

APPLICATION OF GRAPHICAL WORKSTATION SGI INDY AND IBM PC TO RENDERING AND VISUALIZATION OF THE OBJECTS CAPTURED BY A CONFOCAL MICROSCOPE

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Abstract

Kubínová L., J. Janáček, K. Antoš, B. Ježek, M. Indra, D. Jiráček, I. Krekule: *Application of Graphical Workstation SGI INDY and IBM PC to Rendering and Visualization of the Objects Captured by a Confocal Microscope. Acta vet. Brno 1996, 65:253-262.*

The options of several software packages for 3D rendering and visualization of objects captured by a confocal microscope were illustrated by the study of placental terminal villi and tobacco cell chains. Different types of 3D rendering were shown and discussed. It was concluded that software packages developed for solving general problems of medical imaging are well applicable to confocal data.

3D rendering, confocal microscopy, placental terminal villus, tobacco cell chain

The introduction of computed tomography in X-ray medical imaging in the seventies, later into nuclear medicine, magnetic resonance imaging and recently into ultrasound and infrared imaging represented a decisive impetus and objective for the development of medical imaging in the field of 3D object rendering and visualization (see, e.g. Udupa and Herman 1991). The later was enhanced by an interactive manipulation with the display of the object, including rotation, user control of the transparency, colouring, illumination, reflection, etc. of the visualized object, i.e. the organ and its constituents under study. All these attempts were supported by advances in understanding human visual perception (Hersch et al. 1995; Rokita 1996) and achievements in 3D computer graphics (e.g. Glasner 1990), the latter being stimulated by accelerating progress of the computer hardware in general and in its support to computer graphics.

In principle, there are two distinctive types of 3D object rendering, i.e. surface rendering and volume rendering. The first type of rendering, displaying shaded surfaces of the object, produces pictures of the object similar to its images as perceived by the human eye, while volume rendering provides pictures similar to x-ray because optical properties of the material lying on the ray passing through the object into the observer's eye are taken into account. In maximum projection, a simplified version of volume rendering, only the voxel of maximum grey value along the given ray is displayed. The most simple type of surface rendering is represented by wire-frame reconstruction when only sets of contours of profiles of the object and its constituents are displayed. The simplicity of this approach means that the software needed is often home-made in laboratories (e.g. Špaček 1994). The wire-frame visualization is also used in professional software packages for 3D rendering to present on-line results of an interactive manipulation (e.g. rotation) with the object before its surface or volume rendering is actually accomplished.

Rendering and visualization of a tissue captured by a confocal microscope is not complicated when compared with some sophisticated problems of contemporary medical imaging, e.g. multimodal imaging (when images of the same object recorded by different technologies are fused, see e.g. Beolchi and Kuhn 1995) because the series of optical

sections are perfectly aligned. The specific problems encountered in confocal microscopy consist in (i) the limited depth of observation, i.e. limited height of the rendered object, (ii) the lower resolution in z-axis than in x- and y-axes, and (iii) the attenuation of the light intensity with the increasing depth within the optical slice processed and due to the bleaching of the fluorescence dye applied.

In principle, the software and hardware applied to general problems of medical imaging are well applicable to rendering and visualization of the objects examined by a confocal microscope. Often even less sophisticated software and especially hardware are applicable. Therefore, the hardware support of confocal data starts with higher level PCs and sometimes even 'home-made' (non-commercial) software is used. On the other hand, attempts not only to visualize the studied object but also to measure its geometrical characteristics like volume and surface area are encountered in recent applications of confocal microscopy (Howard and Sandau 1992; Kubínová et al. 1996). This is also the reason why stereological methods are beginning to be included in the software processing of confocal data.

We will illustrate the techniques of rendering and visualization of 3D objects examined under a confocal microscope by the study of placental terminal villi and tobacco cell chains to which several software packages run on two types of hardware, i.e. SGI INDY graphical workstation and IBM PC, were applied.

