Three-dimensional reconstruction system for wide area and high magnification light microscopic images with distributed processing system :An application to the nephron of ICGN mouse kidney

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1 Introduction

We have been aiming at developing an integrated three-dimensional reconstruction system [1] for morphological study. The system [2] , developed thus far, includes "Color Image Processing [3] " and "Color Image Handling [4] " applications. Based on the system, we developed further new applications in order to solve specific problems for morphological research, which we often come across when reconstructing the biological structures (body tissues and organs). Spontaneous nephrotic mice (ICGN mice) provide us with such typical difficulties in the reconstruction for the investigation of the pathogenesis [5] .

The mutant mice [6]–[7] show nonproliferative glomerular lesions. Besides, the renal tubules are also compromised. To elucidate the precise processes of the genetical disease, it is necessary to investigate the tubular involvement in terms of three-dimensional structure.

A nephron is a unit of renal function, consisting of a glomerulus and a renal tubule. The renal tubule is subdivided into proximal portion, Henle's loop and distal portion at large. Each has characteristic features and three-dimensional distribution in the kidney. In this case, the morphological characteristics were totally destroyed showing extreme pathological changes from occlusion to high dilatation. The only reliable way of the identification of the location is a three-dimensional approach.

Since the renal tubule starts from renal cortex, going into the renal medulla and coming back to the cortex again, we need a large data storage and retrieval system for the images of wide area to follow up all the way of the renal tubule. To chase the course of the tubule accurately, high magnification is necessary, because the tubules of the ICGN mice are occluded in some parts to be very small in diameter. This increases the quantity of the data and the complexity for reconstruction. To overcome these problems, we developed an integrated application for wide area and high magnification light microscopic images to reconstruct 3-D images from serial sections of the tissues.

Figure 1: Distributed processing system. Main computer is NeXT cube, rendering machine is SPARCserver and LVR control computer is NEWS.

2 Materials and methods

Homozygous ICGN mice (5 months old) were used in this study. After anesthesia with ether the animals were perfused via the left ventricle with 3% glutaraldehyde buffered with 0.1 M Millonig's phosphate at pH 7.4. The kidneys were removed and transversely cut into thin slices (2-4 mm in thickness) across the longitudinal axis of the kidney. The fixation of the tissue blocks fixed with 3% glutaraldehyde was continued in 4% paraformaldehyde buffered with Millonig's solution for 1-2 days. Blocks were then dehydrated with ethanol and embedded in plastic embedding medium (Polysciences) or paraffin. About 500 serial sections were made at 3-5μm in thickness and stained with hematoxylin and eosin.

3 System (Fig.1) and Procedures

The hardware of this system consists of three workstations (NeXT NeXTcube [68040 25MHz], SONY NEWS 3460 [R3000 20MHz], SUN SPARCserver 1000 [SuperSPARC 40MHz, 8 CPU]), laser video-disc recorder (LVR :SONY LVR-3000N), remote control stage of light microscope (DAI-ICHISEIMITSU Co. MICRO SCANNER), light microscope (OLYMPUS : BX50) and TV camera (OLYMPUS Ikegami ITC-380M). The NeXTcube with dimension board which has an Intel i860 (33MHz) dedicated RISC processor for 32-bit color graphics and video capture, is a main computer, controlling all the works: the microscope stage movement, the image recording and retrieval, three-dimensional modeling and rendering and so on. Heavy or hardware-dependent tasks are distributed to other workstations. The NEWS controls directly the LVR via RS232c cable. The SPARC server 1000 renders the three dimensional images. These tasks are controlled by the NeXTcube via TCP/IP protocols.

The first step of the three-dimensional reconstruction is to store the high magnified light microscopic images to the LVR from the serial sections as analog data. This process is semiautomatic. The scan area of each section is determined and sent to the main computer. Then the stage of microscope moves automatically step by step according to the area data, storing each image to the LVR and saving the image information to the main computer hard disk. In addition, the miniaturized whole image of the sections can be reproduced on the computer display as a montage image (Fig.2).

