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STESYS2: Extended STESYS Software for MS Windows

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Summary

The STESYS2 software is a new version of the IBM PC software supporting interactive stereological measurements. In comparison with the previous STESYS, it is enhanced by a number of useful options, e.g. on-line image input *via* a TV camera coupled with a microscope operating under MS Windows OS. The main advantage, when compared with other such software packages, is the design of the STESYS2 as a module of the freeware image processing system Image Tool which provides a user-friendly environment including a number of image processing and preprocessing routines. Capabilities of the STESYS2 are illustrated by a practical example: estimation of the surface area of capillaries in the terminal villi of human placenta by the Sandau spatial grid method.

Key words

Stereology • Software system • TV camera • Grabber • Test grid

Introduction

Interactive methods will flourish in morphometry as long as simple, automatic segmentation of images of biological tissue components is a problem. Contemporary stereology (e.g. Kubínová 1998) has a prominent place among these methods, because it provides unbiased and efficient estimates of geometrical characteristics based on a rigorous mathematical background. For these reasons a number of semi-automatic, computer-assisted stereological systems (Moss *et al.* 1989, Zhao and Browne 1992) have been developed during the past decade. We have already published a description of such a system: STESYS (Stereological TEst SYStems, (Karen *et al.* 1998) comprising IBM PC software under MS DOS. The STESYS system works off-line. It handles images of several different formats stored in various computer media and superimposes stereological test grids on them with the type, size and position chosen by the user. Moreover, the program provides calibration of the images so that the results calculated by hand are immediately available. As the STESYS system has already been successfully applied in different laboratories, updating and further improvement was desirable.

We describe the STESYS2 system updated to operate under MS Windows and enhanced by new options, including a real-time input of image data *via* a TV camera connected with a microscope. Moreover, the STESYS2 has been implemented as an optional plug-in

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module for a freeware image analysis software package – the UTHSCA Image Tool (IT) (developed at the University of Texas, Health Science Center at San Antonio and available from the Internet by anonymous FTP server (ftp//maxrad6.uthsesa.edu). STESYS2 was written as a DLL module of the IT by using C++ in the Borland programming environment.

The STESYS2 module was further enhanced by introducing several new functions such as automatic, density-based evaluation of the interaction of the object's images and the testing grid and visualization of this interaction (i.e. hits or misses). However, the main advantage of its introduction into the IT package is the user-friendly program environment, offering a number of general purpose image processing and preprocessing routines. Moreover, the IT represents an open system, which can be extended anytime by further required procedures.