Materials and Methods

Methods for 3D rendering are illustrated on the following model material:

a) Tobacco (*Nicotiana tabacum* L.) cell chain

Tobacco VBI-0 cell line (Opatrný and Opatrná 1976) was cultured *in vitro* in a liquid culture medium containing 2,4-D growth regulator. Into the culture medium 0.01% solution of fluoresceine diacetate was added which resulted in staining cell interiors by fluoresceine.

b) Terminal villus of human placenta

Full depth tissue sample of placenta was cut into approximately 1 cm³ blocks which were fixed in 4% buffered formaldehyde solution and embedded in paraffin wax. Approximately 100 µm thick slices were cut, mounted on glass slides with 1% gelatine solution, deparaffinated, stained by 0.15% eosin solution and after dehydration and clearing mounted in entellan (Jirkovská et al. 1994).

Both tobacco and placenta specimens were examined under a Bio-Rad MRC 600 confocal laser scanning microscope (CLSM) (excitation wavelength 468 nm for tobacco cells and 568 nm for placental villi) using the inverted microscope Nikon Diaphot. Digitized images of serial optical sections (1 µm apart) of tobacco cell chains and of terminal villi lying completely inside the thick slice were captured using planapochromat oil immersion objectives (40x, NA=1.3 for tobacco cells, 60x, NA=1.4 for placental villi). These images represented input data for 3D rendering.

Different types of rendering were applied:

1) Maximum projection

This simplified volume rendering is offered directly by the COMOS programme, i.e. the control software of Bio-Rad confocal microscopes, run on IBM PC under MS DOS. The new Bio-Rad OS-2 based software - LaserSharp - enables also to rotate projected images. Maximum projection procedure was directly applied on series of optical sections captured by a CLSM.

2) Surface rendering

Shaded surfaces of the studied objects were displayed by using ANALYZE (Robb and Hanson 1991), 3DVIEWNIX (Udupa et al. 1993) and EXPLORER (Halse 1992) software packages run on a SGI INDY workstation under UNIX operation system. The above programmes enable to preprocess and segment the images, to set colour, opacity, reflection, illumination, rotation of rendered objects, etc. In surface rendering it is necessary to segment the regions of interest, i.e. to distinguish boundaries of the object to be rendered. The cell chains and villi were segmented by intensity thresholding.

3) Volume rendering

Volume rendering of both model objects is shown by implementing the ANALYZE software package which enables to choose different types of ray tracing, to set shading, rotation, etc. so that not only the surface but also the internal structure of rendered objects can be seen. Although, in general, volume rendering does not require segmentation, it is possible to define distinct regions to which different colours are assigned to enhance the visual