From a large number of stored images in the LVR, a nephron to be reconstructed is selected

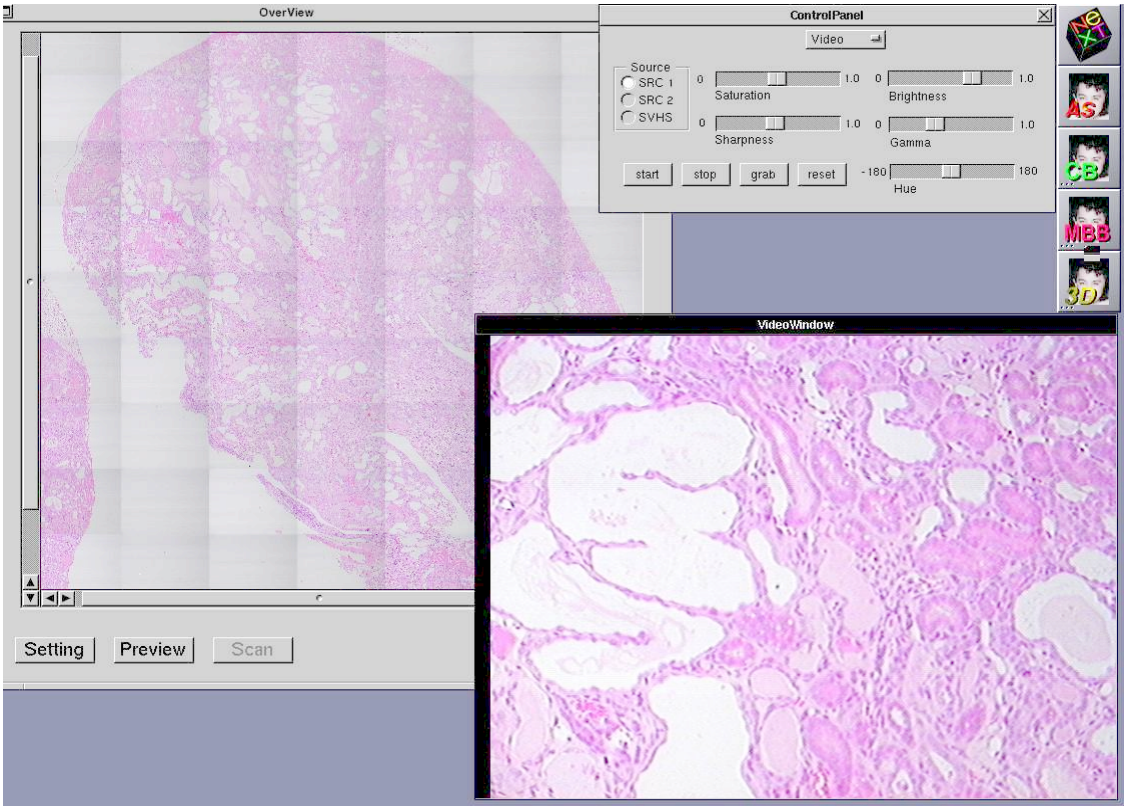

Figure 2: AutoScan.app. The top left window shows a momtage of a section of ICGN mouse kidney. The montage was created automatically. The panel (top right) controls microscope, LVR and NTSC signals. The video window (bottom right) displays an image from microscope or LVR.

and the outline is traced with a pointing device (mouse)(Fig.3). The outline is filled with metaballs [8] automatically by clicking the inside with the mouse (Fig.4). The metaballs are converted into "Image Score", in which the metaballs are clustered, piled up, added with the attributions of the metaballs to make the three-dimensional reconstruction image (Fig.5)[8] .

4 Applications

`Developed programs are as follows.`

1. AutoScan.app (Fig.2)

   This application controls the microscope stage directly via RS232C and LVR indirectly. The LVR control part of the AutoScan.app is a client program, which sends a control message over ethernet to the NEWS workstation, where a server program is run to control the LVR via RS232C. The location of the stage is reproducible within 10 micrometers. Whole montage images of sections can be produced, since the attribute data of each image is saved to the main computer hard disk such as the number of the images of a section, the absolute starting position of the stage, the relative position and the magnification of the image, and the LVR address of each image. When the montage image is clicked with the mouse, the clicked area of the image can be retrieved from the LVR and displayed in any size through analog digital convert.

2. ChainBuilder.app (Fig.3)

   ChainBuilder.app displays images stored in the LVR as analog data or in the hard disk as digital data, and makes outline data of the object of interest to save it as a "chain-file". There