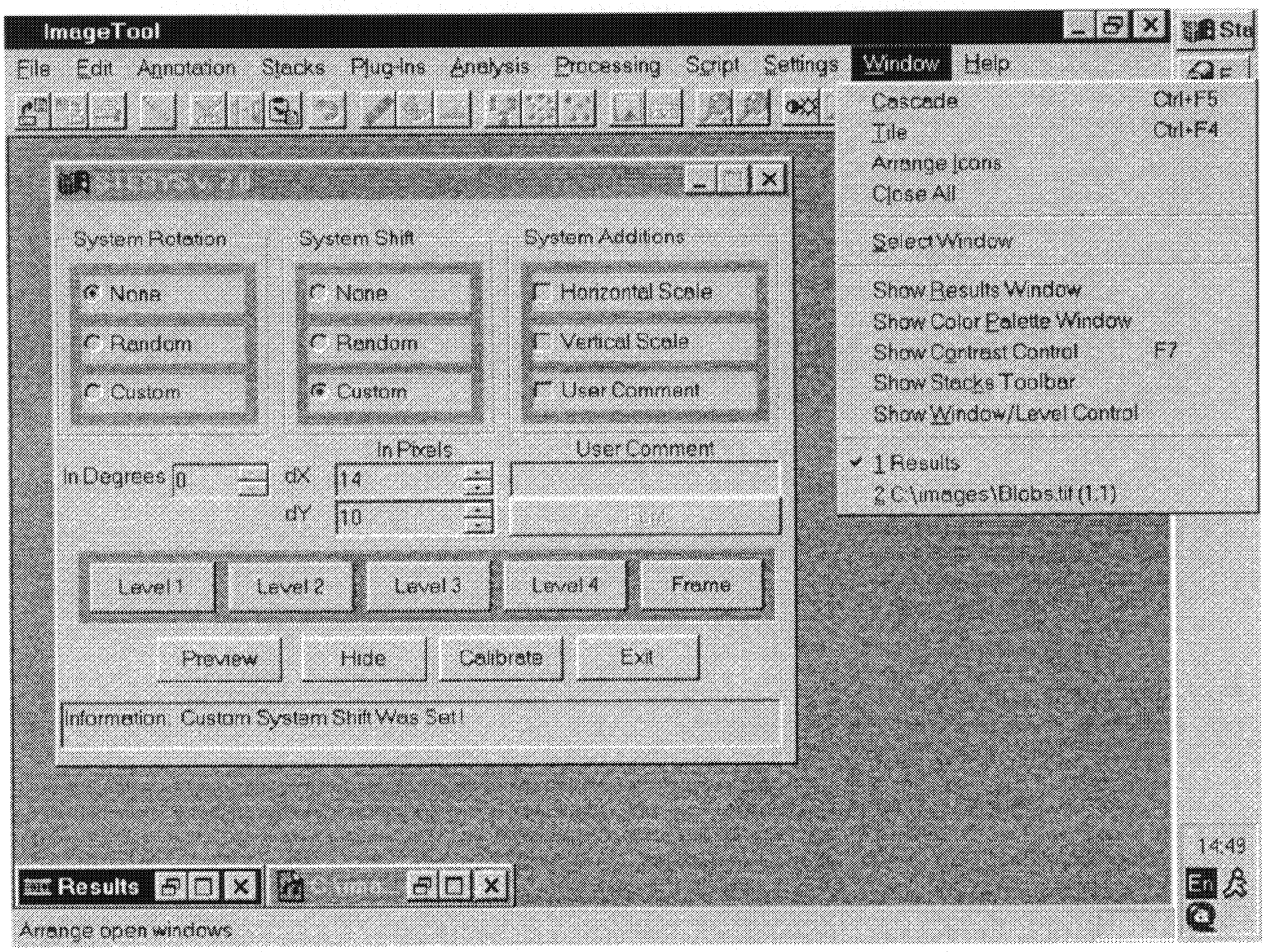

Fig. 1. *The screen showing the IT tool bar in the upper line, (notice the scrolling-down menu of the tagged item: window) and the main (first) control window of the STESYS2 system with usual buttons and set-up tools.*

Description of the system

STESYS2 represents an enhanced version of MS DOS STESYS (Karen *et al.* 1998), now running under MS Windows 95/98/NT, which was accomplished through the IT package. It offers a number of general purpose procedures such as: i) reading and writing data in more than 22 different image file formats including TIFF; ii) displaying image data with zooming and panning; iii) presenting results in a spreadsheet format; iv) threshold-based segmentation of images of objects under investigation; v) interactive segmentation; vi) region of interest (ROI) definition in an interactive mode; vii) image filtering; viii) counting segmented objects; ix) classification of objects, x) image transformations based on mathematical morphology rules.

STESYS2 is a menu-driven and mouse- or keyboard-controlled program in a way usual in MS Windows (e.g. see the main control window in Fig. 1). A scrolling menu is allocated in separate windows.

Image acquisition can be performed either by any TWAIN compatible scanner or by a frame grabber.

Real-time input of image data is accomplished by using a frame grabber, i.e. an IBM PC plug-in board, representing the interface between a video signal source, the PC and its display monitor. Following the recommendation of IT, we have chosen the DT 3155 grabber suitable for current TV standards (RS-170, CCIR, NTSC, PAL), interfaced with the IBM PCI bus and manufactured by Data Translation (USA). The grabber has 4 monochrome inputs, each with 8 bits of data width, with a sampling rate of 30 (25) TV frames per second, and with programmable white and black levels. The grabber is equipped with a 256x8 look-up table (LUT) to correct the intensity scale in real-time, exploiting the display and memory facilities of the computer to present the digital video output in real-time on the computer display. There are two ways of acquiring TV image data by IT: i) a frame by frame manner, controlled by clicking the mouse button, or ii) a series of frames, i.e. stack, input of which is controlled by selected time intervals between consecutive acquisitions or by clicking the mouse.

