Large Volume Imaging of Eye Muscle by 3View® Serial Block Face Imaging

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Three dimensional (3D) nanometric imaging is of growing importance in many areas of biomedical research. Such resolutions are easily achieved by electron microscopes. Attempts to apply this resolution to 3D data sets have traditionally been done by serial sectioning transmission electron microscopy (TEM). Typical issues related to 3D serial sectioning TEM are: labour intensive, error prone, time consuming, registration is lost between successive images, not automated.

In this application note we describe a fully automated method to create a large 3D volume model of a mouse extraocular eye muscle. This powerful technique can also be applied to other biological structures and soft materials in a fraction of the time when compared to traditional methods.

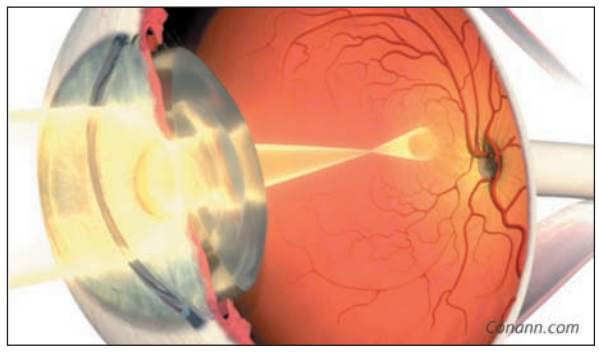

Illustration of the six extraocular muscles and the nerves that control eye movement

Biology

The six extraocular muscles provide precise and fast movement of the eye so that a target stays in view. There are abducens nerves in the muscles that control these muscles. The network of nerve cells in the muscles are of great interest because imperfections in the nerve connections can cause problems such as diplopia (double vision).

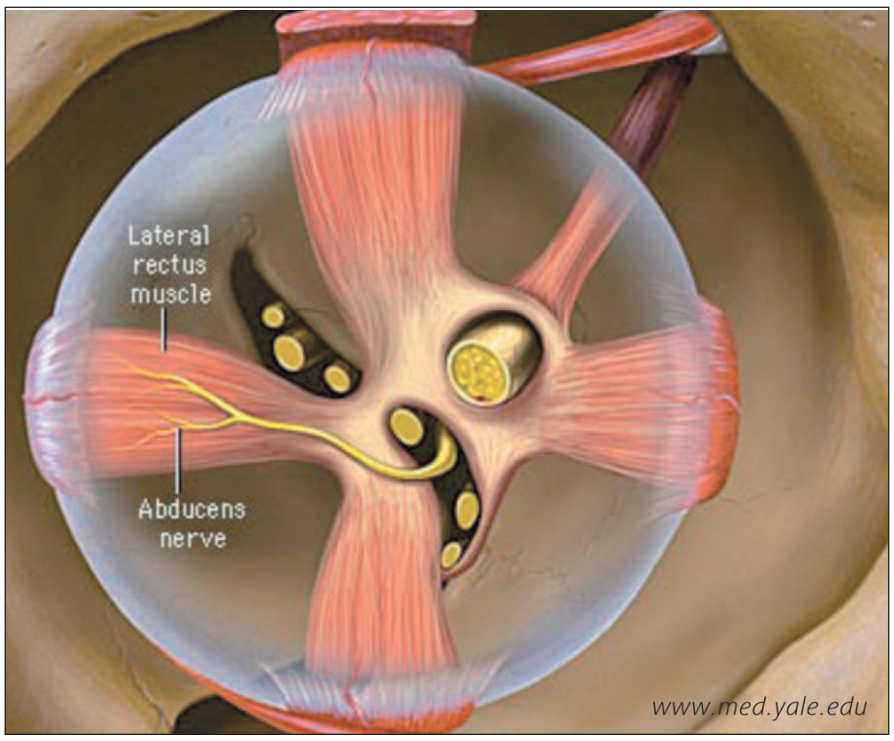

Illustration of the six extraocular muscles and the nerves that control eye movement

Instrumentation

Images were taken using the ZEISS ΣIGMATM VP field emission SEM in variable pressure mode. Serial block face imaging used the 3View® system with a low kV backscattered electron detector from Gatan Inc.

Materials and methods

Biological samples require heavy metal staining to enhance backscattered electron contrast. After staining, the sample is embedded in epoxy resin and trimmed to a square mesa ideally measuring between 0.5 to 0.8mm square and 0.8mm in height.