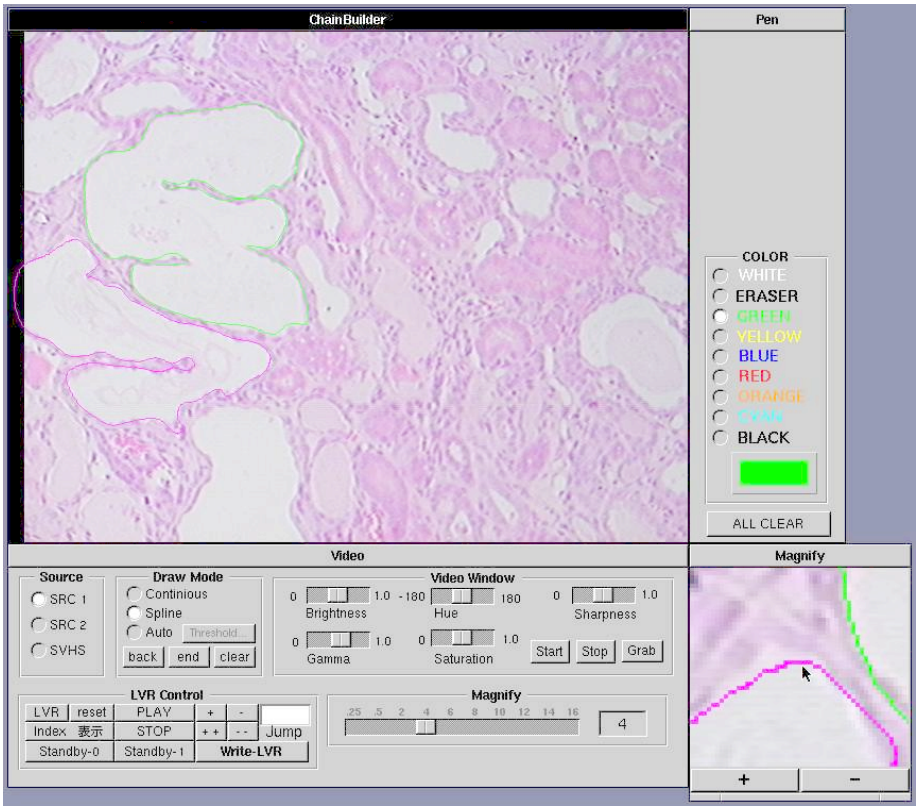

Figure 3: ChainBuilder.app. The outline of the dilated renal tubule has been drawn with a pointing device. The magnified image of the ChainBuilder window is displayed in the Magnify window (bottom right).

are three ways to trace the outline of an object. (1) Manual type: All dots of outline must be drawn with free-hand using the mouse. (2) Semi-manual type: Select the characteristic points of the outline, and the spaces between the points are extrapolated with a spline function. (3) Automatic type: Click the inside of the object of interest, and the outline is automatically drawn according to the difference in color. Though ideal, this is not so useful now, because this often works unexpectedly and needs a lot of manual intervention.

3. MetaBallBuilder.app (Fig.4)

   This software automatically fills the inside of the outline with metaballs by clicking the inside of the outline with the mouse. Until we developed this application, we manually placed the metaballs one by one which was a time-consuming process. A manual correction, however, is inevitable for accurate reconstruction. The metaball data is converted into a source file, the "Image Score" [8] .

4. 3DEditor.app (Fig.5 a.)

   This application edits and manages the Image Score [8] (the shape file of the metaball data made by MetaBallBuilder.app, the cluster file, the attribute file and the staff file), sends the data to the rendering computer via ethernet, and shows the four rendered images from different viewpoints (frontal, lateral, top and any angle) at the same time.

5 Results

Figure 5 b is the three-dimensional reconstruction image produced by this system. It shows the feature of dilated and meandered tubules.

