3View® Application Note

SEM PRODUCTS

An essential biological question is how three-dimensional geometry of cellular structures is organized. Serial block face scanning electron microscopy (SBFSEM) is a new automated technique obtaining serial images using a scanning electron microscope (SEM). Bridging the gap between ultrahigh 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 chamber of the SEM and shaves off less than 50nm of the sample in between imaging. Images are collected using a back scatter (BS) electron detector, which result in a classic TEM like image. The SBFSEM imaging process is completely automated allowing for large volume acquisition in a timely manner without the risk of losing sections.

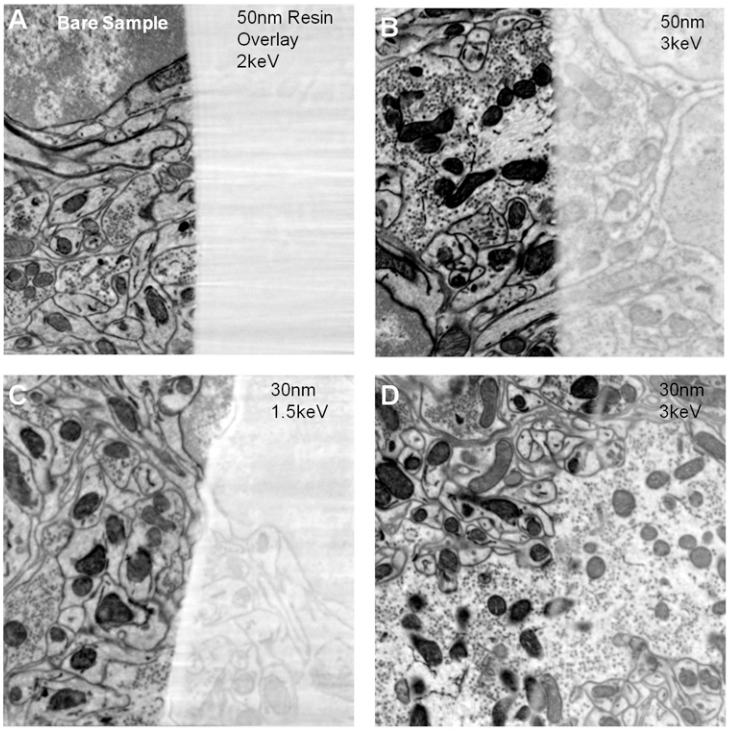


Figure 2. Selecting an appropriate accelerating voltage and slice thickness. Half of the block face was covered with a 30nm or 50nm single section of Durcupan resin cut. (A) 50nm at 2keV, is the desired setting to avoid oversampling. (B) 50nm at 3keV, the accelerating voltage is too high, a signal is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

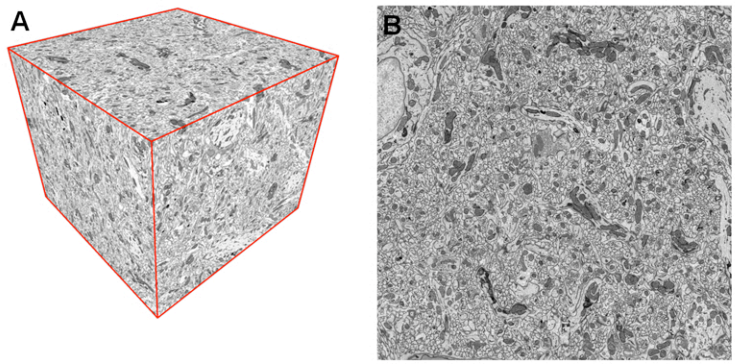


Figure 3. (A) A 25x25x25um volumetric data set containing 500 serial images of mouse brain obtained using 3View® installed on a FEI Quanta™200 FEG SEM. (B) A low magnification overview from the volumetric data set in Figure 1. The original data set was 4096x4096 pixels, 500 serial images, and had a pixel size of 5.6nm.

