A Review Of The Development of Scanning Electron Microscopy At High Chamber Pressure

Vivian Robinson, ETP Semra Pty. Ltd.
244 Canterbury Road, Canterbury, NSW, 2193, Australia

Ever since electron microscopes were developed, it has been the goal of microscopists to observe specimens in their natural state, free from artefacts which can often be introduced through specimen preparation. For most biological specimens, that includes the presence of water. With a pressure of 10^{-4} torr or lower required to operate a scanning electron microscope (SEM), liquid water, which required a pressure of above 5 torr, was clearly a problem.

Although several attempts had been made to examine hydrated specimens in a SEM, the first published results of water imaged in a stable and reproducible manner in the SEM, were presented at the Eighth International Congress on Electron Microscopy in Canberra in 1974 (Robinson, 1974). This represented an increase in the pressure capability of almost 5 orders of magnitude, from less than 10^{-4} torr, to 5 torr.

Separation of the 5 torr water vapour in the specimen chamber from the high vacuum in the electron optics column, was achieved by using a single differentially pumped aperture. Although attempts at using thin films for the separation had been made, they failed because they scattered the electrons too much, even though there was no absorption. (Perhaps that would not pose a problem with some of the thin window materials now available.) Calculations based upon Duschman (1949), showed that the pressure drop across a single 100mm aperture would enable a pressure of below 10^{-4} torr to be sustained in the gun region of the SEM, whilst a pressure of up to 10 torr was maintained in the specimen chamber, providing the pumping speed above the aperture was greater than 10 litre/sec.

Another problem to be overcome was how to form an image? The conventional Everhart-Thornley (E-T) secondary electron (SE) detector, required a pressure of less than 10^{-4} torr to operate. Specimen current imaging was not considered useful because ionisation of the gas molecules could interfere with the adsorbed current. It was decided to use a wide angle scintillator photomultiplier backscattered electron (BSE) detector (Robinson 1974b; 1975a). These detectors could give images with similar signal to noise and resolution as could be achieved with an E-T SE detector.

There was still one further problem to be overcome. How to reduce the path the electrons travelled through the higher pressure, and thus limit the beam scattering and associated loss of image detail? This required two steps; lowering the final aperture to reduce the distance the electrons had to travel; and lowering the temperature of the chamber to make sure the water vapour was never at a higher pressure. The description of the experimental arrangement used in a modified JEOL JSM 2 SEM, was described in greater detail in a few publications. By using a short, less than 5 mm, working distance and a cooled specimen chamber, with the specimen surrounded by an ice and water reservoir, it was possible to produce some good images, up to X2,000, of specimens containing liquid water (Robinson, 1975b; 1976a; 1976b).

This system enabled hydrated specimens to be examined at magnifications from X100 to X2000, with the water present in a stable liquid state The leak rate of the water from the specimen chamber, approximately 10^{-3} torr litre/sec., was sufficiently slow that the specimen would remain hydrated for several hours. The use of a 100 micron aperture and the inability to alter the position of the cross over point of the scan coils, meant that the minimum magnification attainable was X100 times.

Whilst using this technique, it was noticed that all specimens viewed at chamber pressures above approximately 0.1 torr, were free from charging artefacts (Robinson, 1975c). The first explanation was that it was due to residual water in the specimen, rendering it slightly conducting. This was determined to be an inadequate explanation and a new one, in terms of ionisation of the residual gas molecules, was developed. Moncrieff et al (1978), calculated the effect of ionisation due to the incident beam, the BSEs, the charge build up on the specimen, the SEs accelerated by the charge buildup, the positive ions attracted to the specimen, the SEs released by the positive ions impinging upon the surface, and the cumulative effect of these further SEs producing more ions. They also measured these cumulative effects and showed that the elimination of charging artefacts was due almost exclusively to the ionisation mentioned above. Essentially, this established that as long as the gas could be ionised, which was a property of all gases, and the specimen could emit SEs and BSEs, which is a property of all solid and liquid specimens, it was possible to examine a specimen in a SEM, free from charging artefacts, at any accelerating voltage. Should an image still display some intensity fluctuation charging artefacts, it was merely necessary to increase the pressure of the residual gas. This increased the ionisation effect and charging would be eliminated every time.

One other problem was how much did the gas interfere with the electron beam? Moncrieff et al (1979) gave a very good dissertation of the amount of scattering of the electrons by the residual gas molecules. They calculated the elastic and inelastic scattering from nitrogen molecules, and compared the results with experimental observation. They showed that following a single event, an electron would be scattered tens to thousands of microns from the original beam trajectory, and would contribute only to a background signal, which could be removed by subtracting away some of the DC signal level. Those electrons which had not been scattered would continue on to form a beam diameter which had the same Gaussian FWHM diameter as would have existed without any beam scattering. In other words, even if 90% of the electrons in the beam were scattered, the unscattered electrons would still form a beam with the same diameter as if there were no scattering. 90% beam scattering did not mean 90% reduction of resolution, it resulted only in minor a minor deterioration in attainable resolution.

By that stage, the results achieved had established parameters for high pressure SEMs. The next task was to extend the capability to the limits determinable from the knowledge. The results of Moncrieff et al. (1979), showed that as the pressure was increased, shorter path lengths between the final aperture and the specimen were necessary to keep beam scattering to minimum and thus form an usable image. For a pressure of 50 torr, this distance was less than 0.5mm, and 50 torr became the upper practical limit of SEM using this type of technology.