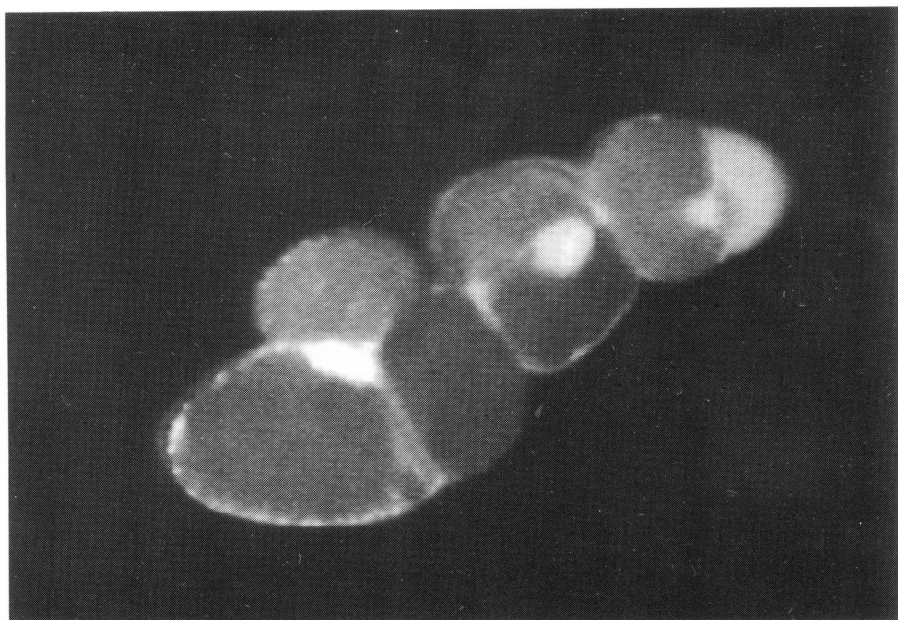


Fig. 1. Tobacco cell chain. One optical section out of 96 optical sections captured by the confocal microscope.

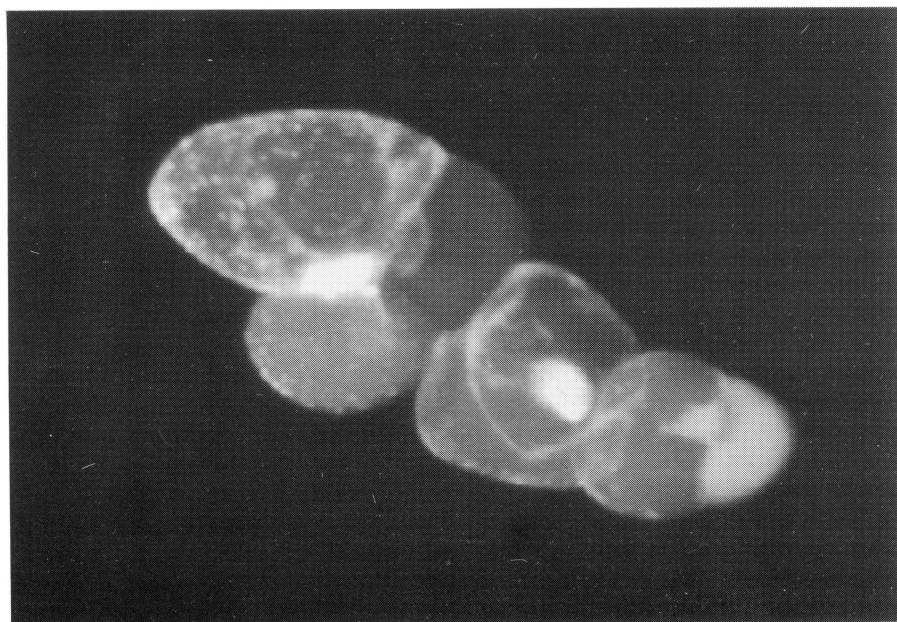


Fig. 2. Tobacco cell chain. Maximum projection of 96 serial sections as obtained by LaserSharp software; a) a view in the direction of z-axis; b) a rotated view.

