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LOW VOLTAGE BACKSCATTERED ELECTRON IMAGING (< 5 KV) USING FIELD EMISSION SCANNING ELECTRON MICROSCOPY

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Abstract

Backscattered electron (BSE) imaging is most commonly performed by applying accelerating voltages of 10kV and above to the specimen. For imaging of surface detail, the application of a lower accelerating voltage results in less beam penetration, spread and overall specimen damage. A field emission scanning electron microscope (FESEM) operated with high emission current (50μA) was used to increase the number of BSE obtainable from imaged specimens. When operated with a high condenser lens current and a lower accelerating voltage (<5kV) the interaction volume of the electron beam with the specimen was minimised. The lower voltage BSE images exhibited enhanced surface detail and contrast. Specimens that normally charged, locally affecting secondary electron (SE) detection, were imaged without such distortions when BSE were used. It was advantageous to use a range of applied accelerating voltages in order to provide the most image information.

Key Words: Field emission scanning electron microscopy, backscattered electron imaging, low voltage.

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Introduction

In scanning electron microscopy (SEM), backscattered electron (BSE) imaging is typically used to provide an image of the distribution of relatively high atomic number material located on or within a sample. When a primary beam electron interacts with one or more atomic nuclei which reverses it's direction of travel with low energy loss (elastic scattering) and causes it to escape from the surface of the specimen it is referred to as a backscattered electron. BSE are emitted from the specimen with energies ranging from 50 eV up to the energy of the interacting primary beam (Everhart *et al.,* 1959). Secondary emitted electrons (SE) have less than 50eV of energy, with the majority having only around 10eV. BSE travel in straight lines at high velocities. Therefore, the majority of these electrons are not attracted to the positive charge on the conventional Everhart Thornley SE detector. However, some do travel in that direction and are detected, providing a BSE derived signal (produced from SE_{II}) within an 'SE' image (Everhart *et al*., 1959).

The amount of BSE produced during the inter-action of the primary beam with the sample increases proportionally to the average atomic number (Z) of the specimen (Palluel, 1947). Variation in the amount of BSE produced leads to an image that exhibits Z contrast. Their source depth depends upon both the energy of the primary beam and the density of the sample. The approximate depth of electron penetration of the primary beam into the specimen can be calculated using electron range equations (Bethe, 1930; Kanaya-Okayama, 1972). BSE images can expose features not only on the specimen surface but also from a significant depth within the material, since the BSE are absorbed to a lesser degree, by the overlying tissue, than the lower energy SE (Abraham and DeNee, 1973). Hence, differential staining of structures on or within a biological sample with heavy metals will provide good BSE contrast when compared to the surrounding lower density material.

The application of BSE imaging to biological samples has been mainly conducted at relatively high primary beam accelerating voltages (>10 kV). This procedure can cause considerable radiation damage to cell surfaces (Pawley and Erlandsen, 1988). When the accelerating voltage applied to the primary beam is lowered, the primary

electron range within the sample is decreased, thereby reducing the interaction volume. The net charge deposition is also reduced (Pawley, 1984, Reimer, 1993). This may actually increase radiation damage due to the same number of electrons being present in a smaller volume. Palluel (1947) demonstrated that the maximum number of BSE are emitted from low atomic number specimens at low accelerating voltages and that the yield does not increase when the primary beam accelerating voltage is increased.

Until recently the relatively poor efficiency of BSE detector systems prevented the investigation of the potential of low accelerating voltage BSE imaging. The only method of obtaining acceptable signal to noise levels in BSE images at lower accelerating voltages from a given specimen, with a particular detector system (held normal to the final lens and specimen), is to increase the probe current at the specimen. This, naturally, results in an increased emission of BSE from the specimen. Probe current can be increased by either using a larger spot size, obtained by decreasing the excitation of the first condenser, or by increasing the emission current at the gun. The latter is preferable because increasing the spot size will lead to reduced resolution (Becker and Sogard, 1979). By increasing the emission current at the gun the spot size can be minimised, while obtaining a significantly increased BSE signal level. The availability of the high emission current setting on a field emission gun instrument makes the exploration of the effects of such operating conditions possible.