Measurements are performed in the following fashion. As soon as the image data are loaded either as a single frame or a stack of frames, data are displayed for inspection in a one frame per window manner (see Fig. 2). Image data can be optionally preprocessed, e.g. low-frequency filtered by using the convolution procedure of the IT package. Another plug-in module which is frequently used along with the STESYS2 system is the segmentation module (Segment Tool). We have developed the latter module which is aimed at segmentation prior to the start of STESYS2. Segmentation can also be performed by using various built-in interactive tools. The resulting polygons representing segmented objects then represent useful input information for STESYS2. The main control dialog is scrolled and partially superimposed on the loaded and/or preprocessed image data on the screen. The main dialog controls four sets of test grids, each of them being determined by the number of choices including the form and size of the test points, grid density and position, color of test points (hitting/missing), shape and size of test lines. The crucial decision, whether an object under study was hit or missed by the test grid, is done either by an interactive regime by clicking one interaction after another, or automatically, taking into account either the information from previously segmented objects, or the image gray level in the tested position (or in its close neighborhood, respectively). Automatic interactive evaluation is suitable whenever an already segmented picture, i.e. binary picture, is processed or if the density based segmentation can be applied. In any case, manual correction of the automatically segmented picture is possible. Interactions (hits/misses) are visualized by changing the color of the particular element of the test grid. The output of the STESYS2 routine is displayed in table showing the number of hits and misses for up to four used test grids.

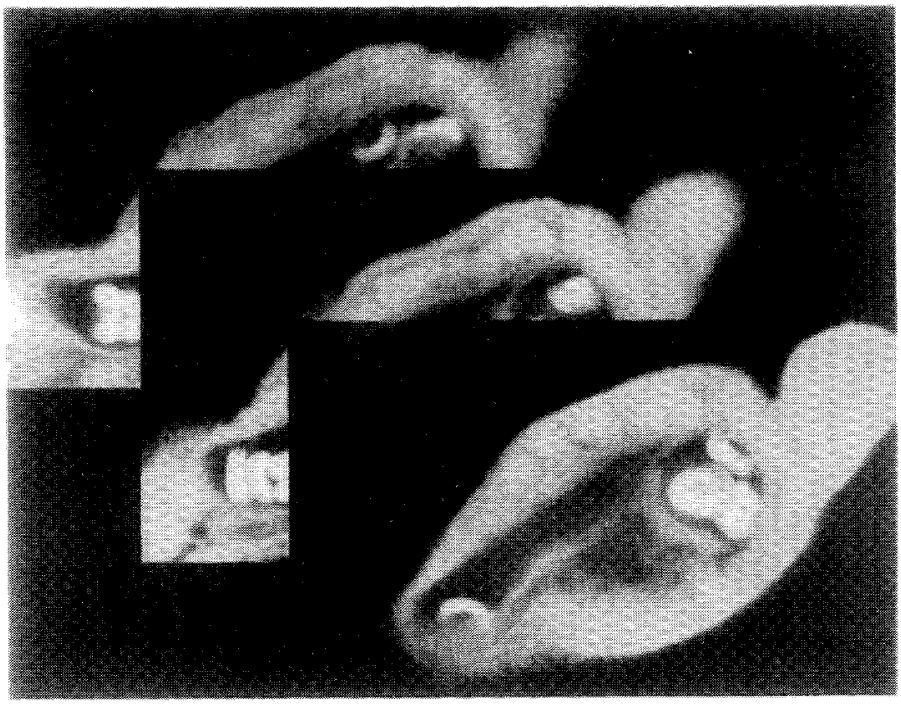

Fig. 2. *An example of the IT display of a stack of input data, i.e. set of windows (serial optical slices of a human placenta villus).*

Example of application

Our setup of the STESYS2 system consists of a student type light microscope Srb&Štýs JN 45 (CZ) and CCD color TV camera manufactured by JVC type TK 1270 (Japan), 752 x 582 pixel matrix, B/W video monitor Minitron type MVT-02 and the already described frame grabber DT3155 which is plugged into the PCI slot of an IBM PC (Pentium, 75 MHz, 64 MB RAM, HD 2GB, graphics controller SVGA). A schematic diagram of the set-up is shown in Fig. 3.