Even then, 50 torr posed great problems for a single differentially pumped aperture. For a pressure of 50 torr to be maintained on one side of a single aperture, with 10^{-4} torr on the other side, an aperture diameter of about 13 micron was required. This placed such a severe limitation on the minimum size of the specimen which could be examined, as to be of little practical use. To overcome this, it was necessary to have an intermediate pressure by using two differentially pumped apertures. Danilatos and Robinson (1979) constructed a system having this capability and showed that this was usable. A pressure of 50 torr was equivalent to saturated water vapour pressure at body temperature, plus a further partial pressure of over 10 torr, for such gases as oxygen and effectively made it possible to examine biological specimens in conditions which were close to those necessary to support cell motility.

By the end of 1979, researchers at The University of New South Wales, Sydney, Australia, led by myself, had developed the following capabilities towards imaging specimens under high vapour pressure conditions:-

a) Established differentially pumped apertures as adequate for pressure separation in an SEM.
b) Established the parameters for the scattering of the electron beam by the residual gas molecules.
c) Established an upper practical working pressure limit of 50 torr, with higher pressures producing too short a working distance requirement.
d) Shown how the ionisation of the residual gas was responsible for the elimination of charging artefacts.
e) Demonstrated the ability to form images of a wide variety of specimens under a wide variety of pressures, with and without hydration.

Neal and Mills (1980) built this type of system in a Cambridge Stereoscan MkII SEM and were able to obtain video images of the adsorption of water into sponge material, as well as other effects. Again, the pressure limitation of 5 torr

meant that it had to be cooled. They gave an extended description of environmental SEM operating conditions. Similar results have also been achieved by others, for example Shah and Beckett (1979).

Having established the parameter for the capability to examine specimens at higher vapour pressures, the next step was to establish reasons for doing it. After all, at this time, most microscopists were intent to look at their specimens in a cleaner vacuum system and to suggest that there were advantages to be gained from going to higher pressures was going against known convention. However, it was the ability to look at insulating specimens at high, above 10kV, accelerating voltages, without charging artefacts which proved to be most valuable. This capability occurred at pressures of approximately 0.1 torr, for most working distances. The amount of beam scattering was generally less than 10%. As 0.1 torr was generally greater than the partial pressure of most oils and waxes at room temperature, this capability enabled these and most other out gassing specimens to be examined at voltages and currents suitable for X-ray analysis, without charging artefacts. This whole situation, including TEM, STEM and SEM controlled environment operation, was reviewed in 1984 (Robinson, 1984).

Interest in this capability was generated by ETP Semra Pty Ltd which, in 1978, manufactured a device which was initially called an environmental cell modification. This was later changed to Charge Free Anti-contamination System (CFAS). This device enabled the specimen chamber to be pumped by a rotary pump to a pressure controllable between 0.05 torr and 2 torr. The aperture remained a few mm inside the final lens and an image was formed by detecting backscattered electrons. Over one hundred of these were sold on Akashi/ISI SEMs. By 1980, Akashi integrated a CFAS into one of their SEMs and called the integrated system WET SEM. Over the next few years, they sold several hundred of these systems, mostly in Japan. Despite many years of my talking to the SEM manufacturers outside Japan, there was very little interest in building this type of instrument. As Akashi increased its market share by actively promoting this technique, the other major Japanese SEM manufacturers followed, JEOL with their LV (Low Vacuum) SEM and Hitachi with their N (Natural) SEM. Initially their sales were limited to the Japanese market, which was perceived as being different from other markets. However, with continued pushing by Mr. Ruscica (Electron Detectors Inc) and myself on how these devices were promoted in Japan and how a similar approach could work in USA, sales started slowly in USA, but soon increased rapidly. AMRAY Inc. realised the potential of this type of instrument and introduced their ECO (Environment Controlled) SEM in 1993. Gresham Camscan introduced their EnVac SEM. When Leica and Zeiss amalgamated to form LEO, their first product was their VP (Variable Pressure) SEM. Philips introduced their CP (Controlled Pressure) SEM in 1996. RJ Lee Instruments Ltd has released their variable pressure SEM.

By 1996, the major SEM manufacturers had all released a SEM which had the capability to examine specimens in a controllable pressure environment in the specimen chamber of their SEM. For some SEM companies, it was noticed that their sales of tungsten filament SEMs were almost exclusively due to this type of SEM. These SEMs all used a single differentialy pumped final aperture inside the final lens as a pressure limiting aperture and a backscattered electron detector to collect a signal to form an image. Although exact sales of this type of microscope are not known, sales by ETP Semra Pty Ltd, of wide angle scintillator type BSE detectors to be included in SEMs of this capability exceed 1500. Not all of this type of SEM are fitted with a scintillator type BSE detector, and I am unaware of the sales of solid state detectors for this purpose. I will leave it to the imagination of the readers to determine how many of this type of SEM have been sold, but as a conservative guess, a figure of 2000 SEMs would not be unrealistic.

While this was occurring, Danilatos continued researching higher pressure capabilities, attempting to image at atmospheric pressure (1981). However, this pressure placed such a severe limitation on depth of focus and working distance that there was no further interest in that work. He also commenced work on a secondary electron (SE) detector capable of operating at higher specimen chamber pressures (Danilatos, 1983). Images obtained with this environmental SE detector have displayed approximately the same resolution capability as those obtained with an efficient BSE detector, from similar specimens.

Much work has been performed on the development of new types of electron guns, for example, the LaB6 and thermal and cold field emission, to obtain greater resolution and through that greater specimen information. The information gained from the ability to examine specimens in their natural state, while not as spectacularly demonstrable as the improvements to gun, is never the less making a quiet revolution to the information which can be achieved from the specimen. It will not be long, given a combination of the higher brightness electron gun and improvements to detector performance, before images from hydrated biological specimens will show as much detail as is currently achieved from dehydrated and gold coated specimens imaged with a conventional tungsten filament. ■

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