Microscopy101

Choosing the Right Accelerating Voltage for SEM (An Introduction for Beginners)

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Historically, most SEM operators used accelerating voltages that were fairly high, quite often in the range of 15–20 kV. Now progress in electron optics has made low-voltage observations a routine mode of SEM operation. The greatly improved range of utilized accelerating voltages provides the SEM operator with additional flexibility and with additional responsibilities for choosing the right SEM settings for image acquisition.

The low-voltage mode of operation is very important in the observation of non-conductive and/or beam sensitive specimens. Because the secondary electron emission coefficient increases as the accelerating voltage decreases, it is possible to find a beam energy where no charging occurs; that is, when the number of electrons (secondary and backscattered) leaving the specimen is equal to the number of incident electrons entering the specimen. This happens for most specimens at accelerating voltages somewhere between 0.4 and 4 kV. Additionally, for beam sensitive specimens, lower electron beam energies (lower kVs) reduce the chance of beam damage. The micrograph of one such specimen is presented in Figure 1a. It is the sticky part of a Post-it® Note that has not been sputter-coated. The micrograph was obtained at 300 V accelerating voltage, and the specimen tilt angle was 75º. On this paper substrate (paper fibers are clearly visible) there are hills of glue. The tops of these hills are flattened because they were attached to the adjacent sheet of paper of a Post-it Notes stack. Figure 1b of the Post-it Note after coating with Au-Pd in a sputter coater shows the sensitivity of the glue to coating: hills are no longer flat topped but rounded, indicating that some glue flow did occur during the coating. For this specimen the low-voltage mode (Figure 1a) is the best mode of observation.

One of the most obvious consequences of decreased accelerating voltage is reduced interaction volume. The secondary electron signal produced from a smaller sized volume, which is closer to the surface, contains more surface information. Figure 2 represents the images of a brand new steel razor blade wiped with alcohol (that is, prepared for trimming TEM blocks). While at an accelerating voltage of 30 kV the blade looks not too dirty (Figure 2a). At 2 kV it displays a surprising amount of contamination (Figure 2b), which was fully- or semi-transparent in the higher-voltage image. But what if contamination effects are of no interest to the researcher? What voltage should the investigator choose?

Not just contamination, but any small details of the surface could be smothered or completely wiped out by a higher accelerating voltage. The micrograph of fractured steel obtained at 2 kV accelerating voltage (Figure 3a) revealed a predominantly cleavage type of fracture, indicating insignificant plasticity during fracture. An important feature of the cleavage fracture is the so-called river pattern, which consists of tiny tear ridges that are clearly visible at 2 kV. Raising the accelerating voltage to 15 kV led to the disappearance of many fine details on the fracture surface (Figure 3b) and made the observation of the river pattern much more difficult. However, at 15 kV, dimples formed by the presence of nonmetallic inclusions in the steel (marked with arrows on Figure 3), are emphasized and are more easily identified. Now, if a researcher is looking for dimples, then, perhaps using higher accelerating voltages is not a bad idea at all. Of course, when the goal is the highest possible resolution