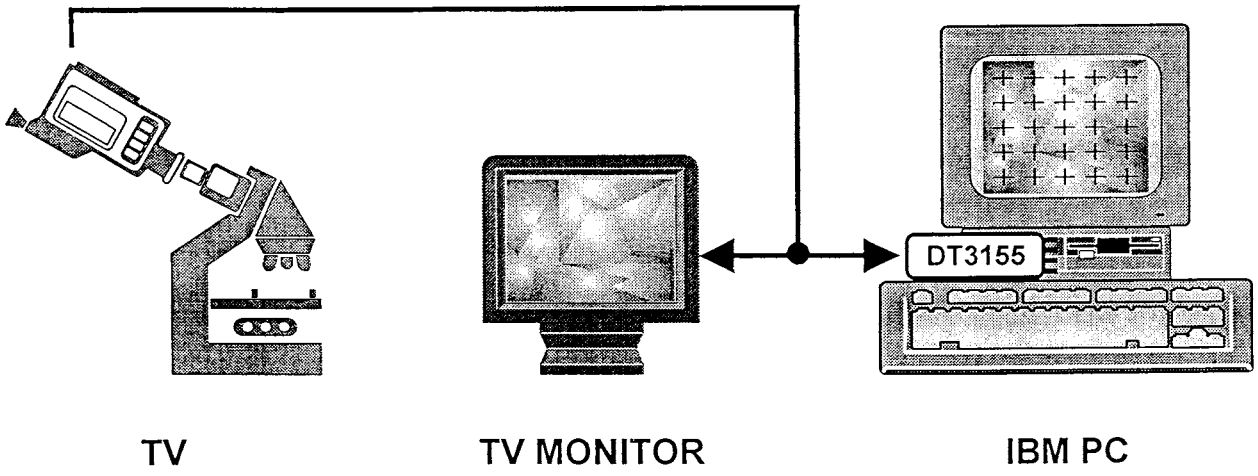

Fig. 3. *A scheme of the hardware set-up of the STESYS2. TV camera feeds video signal into a TV monitor displaying the actual frame of the microscope that can be captured by using the frame grabber DT 3155.*

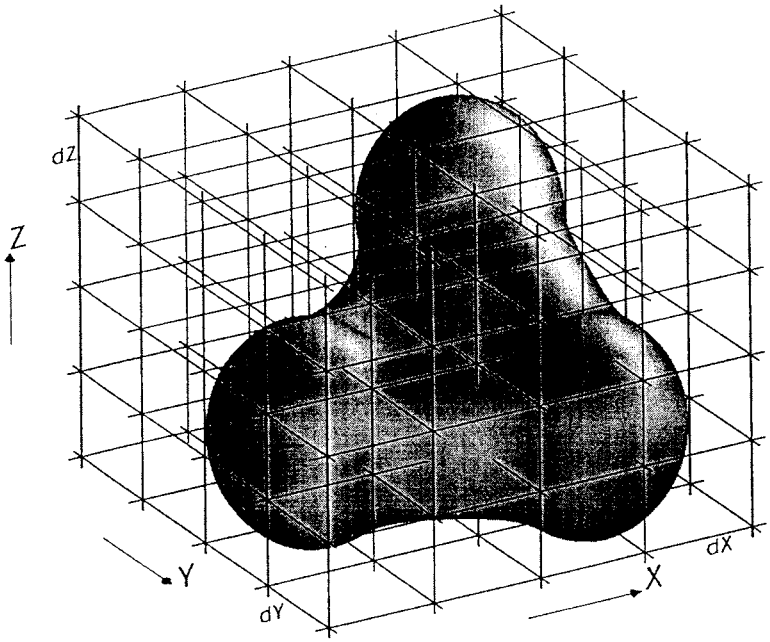

Fig. 4. *A scheme of the spatial grid, the interactions of which with the surface of the object under study make it possible to estimate the surface area of the object.*

To illustrate the application of STESYS2, the estimation of the surface area of capillaries by the method using the spatial grid (Sandau 1987) is shown in Figure 4. This demonstrates the new capacity of browsing through a stack of sections. If a stack of serial sections of the capillaries cut in an isotropic direction is available, it is now easy to apply the Sandau spatial grid method. In our example, a stack of digitized serial sections of a terminal villus of the human placenta recorded by a confocal laser scanning microscope (Bio-Rad MRC 600) was available (Fig. 5).

