



# Low Voltage Scanning Electron Microscopy: Promises and Challenges

## Application Note

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Why do we need a low voltage scanning electron microscope? The simple answer is that “higher voltage” scanning electron microscopes cannot answer adequately some practical issues associated with imaging of certain types of materials. The latter include non-conducting samples whether organic (such as polymers, enzymes, cells, membranes, etc.) or inorganic (such as ceramics, pigments, minerals, composite materials, etc.). These materials usually manifest one or both of these particular problems: (i) charging effect due to accumulation of electrons on the scanned area of sample; (ii) local radiation damage of the sample, induced by energetic electrons through different mechanisms such as decomposition, sputtering, sublimation, ionization, diffusion, or transformation. These effects lie beyond the scope of this note.

Charging effect leads to a degraded image, poor resolution and render EDX analysis worthless. It causes the incident beam to be repelled from the investigated region and gives rise to unstable or distorted SEM images [1]. In conventional high voltage SEM (up to 35kV), charging effects are avoided or minimized for non-conducting materials by coating the sample with a thin conductive layer of gold, carbon, platinum, or gold-palladium. A relatively thick layer of gold may hide some nano-scale features of the sample surface. Some samples, where specimens

cannot be cut or broken for SEM observation, cannot be coated. As this coating can alter the appearance of the sample or hinder its reuse or analysis by other techniques (e.g. atomic force microscopy or Raman). Another option that avoids sample coating in high voltage SEM is using environmental cell conditions which consist in maintaining a low vacuum environment in the sample chamber by allowing water vapor (0–20 torr). E-SEM is particularly helpful when studying biological, wet, or degassing samples. Nevertheless, E-SEM has its own limitations and difficulties. On one hand, there are the technical issues of conditioning a large chamber and the reduced contrast due to the low vacuum the electron beam has to travel through. However, the presence of water in the E-SEM causes more radiation damage to organic and biological samples due to ionization of water molecules and generation of free radicals [2].

The other problem is the radiation effects or damage caused by a high voltage electron beam [2–4]. Conventional SEMs usually operate at an accelerating voltage of 15 kV and FE-SEMs at a voltage that can go up to 35 kV. The high energetic and focused electron beam can cause serious local radiation damage of certain samples. The latter include organic and biological samples and certain inorganic materials such metal sulfides [5].



