EVALUATION OF GEOMETRY OF THREE-DIMENSIONAL OBJECTS RENDERED FROM SERIAL SECTIONS AND IBM PC IMPLEMENTATION OF APPROPRIATE STEREOLOGICAL METHODS

I. KREKULE, L. KUBÍNOVÁ, P. KAREN Institute of Physiology, Academy of Sciences of the Czech Republic, Prague

> Received September 16, 1996 Accepted November 13, 1996

Abstract

Krekule I., L. Kubínová, P. Karen: Evaluation of Geometry of Three-dimensional Objects Rendered from Serial Sections and IBM PC Implementation of Appropriate Stereological Methods. Acta vet. Brno 1996, 65:247-252.

Analytical and stereological methods for evaluation of main geometrical characteristics of 3D objects rendered from serial sections are introduced. Design-based stereological methods for volume and surface area estimation of the above objects are discussed in detail. Examples of practical implementations of these methods in a semi-automatic regime on an IBM PC with and without a frame grabber and a proprietary software are shown.

Geometrical characteristics, volume rendering, surface rendering, stereology, surface area

The main body of medical imaging is represented by 3D rendering of objects from serial sections and their visualization nowadays. These objects (i.e. organs and their constituents) and their serial sections are obtained predominantly by computed tomography of the tissue projections by using various physical modalities (e.g. X-ray, SPECT, NMR, PET, ultrasound etc) and by confocal microscopy (Petráň et al. 1968). Regardless of the quality of the resulting visualization of the objects, including all contemporary options of the interactive manipulation with display of rendered objects, the information thus conveyed represents only a qualitative, though important and unique description of the object under study. However, a rigorous evaluation of the progress of the treatment or pathology requires quantitative information, e.g. geometrical characteristics of the object under study like its volume, surface area, length, width, minimal circumscribed sphere or rotational ellipsoid, coordinates of its center of gravity, moments of inertia etc. Attention should be also paid to topological characteristics and description of mutual spatial arrangement of constituents of the object, e.g. arrangement of neurons and blood vessels etc. Taking into account that precise diagnostic and analytic methods require exact evaluation of the geometry of the objects under study, we are discussing methods for evaluation of geometrical characteristics of objects rendered from serial sections.

Analytical and stereological evaluation of geometry of rendered objects

Analytical methods for evaluation of geometrical characteristics are based on measurements made on the rendered objects, while the modern, design-based stereological methods operate, under strictly defined conditions, with single or serial slices without the necessity to render the object. Stereological methods yield unbiased estimates of geometrical characteristics in general which is not the case of analytical methods. Moreover, rendering represents an estimation of the object from its samples, i.e. serial slices; thus differences, i.e. an error between the original and the rendered object can occur. It should be also taken into account that rendering is a computing intensive procedure, which requires either time or powerful computer hardware (HW). The more precise rendering requires in general more dense sampling, i.e. higher number of slices, while the requirements of



Fig.1. Cavalieri's principle for estimation of the volume of an object from its sections (see text): a) series of sections with a constant intersection distance h: b) example of estimation of the area of a section Si by using stereological point counting method: Area (Si) = M(i).A, where M(i) stays for a number of points of the measuring raster which are inside the section Si (i.e. M(i) = 10 in the shown case); A equals to the area represented by a single point of the measuring raster (e.g. 100 m m/point).

stereology can be often satisfied by evaluation only a few sections. Stereological methods based on mathematical statistics and stochastic geometry (Weibel 1979, 1980; Gundersen et al. 1988a, 1988b; Cruz-Orive and Weibel 1990) are cheaper, faster. simpler. though thev provide just estimates. not measurements, of the required characteristics. For the sake of simplicity we shall compare only evaluation of the volume and surface area of the object by using both discussed approaches.

Both volume and surface area of the object can be estimated by approximating it by suitable analytical model of the object under study, e.g. by a sphere, cylinder etc., and by calculating the corresponding volume and surface area. However. this model-based approach usually leads to biased estimates, since such simple approximation of biological objects is rarely precise enough.

A simple analytical evaluation of the 3D object volume represents sum of all voxels (i.e. volume elements) forming the rendered object. Results are the better the smaller the voxels are, though a certain bias occur due to the part

of voxels on the surface of the object which are not fully assigned to the object. In a similar way but even with a higher bias the surface area of the object can be analytically determined as the sum of the exposed surfaces of all surface voxels (in case of volume rendering). Similarly, the surface area can be determined as the sum of triangles (or polygons) by which the object profile contours in serial sections are connected (e.g. Mercer and Crapo 1988) (in case of surface rendering). Software packages for 3D object rendering and visualization usually offer counting the total number of exposed faces of all surface voxels of the volume-rendered object as the method for surface area determination. Another procedure for evaluation of the surface area is based on recording the values of the indicator function of all neighbours of voxels which form the object surface (Guilak 1994). Another recently proposed method is based on evaluation and erosion of the volume-rendered object by a minimal (elementary) structuring element. The number of voxels representing the shell



Fig. 2. Schematics of the a) spatial grid and b) an example of an image of a tissue section with the spatial grid superimposed.

is related to the surface area of the rendered object (Meyer 1992).