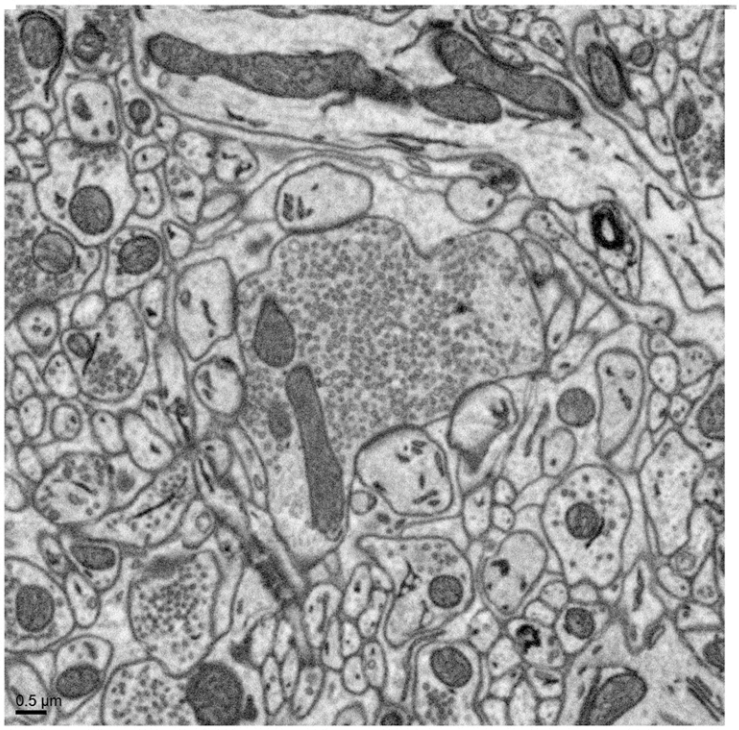


Figure 3. A high magnification image from the region indicated in Figure 2. Note synaptic vesicles, microtubules, and mitochondria cristae.

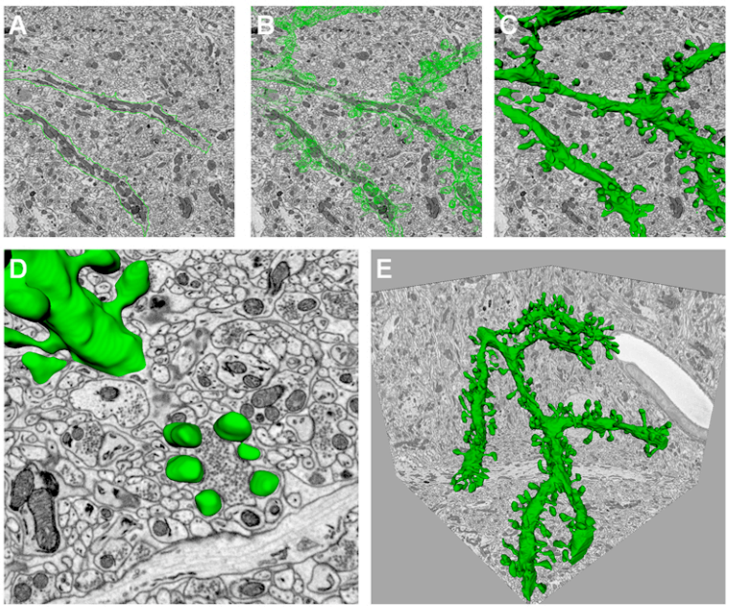
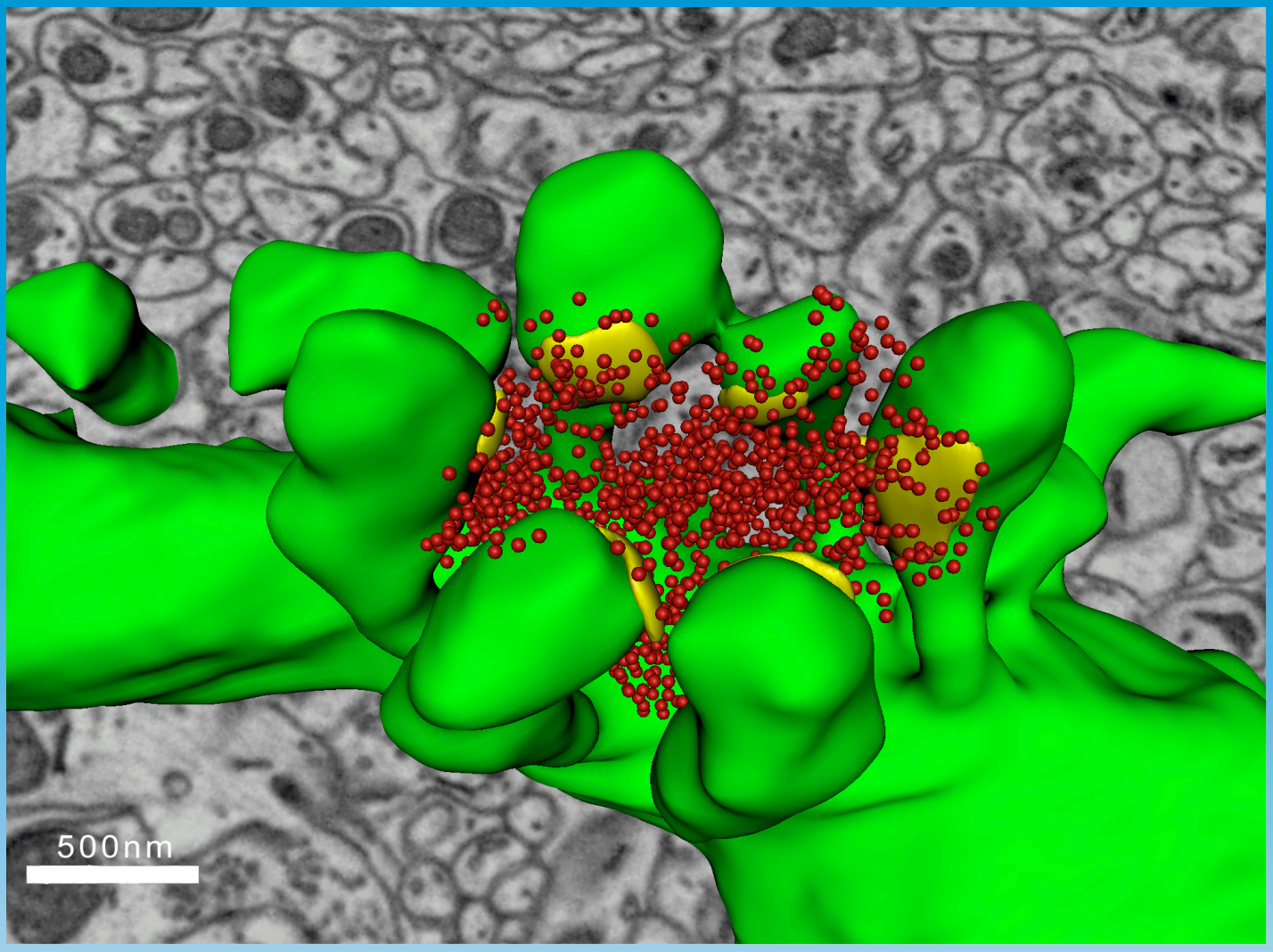
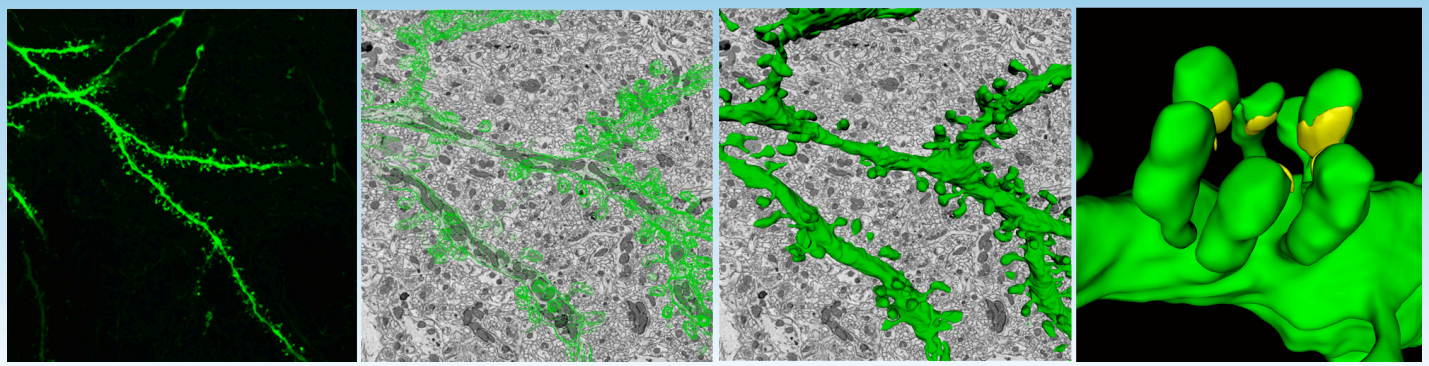


