HREM: High-Resolution Episcopic Microscopy

High-Resolution Episcopic Microscopy (HREM) is a simple method for creating digital volume data and three-dimensional (3D) computer models of organic material. It works with translucent and opaque, native and whole-mount LacZ stained embryos of biomedical model organisms as well as with biopsy material harvested from animals and humans [1-3].

Generation of HREM Data

Materials are processed as for traditional histology, with the exception that eosin is added to the higher ethanols and that resin (JB-4, Polysciences) dyed with eosin (0.4 g/100 ml) is used as embedding medium.

The resin blocks are mounted on a motorized microtome. The microtome has to be modified to allow the block holder to reproducibly come to rest at a "photoposition" after each cut. E.g., the apparatus we operate in Vienna has its photoposition at the upper stopping point of the block holder excursion of a rotary microtome (Microtec CUT 4060E, microTec Laborgeräte GmbH). It is reproducibly reached with an accuracy of approximately +/- 1 µm.

The resin blocks are sectioned in steps of 1 to 5 µm. After each section a digital image of the surface of the block is captured with a digital video camera sitting on the phototube of a magnification optic. The optic is aligned with the photoposition and has a YFP (excitation 500/20 emission 535/30) or red/green/blue triple band filter (excitation 404/20, 494/20, 576/20; emission 457/20, 530/20, 628/28) in its optical path. We operate cameras and optics from Leica, but in principle any type of camera and optics can be used.

Sectioning and image capturing is automated and synchronized by specially adapted software modules (e.g. VisView 2.1.4, Visitron Systems GmbH). The physical resin sections are either discarded or, if thicker than 2 µm, collected for later histological analysis.

Characteristic of HREM Data

By using this simple set up (fig. 1a) series of typically 1 000 to 3 000 single digital section images with pixel sizes of 0.5 x 0.5 µm$^2$ to 3 x 3 µm$^2$ are produced. In the
images the tissues appear similarly contrasted as in images captured from histological sections stained with eosin/hematoxilin (fig 1b) and - optionally - LacZ.

But there are some fundamental differences between images captured from histological sections and volume data derived from histological serial sections on the one hand and HREM images and volume data on the other hand.

Firstly, histological sections and digital images captured from those show non-affine distortions introduced by the sectioning, section processing and section mounting processes.

HREM images do not show such artefacts [4].

Secondly, a series of a few thousand HREM images is inherently aligned and is produced within a few hours in an automated way. In contrast it takes weeks and a lot of user interactions to produce a series of more or less precisely re-aligned digital images from a few thousand histological sections.

Thirdly, the distance between HREM sections can be routinely as low as 1 µm. Histological sections routinely have a thickness of 5 to 7 µm. Therefore the resolution (voxel size) of volume data produced from histological section series is much lower than that of HREM data [5]. E.g. given a pixel size of $1 \times 1 \, \mu\text{m}^2$ in single digital images, the voxel size of HREM data can be as low as $1 \, \mu\text{m}^3$, while it is 5 to 7 $\mu\text{m}^3$ in volume data derived from histological sections.

**Visualization of HREM Data**

HREM volume data can be immediately analyzed with orthogonal and oblique virtual resectioning tools. LacZ labelled tissues and tissues with high eosin uptake can be quickly and effectively visualized by using volume rendering algorithms (fig. 2, 3). Tissues with lower contrasts require the time demanding generation of binary data and surface rendered 3D models (fig. 3).

**Applications of HREM**

HREM is a relatively "young" 3D imaging method, which is still under construction. Nevertheless it has proofed to be an excellent tool in a broad variety of research fields. For once it has proofed its usefulness and superiority to alternative imaging methods in a number of studies involving 3D visualization of embryos of biomedical model organisms and their organs and structures [2, 6-13]. Hence, it was chosen as the prime visualization method for screening the phenotype of prenatally lethal E14.5 mouse embryos produced in the Deciphering the Mechanisms of
Developmental Disorders (DMDD) project [14] - a project, which is embedded in the International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org), which in turn is part of the International Mouse Knockout Consortium (IMKC, www.mousephenotype.org).

In addition to the increasing use of HREM for visualizing embryos, it was very recently also employed for visualizing tissue samples of adult biomedical model organisms [15, 16], human skin biopsies [17] and biological materials used in modern medicine [16]. The highly encouraging results lead us to the conclusion that HREM has the potential to become a valuable tool in various fields of clinical research within the next decade.

References

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