the electron source from the specimen chamber at higher pressure. The maximum water vapour pressure was 665 Pa (5 torr), and liquid water was maintained by cooling the chamber and surrounding the specimen with an ice/water reservoir. A solid-state backscattered electron (BSE) detector was used, with reasonable resolution up to a magnification of 2000x. The presence of the aperture restricted the scan range of the electron beam such that the field of view was limited and the minimum magnification was 100x (Robinson, 1996).

At that time, the goal was to enable biological specimens to be imaged without specimen preparation. It was incidentally observed that imaging uncoated insulators at pressures above ~10 Pa seemed to reduce the effects of charging. Early explanations for this centred on the proposition that a film of liquid water was responsible for conferring the necessary conductivity. Of course, this could not explain why imaging with gases other than water had a similar effect. Moncrieff *et al.* (1978) then proposed that it was the collisions between electronic species and gas molecules, resulting in the production of positive ions (see von Engel, 1965), and the attraction of these ions to the negatively charged specimen, which was the mechanism for the observed charge 'neutralisation'.

Moncrieff and co-workers went on to calculate the effects and amount of scattering of primary electrons in, for example, nitrogen gas (Moncrieff *et al.*, 1979). An important conclusion of this work was that, although some primary electrons may be scattered tens to thousands of microns away from their original trajectories, the electrons forming the focused probe maintain a beam of the same diameter as would be formed in high vacuum. This is a vitally important and often misunderstood concept. Meanwhile, the scattered electrons reduce the total current in the focused probe while adding a uniform component to the overall background signal.

In 1978 Robinson began to commercialise this new technology with his company ETP Semra Pty Ltd, manufacturing a device called an environmental cell modification and later called the charge-free anti-contamination system (CFAS). They were mostly sold in Japan, via Akashi/ISI SEMs, and had a pressure limit of 266 Pa (2 torr). In 1980, Akashi/ISI integrated the CFAS and launched WET SEM.

Ultimately, Robinson and co-workers were aiming to work at physiological pressures and temperatures. Observation of liquid water at body temperature (~37 °C) requires a vapour pressure of water above 6.65 kPa (50 torr) and a gas path length[5] no greater than 0.5 mm. Since it becomes physically difficult or impractical to work with a hydrated specimen any closer than this to the pressure-limiting aperture, these criteria define the upper pressure limit. Now, in order to maintain the pressure differential between just two zones separated by one aperture, for a chamber pressure of 6.65 kPa, the aperture size would need to be reduced to ~13 μm (Robinson, 1996). This places a very large restriction on the field of view. Hence, to improve the situation, Danilatos and Robinson (1979) introduced a two-aperture system, having three differentially pumped zones so that the aperture did not have to be reduced in size. At the same time, Shah and Beckett (1979) were obtaining similar

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[5] The distance a primary electron has to travel through a gas, defined as the distance between the final pressure-limiting aperture and the surface of the specimen.

results in the UK, using the acronym MEATSEM (moist environment ambient-temperature SEM), and Neal and Mills (1980) built such a system using a Cambridge Stereoscan Mk II.

The next important milestones occurred in the 1980s when Danilatos, working with Electroscan, developed environmental SEM (ESEM). ESEM had a pressure limit of 2.66 kPa (20 torr)[6] and incorporated a secondary electron (SE) detector that could be used in a gaseous environment, utilising the ionisation cascade of secondary electron signals (Danilatos, 1990b). These two features represented a huge increase in the range of commercially available imaging capabilities.

In the 1990s, several other instruments became available in a growing number of geographical areas. These included the low-vacuum SEM, (LVSEM, JEOL); natural SEM (NSEM, Hitachi); environment-controlled SEM (ECO SEM, Amray Inc); and the EnVac (Gresham Camscan). In 1995 the high-tech companies Leica and Carl Zeiss pooled their electron microscopy resources in an independent joint venture, LEO Electron Microscopy Ltd, and introduced variable pressure SEM (VPSEM), later adding an extended pressure (EP) mode. Variable/extended pressure SEM then became products incorporated into Carl Zeiss SMT in 2001. Meanwhile, in 1996, controlled pressure SEM, CPSEM, was introduced by Philips Electron Optics. During that same year, Philips acquired Electroscan and its ESEM technology group, and subsequently merged with FEI, which became known simply as FEI Company in 2002.

