Measurement of capillary length from 3D microscopic image data by methods of image analysis and stereology

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Studies of the microvascular bed, i.e. an interconnected net of thin capillaries, characterized by the capillary length or length density, is of interest in many biomedical studies. However, a proper measurement of length is a difficult task as its unbiased estimation from practically two-dimensional thin histological sections requires cutting such physical sections in randomized direction, which is often technically demanding, inefficient and sometimes impossible. However, if three-dimensional (3D) image data of the studied microscopic structure are available, as it is in the case of confocal microscopy or transmission electron tomography, methods for length estimation that do not require randomized cutting of physical sections can be applied. In our study we focused on such methods, which appear to be suitable for the evaluation of capillaries in different organs.

We tested several methods for the length estimation on two specific examples of capillary network: 1. Capillaries in two different rat brain regions, forming a spatial net of tubules, whether more dense, as it was in the case of cortex, or less dense, as in corpus callosum region. 2. Capillary bed of individual rat skeletal muscle fibres forming an anisotropic net od tubules, with axis of anisotropy going parallel to the axis of the muscle fibre. In both examples we used 3D images of capillary network acquired by a confocal microscope.

We compared three types of methods: 1. Stereological methods for the length estimation based on computer generation of isotropic uniform random virtual test probes in 3D, either in the form of spatial grids of virtual "slicer" planes (Fig. 1a) using global spatial sampling [1,2], or sphere probes [3] (Fig. 1b). 2. Automatic method, exploiting a digital version of the Crofton relations using the Euler characteristic of planar sections of the binary image in three perpendicular principal directions [4,5]. A suitable 3D image pre-processing for compensation of inhomogeneities in image contrast, removal of image noise and enhancement of the relevant features by filtering, followed by segmentation of capillaries was applied before using this method (Fig. 2a). 3. Interactive "tracer" method for the length measurement based on manual delineation of axes of capillary segments in 3D using visualization of the confocal image stack in three orthogonal sections so that each capillary segment was represented by a chain of line segments in 3D [6] (Fig. 2b). We implemented all algorithms and methods as special plug-in modules in *Ellipse* (ViDiTo, Slovakia) software environment.

The presented methods were compared from the point of view of their practical applicability, efficiency and precision, with respect to the arrangement of the capillary

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network under study, taking into account different types and arrangements of spatial grids in interactive stereological methods, as well as sources of possible errors due to technical processing of specimens, noise in acquired images, aberrations in microscope system applied, and biased sampling during the relevant biological experiment.

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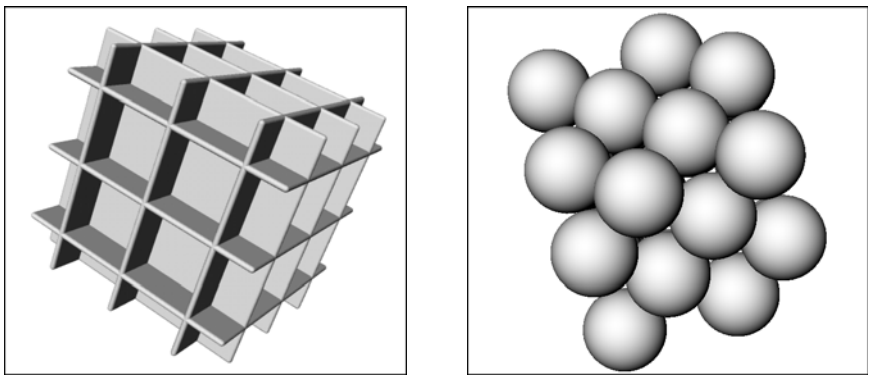
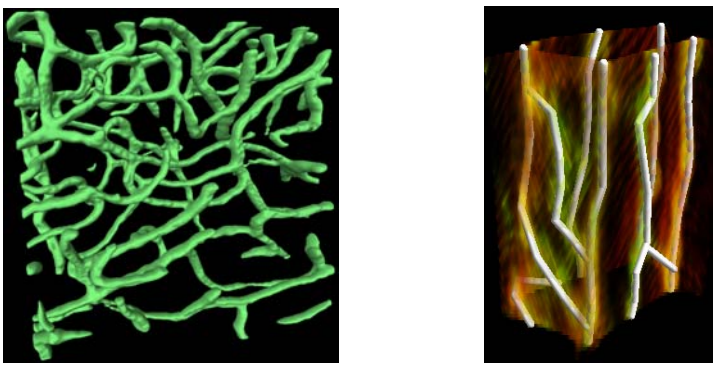


(a) (b)

Figure 1. Spatial grids of test probes in 3D used for length estimation. (a) Orthogonal triplet of slicer probes. (b) Spherical spatial grid.



(a) (b)

Figure 2. Two types of capillary network used for testing methods for length estimation. (a) Surface rendering of capillaries in the rat brain cortex after image pre-processing by Gaussian and Lipschitz top-hat filters and segmentation by thresholding. (b) Volume rendering of a stack of confocal microscopy images of rat skeletal muscle fibre with adjacent capilaries delineated by tracer method.