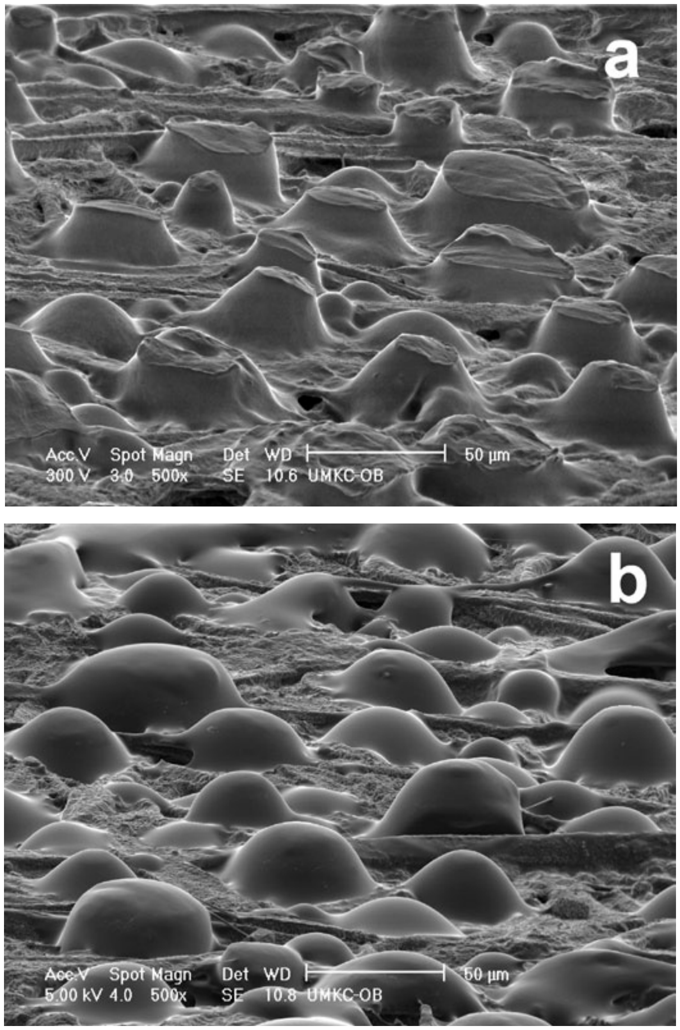

Figure 1. Adhesive on Post-it® Notes. (a) uncoated at image at 300 V accelerating voltage, (b) sputter-coated with AuPd and imaged at 5 kV. Bar = 50 µm.

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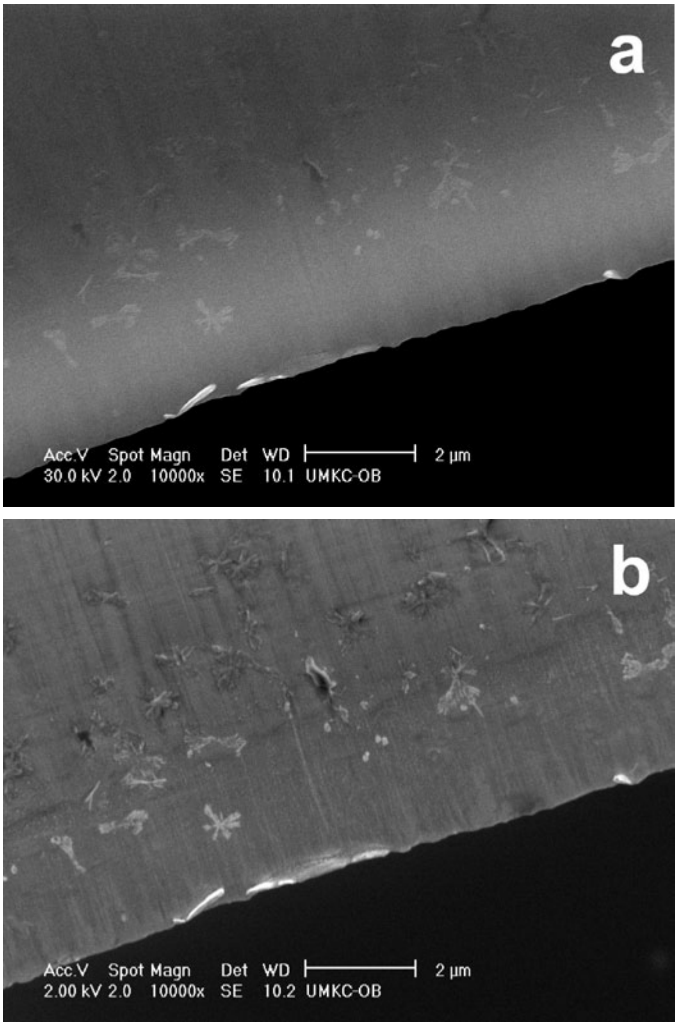

Figure 2. Steel razor blade wiped with alcohol and imaged at 15 kV (a) and 2 kV (b). Bar = 2 µm.

for specific details, then the task becomes very specimen- and microscope-dependant, and the right accelerating voltage should be chosen on a case-by-case basis.

