Optimizing 3D Electron Microscopy Results at Low kV

3View Technical Note

Over the past several years, a new generation of three-dimensional electron microscopy (3DEM) tools has been developed for the scanning electron microscope (SEM). These new techniques are capable of imaging at spatial resolutions over an order of magnitude better than optical microscopy. Serial block-face scanning electron microscopy (SBFSEM), a leading 3DEM technique, continues to push the 3D resolution limits by acquiring serial images from volumes (slices) thinner than 15 nm. Imaging uses backscattered electrons with a low kV optimized detector. Special considerations are necessary to maximize spatial resolution in all three axes: primarily, the accelerating voltage must be matched to the slice thickness. SBFSEM samples are plastic embedded biological samples, stained with heavy metals such as lead and uranium. One of the potential limitations in acquiring an SBFSEM image is sample charging, which can be compensated by imaging with low pressure of water or nitrogen gas. Charging effects and the need for compensation are greatly reduced or eliminated when imaging takes place at low accelerating voltage.

Two Monte Carlo simulations were run to determine the penetration depth of the electron beam at two different beam energies. We confirmed the Monte Carlo simulations with experimental data by overlaying a blank epoxy section on top of a block-face specimen. Figure 1A is an image acquired with an accelerating voltage of 3.0 kV; the left-side of the image is covered with a 50 nm thick section overlaid on top of the right side of the image. At 3.0 kV, electrons have enough energy to penetrate the blank resin section and generate a signal from the block-face below. Figure 1B is the same experimental setup with images acquired with a beam energy of 2.0 kV. At 2.0 kV, the electrons do not have enough energy to penetrate the 50 nm section, and therefore no signal is generated from the block-face below. Both of the attached Monte Carlo simulations confirm the electron penetration showing the backscattered electron trajectories in red.

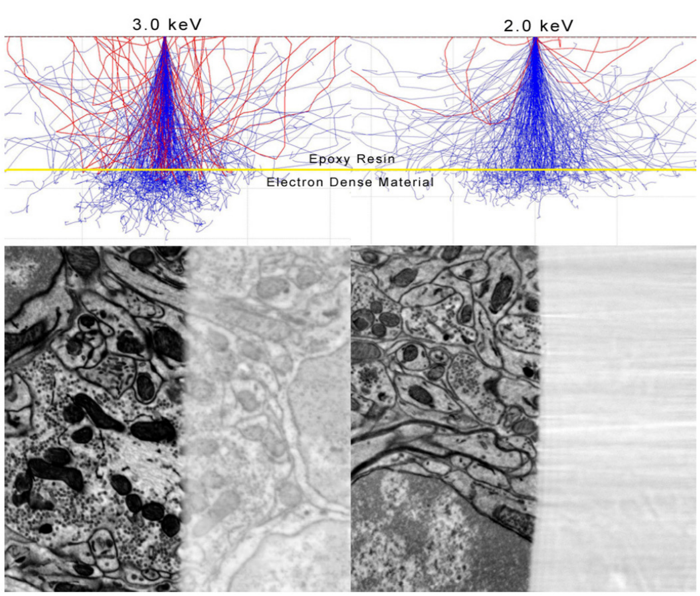

Figure 1. Monte Carlo simulations with corresponding SBFSEM images, demonstrating signal depth through a 50 nm slice of blank resin on top of a block-face.

Understanding the penetration depth is important when collecting serial images thinner than 50 nm because lower and lower accelerating voltages are required to maintain z resolution better than the slice thickness. Two additional Monte Carlo simulations for cutting 15 nm and 5 nm suggest corresponding beam energies to be 1.0 kV and 0.5 kV, respectively (Figure 2).

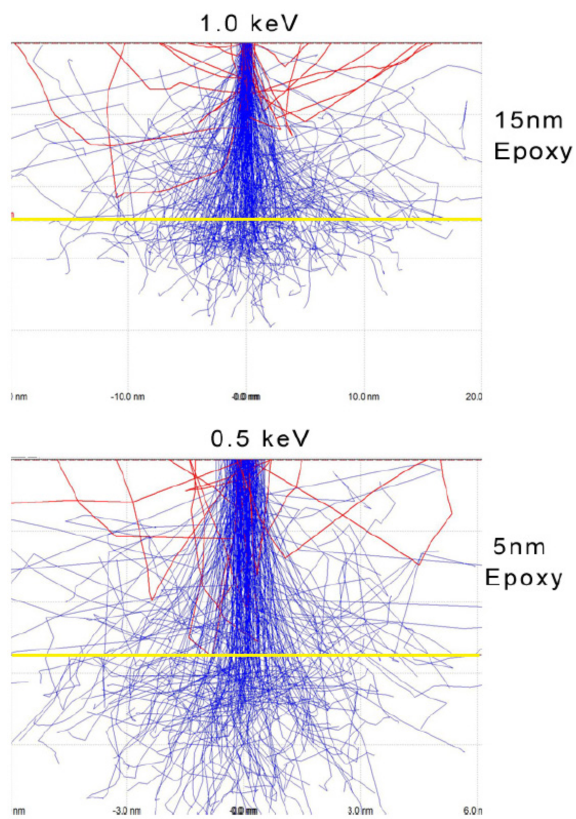

Figure 2. Monte Carlo simulations demonstrating the depth of the backscattered electron signal at 1.0 kV and 0.5 kV. The signal depth at 1.0 kV does not exceed 15 nm, while the signal depth does not exceed 5 nm at 0.5 kV.