Increase in BSE emission, obtained by this method, has been applied to produce a more useful signal-to-noise ratio during BSE imaging of biological specimens. BSE imaging in such a mode has already been reported for fixed, stained and embedded cells at varying accelerating voltages, including low beam potentials (Richards and ap Gwynn, 1995). It has also been used for viewing the glass knife planed surfaces of resin embedded biological sample blocks with the FESEM (Richards and ap Gwynn, 1996). The images obtained closely resemble those obtained of thin-sectioned material by conventional transmission electron microscopy. This paper describes the advantages, in many situations, of low voltage BSE imaging of bulk biological and non-biological surfaces over SE imaging.

Material and Methods

BSE imaging at high emission currents with an FESEM (Richards and ap Gwynn, 1995) was applied to various specimens at both typical (>10 kV) and low accelerating voltages (<=5 kV). Biological samples were coated with 8 nm 80/20 gold/palladium (as measured with a quartz thin film monitor, positioned at a fixed place relative to the specimen) in a Baltec MED 020 unit (BALTEC, Balzers, Liechtenstein). Non biological samples were not coated. A Hitachi S-4100 FESEM (Prophysics AG, Zürich, Switzerland) was operated at a high emission current (50 μA), while fitted with an Autrata yttrium aluminium garnet (YAG) scintillator type BSE detector, with a central conical shaped 2mm bore (Institute of Scientific Instrumentation, Prague, Czech Republic). Images were saved on a digital image acquisition system (Quartz PCI, Quartz Imaging Corporation, Vancouver, Canada). The microscope was operated at varying accelerating voltages between 1 kV and 30 kV. The working distance used was set at 10mm from the final lens, for optimal BSE collection.

The largest condenser aperture (100 μm) and the maximum condenser lens current (setting C18) were used resulting in a small spot, with a high current density, thus maximising resolution. At low accelerating voltages, when the signal level fell below the functional contrast threshold for the detector system, the first condenser lens current was reduced (settings C16, 14, or 12). This adjustment increased the available probe current at the specimen surface to a useable level, allowing sufficient information to be acquired for BSE imaging. By selecting a suitable accelerating voltage, it was possible to control the maximum depth in a specimen from which the BSE emerged and therefore select the information to be imaged.

The probe current was also measured at an emission current of 50 μA with the condenser lens current at maximum (setting C18) and the other microscope parameters as above. This measurement was performed from 1-30 kV at 1kV intervals using a Faraday cup and a probe current meter (KE Developments Ltd. Cambridge, UK) attached to the specimen stage.

Results

BSE imaging with the FESEM, using high emission currents at low accelerating voltage, resulted in high probe currents at the specimen surface (Fig. 1). This re-sulted in a higher production of BSE, when compared to signals obtained when more usual aperture and emission settings were used. Furthermore, the high probe currents resulted in the production of detectable amounts of BSE from smaller interaction volumes. The smallest probe diameter was maintained by using the highest available first condenser lens current settings. This mode of operation enhanced the surface contrast and detail obtainable from a variety of samples (Figs. 2-4).

Low voltage SE imaging (<5 kV) with the FESEM provides detailed surface topographical information about specimens. However, localised electrical charging can occur, especially in biological samples with many protruding fine projections of their surfaces. Rabbit lung epithelium tissue is a good example of such a specimen (Fig. 2a). The cilia and microvilli, of this highly structured surface, displayed charging. Adequate images were not obtainable even when low beam accelerating voltage had been applied.

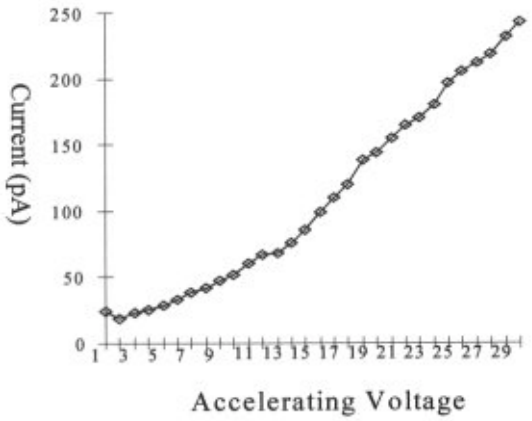

Figure 1. Distribution of probe currents at the specimen surface at increasing accelerating voltages using 50µA emission current, the largest condenser aperture (100µm) and the maximum condenser lens current to minimise the spot size (setting number 18) at a working distance of 10 mm.

