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A novel technique of three-dimensional reconstruction segmentation and analysis for sliced images of biological tissues*

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Abstract: A novel technique of three-dimensional (3D) reconstruction, segmentation, display and analysis of series slices of images including microscopic wide field optical sectioning by deconvolution method, cryo-electron microscope slices by Fourier-Bessel synthesis and electron tomography (ET), and a series of computed tomography (CT) was developed to perform simultaneous measurement on the structure and function of biomedical samples. The paper presents the 3D reconstruction segmentation display and analysis results of pollen spore, chaperonin, virus, head, cervical bone, tibia and carpus. At the same time, it also puts forward some potential applications of the new technique in the biomedical realm.

Key words: Sliced images, 3D reconstruction and analysis, 3D segmentation, Chaperonin, Virus
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INTRODUCTION

The past decades witnessed unprecedented advances in the imaging science fields (Yelbuz *et al*., 2002; Campagnola *et al*., 2002; Sharpe *et al*., 2002). The technique of 3D image reconstruction and analysis plays a significant role in the basic research of life science, which developed from 2D image processing but provides more information than 2D image, and has rapidly expanded to molecular and cellular field (Guo *et al*., 1995; Trehan *et al*., 2003). Despite the rapid development of the new field, many challenges are still waiting for resolution (Paddock, 2002). Rare 3D image analysis techniques can be used for macroscopic and microcosmic fields. This paper presents a new method of 3D segmentation and analysis, by which the tomograms of the cellular and sub-cellular inner structure of tissue can be displayed, segmented and analyzed in 3D mode.

METHODS

3D display

The 3D display method we used is volume display in real space based on light projection algorithm. Firstly, each point of the volume data is evaluated with the color and transparency values, and then the viewed plane and observation direction are determined. Then the reference frame is transferred into the 3D image coordinates, and the illumination of each point can be calculated by the method of light projection model. Finally, the 3D image can be synthesized by summing up the contributions of all points to each pixel of the image.

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3D segmentation and analysis

We developed a new method called "virtual dissection" (Li *et al*., 2001), which controls the volume projection in 3D space by Open GL interface. The function of the segmented line and the angle of segmented volume are properly resolved by applying circular cylindrical coordinates which are unified by differential and light tracing methods. The segmentation of any part and in any way can be realized in 3D volume while complicated calculation was avoided. Even more important, accurate measurement of area, volume, distance and angle in 3D mode can be done by analyzing the characteristics of the 3D volume and summing up the pixels.

RESULTS AND DISCUSSION

3D analysis of optical section

The devices for taking pictures are: Nikon TE300 microscope, CCD camera (IMAC-CCD30 768×527), and computer for image displaying, storing, processing and analysis. After 2D image acquisition, the optical section can be obtained by removing the defocused background by deconvolution methods. The 3D segmentation of pollen spore is shown in Fig.1 where the thickness of each section is 1.0 μm. The segmented part can be moved and quantified. The measurement results of the segmented parts A1 A2 are given in Table 1.

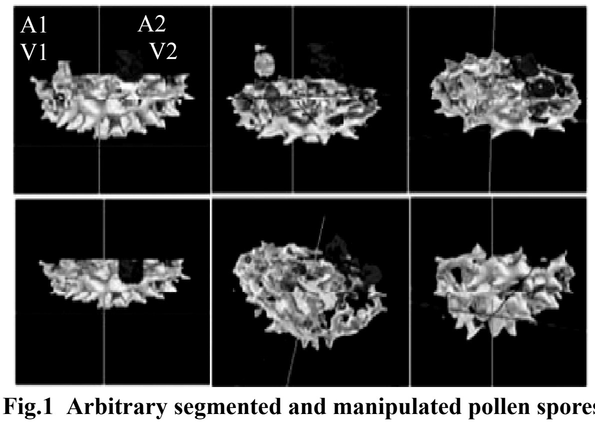


Fig.1 Arbitrary segmented and manipulated pollen spores

Table 1 The results of 3D measurement of reconstructed and segmented pollen spore