Both the volume and the surface area can be well estimated directly from serial sections by using stereology. The Cavalieri's principle (known from 17th century, see e.g. Michel and Cruz-Orive 1988) is the most appropriate stereological method for volume estimation in this case: volume of the object is estimated as the sum of volumes of elementary general cylinders into which the object is partitioned by sections, i.e. as the sum of the multiples of the area of individual sections and particular inter-section distance, (i.e. the sum of area of all sections multiplied by the inter-section distance), provided the first slice was chosen in a uniform random (UR) manner. The area of the individual sections can be estimated by the number of pixels (i.e. picture elements) it contains or the point counting stereological method can be advantageously applied. This method is based on counting test points lying within the object profiles. The point raster should be superimposed in UR manner on the picture of the given section (Fig. 1). The described stereological procedure is very simple when compared

with analytical methods requiring the object to be rendered first of all. Moreover, it can be accomplished in an interactive regime which can be advantageous when considering the segmentation (thresholding) otherwise necessary for the surface rendering.

Stereology offers a couple of methods for estimation of the surface area of an object out of which the spatial grid (S and au 1987) will be discussed because it is suitable if serial sections are available. It is based on application of a regular spatial grid positioned in an isotropic uniform random (IUR) manner with respect to the object under study. Intersections of lines connecting nodes of the grid with the surface of the object are counted. The intersections are evaluated by comparing profiles of consecutive slices with the superimposed raster representing projection of the spatial grid (Fig. 2). Another stereological method for the surface area estimation using series of perfectly registered sections is based on evaluation of intersections of the surface with spatial cycloids, i.e. the method of vertical spatial grid (Cruz-Orive and Howard 1995). It has higher demands on the grid design when compared with the described spatial grid and thus remains predominantly in the realm of the research until now.

Semi-automatic computer supported stereology

In essence, stereological measurements require study of interactions (intersections) of a measuring system, i.e. raster with the image of the section or projection of the studied



Fig. 3. a) Schematics of a system implementing semi-automatic stereology. It is based on a TV camera and IBM PC enhanced by a frame grabber (FG) with a graphical overlay (GO) into which the measuring raster is loaded by a PC software (e.g. STESYS - see text); b) an example of measuring raster (cycloids for surface area measurement in vertical sections) generated by the system STESYS.

object. Therefore, generation of a suitable raster-measuring system which can be superimposed on the chosen picture of the object are main prerequisites of stereological measurements. These measurements can be accomplished even by a naked eye, which is advantageous whenever an automatic delineation (segmentation) of the object is difficult for whatever reason. The generation of the measuring system can be accomplished by most computer graphics editors. The superimposition of the raster on the picture of the object studied can be easily done on the screen of the computer display. The picture of the object under study is either stored in a computer memory or is captured in the real-time by using a TV camera with a standard video signal output. This output is usually not compatible with the input of the computer display. The frame grabber, i.e. a plug-in card of the IBM PC was designed to store a TV picture (i.e. frame) in the real-time, to process (enhance) it and to provide output TV video signal at the same time. It also generates graphical overlay and mixes pictures from various sources, i.e. provides the necessary superimposition of the measuring raster on the picture of the studied objects. The interactions of the measuring raster with the studied object picture are analyzed either interactively (semi-automatically), i.e. by the naked eye, or automatically. In both cases the evaluation of the interaction can be processed (formula solved) by the computer. Systems for stereological, computer supported measurements in semi-automatic regime are available commercially, e.g. CAST- grid (Olympus DK A/S); Digital Stereology (Kinetic Imaging); Stereology Toolbox



Fig. 4. Schematics of a system implementing semi-automatic stereology based on a TV camera and IBM PC enhanced by a transcoder (TT) which transforms the signal of the VGA display controler into TV video signal, thus the measuring raster can be developed by using an elementary display based computer graphics. The TV camera is synchronized by the transcoder.

(Morphometrix) as well as developed by a number of laboratories, e.g. STESYS (Karen et al. - in preparation). The main parts of these system, represent raster generation, object picture display and handling procedures. Images of the sections of the analyzed object can be supplied by other instruments (sources) like a confocal microscope or computer tomography systems.

Schematics of an IBM PC based system with a TV camera and frame grabber which implements stereological procedures in a semi-automatic regime is shown in Fig. 3. A more simple system in which the expensive frame grabber is replaced by a simple video transcoding plug-in board (Fig. 4) known from multimedia applications was already published elsewhere (Krekule et al. 1995). It allows to apply basic computer graphic tools of the computer system to a raster generation under the direct display control, while its price is significantly lower when compared with systems based on frame grabber.

