

DEBBIE J. STOKES

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

 **WILEY**

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Principles and Practice of Variable Pressure/Environmental Scanning Electron Microscopy (VP-ESEM)

Debbie J Stokes

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This book is dedicated to the memory of Ralph Knowles, who tragically died in June 2008 as this book was going to press.

Ralph played a major role in the development of many aspects of ESEM technology, from its commercial realization with Electroscan Corp., through the transition to FEI-Philips and FEI Company, where he had recently been appointed Director of R&D, North America. His technological achievements in various areas of electron microscopy were widely recognized and his many years of experience, dating back to his time at Cambridge Instruments in 1975, highly valued.

Ralph was an inspiration to many, all around the globe. I was fortunate enough to meet Ralph and others from Electroscan during the heady, early days of ESEM, all sharing 'the passion'. This led to more than a decade of Ralph's unerring support, mentoring and friendship. When considering whether to undertake writing this book, I immediately turned to Ralph for words of wisdom and guidance. His advice was to try to keep the details as generic as possible, to be accessible not only to the users of 'ESEM' instruments but to those of other manufacturers. His philosophy was this: all of these microscopes rely on a chamber gas for their operation, the rest is a just variation on the theme. That is why this book is firmly centered on the physical effects of performing electron microscopy in a gas environment and genuinely tries to avoid any commercial reference. This is a tribute to Ralph's personal integrity and reflects my own wish to help as many people as possible, as far as I am able.

Preface

Scanning electron microscopy (SEM) is a technique of major importance and is widely used throughout the scientific and technological communities. The modern scanning electron microscope is capable of imaging details of the order of tens of Ångströms (i.e. sub-nanometre), subject to the limits of electron-specimen interactions. However, for a long time it has been apparent that the high-vacuum SEM needed to develop in respects other than increased resolution. Hence, the advent of SEMs that utilise a gas for image formation while simultaneously providing charge stabilisation for electrically nonconductive specimens. Some instruments also allow for thermodynamic stabilisation of hydrated specimens. These microscopes are known by terms such as ‘environmental’, ‘extended pressure’ and ‘variable pressure’ SEM, amongst many others, depending upon manufacturer. There is ongoing discussion in the microscopy community as to adopting a generic term to encompass all of these instrument types. For the time being, I propose to use the term variable pressure-environmental SEM (VP-ESEM), with the proviso that this is merely for the sake of convenience.

Our knowledge of the physics of VP-ESEM has only now matured to a level where we can start to collect the concepts together in a dedicated book. There are undoubtedly developments still to come, and the future will bring books that tackle the scientific and technological aspects in much greater depth. What I hope to achieve with this book is a guide that will help those that are just starting out with VP-ESEM, as well as those with more experience looking to gain a deeper appreciation of the concepts.

The principles and applications have been outlined in a generic way, applicable to readers familiar with any of the types of VP-ESEM on the market, irrespective of manufacturer. The aim is to provide a practical overview: the reader is then referred to appropriate sources in the literature should they wish to obtain further information about the

inherent physics and chemistry of a particular process or phenomenon. A considerable amount of effort has gone into recognising the work of all those that have contributed to the beginnings, development and growth of this subject. A daunting task in itself. I know that a lot of very interesting and useful work has been carried out, and reported at conferences and meetings, that may not have made it into this book.

One of the biggest difficulties in the field of VP-ESEM is that there is no simple rule that defines which parameters to use. Every specimen and its imaging history will be different and many of the operating parameters are interdependent as well as specimen-dependent. These are the factors that make VP-ESEM so interesting and powerful. With that in mind, an effort has been made to supply a quantitative background to the physics of VP-ESEM, designed to be of help in deciding which set of conditions are appropriate for a given specimen or experiment. I strongly encourage all users of the VP-ESEM to freely experiment for themselves and, using the information contained here and in the literature, to consider the effects of operating conditions on image formation and microanalysis. This truly is the best way to get maximum information from a given specimen.

On a personal note, I would like to acknowledge the primary mentors from my early days in this field at the Cavendish Lab, namely Steve Kitching, Brad Thiel and Athene Donald. I am also very grateful to numerous people for their help and advice during the preparation of the manuscript, particularly JJ Rickard, Tony Edwards, Milos Toth, Ralph Knowles, Matthew Phillips, Gerard van Veen, David Joy, Joe Michael and Andrew Bleloch, and to the staff and Executive Committee of the Royal Microscopical Society, especially its current President, Mark Rainforth. A special mention and thanks go to Richard Young for reading the entire manuscript and giving much-needed support and encouragement. Thanks also to those that supplied images and diagrams particularly David Scharf for permission to use the image on the front cover. Last but not least, I'd like to acknowledge the forbearance of my sons Matthew and Oliver.

Debbie Stokes
Cambridge, May 2008

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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.

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

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.

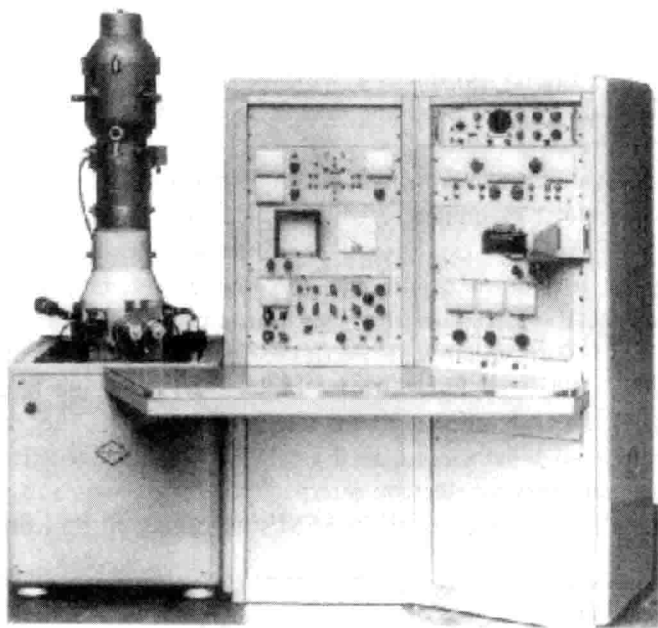


Figure 1.1 The first commercially available scanning electron microscope, the Cambridge Instruments Stereoscan Mk1, 1965

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 resolution³ 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

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 ($\sim 37^\circ\text{C}$) requires a vapour pressure of water above 6.65 kPa (50 torr) and a gas path length⁵ 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 $\sim 13\ \mu\text{m}$ (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

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.