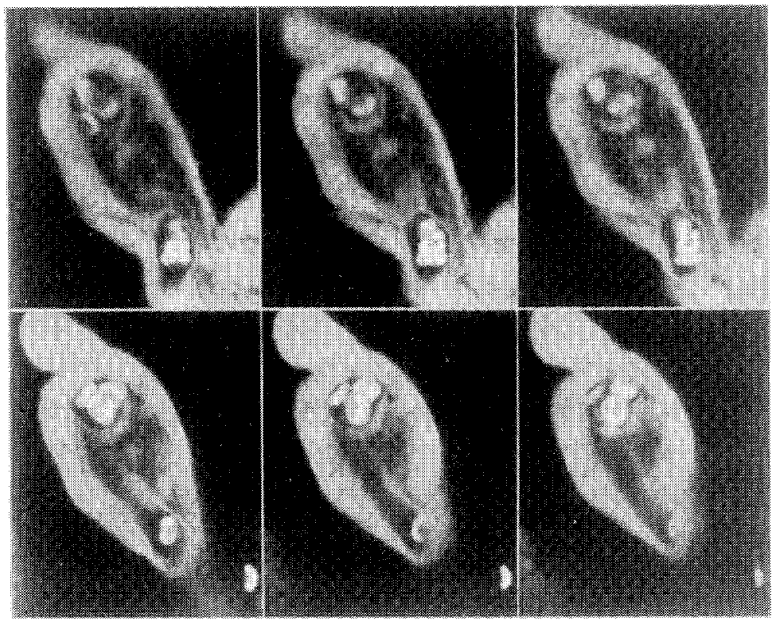

Fig. 5. *An example of six serial optical sections of terminal villus of human placenta captured by confocal laser scanning microscope (Bio-Rad MRC 600). The distance between sections is 2 μm.*

The spatial grid method as proposed by Sandau (1987) is a stereological method for estimating surface area based on interactive counting of intersections between the surface and a spatial grid of lines consisting of three perpendicular sets of equidistant parallel lines intersecting at the knots of the grid. The practical implementation consists of superimposing a square or rectangular net on serial sections of a stack of perfectly registered sections consecutively and counting intersection points of the surface with the line grids parallel to the x-, y- and z-axis. The surface area (S) can be estimated by:

$$estS=2/3.(I_x d_y d_z+I_y d_x d_z+I_z d_x d_y) \quad (1)$$

where d_x, d_y, d_z are distances between neighboring parallel lines of the grid in the direction of the x-, y- and z-axis, respectively, and I_x, I_y, I_z are numbers of intersection points of the surface with the line grids parallel to x-, y- and z-axis, respectively.

In our case of estimating the surface area of the capillary bed in a terminal placental villus, the intersections of the capillary surface with the spatial grid are counted easily not only in the x- and y-direction (i.e. intersections between the net of test lines and capillary contours on x-y sections), but also along the z-direction, as the STESYS2 allows focusing through the serial sections and can thus look along the test lines of the spatial grid parallel to the z-axis and judge where the specific test line is located with respect to the capillary surface (Fig. 6). The STESYS2 options such as setting the distances d_x, d_y, d_z position of the spatial grid and color of the grid are useful in practice.

Further development

Disector, a classical stereological procedure for unbiased counting or sampling of particles (Sterio 1984), will soon be added to the module of STESYS2. Further development of the STESYS2 will also be aimed at simplification of the measurement with real-time video input by introducing macroinstructions handling data acquisition, display and their measurement in a single command. Finally, we assume that new stereological procedures such as "fakir" (Kubínová and Janáček 1998) for estimating the surface area and "slicer" (Larsen *et al.* 1998) aimed at the estimation of the length of a 3D curve will become part of the STESYS2 in the near future.

Conclusions

The STESYS2 system represents a suitable and time-saving stereological tool, enabling a number of practical measurements in morphometry with minimal cost, since it can also be applied in an off-line regime without a real-time image input. In the more powerful application STESYS2 allows for a real-time video input *via* a TV camera focused on the microscope field of observation. Considering the limited hardware