The micrograph of a cell culture obtained at 1 kV accelerating voltage (Figure 4a) looks fine, and only a comparison with the micrograph obtained at 4 kV (Figure 4b) shows the drawbacks of the lower-voltage image. The most striking difference is that the 1 kV image could not make a distinction between the cell surface and the substrate surface, displaying them at the same brightness level. When the voltage was increased to 4 kV, the difference between cells and substrate became clearly visible, cell attachments (fine details on cells edges) became far more noticeable, and the obtained micrograph was overall much better suited for cell culture examinations. A further increase in accelerating voltage (15 kV, Figure 4c) did not yield additional improvements. On the contrary, the edge effect, more pronounced at higher voltages, made some image feature edges extra bright, thus decreasing the overall image quality. Thus, for cell culture studies, this procedure provides a method for finding a suitable accelerating voltage for specimen observation.

Special imaging needs of a researcher can be met with specific choices of accelerating voltage. For example, when just the shape of a yeast colony is of interest, then the 30 kV accelerating voltage can give rather interesting pictures of specimens with white colonies on a black background (Figure 5); however, 30 kV is an inferior choice for the observation of individual yeast cells.

Similarly to the yeast colonies, bone cells (osteocytes) are highlighted in Figure 6a at the accelerating voltage of 15 kV (original magnification 200×). The sample was a mouse bone embedded in acrylic resin, polished, and slightly etched. Etching removed a thin layer of bone mineral, leaving behind the resin casts of cells and their dendrites (to be precise, they were not exactly cell casts, but casts of the slightly bigger lacunae). Highlighting the cells with the help of the edge effect is useful for cell identification and location, but taking pictures of individual cells at higher magnifications is better done with a minimized edge effect at lower accelerating voltages, such as 5 kV (Figure 6b, original magnification 5000×). So, the selection of the accelerating voltage for this type of specimen is magnification-dependant: 15 kV for lower magnifications and 5 kV for higher ones (until hollow magnification begins).

Of course, SEM is used not only for the study of specimen topography but also for the study of its composition. This is often done with the help of the backscattered electron (BSE) signal, but in many cases it is possible to combine the topographical and compositional information in a single secondary electron picture. Secondary electrons are produced by both incident electrons when they enter the specimen and by BSE when they