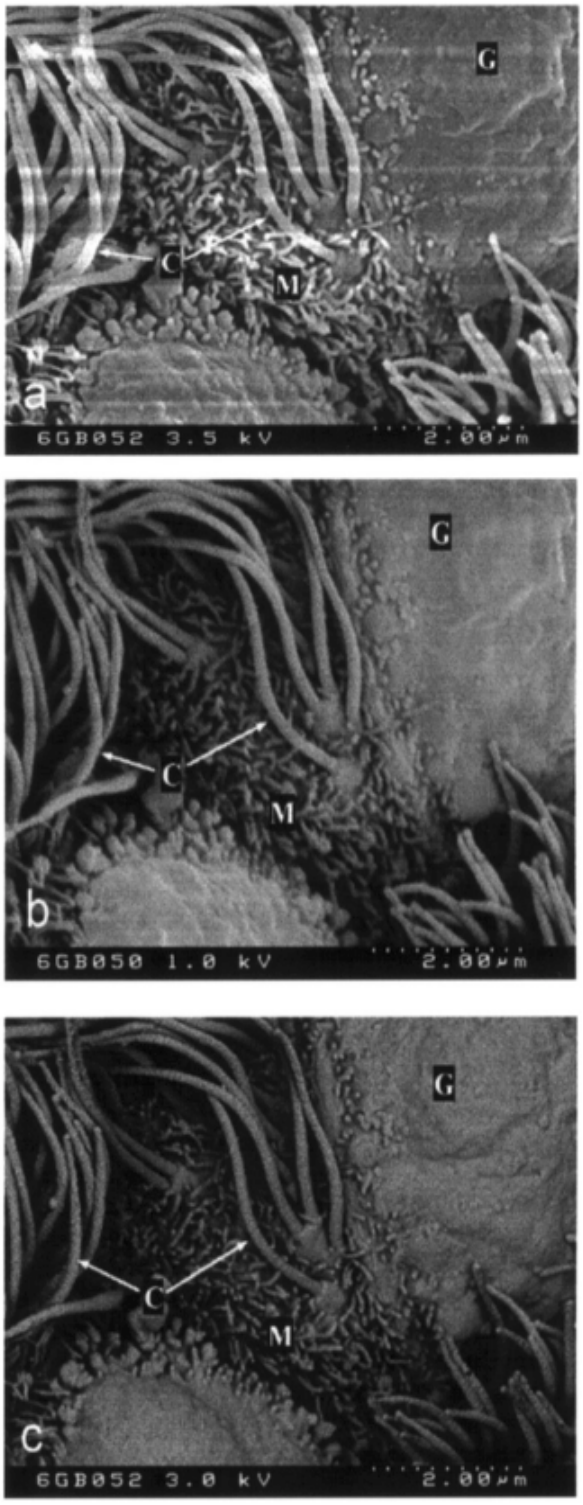

Figure 2. Images of the epithelium of a rabbit lung bronchus containing goblet cells (G), cilia (C) and smaller microvilli (M). (**a**) SE image at 3.5 kV, 10µA emission current, condenser current setting C12 and final lens aperture 20µm. At these normal operating conditions with this type of sample charging across the specimen surface can not be avoided. (**b**) SE image with microscope operating conditions as (a) but at 1 kV. The lower kV removes the overall charging, though there is some on the goblet cell. There is also less clarity and topological information in the image. (**c**) HCBSE image at 3 kV, 50µA emission current, condenser current setting C18 and final lens aperture 100 µm. Detail of the specimen surface is evident with high clarity and contrast. Sample charging effects are also removed.

Lowering of the accelerating voltage even further minimised the overall-charging problem (Fig. 2b), but led to an increase in chromatic aberration and, as a result of this, a decrease in the attainable resolution.

When high emission current (HC) BSE imaging was used, these complex samples were imaged without charging effects (Fig. 2c). Details of the specimen surface were evident with high clarity and contrast. At low accelerating voltage (<5 kV) the BSE detector was seen to provide improved topographical contrast compared to the SE image.

