Generating 3D Models of Skin Cells Using SBFSEM
3View Application Note

The 3View® system is one of a new generation of three-dimensional electron microscopy (3DEM) systems that have been developed for the scanning electron microscope (SEM). 3View performs serial block-face scanning electron microscopy (SFBSEM) and has pushed the performance envelope of 3DEM in both the research and clinical fields. A recent study from the School of Life Sciences and Biotechnology at Korea University was published in the Journal of Electron Microscopy. The paper reported results corroborating the effects of laser treatment for melisma. The group used Gatan 3View data to search for melanosomes, as well as generate 3D models of melanocytes, results from which were displayed on the cover of the journal.

Based on groundbreaking work performed by Winfred Denk in MPI Heidelberg[1], the 3View system allows automated acquisition of 3D ultrastructure by sequentially imaging a freshly cut, resin-embedded block-face. An ultramicrotome is mounted inside the SEM, and a diamond knife is used for removing material to expose a new surface to be imaged. By removing the need to cut and collect ultrathin sections, it can turn an embedded sample into thousands of images overnight. Unlike serial section transmission electron microscopy (TEM), the acquired images are aligned at the point of acquisition. The user can view and browse the 3D image data live during the acquisition.

Melasma is a common dermalogical skin disease consisting of a dark skin discoloration that appears on sun-exposed areas of the face. J. Y. Mun et al. examined structural modifications of melanocytes after exposure to a Q-switched Nd:YAG laser using SBFSEM (Figure 1). Melanocytes are located in the bottom layer of the epidermis, and their dendrites are scattered throughout all layers of the epidermis (Figure 2).

Although the dendrites of melanocytes are too small and too deep to be resolved by 3DEM, the 3View system can slice down to a region of interest, uninhibited by structures that are too deep for confocal or two-photon microscopy. In this experiment, the 3View system collected 500 serial images with a 28 x 28 µm field of view and an isotropic voxel size of 50 nm (Figure 3). Collecting the data with isotropic voxels allowed the data to be viewed from any orientation without distortion (Figure 4).

Compared to serial section TEM throughput, the 3View system is collects high-resolution volumes previously unattainable with conventional techniques. Using 50 nm isotropic voxels, it can collect a 100 – 200 µm³ dataset in a 24 hour period. The 3View system is typically capable of resolving subcellular structures such as cristae within the mitochondria and desmosomes, but it can also cover large fields of view using the stage montage feature in the DigitalMicrograph® software.
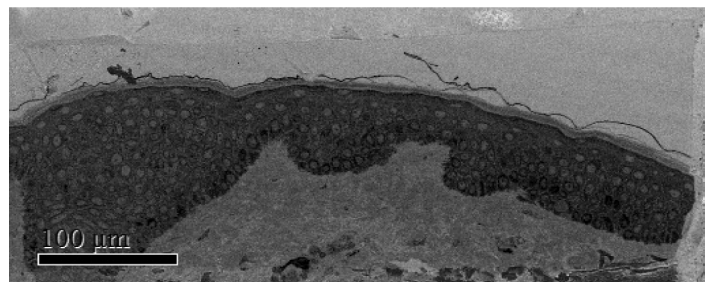

Figure 1. Low magnification SBFSEM image displaying the entire block-face sample with a field of view of 200 x 500 µm.
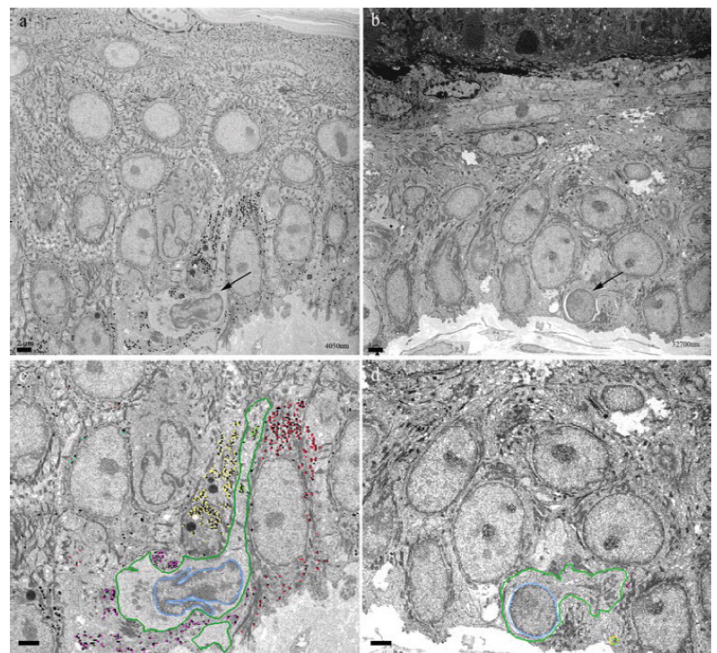