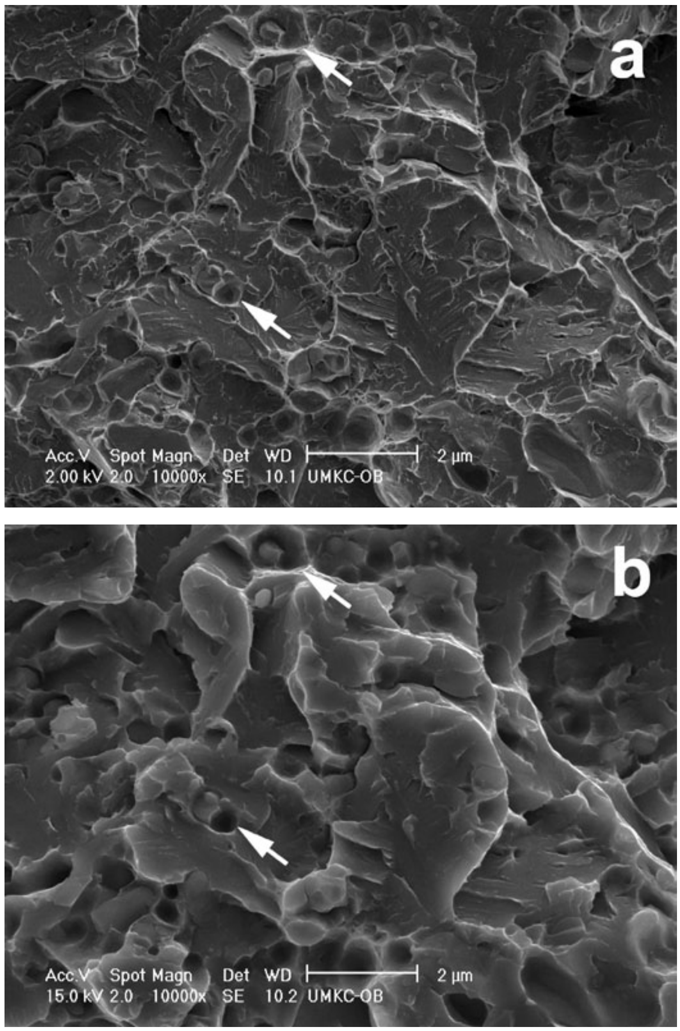

Figure 3. Steel fracture imaged at 2 kV (a) and 15 kV (b). Bar = 2 µm.