HC-BSE imaging was also seen to improve the imaging of 'everyday' normal samples such as cultures of Chinese Hamster ovary cells (Fig. 3). The SE image at 1kV did not have charging artefacts (Fig. 3a) and the surface detail appeared fairly clear. However, when the HC-BSE image was prepared it provided much more surface contrast and fine detail, such as small pores and crinkling in the membrane (Fig. 3b).When HC-BSE, at low accelerating voltage, imaging was applied to non-biological samples improved results were similarly obtained. Imaging the surface of a titanium orthopaedic implant at an accelerating voltage of 5 kV (Fig. 4), with both SE and BSE detection, showed that some information was lost within the SE image as a result of the overproduction of SE at surface edges. The contrast and detail was also inferior in the SE image, when it was compared to the HC-BSE image.

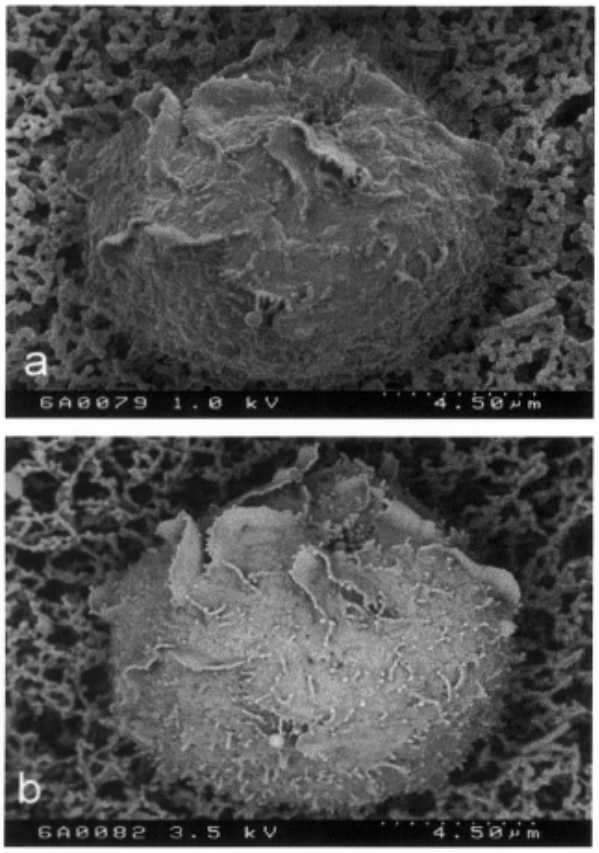

Figure 3. A Chinese hamster ovary cell on Millipore filter. (**a**) SE image at 1 kV, 10 µA emission current, condenser current setting C16 and final lens aperture 20 µm. (**b**) BSE image of the same cell as (a) at 3.5 kV, 50µA emission current, condenser current setting C16 and final lens aperture 100 µm. The surface blebs and filopodia are more distinct than in the SE image and more surface detail such as membrane pores is exposed

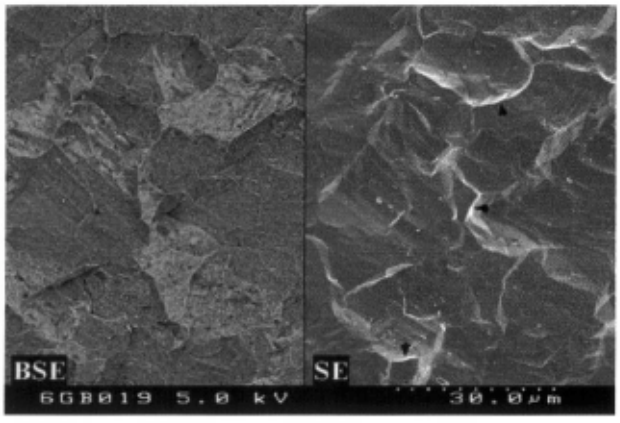

Figure 4. The surface of a titanium orthopaedic implant at 5 kV. On the right side is the SE image with some information lost by overproduction of SE at the edges of the topology (arrowheads). The contrast and detail is much clearer on the BSE image on the left.

Discussion

There are two classes of BSE. BSE_I are emitted at high elastic-scattering angles, emerging in close proximity to the beam impact area. This signal is sensitive to the surface topology of the specimen. BSE_{II} come from a greater depth within the specimen and undergo multiple accumulative elastic interactions, spatially disconnecting them from the primary beam impact area (Fig. 5a). Ultimately BSE_{II} emerge from the surface at a distance from the beam impact point.

