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3D RECONSTRUCTION OF SURFACES CAPTURED BY CONFOCAL MICROSCOPY

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ABSTRACT

A method for construction of triangulated surfaces from 3D images is described. 3D reconstructions of tobacco cell chain surface and capillary bed in terminal vilus of human placenta are presented.
Key words: confocal microscopy, Crofton formula, image analysis, surface area, tobacco cell chain.

INTRODUCTION

Imaging methods usually yield 2D projections or cross-sections of objects under study, containing only partial information on its shape and dimensions. Construction of 3D representation of objects from such partial data enables better understanding of shape and topological properties via visualisation and enables direct measurements of object geometrical characteristics. Computer modelling and visualisation represents fast and a convenient method for 3D reconstruction (Udupa, 1991). Recent development of computer technique makes it easy to visualise fairly complex objects by using personal computers and also to make them available world-wide via Internet.

3D objects are digitally represented either in raster volume or vector format. Raster volume format consists of parallelepiped matrix of volume elements (voxels). Binary (0,1) volume can be obtained from 3D volume of grey voxels, originated e.g. by digitisation of registered parallel sections, by identification of voxels belonging to the object, i.e. segmentation. Vector format consists of simple geometrical objects, surfaces can be represented as union of triangles. Objects in vector format can be easily combined, coloured, made transparent, illuminated, and their position and orientation may be changed on-line (Wood, 1992).

Thin sections obtained by confocal laser scanning microscopy represent an almost ideal source of data for 3D reconstruction (Pawley, 1995). The specific problems encountered in confocal microscopy (CLSM) consist in (i) the limited depth of observation (ii) the lower resolution in Z-axis than in X- and Y-axis (iii) the attenuation of the light intensity with the increasing depth within the physical slice processed and due to the bleaching of the fluorescence dye applied

Quantitative properties of surfaces depend critically on resolution and signal to noise ratio of digitisation: lowering resolution or blurring always decreases while noise always increases the surface area of the object under study. Therefore it is a complicated task to make representative and efficient 3D reconstruction of surfaces from fluorescence images captured by CLSM. Cost-effective routine processing requires easy, flexible and robust methods. One of such methods for identification and triangulation of surfaces from fluorescent images captured

by confocal laser scanning microscope is presented in the paper. The method consists of pre-processing of grey image by digital filtering, segmentation by thresholding, processing of binary image by tools of mathematical morphology and isosurface generation.