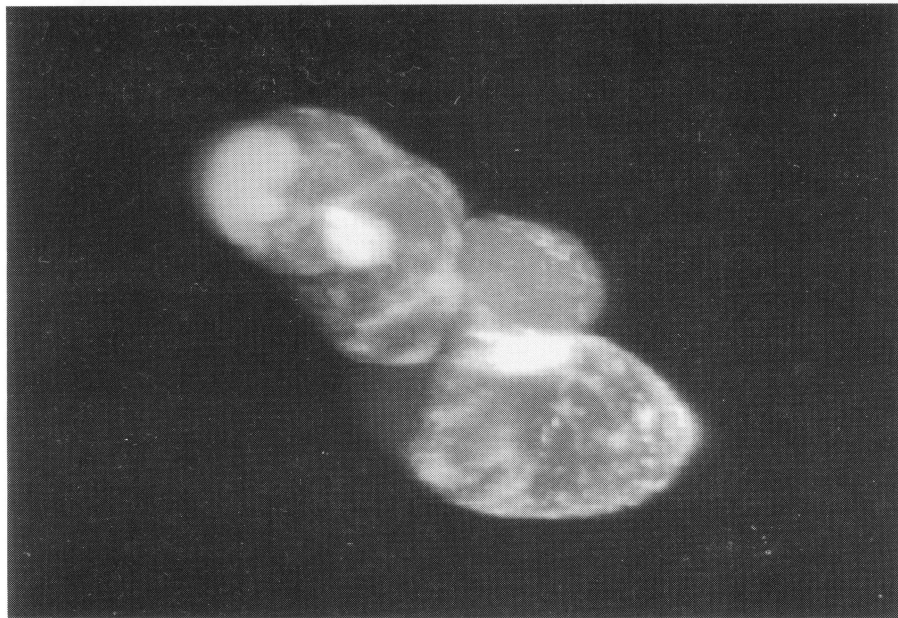


Fig. 2b) a rotated view.

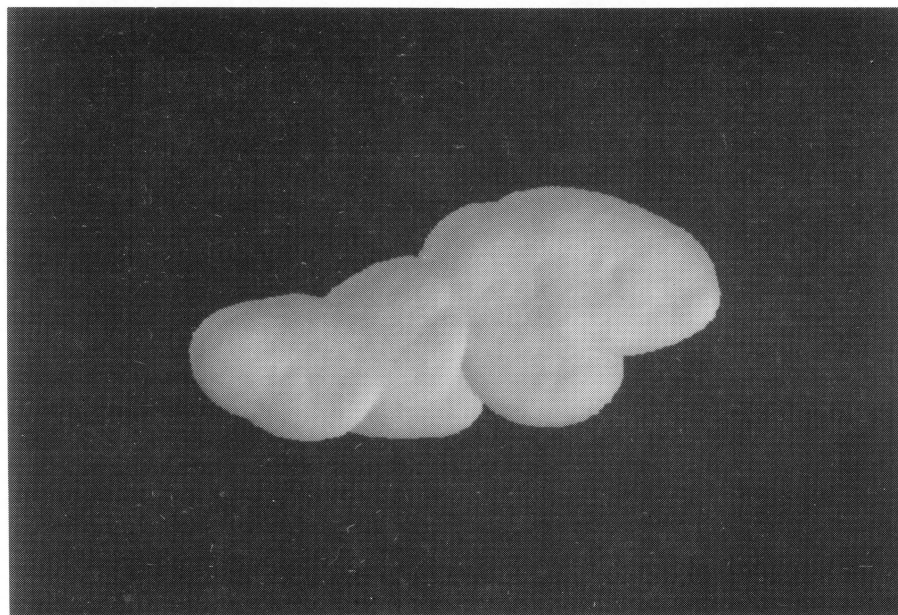


Fig. 3. Surface rendering of tobacco cell chain; a) by 3DVIEWNIX software package.