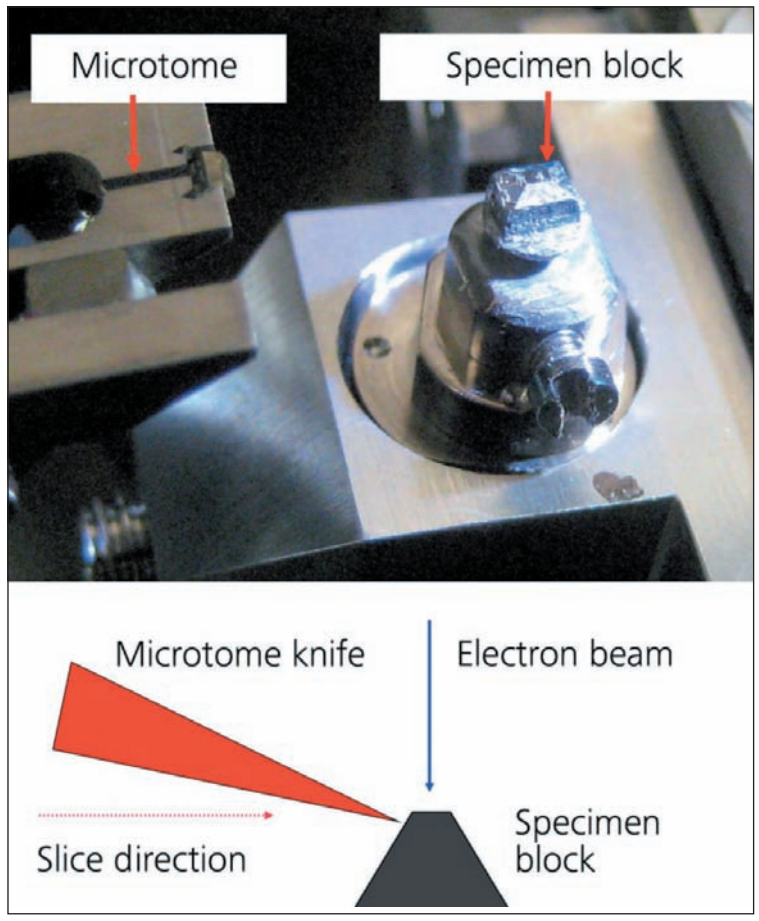

Figure 1. Image and schematic of the Gatan microtome

The ΣIGMATM VP is an advanced analytical field emission microscope that has been developed to incorporate the 3View® ultramicrotome. The technique involves the sequential removal of material from the resin block using the in-chamber microtome (15-100nm thick slices) followed by imaging of the freshly exposed block face. This process is automated and generates a stack of 1000+ images which maintain Z-registration. The stack of 2D SEM images is used to create a 3D model of the structure.

Figure 2. ΣIGMATM VP with the 3View® module

Methodology

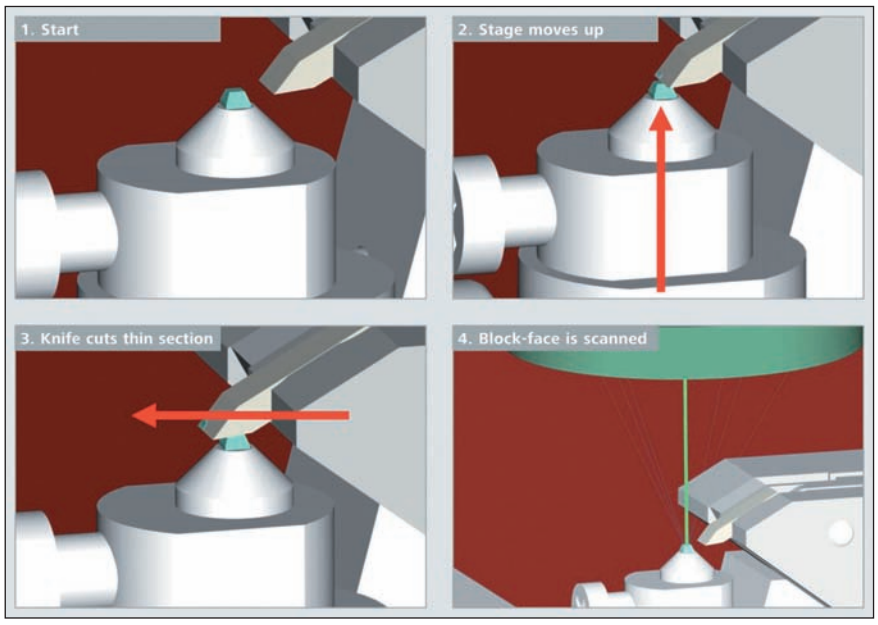

Figure 3. Schematic of serial block face imaging procedure

Serial block face imaging procedure:

1. Sample is set to the height of the knife.
2. Stage moves 15-100nm up.
3. Knife removes surface layer.
4. Fresh block face is imaged.
5. Repeat steps 2-4.