Figure 4. Segmentation. (A) Tracing out cell of interest. (B) Combining traces from serial images. (C) Rendering wireframes into a surface model. (D) A higher resolution example. (E) Complete model of a dendrite with in the data set.

Serial block face scanning electron microscopy (SBFSEM) is an automated technique of obtaining serial images using a scanning electron microscope (SEM). 3View and SBFSEM imaging is a straight forward method to collect and address biological questions where three dimensional analysis is required. 3View complements the high resolution and small volume techniques such as tomography by revealing 3D ultrastructure of large volumes. Unlike serial sectioning, 3View does not require the user to master difficult techniques to produce complete data set, 3View does not loose sections and human errors is eliminated because the process is completely automated.

Complement Confocal with Ultra Resolution





Top: A 3D reconstruction of a dendrite from a 15,625 µm³ (25 x 25 x 25 µm) volumetric data set containing 500 serial images of mouse cerebellum generated by Gatan 3View®. Dendrite structure (green), buttons (yellow), and vesicles (red). Bottom Left: Confocal image of a dendrite. Middle left: 3View® image showing wire frame traces. Middle right: Wire frame traces rendered into a volumetric model. Bottom right: Ultra resolution dendritic spine model with synapses. Sample courtesy of Tom Deerinck and Dr. Mark Ellisman, National Center for Microscopy and Imaging Research, University of California, San Diego. Serial images were segmented using Imaris to create a 3D model of a neuron of interest.

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Serial Block Face Imaging in the SEM

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