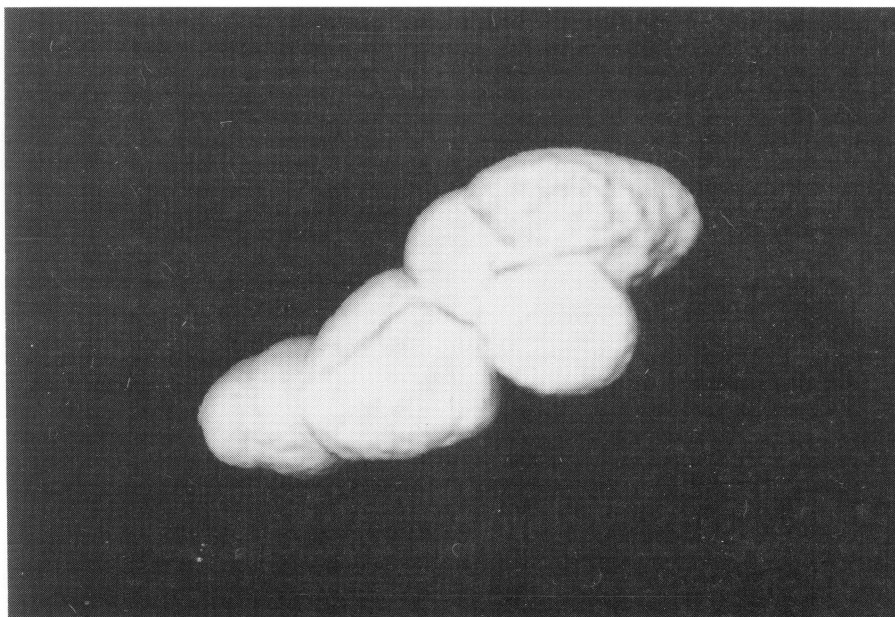


Fig. 3 b) by EXPLORER software package;

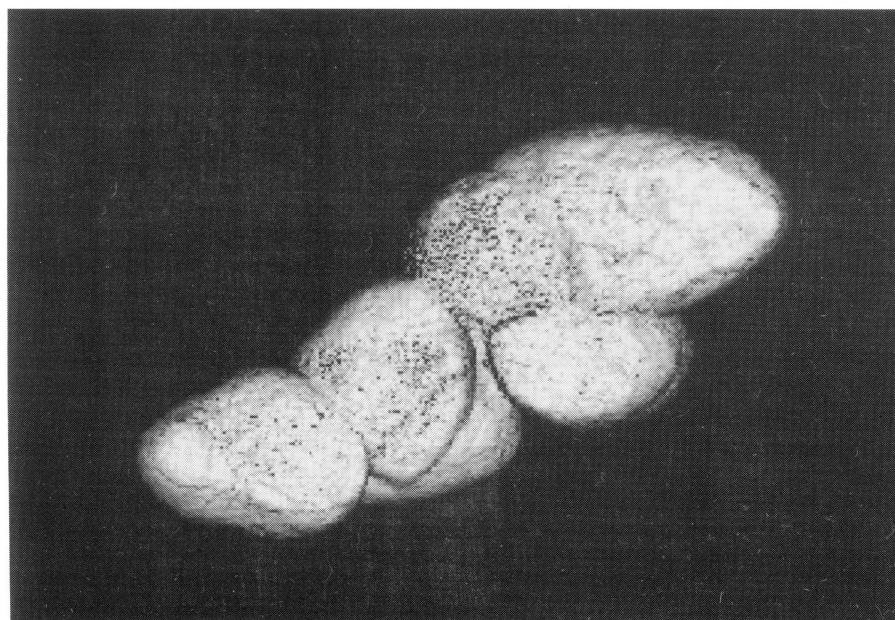


Fig. 3 c) by ANALYZE software package.

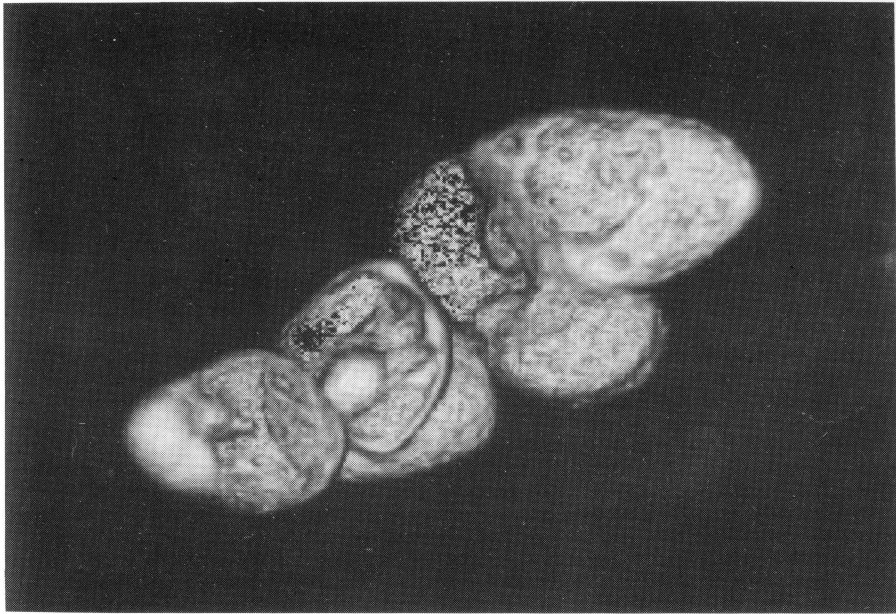


Fig. 4. Volume rendering of tobacco cell chain by ANALYZE software package.