SEM configuration

Developments in FE-SEM design permit operation in variable pressure mode (VP) which allows the introduction of a small quantity of charge neutralising gas (typically 20 Pa) into the chamber. VP helps to neutralise charging as the freshly microtomed resin block face is non-conductive. The field emission GEMINI® column on the ΣIGMATM VP combines high spatial resolution at low kV with signal stability. Low kV (typically 2-4 kV) is important to match beam interaction depth to the thickness of material removed by the microtome. A special low kV backscattered electron detector, developed by Gatan, is used to optimise signal quality.

Results

Figure 4 is an example of a conventional BSD image from a mouse extraocular muscle acquired with 3View® on a ZEISS ΣIGMATM VP. The contrast is reversed to produce a 'TEM-like' image. The extraocular muscles are the large grey cells with small islands of mitochondria. The dark rings are the myelin sheath that encapsulate the peripheral nerve cells. The peripheral nerve network goes off in many directions and it is difficult to visualise the 3D network of nerves in this image.

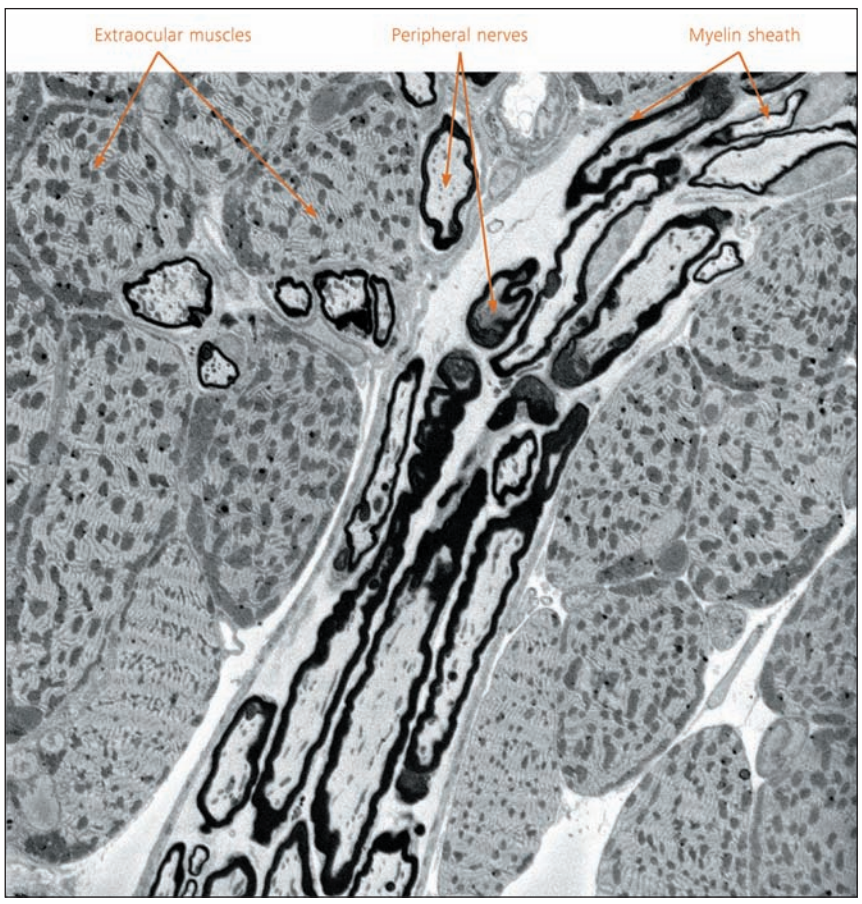

Figure 4. Conventional 2D image from the dataset used to reconstruct the 3D volume in Figure 5. 100 micron field width. Original imaging conditions were 4 keV, 20 Pa chamber pressure

Figure 5 shows 1000 conventional images stacked together to yield a 3D volume image. Any feature inside the volume can be reconstructed to display the 3D shape. The myelin sheath have been reconstructed throughout the image stack and are shown in red. The 3D nerve network, nodes and bends are clearly shown within the matrix of extraocular muscle cells.