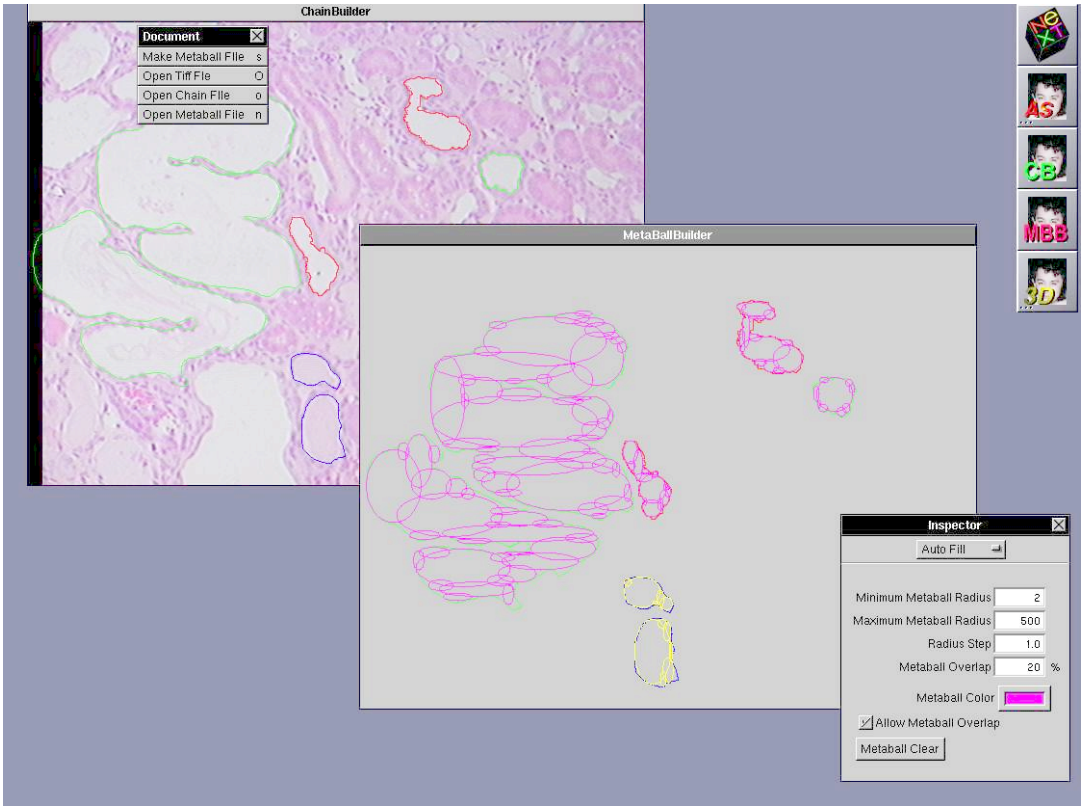

Figure 4: MetaBallBuilder.app. It can fill the ellipses in the closed curve. These ellipses will be metaball.

6 Discussion and conclusion

The characteristic point of this system is user (morphologist)-oriented. The high-magnified images of interest are immediately retrieved from the LVR by pointing the montage image. This enables us to check the consistency between the original two-dimensional data and the reconstructed image with ease. The methods of getting the outline and the data conversion into metaballs are much improved and the management of the image data and the Image Score is greatly facilitated for three-dimensional reconstruction.

Although much improved, this system still has weak points. The reconstruction method is very complicated, time-consuming and needs lots of manual intervention. Furthermore, if the section becomes warped or rotated, the reconstruction becomes difficult. About this point, the distortion is adjusted by researchers. Therefore, keeping this in mind, we must deduce biological conclusions from the reconstructed image.

The quality of the reconstructed image and the slow rendering speed will be improved in the future. We think that the concept and the integration of the system is most important. We have been progressively developing this system, all the while keeping an ideal and system in mind. Because this system uses NEXTSTEP and distributed processing, we hope this system is promising. Nonetheless, three-dimensional reconstruction still requires very complicated work. So, continuous efforts must be made on developing the ideal system for morphological study.

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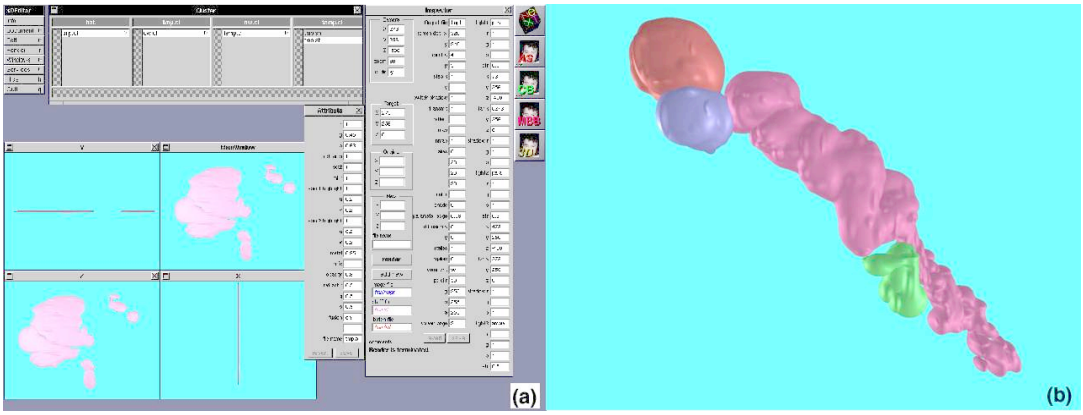

Figure 5: (a) 3DEditor.app. It produces four rendering images at the same time. (b) The reconstructed three-dimensional image of renal tubules of the ICGN mouse.

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