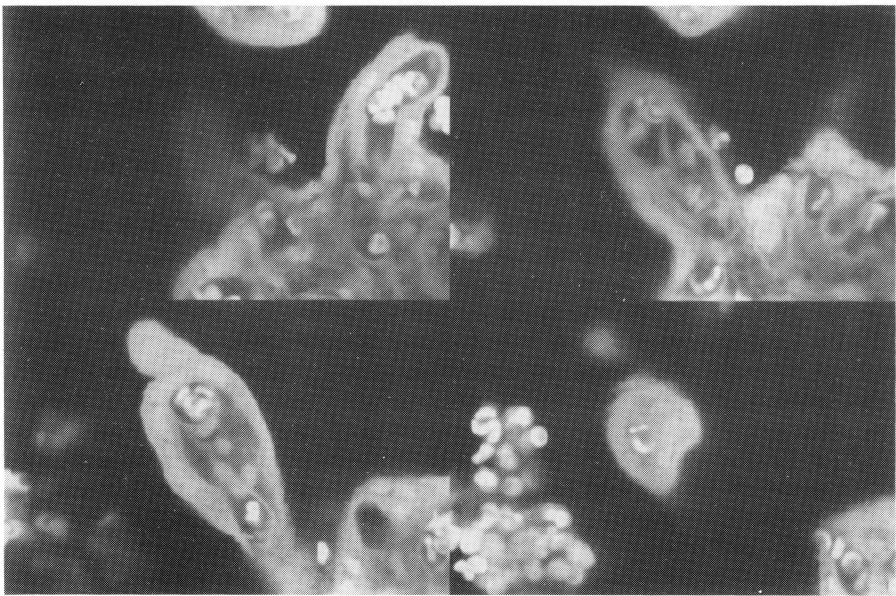


Fig. 5. Terminal villus of human placenta. Four serial sections (10 μm apart) from 48 optical sections captured by the confocal microscope.



Fig. 6. Terminal placental villus. Maximum projection of 48 serial sections as obtained by LaserSharp software.

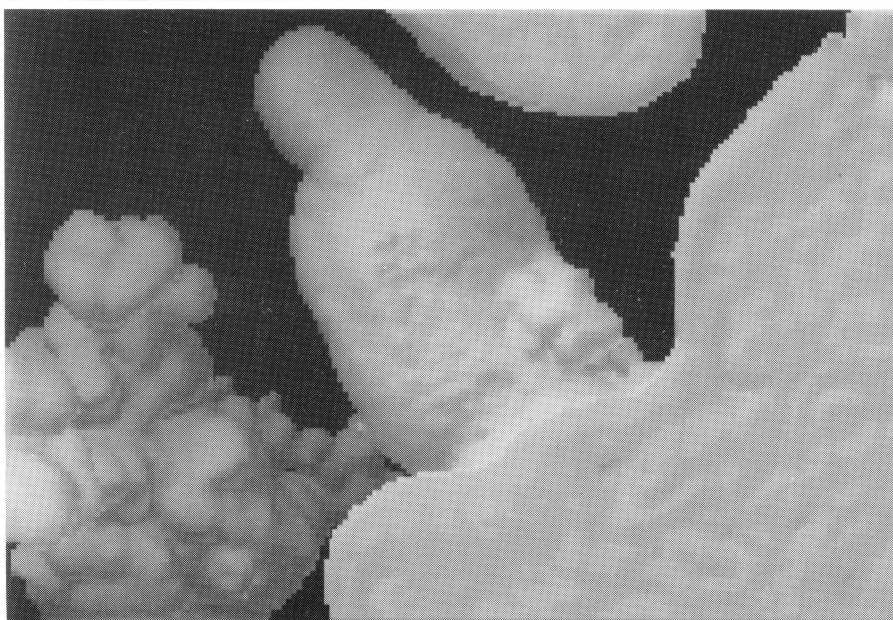


Fig. 7. Surface rendering of terminal placental villus by 3DVIEWNIX software package.

