Application Example

Serial Block Face Imaging – 3D approach to cell biology

The facility of integrated Gatan 3View2XP ultra-microtome in the TESCAN microscope offers robust solution to the Serial Block Face Imaging (SBFI). We have performed the structural study of the human stem cell colonies and mouse liver tissues, resulting in their 3D reconstruction.

Introduction

The techniques designated to the 3D ultra-structural imaging are gaining importance in current bio-applications. One of the most effective is the serial block face imaging (SBFI). This method was founded on invention of the remotely operated microtome of Steve Leighton (Woods Hole, 1982)[1] and further developed primarily to biological research by Dr. Denk, MPI, Heidelberg[2].
The SBFI is automated technique based on the imaging of sample block faces that are automatically renewed by repeated cutting off of the sample surface. The cutting is done by means of the ultra-microtome positioned in the SEM chamber[2]. The process is followed by creating a three-dimensional dataset of the selected volume. The SBFI method allows easy collection of huge amount of quantitative data and overcomes the bottleneck of traditional composition of transmission electron microscopy data with extremely demanding sample preparation and demanding data mining in general[2]. The SBFI technique provides the unique insight into the cell and/or tissue architecture and enables yielding of the crucial knowledge for understanding of pato/physiological functions on all levels of an organism.

▲ **Fig. 1:** The Gatan 3View2XP ultra-microtome integrated in TESCAN MAIA3 XMU SEM.

SBFI

The 3View2XP ultra-microtome as integrated in TESCAN MAIA3 XMU SEM is depicted in the Fig. 1. This integration enables fully automatic and comfortable control of experiment setup permitting high efficiency for all operators.
The FEG SEM assure high beam stability in order of days, which is essential to collect large datasets for 3D reconstructions. The ultra-high resolution objective is suitable for subcellular details observation. The low energy detectors are in aid of a fast and low-noise imaging at low energies. The fast electrostatic beam blanker reduces sample degradation. The variable pressure option assist the charge neutralization. The collection of above mentioned features meats the high fragility of biological samples permitting their best preservation.
Integration standard offers intuitive control of the ultra-microtome in few simple steps, i.e. adjustment of the cutting speed and number of cutting cycles. Thickness of slices for rough approach can be set up to 200 nm. The working slice thickness is adjustable down to 15 nm. Multiple ROI and stage montage allows effective data capturing.
Moreover, possibility of the chamber door interchange enables to utilize full potential of SEM equipment.

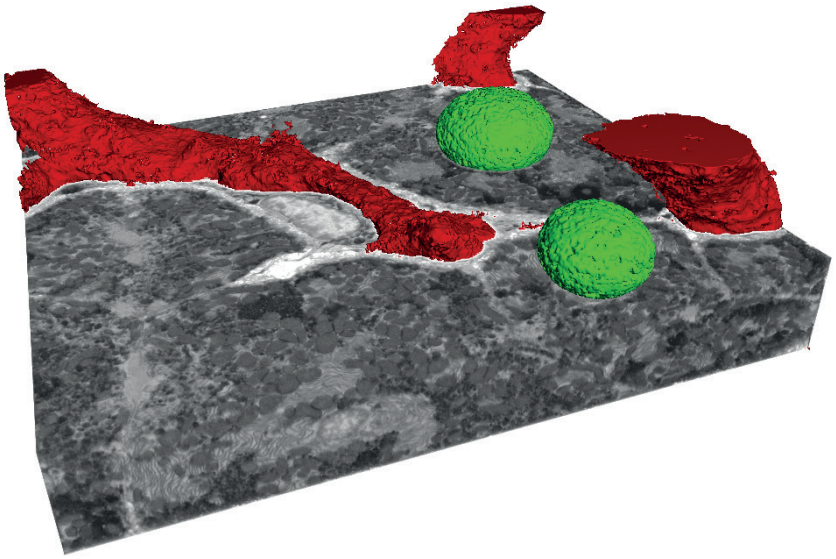

Fig. 2: Volume reconstruction of a mouse liver tissue as obtained from 500 slices, 20 nm thick, and field of view 65 µm, HiVac. The green spherical caps show the cells nuclei, the red protrusions represent sinusoids. The dataset is processed in ORS software. Sample was provided by Gatan Inc.

Experiments and Results

The sample imaging was done in the backscattered mode. Contrast of samples has crucial impact on an image quality, thus the Ellisman staining protocol adapted to enhance the contrast for SBFI has been used[3]. Volume reconstruction of a mouse liver tissue offers distinct view of both subcellular arrangement and sinusoidal blood vessels, see Fig. 2.

The Fig. 3 depicts cut through the human embryonic stem cell (hESC) colony whose reconstruction is provided for the submicroscopic analysis of cell structure, cells arrangement in colony and cell junctions. The post processing and visualization was done by means of the ORS Visual software

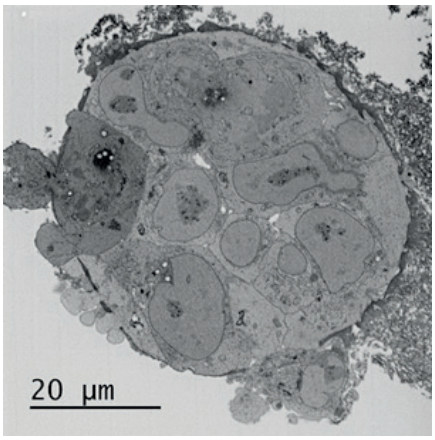

Fig. 3: The human embryonic stem cell colony acquired in BSE mode during SBFI. UniVac mode; 50 Pa, 5 kV, field of view 65 µm. Sample by courtesy of Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno[4]

Acknowledgements

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References

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