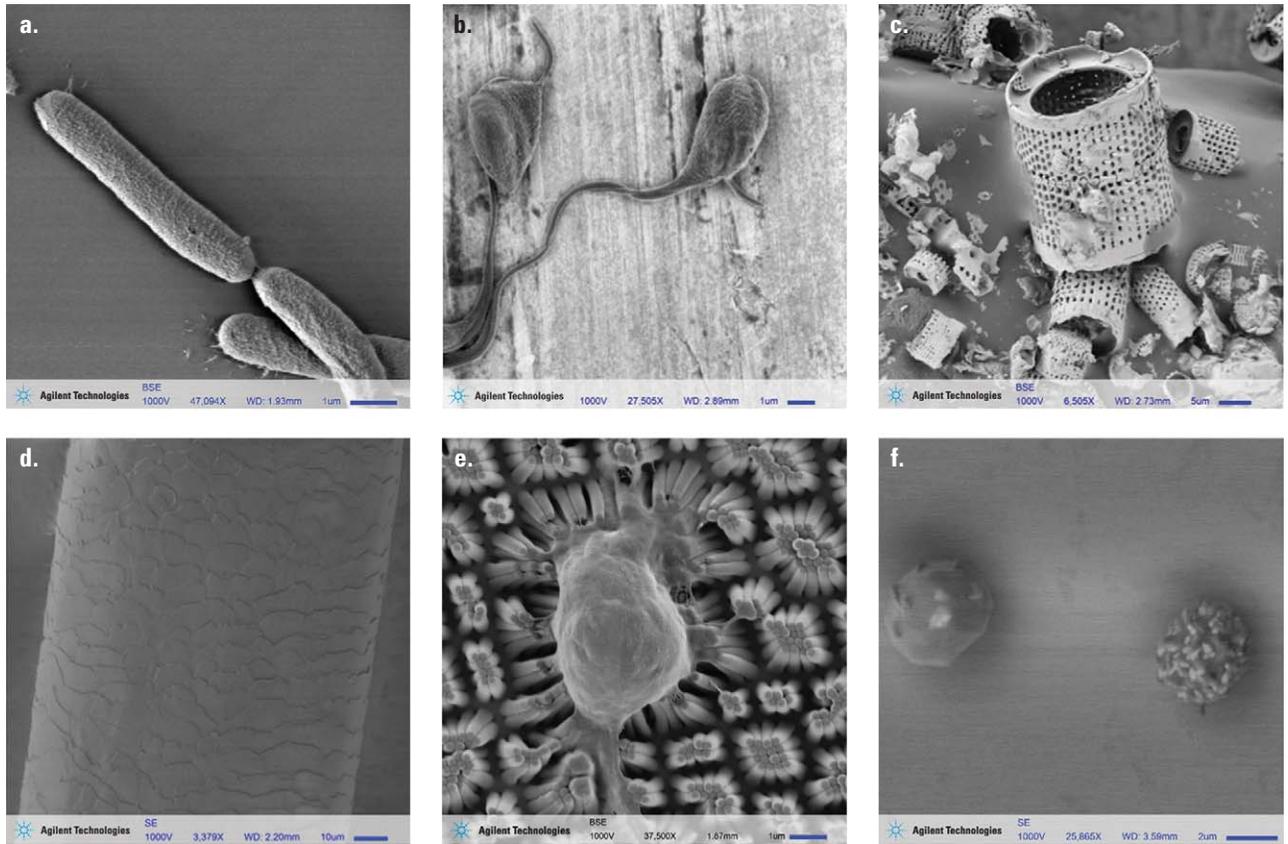


Figure 1. Images obtained with Agilent 8500 for: (a) bacteria; (b) cells with flagella; (c) diatoms; (d) gray human hair; (e) cells grown on PDMS pillar substrate ; (f) human blood cells, respectively.

