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COMPUTER PRIMER FOR STEREOLOGICAL APPLICATIONS

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ABSTRACT

An open semi-automatic, IBM PC-based, image analysis system implementing design-based stereological methods is described. It evaluates geometrical characteristics of structure (e.g. tissue constituents) observed in a light microscope. The system was developed by using already available HW and SW components.

Key words: open system, semi-automatic image analyzer, stereology

INTRODUCTION

Difficulties connected with the automatic segmentation of images of biological tissues observed through a light micro scope have stimulated the development of semi-automatic image analysis, based on manual segmentation (e.g. Lum & Jones 1980). Therefore, an interactive, hand-controlled computer graphic input, (e.g. light pen, graphic tablet etc.) became an important part of standard semi-automatic image analyzers (SIAs). These devices make it possible to load the contours of a studied sample or its part into the computer and to benefit simultaneously from vision, knowledge and experience of the human operator who manually traces contours of the analyzed structure. Most SIAs apply the same procedures to the segmented image as the automatic image analyzers do, inclusive the noise prone A-D conversion of image data into the pixel matrix. Except for the above interactive graphic input, the HW of the SIA usually consists of an image display and a PC interfaced with a TV camera. This makes up an inexpensive system which has proved useful in practice.
However, a more recent, simple and inexpensive approach to the SIA design offers implementation of the design-based stereological methods estimating geometrical characteristics of studied objects without the need to segment the analyzed images, as first suggested by Moss et al (1969). An appropriate test system, e.g. point grid, system of counting frames, etc. (Weibel 1980) is only superimposed on the tissue picture (see Fig. 1) and simple counts of particles in frames or their hits with the grid are evaluated manually (e.g. test points lying in the picture of the analyzed particles are counted) to obtain unbiased estimates of chosen geometrical characteristics of the observed structure. The HW of the SIA implementing stereological methods is more

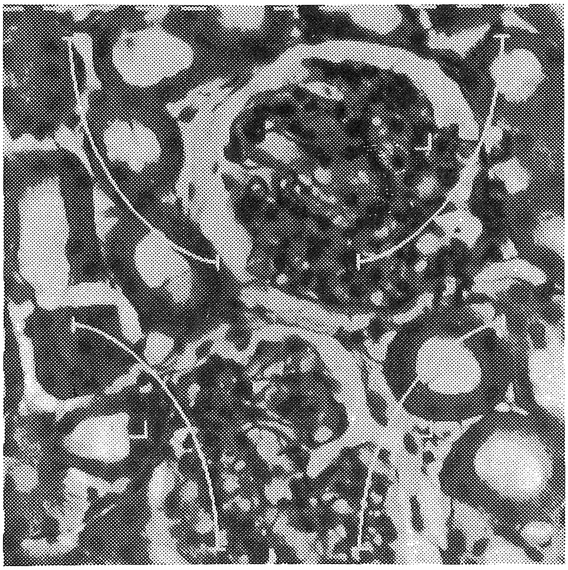

Fig. 1. Picture of the display screen depicting image of a tissue (rat kidney) and the measuring raster (used for point counting and surface area estimation) superimposed.

simple when compared with the standard one because an interactive graphical input is redundant. The image display should be equipped with a separately controlled graphic overlay to provide required measuring rasters. The raster can be superimposed either on the image of the analyzed tissue or on its display (Krekule and Gundersen, 1989). The former solution requires modifications of the light microscope (e.g. Leitz Microvid, 1988), while the latter, more popular one, usually needs a frame grabber as the interface between the TV camera and the PC. The latter approach was used in our design.