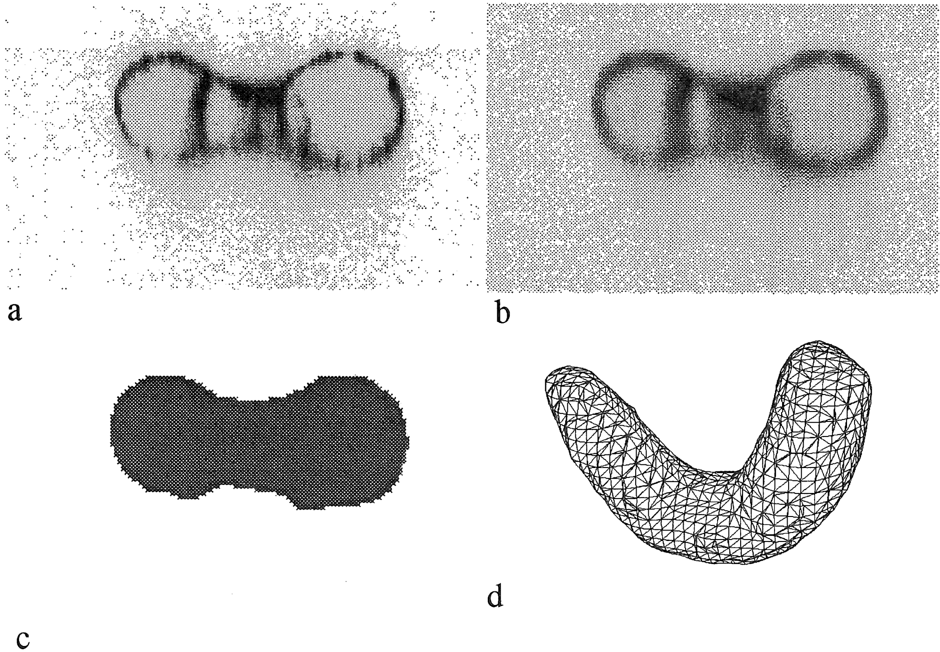

Figure 1: Volume processing of tobacco cell chain
a) FDA fluorescence image captured by CLSM MRC-600 (BioRad), resolution 1µm x 1µm x 1µm, 8 bits per voxel, section in YZ plane.
b) Grey volume processed by 2D closing in XY plane and 3D Gaussian filter, section in YZ plane.
c) Binary volume obtained by segmentation by thresholding and processed by 3D closing, 3D holes filling and 3D opening by reconstruction.
d) Isosurface obtained from binary volume resampled to 4µm x 4µm x 4µm grid and smoothed by 3D Gaussian filtration.

MATERIAL AND METHODS

Image processing algorithms were implemented as modules in graphical programming environment IRIS Explorer (Iris Explorer documentation, http://www.nag.co.uk/visual/IE/iecbb/DOC/Index.html, 1996) running on Indy (SGI, USA) workstation. The graphical environment enables quick building of applications by connecting programme modules. It also makes programming of image processing algorithms easier by providing devices for user interface and data flow handling. Original Explorer modules for displaying images, for generation of isosurface by marching cube algorithm (Lorensen and Cline, 1987) and for rendering of 3D scenes were used. Custom-made modules for mathematical morphology filters (Serra, 1982), for hole filling and opening by reconstruction, for smoothing by Gaussian filter,

for surface area measurement by Crofton formula, for reading data in internal format of confocal microscope MRC 600, for orthogonal slicing and projecting of image volumes and for overlaying of binary and grey images were written in C language. Mathematical morphology filters for erosion, dilation, opening and closing of grey and binary images and volumes use quadrate lattice with sequence of 4,8,4 neighbourhoods in 2D and cubic lattice with cubooctahedral 18 neighbourhood in 3D. Modules for hole filling and opening by reconstruction in 3D use cubic lattice with 6, 18 or 26 continuity. Module for smoothing by Gaussian filter uses 3^3 neighbourhood. Module for surface area measurement by Crofton formula uses counting of intersections in 13 main direction of cubic lattice (Meyer, 1992).