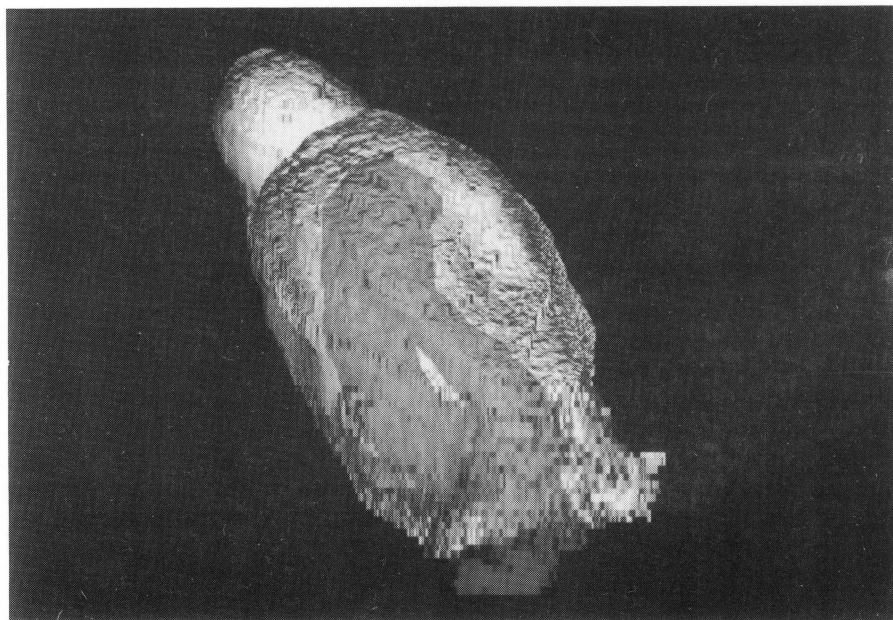


Fig. 8. Volume rendering of terminal placental villus by ANALYZE software package. Capillary bed is marked by dark grey.

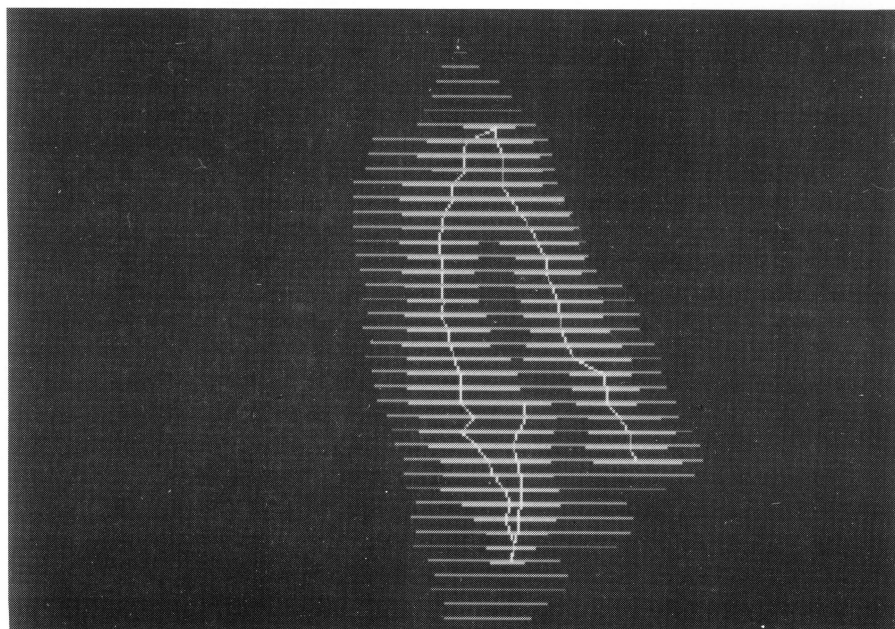


Fig. 9. Wire-frame reconstruction of terminal placental villus. the villus is marked by dark grey, capillary by medium grey and the line showing the capillary course by light grey.