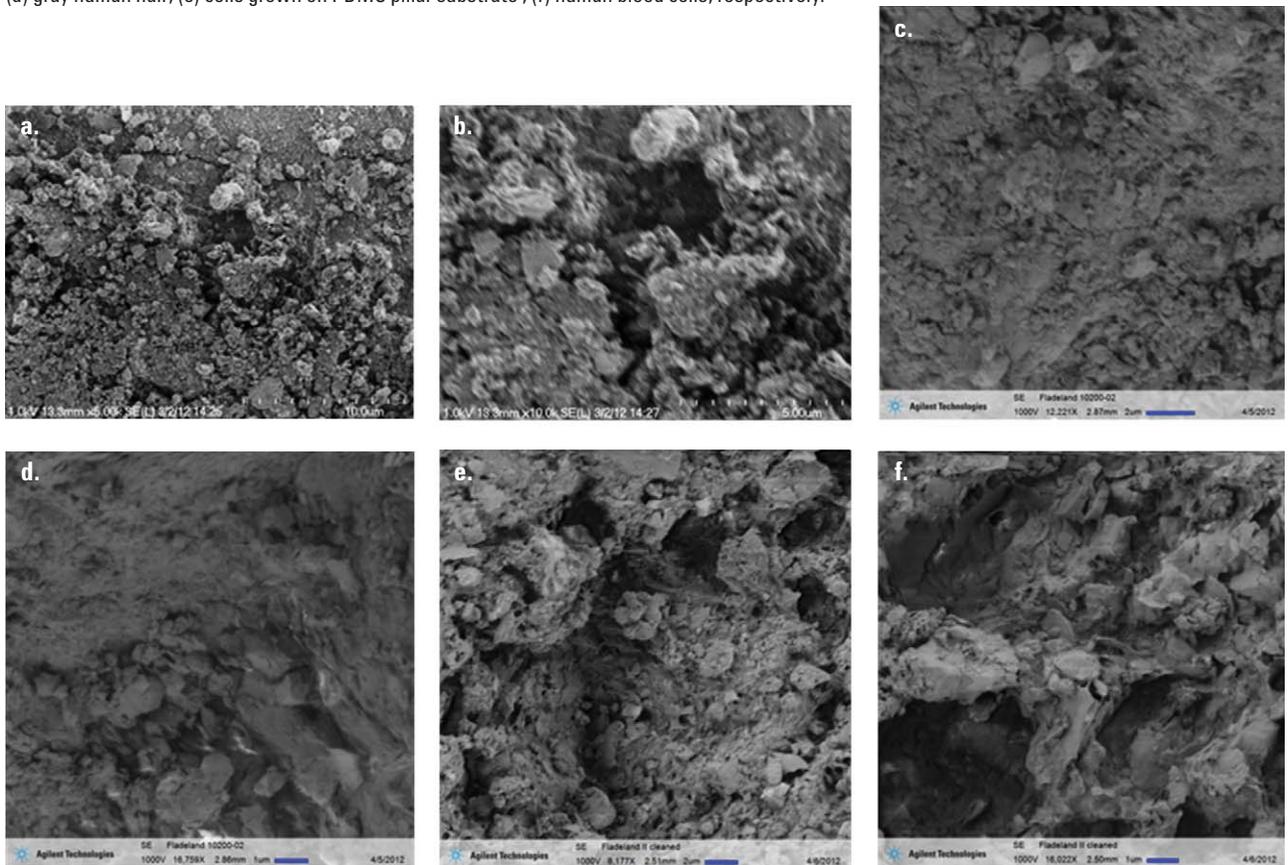


Figure 2. SE images obtained for the "as is" Fladeland rock sample II. (a and b) with a cold emission field SEM in low voltage mode; (c and d) obtained with the Agilent 8500 FE-SEM; (e and f) with Agilent 8500 for the acetone-cleaned sample.

To cope with both problems, the alternative is to use a low voltage scanning electron microscope. FE-SEM uses an accelerating voltage as low as 0.5–2 kV. This substantially minimizes the charging effect and radiation damage, while avoiding coating of the sample and maintaining a high contrast and high resolution imaging. Agilent 8500 FE-SEM in this case is a great and appropriate choice [6–7].

In this short application note, we show examples where Agilent 8500 FE-SEM provides high resolution images of these very sensitive organic and biological samples (Figure 1). The 8500 FE-SEM uses a miniature electrostatic electron beam column combined with a thermal field emission electron source. The system’s thermal field emission electron source, meanwhile, provides high brightness, high stability, small virtual source size, and low energy spread.

For comparison, we show here SE images obtained with a cold field emission high voltage SEM, which can operate also in low voltage mode. In the first case the materials investigated with both microscopes is a natural rock sample studied “as is” without any further preparation. It is evident that the images obtained with Agilent 8500 (Figures 2c and d) are superior in resolution and contrast to those obtained with the cold field emission SEM instrument (Figures 2a and b). A fast sample cleaning with acetone has dramatically improved the image resolution and contrast (Figures 2e and f).

In the second case, the material studied was a quartz sand consolidated through “biotically-precipitated” calcium carbonate (calcite) using an enzyme. Whereas the cold field emission SEM (Figure 3a) fails to resolve many morphological features

of the reaction products (such as quartz surface indentation, calcite nucleation sites, or growth features), the Agilent 8500 was able to clearly show these features (Figure 3b and Figures 4a–d). This gives strong indications to the reactions mechanisms.

Resolution in an SEM is determined by the spot size or the electron beam size, which is determined by both the quality of the lens used for focusing and the performance of the electron gun (or gun brightness). Smaller working distance (WD) in Agilent 8500 (1.9–3.5 mm) also improves resolution compared with FE-SEMs operating in low voltage mode which operate at a WD of 13–20 mm. This may explain the observed difference between these two SEM instruments.