One advantage of imaging at low accelerating voltages is that charging becomes less of a potential problem and non-existent when the beam energy is 1.5 kV and lower, as demonstrated in Figure 3. Minimizing charging allows image acquisition in high vacuum, which boosts the signal to noise ratio, producing higher-quality images in a shorter period of time. With appropriate staining, dense biological samples such as brain or liver tissue are less problematic in high vacuum even at voltages as high as 2.0 kV. Samples with large fields of open unstained resin, such as plant or cell monolayers, experience charging much more easily, and therefore, benefit from imaging at low accelerating voltages. Figure 3 is an example of imaging in high vacuum at a 1.4 kV with minimal charging artifact.

The Gatan backscattered electron detector has been optimized for low accelerating voltages and produces images with a high signal to noise, even when imaging at or below 1.0 kV. Figure 4 demonstrates the image quality with the Gatan detector at 1.0 kV and 0.9 kV.

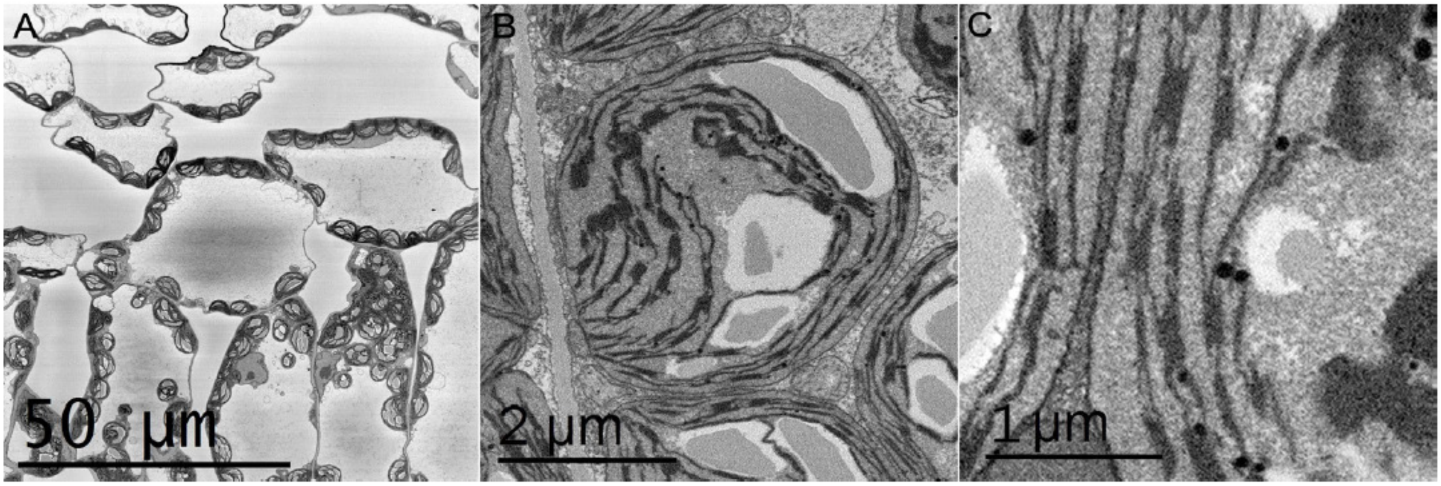

Figure 3. Arabidopsis SBFSEM images acquired with a 3View® system on a Zeiss Merlin in high vacuum at 1.4 kV. Image A was acquired with 50 nm pixels, image B with 10 nm pixels, and image C with 5 nm pixels.

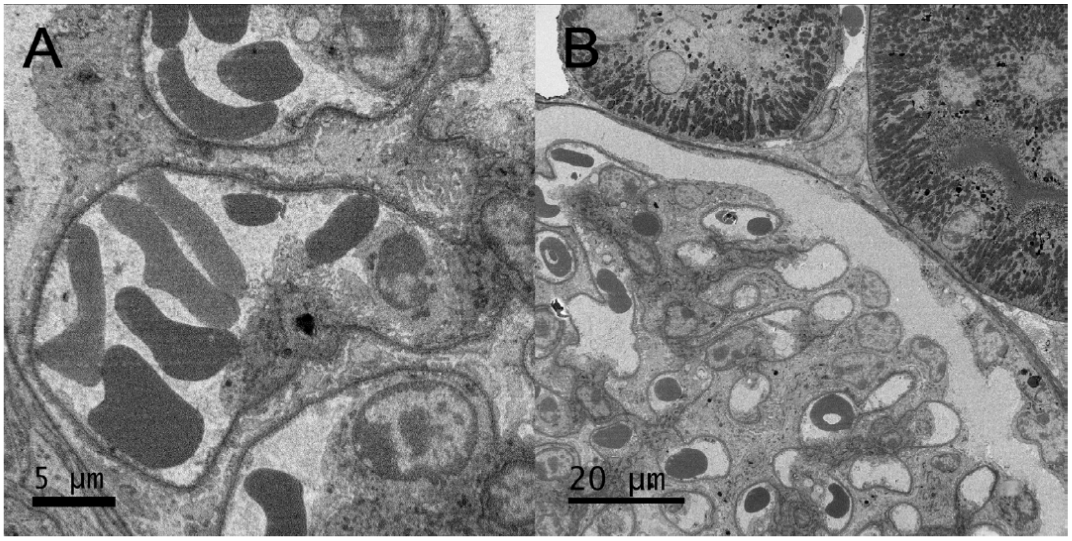

Figure 4. Mouse kidney SBFSEM images acquired with a 3View system on an SEM in high vacuum. Image A is an extract of a 4k x 4k image acquired at 900 V. Image B is an 8k x 8k image acquired with 10 nm pixels at 1.0 kV.

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