The beam interaction volume is reduced at lower accelerating voltages. As a result, the BSE_{II} originate from areas closer to the beam impact area. This signal is therefore more sensitive to the surface topology at lower rather than at higher accelerating voltages (Fig. 5b). The low energy spread of the more coherent FESEM beam also decreases the interaction volume, concomitantly increasing the topographical contrast at low accelerating voltages. The signal to noise ratio is also increased at low accelerating voltages because the proportion of BSE_I emitted at high elastic-scattering angles increases.

Between 5-30 kV the electron penetration range into the specimen is much greater than the diameter of the primary beam. Primary electrons lose energy at the rate of several eV per Angstrom of distance traveled through the specimen (Joy, 1995). The total distance traveled at low accelerating voltages is limited to a few tens of nanometers, rather than several micrometers, which is typical when high-energy accelerating voltages have been used. Theoretically, below about 1.5 kV, the depth within the specimen from which BSE derived information emerges becomes less than that for SE and therefore carries more surface detail information (Joy, 1987).

As the interaction volume decreases, the SE signal becomes more sensitive to layers of hydrocarbon contamination on the specimen surface produced during interaction of the primary beam with the specimen (Katani *et al.,* 1991). BSE are affected to a much lesser extent by these contamination layers, due to the fact that they have a significantly higher energy than SE. At specimen edges the beam-specimen geometry results in increased SE emission that tends to obscure surface topology. Since BSE are not affected by subtle changes in surface geometry, the edge effect is suppressed - which makes image interpretation easier.

The position of the annular BSE detector above the specimen, below the lens, helps with improving the signal by detecting the high angle take-off BSE. Maintaining a high emission current sustains a high probe current at the specimen surface that therefore allows the production of a strong BSE signal for detection. If the BSE detector system is to one side of the specimen, then tilting the specimen surface towards the system will also increase the collection of the BSE signal.

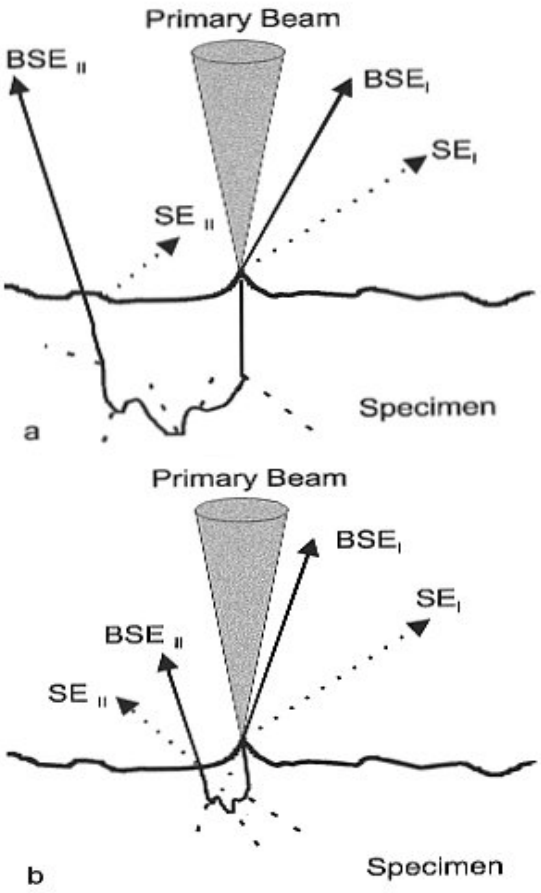

Figure 5. Excitation of SE and BSE from within a specimen by the primary beam. SE and BSE trajectories are shown. (**a**) High accelerating voltage applied to the primary beam. BSE_I emerge from close proximity to the beam impact area. BSE_{II} come from a greater depth from within the specimen after undergoing multiple accumulative elastic interactions, ultimately emerging spatially disconnected from the point of impact of the beam. (**b**) Low accelerating voltage applied to the primary beam. The primary electrons penetrate less into the specimen and therefore the BSE_{II} emerge closer to the beam impact area and are more sensitive to the surface topology than at higher accelerating voltages.

Conclusion

BSE imaging at low accelerating voltages results in enhanced specimen surface contrast and detail. More scattering events occur close to the surface containing high-resolution information. The method decreases the depth of specimen radiation damage and allows for a clear visualisation of non-conducting specimens where local charging is visualised using SE imaging. The edge effect, observed with SE imaging, is also suppressed with the low voltage BSE imaging. This imaging method can also be used with other BSE detectors and thermionic emission SEMs, provided high beam emission currents are used. The use of more than one accelerating voltage, applied to a sample, is advocated for obtaining as much information from that sample as possible.