Using Agilent 8500 FE-SEM, it is possible to image, with high resolution and excellent contrast, insulating

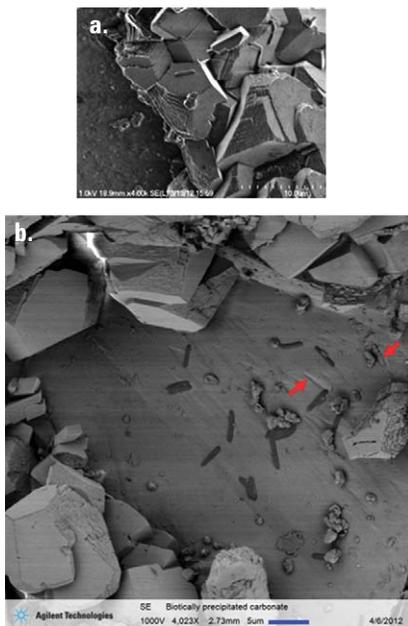


Figure 3. SE images obtained for a quartz sand consolidated with biotically-precipitated calcium carbonate. (a) SE image obtained with a cold field emission SEM in low voltage mode; (b) SE image obtained with the Agilent 8500 FE-SEM. Images are adjusted to the same area scale. Quartz surface indentation as a result of chemical reaction (red arrows) are visible in image (b), but not resolved in (a).

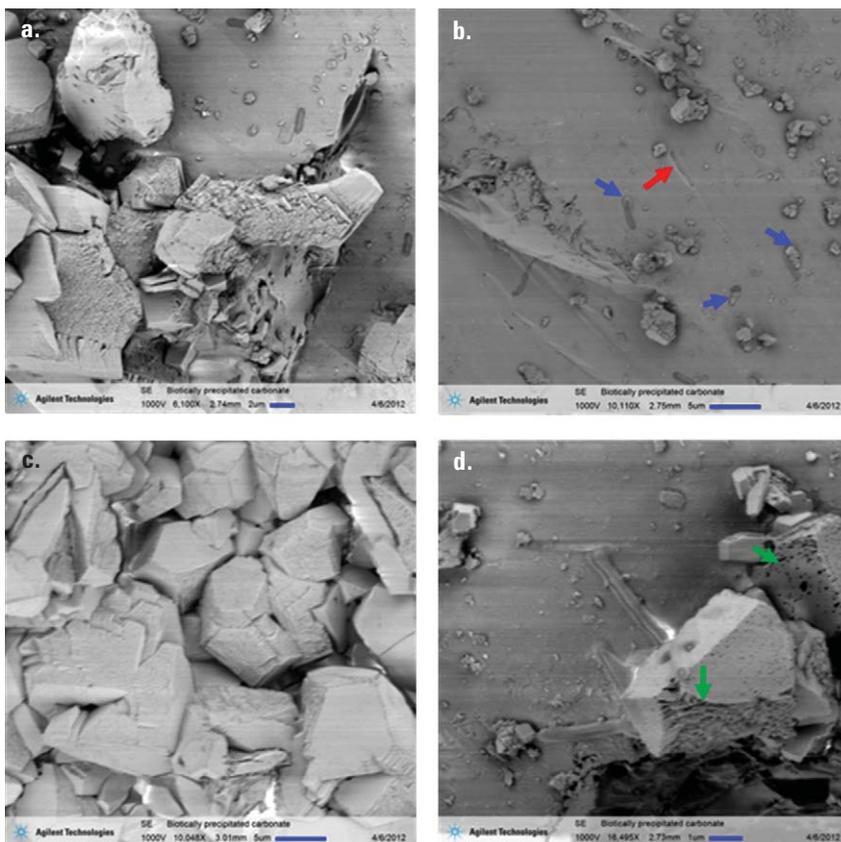


Figure 4. SE images obtained with Agilent 8500 FE-SEM for a quartz sand sediment consolidated with biotically-precipitated calcium carbonate (calcite): (a) growing calcite crystals; (b) indentation of quartz surface (red arrow) and calcite nucleation (blue arrows) where enzyme molecules adhere to quartz surface; (c) well grown and cementing calcite crystals; (d) high magnification image showing growing calcite crystals to be layered and porous (green arrows).

inorganic materials without the need for a metal coating. Furthermore, Agilent 8500 FE-SEM allows high resolution imaging of organic samples without coating or radiation damage to the samples. Otherwise, these analyses are not as feasible using other FE-SEMs: cold field emission SEM operating at

low voltage and E-SEM, respectively. It is expected also that Agilent 8500 FE-SEM would be even more helpful in studying other sensitive organic or biological samples such as cell membranes, bacteria, biofilms, and biogels, when compared to other FE-SEMs currently available.

## References

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