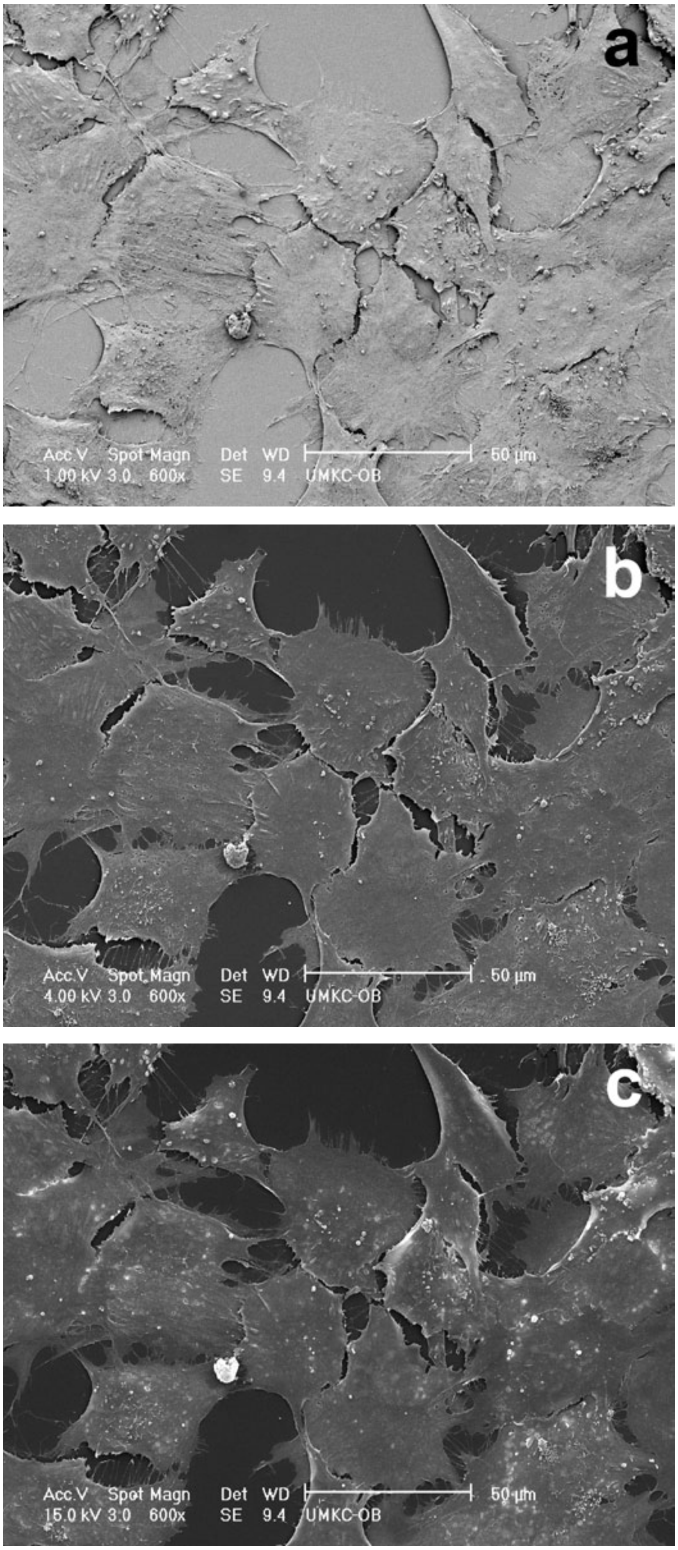

Figure 4. Cell culture observed at 1 kV (a), 4 kV (b), and 15 kV (c). Bar = 50 µm.

leave the specimen. Therefore, the secondary electron signal under the right conditions can carry information about both topography and composition. The micrograph of a fractured dental composite (used for dental restorations) taken at 2 kV accelerating voltage presents nicely detailed surface features (Figure 7a). The dental composite consists of a resin matrix and filler particles, and these materials

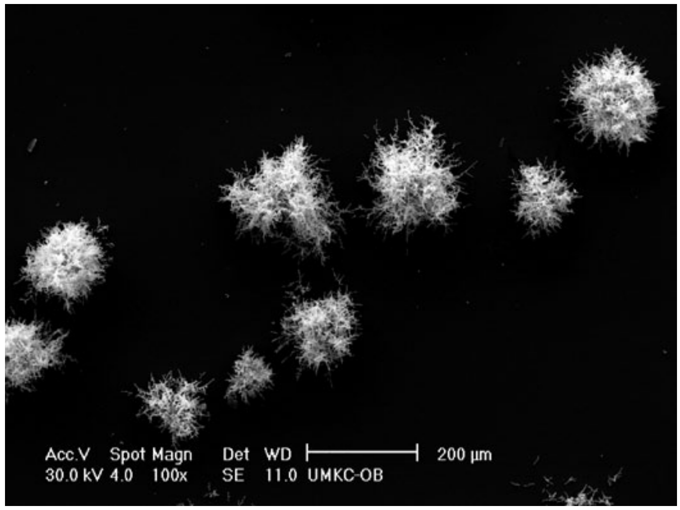

Figure 5. Yeast colonies observed at 30 kV. Bar = 200 µm.