Thus, imaging in a gaseous environment became a global phenomenon and now occupies a large fraction of the SEM market. However, something that is clear from the above discussion is that the marketing strategies of the various companies involved have resulted in myriad names and endless confusion. There is no standard, generic term, and this makes it very difficult to talk about the technology without using a specific brand or trademarked name. However, two terms that have emerged as the most appealing are 'variable pressure' and 'environmental'. These terms tend to be used in different contexts: variable pressure suggesting use of a gas for charge control; environmental suggesting that there is an additional need for some specific gas, pressure and/or temperature. Often, the distinction between the two becomes clear from the context of the experiment. Ideally then, it would be more practical to create a single, descriptive term, rather than having to explicitly draw a distinction between techniques that essentially use the same basic

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[6] The noncommercial version was actually capable of working up to a pressure of 6.65 kPa (50 torr) with a 300 μm aperture.

technology. Hence, in order to simplify the use of acronyms, the generic terminology to be used in this book will be VP-ESEM.

It should be noted that there are several different approaches to signal detection in VP-ESEM, often manufacturer-dependent, for historical reasons. Several of these are summarised below, along with a small selection of literature describing the theory and practice. Irrespective of detection mode, the gas environment is central to all of these methods.

- Detection of the ion signal via the induced specimen current (Danilatos, 1989a; Farley and Shah, 1991; Mohan *et al.*, 1998)
- Collection of the gas-amplified secondary electron signal (Danilatos, 1990a; Thiel *et al.*, 1997; Toth *et al.*, 2006)
- Gas luminescence in which photons generated in the gas are collected and photo-multiplied (Danilatos, 1989b; Morgan and Phillips, 2006)
- Use of a conventional Everhart–Thornley secondary electron detector, physically separated from the higher pressure specimen chamber (Jacka *et al.*, 2003; Slowko, 2006)

### 1.2.2 Leaps and Bounds

From the earlier work discussed in Section 1.2.1, a number of refinements to the theory and practice of VP-ESEM were set to follow, as the range and quality of experiments began to increase.

The processes underlying gas cascade signal amplification were investigated in more detail (Meredith *et al.*, 1996; Thiel *et al.*, 1997) and the properties of different imaging gases were being explored (Fletcher *et al.*, 1997; Fletcher, 1997; Stowe and Robinson, 1998). Calculations were being made and experimental measurements performed to assess the primary electron beam profile, taking into account electrons that are deflected to form the beam 'skirt' (Mathieu, 1999; Wight *et al.*, 1997; Gillen *et al.*, 1998; Phillips *et al.*, 1999; Thiel *et al.*, 2000; Wight and Zeissler, 2000; Tang and Joy, 2005). As a corollary to this, work was going on in earnest to determine to what extent the presence of a chamber gas influences the results of X-ray microanalysis, particularly for quantitative work. Theories were postulated and algorithms were written to correct for the spurious X-rays generated by scattered primary electrons (Griffin and Nockolds, 1996; Bilde-Sorensen and Appel, 1997; Doehne, 1997a; Gauvin, 1999; Mansfield, 2000; Newbury, 2002; Le Berre *et al.*, 2007).

Meanwhile, several experiments had already begun to suggest that there may be some novel contrast mechanisms available in VP-ESEM, like those found in high-vacuum SEM (such as voltage contrast) but with the added complication that the effects were dependent on specific VP-ESEM operating conditions (Clausen and Bilde-Sorensen, 1992; Horsewell and Clausen, 1994; Harker *et al.*, 1994; Meredith and Donald, 1996). From about 1997 onwards, there was a great deal of activity in the VP-ESEM community following the presentation of findings by Griffin (1997) and Doehne (1998). Whilst observing highly polished mineral specimens, they observed that particular features were only visible under certain conditions, attributed to a charge-related mechanism (Baroni *et al.*, 2000). At around the same time, work was being carried out to explain the contrast observed in heterogeneous liquids (Stokes *et al.*, 1998; Stokes, 1999), and a similarly transient effect was noticed in these systems, too (Stokes *et al.*, 2000).