perception. This approach was used to distinguish villus and its capillary bed when the masks of capillary and villus profiles were delineated interactively by a mouse in each section image.

4) Wire-frame reconstruction

Wire-frame reconstruction of the villus and its capillary bed was accomplished by a PC-based, Q-basic programme developed in our laboratory. Images of villus optical sections were printed by a laser printer and the villus and capillary contours were then traced by using a graphical tablet DG-1 (manufactured by the Czechoslovak Academy of Sciences). A distinct visualization was achieved, the villus and its capillary bed having been colour distinguished and the rotation of the wire-framed object having been easily accomplished. The analysis of capillary arrangement was enhanced by implementing a procedure reducing the capillary into a line.

Results and Discussion

Different types of 3D rendering of a tobacco cell chain (Fig. 1, 2, 3, 4) and a terminal placental villus (Fig. 5, 6, 7, 8, 9) were made. The maximum projection can be useful if the internal structure of the studied object is simple (Fig. 2). Objects with a more complex structure like the placental villus with its capillary bed cannot be visualized distinctly in this way (Fig. 6). For this purpose the volume or a wire-frame reconstruction are more suitable. Wire-frame reconstruction (Fig. 9) is more simple and less hardware and software demanding while the volume rendering of the object (Fig. 8) retains more information on its internal structure, e.g. the relationships between the villous core, trophoblast and vessels. Surface rendering (Fig. 3, 7) is suitable especially if the shape of the object is of interest.

Conclusions

Future trends in rendering and visualization of 3D objects from serial sections captured by a confocal microscope will be most likely, in accordance with general trends in medical imaging, aimed at higher interaction between the system and the user resulting in interactive navigating through tissue in 3D, enhancing the interactive manipulation with the object, combination of scales and combination of the real object with virtual reality environment. Rigorous methods for measurements of geometrical characteristics of the object and its components should be implemented into software packages for 3D rendering as well.

It can be also assumed that implementation of the picture deconvolution based on the point spread function of the optical system which improves the image quality will become a standard part of the confocal software package in the future.

Multimodal imaging might be found interesting also in the realm of microscopy, e.g. if confocal microscopy were applied in combination with the new scanning probe microscopes.

In conclusion, it should be mentioned that:

- i) Practically all software packages developed for solving general problems of medical imaging are well applicable to confocal data.
- ii) In this context, it is not efficient to develop special software for rendering and visualization of confocal data.
- iii) The software support should contain implementation of stereological methods, at least in an interactive regime.
- iv) Software for wire-frame visualization and rendering can be useful in the domain of confocal data.
- v) Hardware support of rendering and visualization of confocal data at the level of high-end IBM PC or low-end graphical workstation is quite adequate until now.

Rekonstrukce trojrozměrných dat získaných konfokálním mikroskopem: ztvárnění objemu i povrchu a jejich vizualizace s využitím IBM PC i pracovní stanice SGI INDY

Na příkladech studia terminálních klků placenty a řetízků tabákových buněk byly předvedeny možnosti několika programových systémů při trojrozměrné rekonstrukci a vizualizaci objektů studovaných konfokálním mikroskopem. Dále byly demonstrovány a diskutovány některé typy trojrozměrných rekonstrukcí. Ukázalo se, že programové systémy vyvinuté pro řešení obecných problémů medicínského zobrazování jsou dobře použitelné pro zpracování dat z konfokálního mikroskopu.

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