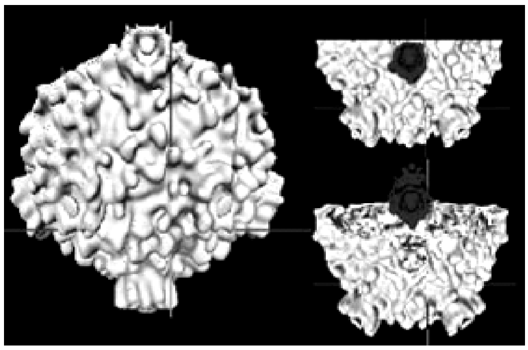
Item	A1	A2	V1	V2
Surface area (μm^3)	18.1	23.3		
Volume (μm^3)			97.3	127.7

3D analysis of tomogram by ET

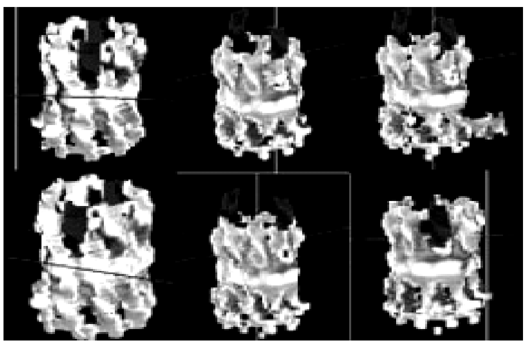
The results of 3D reconstruction and arbitrary segmentation of Bombyx mori Cytoplasmic Polyhedrosis virus (CPV) are shown in Fig.2a whose tomograms were collected by Fourier-Bessel synthesis (Crowther, 1971). The result of 3D reconstruction of chaperonin is shown in Fig.2b whose tomograms were collected by electron tomography (Nitsch *et al*., 1998). Arbitrary parts can be segmented from the whole. Important information about super macromolecular organization and interactions can thus be obtained by analyzing the sub-part's spatial relationships inside a whole. The technique can also be useful for determining the 3D conformation of macromolecular and molecular modelling in the future.

3D analysis of X-ray CT

The application of 3D segmentation in orthopaedics is very useful for computer-aided surgery plan and biomechanical analysis (Li *et al*., 2003).



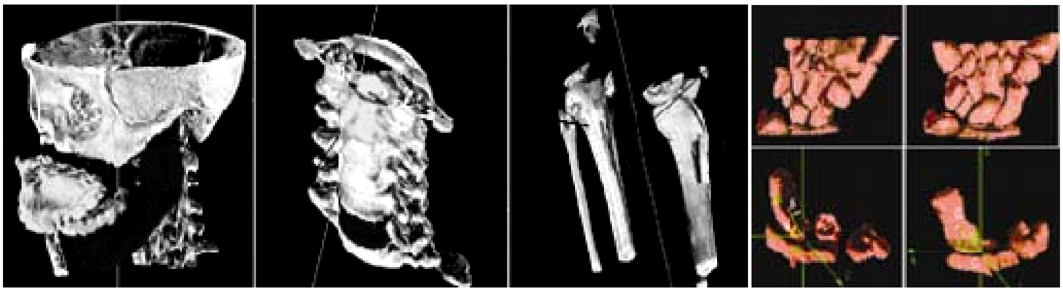
(a)



(b)

Fig.2 3D images reconstruction and segmentation to microscopic specimen by cry-electron microscopy (a) Reconstruction and segmentation to 207×207×104 CPV; (b) Reconstruction and segmentation to 256×256×30 chaperonin

The results of using the technique in Computer Aided Cranio-Maxillofacial Surgery System are shown in Fig.3a, the 3D segmentation of cervical bone is shown in Fig.3b, the 3D segmentation of tibia is shown in Fig.3c and the results on carpus are shown in Fig.3d. The technique can be applied to relatively bigger tissues such as those of the head or tibia and smaller tissues such as those of the carpus.



(a) (b) (c) (d)

Fig.3 Reconstruction and arbitrary segmentation of human organs (a) 3D reconstruction and arbitrary segmentation of 256×256×94 CT bone images; (b) 3D reconstruction and arbitrary segmentation of 256×256×56 CT cervical bone; (c) 3D reconstruction and arbitrary segmentation of 256×256×120 CT tibia; (d) 3D reconstruction and arbitrary segmentation of 256×256×47 CT carpus

CONCLUDING REMARK

Software has been developed based on the new technique of 3D segmentation and analysis presented in this paper. The 3D inner structure can be clearly displayed and measured accurately by this method. The new technique can be used as a common method for 3D reconstruction, display, and analysis of both macroscopic and microscopic sectioning of images, and can also be widely used for medical applications such as computer-aided surgery, virtual reality and minimally invasive medical procedures and so on.

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