The potential for VP-ESEM in the study of life science specimens was also being further explored and assessed, and methodologies were being developed that enabled delicate, hydrated biological materials to be observed without laborious preparation (Farley *et al.*, 1988, 1990; Gilpin and Sigee, 1995; Stokes, 2001).

This growing body of emerging observations prompted a series of 'roadmap' meetings in Australia (1999 and 2001 – Figure 1.3) and the USA (2005), aimed at gathering some of the most active members of the

**Figure 1.3** Part of a roadmap meeting in the Blue Mountains, Sydney, Australia, 2001. Left to right: Dominique Drouin, Debbie Stokes, Ralph Knowles and Milos Toth. Photograph courtesy of Matthew Phillips, University of Technology, Sydney

community together, in the interests of understanding and advancing this new technology and its applications. And, as the pieces of the jigsaw began to fall into place, it was recognised that something was missing: other than being useful for charge control, what effects were the positive ions generated in the gas cascade process having on the system as a whole? Extensive investigations began that added vital extra detail and further helped to explain some of the charge-related phenomena previously seen (Craven *et al.*, 2002; Toth *et al.*, 2002a; Toth *et al.*, 2002b).

Various aspects of VP-ESEM fundamentals and applications can be found in a number of review articles, for example Newbury (2002); Donald (2003); Stokes (2003b); Muscariello *et al.* (2005); Thiel and Toth (2005), in a special issue of *Microscopy and Microanalysis* (Multi-authors, 2004) and in a few book chapters (Doehne, 1997b; Baker *et al.*, 2003; Stokes, 2003a; Donald, 2007; Griffin, 2007).

It is notable that the annual number of peer-reviewed journal publications has grown steadily over the past decade or so (see Figure 1.4). Given the wide range of names and acronyms in use, numerous variations have been input as search terms in compiling this data. In addition, a wealth of conference papers has been presented in this field. However, conference abstracts are not reflected in the graph.[7]

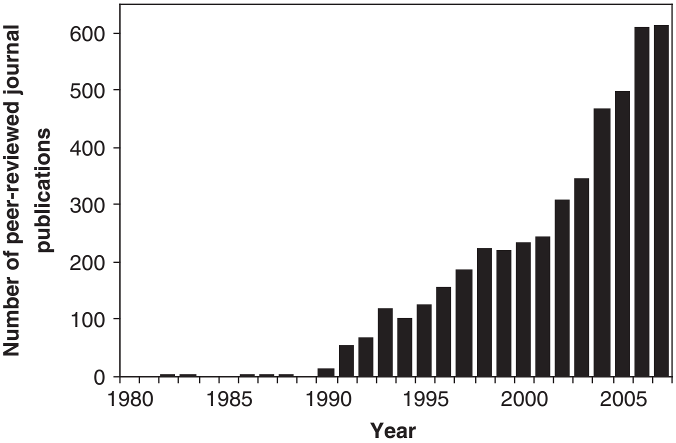

**Figure 1.4** Graph to show the number of peer-reviewed journal publications on the subject of 'VP-ESEM'. Source: ISI Web of Knowledge

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[7] Indeed, it should be noted that reference to conference abstracts has been kept to a minimum throughout this book, given the difficulty in obtaining such material and the often brief, speculative nature of the work reported.

It is gratifying indeed to see that a significant range of applications has now been reported. Many of these will be outlined in Chapter 6. Despite a great deal of early scepticism from the SEM community, ironically similar to the misgivings of the TEM community when the idea of SEM first came along, VP-ESEM has become established as a tremendously versatile technique and the instrument has firmly taken its place as a member of the electron microscopy family.

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