Conclusion

Contemporary stereology provides methods for unbiased estimation of a number of geometrical characteristics of an object rendered from its serial sections. Main advantages of stereological applications consist in: i/ avoiding the otherwise necessary rendering of the object to obtain its geometrical characteristics, ii/ avoiding an automatic object segmentation as a prerequisite for automatic rendering, iii/ simple implementation, especially in a computer-assisted, semi- automatic regime. This is also the reason why some commercially available software packages for medical imaging already include stereological procedures, e.g. Analyze (Robb and Hanson 1991).

The contemporary interest of stereology represents description of the spatial arrangements of constituents of the object under study by using second-order characteristics and models of point, fiber and particle processes and dispersion, distance and polygonal methods.

Určení geometrických charakteristik 3D objektů ztvárněných ze sériových řezů a implementace odpovídajících stereologických metod na IBM PC

Úvodem jsou shrnuty analytické i stereologické metody pro určení geometrických charakteristik trojrozměrných objektů, ztvárněných ze seriových řezů. Podrobně jsou pak diskutovány nestranné stereologické metody pro odhad těchto charakteristik. Další část pak uvádí příklady praktické implementace těchto metod pro poloautomatický režim práce sestav IBM PC se speciálním uživatelským programovým vybavením a to jak s použitím samostatné obrazové paměti (frame grabber), tak i bez ní.

Acknowledgement

Financial support of the described work by grants (Grant Agency AV ČR, grant A5011504 and Grant Agency CR, grant 304/941/1202) is gratefully acknowledged.

References

- CRUZ-ORIVE, L. M., WEIBEL, E. R. 1990: Recent stereological methods for cell biology: a brief survey. Am. J. Physiol. 258: 148-156
- CRUZ-ORIVE, L. M., HOWARD, C. V. 1995: Estimation of individual feature surface area with the vertical spatial grid. J. Microsc. 178:146-151
- GUILAK, F. 1994: Volume and surface area measurement of viable chondrocytes in situ using geometric modelling of serial confocal sections. J. Microsc. 150:117-136
- GUNDERSEN, H. J. G., BENDTSEN, T. F., KORBO, L., MARCUSSEN, N., MOLLER, A., NIELSEN, K., NYENGAARD, J.R., PAKKENBERG, B., SORENSEN, F. B., VESTERBY, A., WEST M. J. 1988a: Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96: 379-394
- GUNDERSEN, H. J. G., BAGGER, P., BENDTSEN, T. F., EVANS, S. M., KORBO, L., MARCUSSEN, N., MOLLER, A., NIELSEN, K., NYENGAARD, J. R., PAKKENBERG, B., SORENSEN, F. B., VESTERBY, A., WEST M. J. 1988b: The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. APMIS 96:857-881
- KAREN P., KUBÍNOVÁ L., KREKULE I.: Semi-automatic, computer-assisted digital stereology for morphometrical measurements using the STESYS system software. J.Neurosc. M. (in prep.)
- KREKULE, I., KUBÍNOVÁ, L., KAMINSKI, J., KAREN P. 1995: Computerized system for semi-automatic stereological analysis. Med. Tech. 40: Suppl. 2:193-95
- KREKULÉ, I., KUBÍNOVÁ L., KAMINSKY, J., KAREN P. 1995: Simple hardware for semiautomatic stereological analysis. Acta Stereol. 14/2:197-200
- MERCER, R. R., CRAPO, J. D. 1988: Structure of the gas exchange region of the lungs determined by threedimensional reconstructions. In: Toxicology of the lung (Ed. by D. E. GARDNER, J. D. CRAPO, E. J. MASSARO), pp. 43-70. Raven Press, New York
- MEYER, F. 1992: Mathematical morphology: from two dimensions to three dimensions. J. Microsc. 165:5-28
- MICHEL, R. P., CRUZ-ORIVE, L. M. 1988: Application of the Cavalieri principle and vertical sections method to lung: estimation of volume and pleural surface area. J. Microsc. **150**: 117-136
- PETRÁŇ, M., HADRAVSKÝ, M., EGGER, M. D., GALAMBOS, R. 1968: Tandem-scanning reflected-light microscope. J.Opt. Soc. Am. 58: 661-664
- ROBB, R. A., HANSON, D. P. 1991: A software system for interactive and quantitative visualization of multidimensional biomedical images. APESM 14:9-30
- SANDAU K. 1978: How to estimate the area of a surface using a spatial grid. Acta Stereol. 6:31-36
- WEIBEL, E. R. 1979: Stereological methods. Vol. 1: Practical methods for biological morphometry. Academic Press, New York
- WEIBEL, E.R. 1980: Stereological methods. Vol. 2: Theoretical foundations. Academic Press, New York

Address for correspondence: Ing. Ivan Krekule CSc. Institute of Physiology Czech Academy of Sciences Vídeňská 1083 142 20 Praha 4-Krč Czech Republic Tel.(02) 475 1111