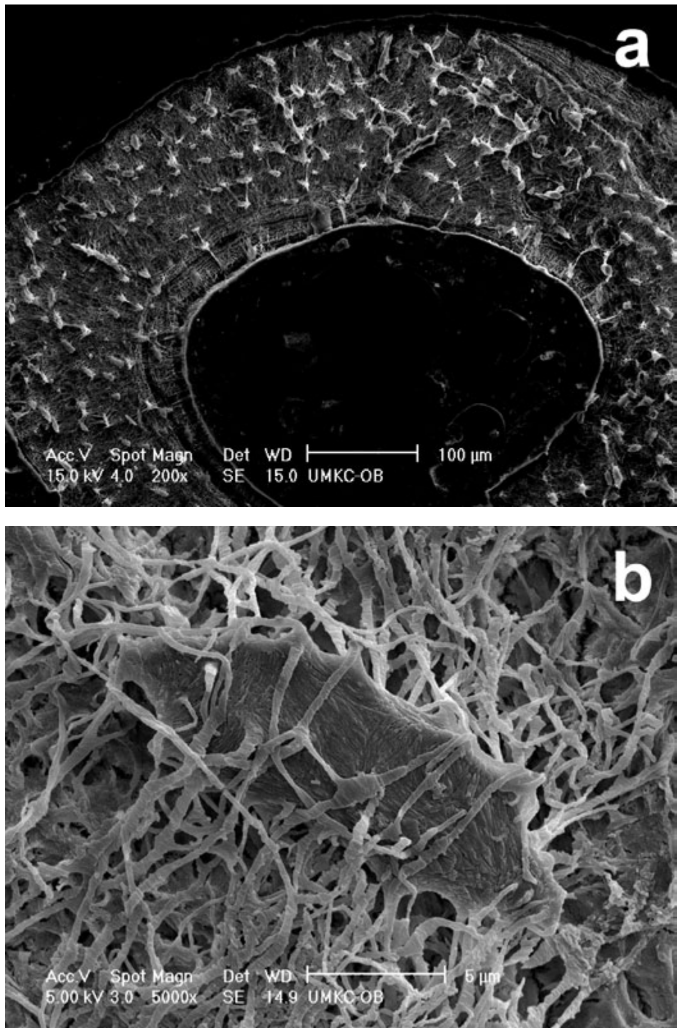

Figure 6. Mouse bone, resin embedded, polished, and etched. (a) Imaged at original magnification of 200× and 15 kV. Bar = 100 µm. (b) Imaged at original magnification of 5000× and 5 kV. Bar = 5 µm.

have significantly different mean atomic numbers. Raising the voltage to 20 kV increases the number of BSEs contributing to the image signal, especially from the filler particles

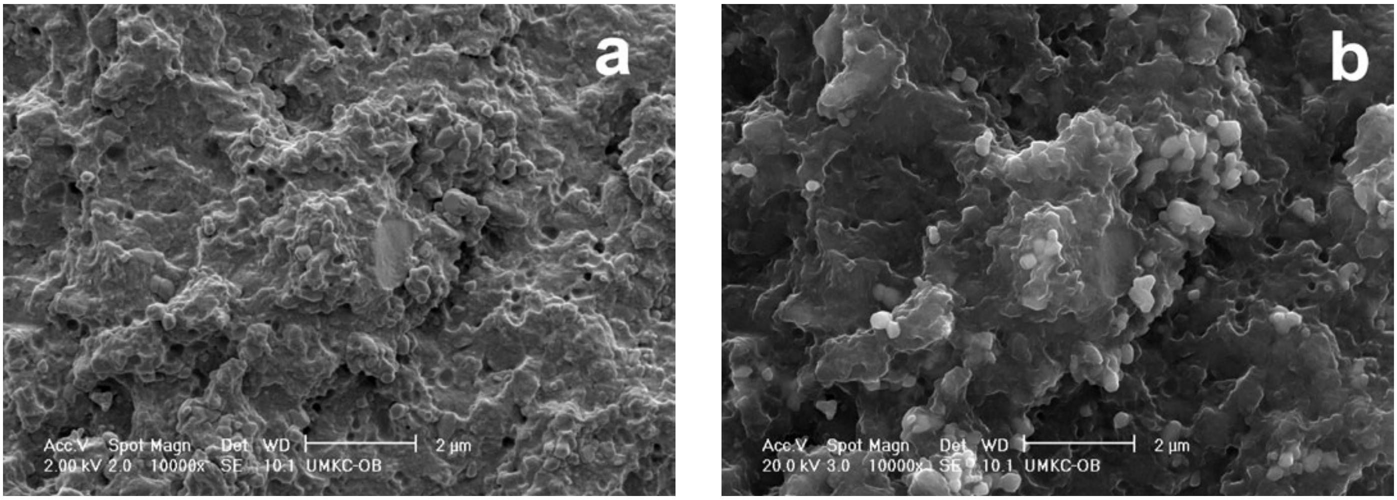

Figure 7. Fracture surface of dental composite observed at 2 kV (a) and 20 kV (b). Bar = 2 µm.

that have a higher mean atomic number. At 20 kV the filler particles are highlighted (Figure 7b), but the trade-off is a somewhat less sharp image of the surface because of the larger interaction volume. Again, to choose the value of accelerating voltage, the researcher should decide what is more important: fine surface information or additional compositional information. Of course, it is always possible to acquire two images: one at a lower voltage in secondary electrons and one at a higher voltage in BSE [1–3].

References

[1] All micrographs presented in this paper were obtained with Field Emission SEM XL30 (FEI, Hillsboro, OR).
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