

# MEASUREMENT OF SURFACE AREA OF MICROSCOPIC STRUCTURES USING IMAGE ANALYSIS AND STEREOLOGY

Lucie Kubínová, Jiří Janáček

Department of Biomathematics, Institute of Physiology, v.v.i., Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-142 20, Praha, Czech Republic

E-mail: [kubinova@biomed.cas.cz](mailto:kubinova@biomed.cas.cz)

**KEY WORDS:** 3D image analysis, confocal microscopy, surface area, stereology

Proper measurement of surface area from practically two-dimensional (2D) thin histological or ultrathin sections is a difficult task as its unbiased estimation requires randomized direction of cutting such sections, which is often technically demanding, inefficient and sometimes impossible. This problem can be solved by applying methods for surface area measurement based on evaluation of three-dimensional (3D) image data, acquired, e.g. by confocal microscopy or transmission electron tomography.

We developed software implementation of a number of methods for surface area estimation based on evaluation of 3D image data, namely interactive stereological methods and automatic or semi-automatic digital methods [1]. All methods were implemented in special plug-in modules of *Ellipse* SW environment (ViDiTo, Košice, Slovakia). The stereological methods were based on using computer-generated, properly randomized virtual linear test probes in the form of spatial grids of “fakir” straight lines or cycloids [2], and interactive marking of intersections of these test probes and surface of microscopic structures under study. We studied variances of the relevant surface area estimators using several arrangements of spatial grids of lines. Further, we implemented the automatic digital methods, based on Crofton formula or surface triangulation which were applied to automatically or semi-automatically segmented images. We checked the sensitivity of these methods to image pre-processing, which is usually necessary for successful segmentation of structures under study.

We evaluated the usability, time efficiency and precision of above methods for surface area estimation on microscopic structures of different shapes and arrangements, such as muscle fibres, plant cells, or chloroplast thylakoid membranes. We conclude that there is no absolutely universal method which would be optimal for all types of structures. Automatic methods are faster than interactive methods but require automatic segmentation of analyzed objects and they are sensitive to resolution and processing of microscopic image data, e.g. to smoothing which is used for reduction of noise in acquired images. Therefore, they require careful testing and adjusting to the given type of microscopic structure.

This study was supported by the Academy of Sciences of the Czech Republic (grants A100110502, A600110507, and AV0Z 50110509), by the Ministry of Education, Youth, and Sports of the Czech Republic (grant No. LC06063), and by the Grant Agency of the Czech Republic (grant No. 304/09/0733).

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