Figure 5 dataset details:

- 100 x 100 x 100 micron 3D dataset
- 1000 slices
- Voxel size 100nm
- Microtome set to remove 100nm/slice
- Automated overnight run

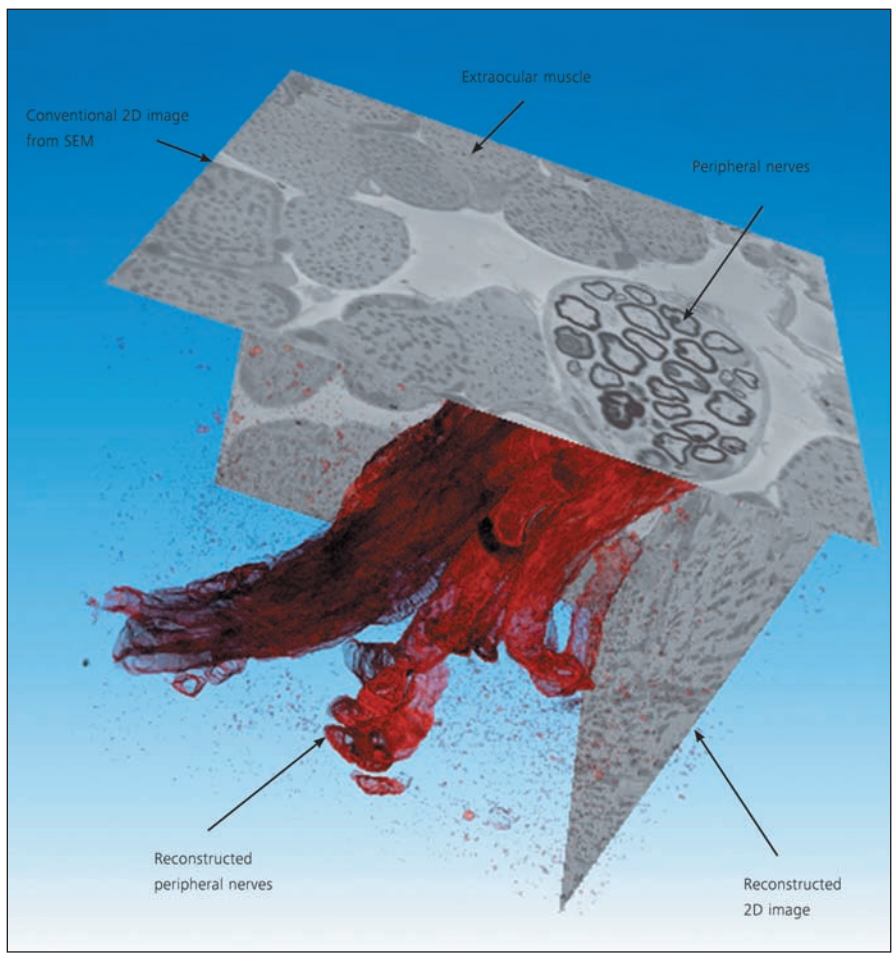

Figure 5. 100 x 100 x 100 micron reconstructed mouse extraocular muscle. Reconstructed peripheral nerves are painted red and show the intricate detail of the nerve network, nodes and bends

Conclusions

- Fully automated procedure
- Quickly collects a stack of images for 3D reconstruction
- The 3D model shows the intricate detail and network of muscle and nerve that would not be visible in 2D
- Solves traditional problem of visualising a whole cell from start to finish
- Variable pressure is vital for charge compensation of non-conducting specimens
- Excellent low kV performance is required to obtain high resolution images
- 3D information from the high resolution that the ΣIGMATM VP collects is complementary to the 2D images from a transmission electron microscope at higher magnifications

Application area:

- 3 Dimensional
- Large volume imaging
- Soft material 3D imaging

Recommended instrument type:

- ΣIGMATM VP or MERLIN®
- Variable pressure recommended
- 3View® package from Gatan Inc.

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The Institute of Ophthalmology aims to further the understanding of the processes of vision and to develop new diagnostic and therapeutic strategies for the benefit of patients worldwide.