Figure 2. SBFSEM images of the epidermis layer. (A) and (C) images of pre-laser treatment. (B) and (D) images of post-laser treatment. The images (A) and (B) show one section among over 500 serial sections. Arrow in (A) and (B) represent melanocytes. In (C) and (D), dark green represents melanocytes, and light blue shows nucleus. Yellow, purple, red, and cyan dots show melanosomes. The images (C) and (D) show a decrease in the volume of melanocyte and the number of melanosomes after laser treatment. The size bar is 2 µm.
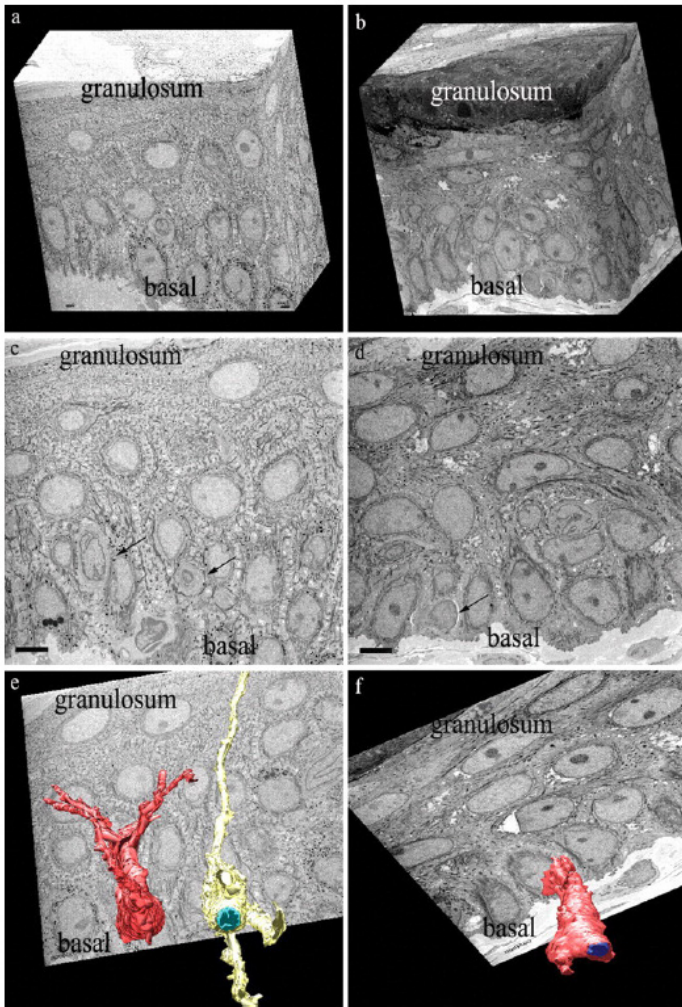
Figure 3. 3D structures of melanocytes in the epidermis layer of melasma patients. (A), (C) and (E) images of pre-laser treatment, and (B), (D), and (F) images of post-laser treatment. The 3D volume of over 500 serial images using the 3View system was visualized by IMOD (E) and (F). (C) and (D) one of the serial images for 3D reconstruction of (E) and (F). The size bar of (C) and (D) is 5 µm. The images (E) and (F) show the surface model of melanocytes showing cell body and their dendrites. Each nucleus was assigned a separate color (E, cyan blue; F, blue). Melanocytes in melasma patients have many dendrites and stretch from the basal layer to the granulosum layer (E). After laser toning treatment, the 3D structure of melanocytes contained fewer dendrites (F).

Figure 4. Raw data displayed with the 3D visualization tool in the DigitalMicrograph software. (A) A 3D visualization of the complete dataset displaying the complete volume. (B) A 3D extract of displaying a specific region of interest. (C) Is the same region of interest thresholding out the melanosomes in red and displaying ortho-slices in all three planes.

Credits

[1]3View is a product based on work performed by W. Denk and H. Horstmann, Mak-Planck Institute for Medical Research, Heidelberg, Germany. *"Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure."* Plos Biology, 2004.2(11):p. 1900-1909.

Figure 2 and Figure 3 are from the publication: Mun J. Y., Jeong S.Y., Kim J.H., Han S.S., Kim I.H. *"A low fluence Q-switched Nd:YAG laser modifies the 3D structure of melanocyte and ultrastructure of melanosome by subcellular-selective photothermolysis."* J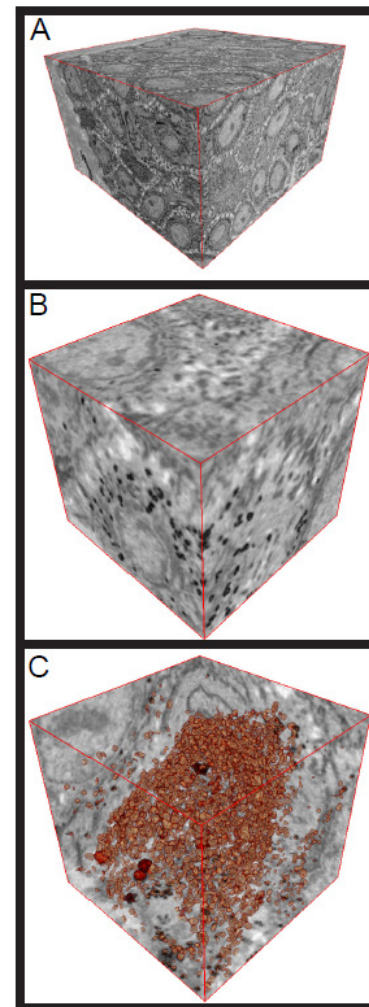 Electron Microsc (Tokyo). 2011;60(1):11-8. Epub 2010 Oct 11.