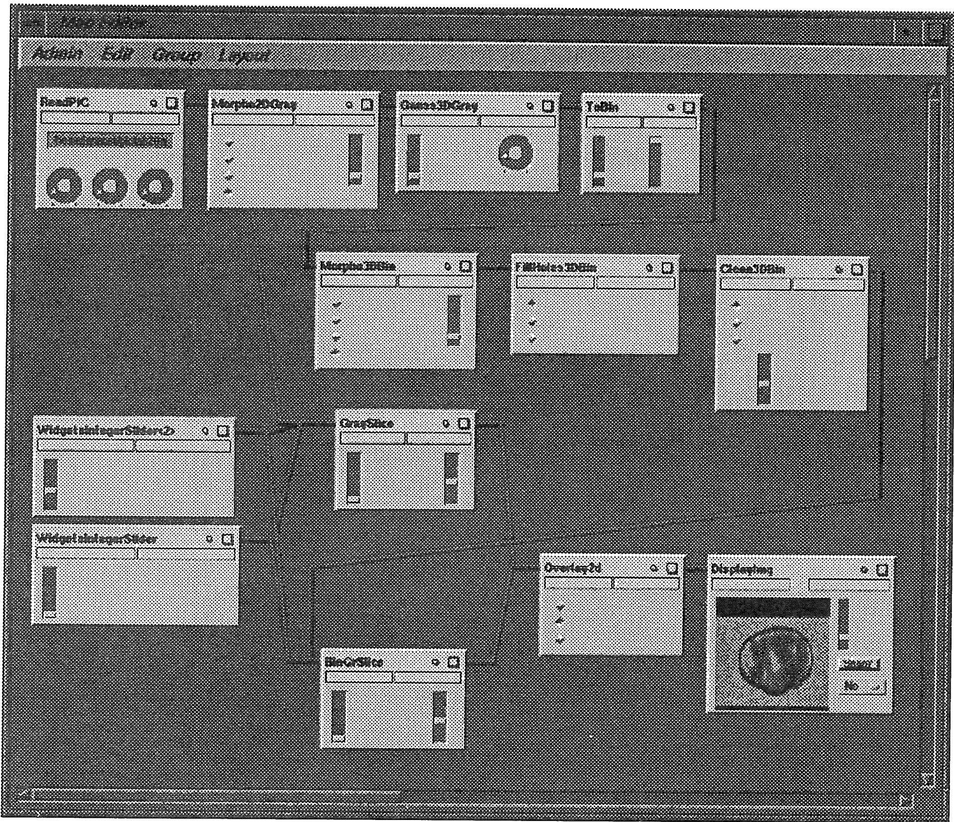

Figure 2: 3D processing and interactive segmentation of tobacco cells in IRIS Explorer. The boxes represent the programme modules, the lines represent data flows. The orthogonal section through original image volume together with resulting binary volume is displayed as overlay image.

Thin optical sections of tobacco cell chain, VBI-0 strain (Opatrný and Opatrná, 1976), were obtained as fluoresceine diacetate (FDA) fluorescence images captured by a confocal laser scanning microscope (CLSM) MRC 600 (BioRad). Greyscale 8bit volume of size 98µm x

141μm x 82μm was resampled to spatial resolution 1μm x 1μm x 1μm, and processed by 2D closing in XY plane and by 3D Gaussian filter to obtain closed image of cell chain surface and to decrease noise. Binary volume was obtained by segmentation by thresholding and was processed by 3D closing and 3D holes filling to fill in the cell interiors and 3D opening by reconstruction to remove artefacts originated from noise. The total volume and surface area was measured. The binary volume was resampled to resolution 4μm x 4μm x 4μm, smoothed by 3D Gaussian filtration and the isosurface was detected (Figures 1, 2).

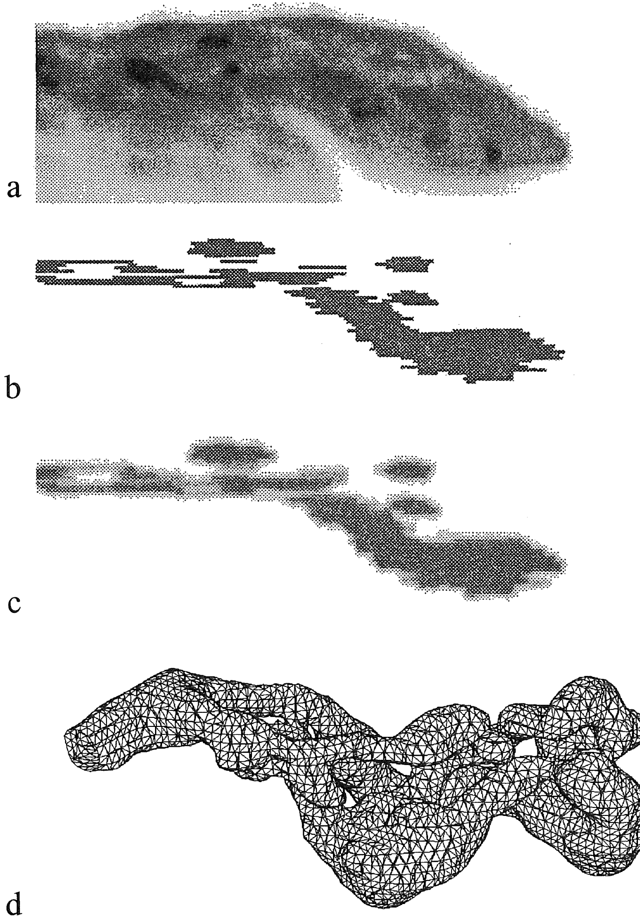

Figure 3. Volume processing of capillary bed in terminal vilus of human placenta
a) The image captured by CLSM, resolution 1μm x 1μm x 1μm, section in YZ plane.
b) Contours of capillaries: drawn interactively in XY plane, section in YZ plane.
c) Grey volume processing by 3D Gaussian filtration.
d) Isosurface obtained from binary volume resampled to 2μm x 2μm x 2μm and smoothed by 3D Gaussian filtration.