SYSTEM DESCRIPTION

Unlike the already published stereological SIAs (e.g. Moss et al., 1989; GRID, 1990) which differ by a set of available rasters and the convenience of manipulation with them and by data recording and processing, the described system was designed as an open system with respect to the set of measuring rasters and image data sources, inclusive a TV camera. The cost efficacy of the described design was achieved by exploiting already available parts of the system: i/ a simple, though adequate frame grabber which provides minimum of redundant functions and ii/ the SW: vector-based graphic editor GSE (Karen and Krekule, 1994) which provides all measuring rasters displayed through the graphic overlay of the frame grabber. A set of already generated measuring rasters is stored on HD and can be superimposed on the picture of the analyzed tissue provided by the TV camera looking through the light microscope and presented on the screen of the image display. Mutual position of the raster and the analyzed image is SW controlled. New, rasters can be readily designed by using the GSE which forms a part of the SW system.
The commercially available frame grabber ZOB1 which handles color picture matrix was used. It is supported by a set of commands for

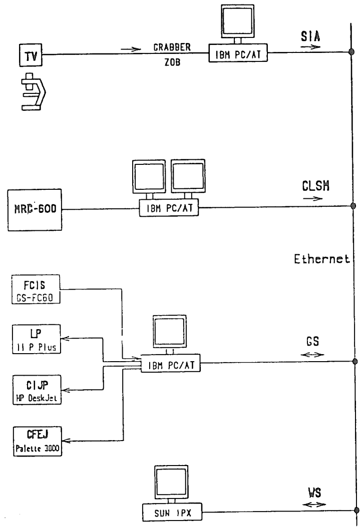

Fig. 2. Block scheme of the open, semi-automatic stereological image analyzer and I/O image devices connected via Ethernet (CFEJ: colour film exposition control unit; CIJP: colour ink-jet printer; CLSM: confocal laser scanning microscope; GS: graphic station; FCIS: flat-bed colour image scanner; LP: laser printer; SIA: semi-automatic image analyzer; WS: Unix workstation.

basic manipulation with input image data, e.g. grey or RGB palette modifications, gamma corrections, averaging, zoom, thresholding, edge enhancement, intensity profiles presentation, arithmetic operations, rotation, statistics evaluation, etc.

RESULTS

The described system was materialized by using IBM PC/AT (16MHz, 1MB RAM, 40 MB HD) enhanced by the ZOB1 frame grabber (Vidis s.r.o.; Prague, 512x512 pixels, 12 bits each: 8 bits = grey or color palette, 4 bits = graphic overlay), CCD TV camera (type PTK 256, Tesla Piestany, 256x256 B/W pixels) and a B/W TV video monitor. Sets of measuring rasters, e.g. point and line test systems, systems of unbiased counting frames (Gundersen 1978) and the spatial grid (Sandau, 1987), are available at the present time. The position of the reference point of the measuring raster can be specified beforehand, or determined by the output of a SW random number generator. Some of these rasters were used in a pilot study evaluating the volume and outer surface area of terminal placenta villi and the volume and inner surface area of their capillary bed (Kubinova et al., 1994).
The image data format of the described frame grabber was made compatible with a number of image I/O devices including a flat-bed color image scanner, laser printer, ink-jet printer, film exposition control unit, altogether comprising a graphic station. Image data from the SUN RISC IPX workstation and from the IBM PC which controls the confocal laser scanning microscope (Bio-Rad MRC 600) are also accessible via a LAN, and so processing of image data from various sources is possible. Moreover, a hard copy of either input or output data can be provided (see Fig. 2).

CONCLUSION

Despite the progress in computer HW yielding improved power/cost

ratio and stimulating implementation of very complex algorithms for segmentation of particles, the automatic segmentation of images of biological tissue constituents is, in general, still behind the near time horizon. Moreover, attempts to analyze geometrical characteristics based on 3D computer reconstruction of the specimen have already shown that the evaluation of these characteristics on serial sections can be done in a more simple and stable manner by using the design-based stereology rather than by applying models on the volume or surface reconstructions of the analyzed specimens.
Although the stereological methods yielding estimates of geometrical characteristics of biological structure were already successfully implemented in the automatic image analysis (e.g. Zhao et al, 1992; Zhao et Brown, 1992; Fenestra, 1992), the stereological semi-automatic image analyzer, thanks to its simplicity and cost efficacy, will assist the morphometrical analysis of biological tissues both in research and clinics.

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