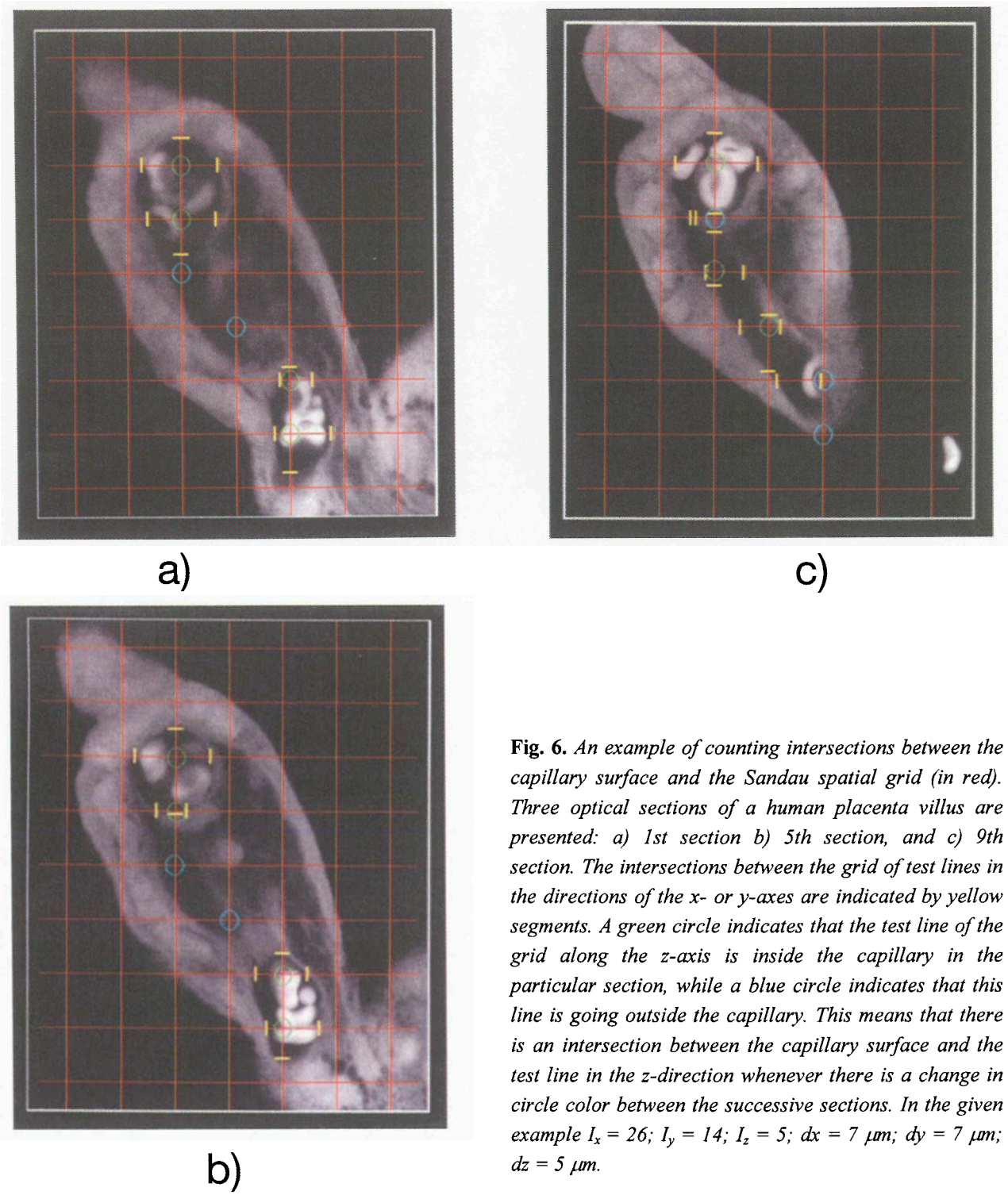

Fig. 6. *An example of counting intersections between the capillary surface and the Sandau spatial grid (in red). Three optical sections of a human placenta villus are presented: a) 1st section b) 5th section, and c) 9th section. The intersections between the grid of test lines in the directions of the x- or y-axes are indicated by yellow segments. A green circle indicates that the test line of the grid along the z-axis is inside the capillary in the particular section, while a blue circle indicates that this line is going outside the capillary. This means that there is an intersection between the capillary surface and the test line in the z-direction whenever there is a change in circle color between the successive sections. In the given example* $I_x = 26$; $I_y = 14$; $I_z = 5$; $dx = 7$ *μm*; $dy = 7$ *μm*; $dz = 5$ *μm*.

requirements and the application of the freeware software, the STESYS2 system is comparable with a number of commercially available systems of this kind, like the system manufactured by Olympus (Denmark) (C.A.S.T. 1999) or Digital Stereology by Kinetic Imaging (U.K.) or the "Stereology Toolbox" by Morphometrix, (USA).

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Reprint requests

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