Optical sections of terminal vilus of human placenta were obtained as images with lateral resolution 0.275μm captured by CLSM MRC-600 (BioRad) (Jirkovská et al., 1994). Total volume size was 126μm x 111μm x 66μm. Contours of capillaries were drawn interactively in XY plane. The resulting volume was resampled to resolution 2μm x 2μm x 2μm, processed by 3D Gaussian filtration and triangulated by isosurface detection (Figure 3).

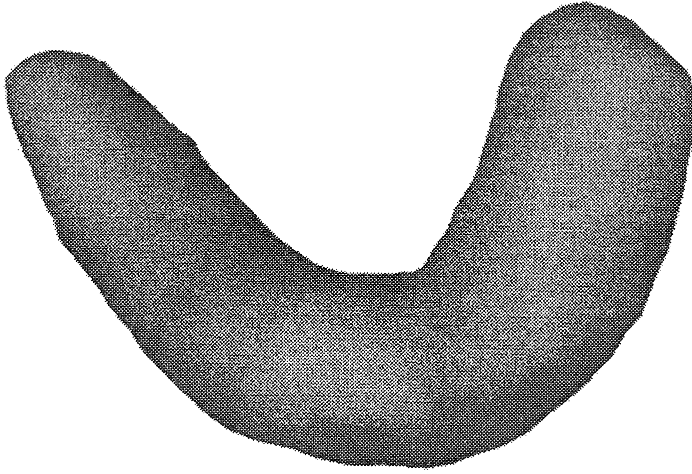

Figure 4: Surface rendering of tobacco cell chain

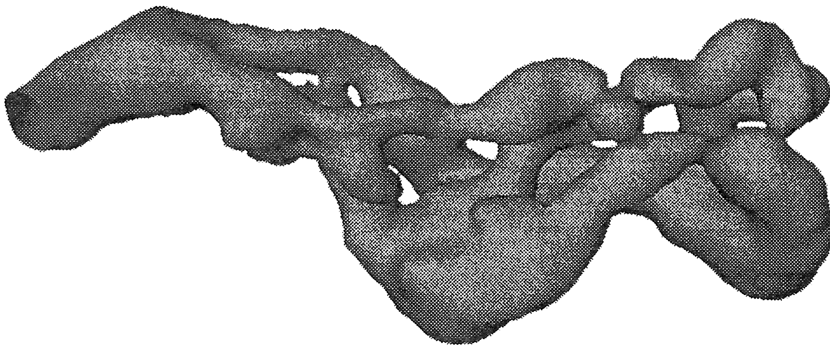

Figure 5: Surface rendering of capillary bed of terminal villus of human placenta

RESULTS

3D reconstruction of tobacco cell chain (Figure 4) consists of 3352 triangles. The total area of triangles is 18112 μm^2 which is close to the estimate of surface area 18668 μm^2 measured on binary volume with resolution $1\mu m \times 1\mu m \times 1\mu m$ by the digital Crofton method.

Reconstruction of capillaries in terminal vilus of human placenta (Figure 5) consists of 13423 triangles. Arrangement of the capillary bed can be seen in the picture.

DISCUSSION

The method yielded satisfactory results in the presented examples, however the results of reconstruction in any individual case depend heavily on tuning of the sequence of operations, which must also use an apriori knowledge on the object under study. In the case of tobacco cells we are using the fact that the cell surface is closed and relatively smooth. Resolution of the image volume during processing must be high enough to detect details under study but not so high as to produce unmanageable number of surface components. Too high resolution can also yield close but jagged approximation that largely overestimates the surface area.

Marching cubes algorithm that we used for surfaces triangulation is perhaps not the only nor the best possible choice. It is sufficiently general and easy to implement, but generates triangles that strongly differ in size and the resulting surfaces can contain holes.

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