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Discussion with Reviewers

I. Müllerova: The producers of the SEMs are going down with the energy of primary electrons to, say, about 200eV with a resolution of several nm, and they are considering to go even to lower energies with a high resolution. What can be the main applications for biologists in such a low energy range in the SEM?
Authors: If these high resolutions could be obtained at such low energies, with low chromatic aberration, this would be a breakthrough for 'real' surface imaging of specimens in the SEM. This would remove the requirement for coating non-conductive specimens, such as the biological ones highlighted in this paper as well as polymeric materials, which are very sensitive to radiation damage. It would also be of benefit for imaging surface oxides on metallic implant materials that normally are affected by charging artefacts at discontinuities. In the technique presented here where, by normal nomenclature low voltage is used between 3-5 kV in the BSE mode there are still many electrons being directed into a small volume, which can cause radiation damage to the specimen. The very low voltages you mention should minimise any possibility of specimen damage.

I. Müllerova: Do you think that some types of biological applications of the imaging of specimens in transmission electron microscope (TEM) could be done in SEM at low energies without all complicated preparation techniques? If yes, which types of applications?
Authors: We have already published that this HCBSE technique can be used for imaging TEM blocks and sections in the SEM (Richards RG and ap Gwynn, 1996). This technique does not work well at low accelerating voltages though, since surface imaging of the resin occurs rather than the stained embedded structure within. Electron-optical 'sections' through embedded stained cells have also been performed (Richards and ap Gwynn 1995). There is work currently underway to attempt to remove slices of information from overlying 'sections' using deconvolution methods, so that applying a series of increasing accelerating voltages could provide images which would be similar to having collected images of serial sections with a TEM. We also have ideas for applying this method towards the field of low voltage X-ray microanalysis of samples.

S. Chapman: You state that 10 mm is the optimum position for BSE. Did you conduct experiments and could you provide a graph that demonstrates the position for maximum BSE?
Authors: For the set-up of the microscope and BSE detector we have, a working distance from the final lens to the specimen of 10 mm is optimal. Observing the line trace at various working distances tested this. We do believe that if it were possible to move the BSE detector closer to the final lens (at present around 3 mm, to say 1 mm) then the optimal working distance would be slightly less.

S. Chapman: Is there a change in information with working distance as one might expect a contrast variation with such a change? Moving closer to the detector would not more electrons be lost through the incident beam aperture, a narrow scattering angle? In a similar fashion would not electrons be lost through too wide a scattering angle at longer working distances?
Authors: Yes, again for the set-up of the microscope and BSE detector we have, moving the specimen from the optimal working distance of 10 mm either towards, or away from the detector lowers the contrast. We believe this is due to the reasons suggested above by the reviewer. A smaller central bore hole and a larger detector area would probably increase the BSE signal, though the minimum useable magnification would be increased with a smaller bore.

S. Chapman: With conventional imaging the resolution and signal differences between 5 mm and 10 mm working distance are considerable, do you have any idea of the balance between resolution and signal efficiency at these levels for BSE?
Authors: Unfortunately, we have not performed tests in this area. We think David Joy may be working in this area. It is likely that if we could move the detector closer to the final lens that the resolution would increase. We know that, with other detector systems we have tested in this microscope, the smaller the central bore in the detector, the better the final image contrast and resolution. This is explained by the reasons the reviewer has pointed out in the previous question.

T. Maugel: Since this technique produces enhanced resolution and clarity of detail compared to SE imaging, can you comment on the application of this technique to the localisation of very small (< 5 nm) immunogold particles ?
Authors: HCBSE imaging has been used to look at silver enhanced 1 nm gold particles (final size around 50 nm) both from the surface of fibroblasts at around 4 kV to 6 kV with enhanced signal from the particles. The same gold particles have been viewed on the undersurface of the cells after embedding in resin and removal of the substrate at higher accelerating voltages, again with good discrimination from the cell (Richards *et al*., 1997). At present the technique has not been tried directly on 5 nm gold particles, but we have visualised 10nm particles on biological samples without silver enhancement using this method.

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