3View Application Note

An essential biological question is how the three-dimensional (3D) geometry of cellular structures is organized. Serial block-face scanning electron microscopy (SBFSEM) is an automated technique obtaining serial images using an SEM. Bridging the gap between ultra-high-resolution tomography and fluorescence microscopy, SBFSEM allows a streamlined and automated 3D data acquisition process. A microtome equipped with a diamond knife is mounted inside the SEM chamber and shaves off less than 50 nm of the sample in between imaging. Images are collected using a backscattered electron detector, which results in a classic transmission electron microscope like image. The SBFSEM imaging process is completely automated, allowing for large volume acquisition in a timely manner without the risk of losing sections. The 3View® system complements the high resolution and small volume techniques such as tomography by revealing the 3D ultrastructure of large volumes. Unlike serial sectioning, the 3View system does not require the user to master difficult techniques to produce complete data set; it does not lose sections and eliminates human errors.

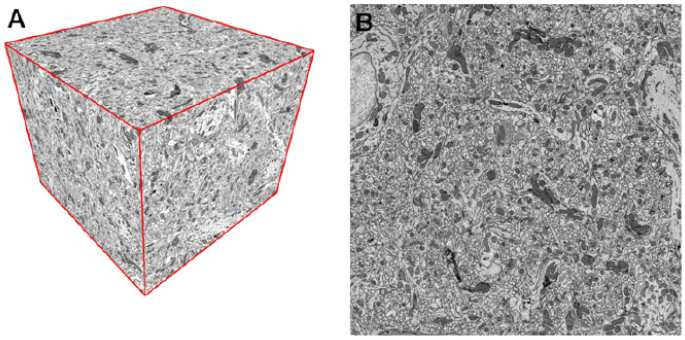

Figure 2. (A) 25 x 25 x 25 µm volumetric data set containing 500 serial images of a mouse brain obtained using the 3View system installed on an FEI Quanta 200 FEG SEM. (B) A low magnification overview from the volumetric data set in Figure 3. The original data set was 4k x 4k pixels, 500 serial images, and had a pixel size of 5.6 nm.

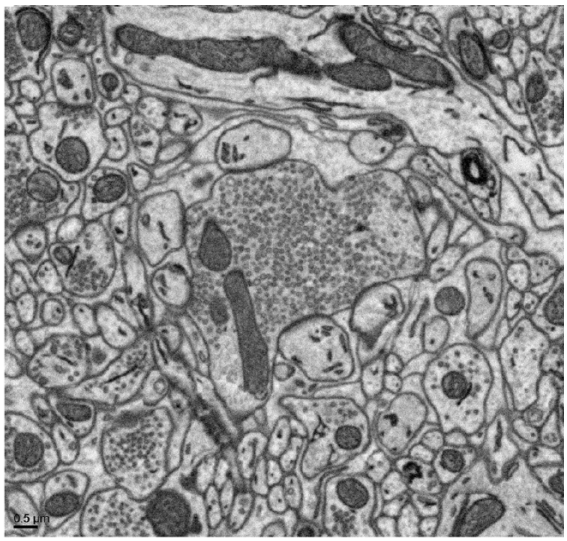

Figure 3. A high magnification image from the region indicated in Figure 2. Note synaptic vesicles, microtubules, and mitochondria cristae.

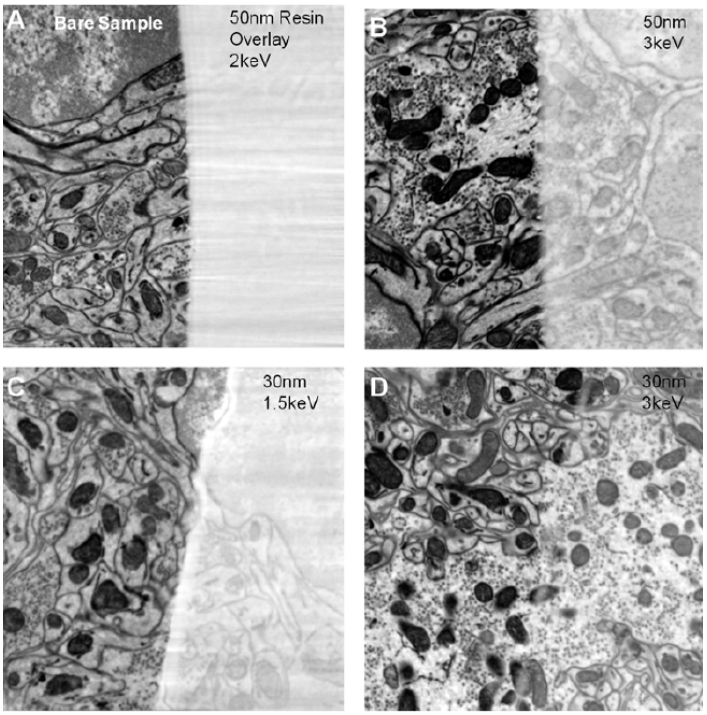

Figure 1. Selecting an appropriate accelerating voltage and slice thickness. Half of the block-face was covered with a 30 nm or 50 nm single section of Curcupan resin cut. (A) 50 nm at 2 keV, is the desired setting to avoid oversampling. (B) 50 nm at 3 keV, the accelerating voltage is too high, a signal is detected through the blank section. (C) 30 nm at 1.5 keV, a weak signal is detected through the section. (D) 30 nm at 3 keV, a strong signal is detected through the section.

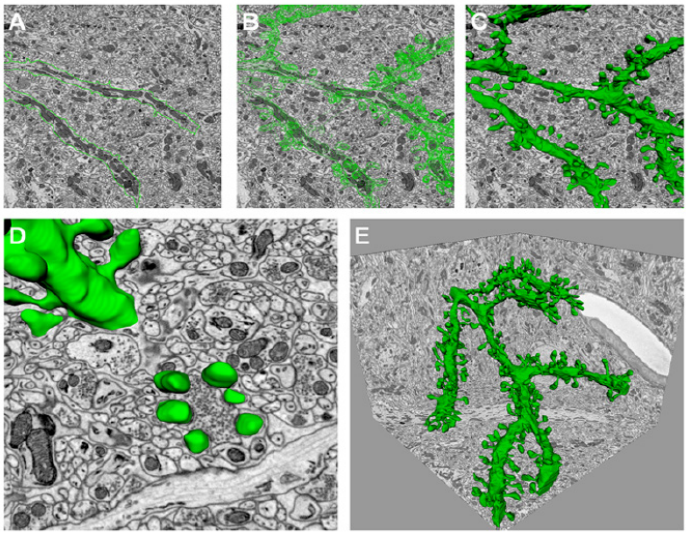

Figure 4. Segmentation. (A) Tracing out the cell of interest. (B) Combining traces from serial images. (C) Rendering wireframes into a surface model. (D) A higher resolution example. (E) Complete model of a dendrite within the dataset.

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