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Stereographic Semitransparent Images Reconstructed by Computer Graphics from Serial Microscopic Sections

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Contour lines of multilayered structures were extracted from microphotos of serial sections of a 10.5 day mouse embryo using a tablet and a personal computer. As the reference for reconstruction, a pair of base points were set on each microphoto. The hierarchical data structure was newly introduced in order that multilayered structures could be dealt with. The data were transferred to a minicomputer, and the contour planes were reconstructed by small triangular patches. Then calculation for a semitransparent display was performed. For an effective semitransparent display, the decrement of light, and the function of the angle between the line of sight and the surface normal were introduced beside the transmission coefficient. The reconstructed images were very comprehensive.

INTRODUCTION

Three-dimensional reconstruction from serial microscopic sections is important to make the structure of living organism easily understandable. But it is time-consuming to reconstruct an three dimensional structure. One of the traditional methods to reconstruct a specimen from serial microscopic sections is to draw contour lines of various structures, on wax plates, to cut on the outermost contour line, and to pile up the cut pieces. Recently, computer graphics have become available to aid such reconstruction. But most images thus reconstructed are those with either discontinuous contour planes or opaque contour planes. It is, therefore, rather difficult to deal with multilayered contour lines. Our procedure reported in this paper can present colored semitransparent stereoscopic images reconstructed by a computer, and can be applied widely to various field of research work and education.

MATERIALS and METHODS

Microscopic sections: A 10.5 day mouse embryo (4.4 mm in crown-rump length) was processed to 630 serial microscopic cross sections (7 μm in thickness) and stained with hematoxylin-eosin. If precise measurement is to be made on a reconstructed image, sections must be provided with some adequate absolute references. But it was desired that conventionally processed and preserved sections could be utilized, so no special absolute reference system was adopted.

The sections were photographed under a constant magnification (x10) with a constant exposure. Just after processing, we marked the photographic negatives for identification with a needle on their emulsion side.

Data structure: A characteristic "hierarchical" data structure was introduced in order that multilayered structures of living organism could be dealt with (Fig. 1) (Nakamae et al.: 1985).

On a microscopic section, each "contour line" is composed of a series of "contour dots". Contour lines assemble to form each "sub-object" which is a part of a certain structure with neither branching (bifurcation) nor confluence. Two different sub-objects, therefore, fuse at the plane of confluence to form the third sub-object, or vise versa. Several sub-objects which are continuous to each other make a certain structure which belongs to a certain "element". An element is an independent structure to each other with a certain character. Several elements assemble to form an "object", which is a multilayered structure as a whole to be reconstructed. For example, the respiratory system (larynx and trachea) is a sub-object which join with another sub-object, the digestive tract (esophagus) to make the third sub-object, pharynx. These three sub-objects belong to the same element.

Base points: On each photographic positive print (75 times magnification on the 8 x 10 format), two relative base points were put as follows (Fig. 2) as the reference for alignment of cross sections.

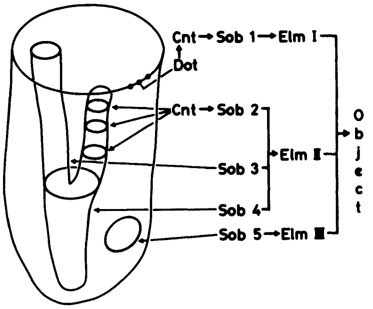

Fig. 1. Hierarchical data structure.
Dot: Contour dots.
Cnt: Contour lines.
Sob: Sub-objects.
Elm: Elements.
Object: The whole to be reconstructed.

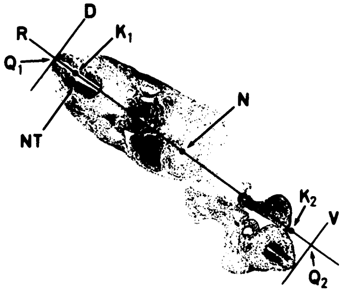

Fig. 2. Relative base points on a microphoto of a section.
NT: Trunk neural tube.
R: The line passing through the middle of NT (see text).
D, V: Perpendicular lines to R tangent to the section.
$Q_{1,2}$: Intersections of R with D or V.
N: Middle point between Q_1 and Q_2.
K_1, K_2: Relative base points.

1. A line (R) was drawn passing through the middle of the roof and the floor plates of the trunk neural tube (NT).

2. Two perpendicular lines to R were drawn from the dorsal and the opposite margins of the trunk. The intersections with R were Q_1 and Q_2.

3. The middle point between Q_1 and Q_2, N, was put.

4. On R, the two points (K_1 and K_2), 80 mm apart from N towards Q_1 and Q_2, were put to be the relative base points.

Input of the data of contour lines: According to the "contour sampler" program in disk BASIC, respective contour lines on each photographic print were extracted as a series of dots on a digitizer (Frontec, DT-1000) connected to a 16 bit personal computer (NEC, PC-9801). The interval between contour dots could be chosen from 1 mm to 5 mm by 1 mm step, in compliance with the curvature of a contour line. Each contour line was identified by a numerical code specific to each sub-object, combined with the number of the section. Then, the relations between contour lines, sub-objects and elements, including their branching or confluence, were specified in a sub-object table by means of numerical or alphabetical codes. These data were stored in 8 inch floppy disks.

Conversion and transfer of files: A file of the data in the disk BASIC format mentioned above were converted into those in the IBM format. Then, they were transferred to a mini-computer (TOSBAC, DS-600), rearranged in order, and subjected to computation for reconstruction.

Reconstruction of contour planes: Contour planes were reconstructed with small triangular patches generated automatically between the neighboring contour dots on the vertically adjacent contour lines after Christiansen's method (Christiansen et al.:1978) with some modification in order that more natural images could be reconstructed automatically (Nakamae et al.: 1985). A gap branch (Fig. 3), namely a bifurcation or a confluence, can be processed in the same way by horizontal triangular patches with the four terminal points specified automatically (Nakamae et al.:1985).

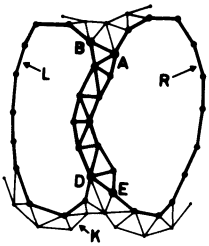

Fig. 3. Contour lines on the vertically neighboring sections showing a gap branch seen from above.
K: Lower contour line.
L, R: Upper contour lines.
A, B, C, D: Terminal points for horizontal triangular patches between L and R.
See element II in Fig. 1 also.

Calculation for a semitransparent display: After specification of the location of an imaginary light source and that of an eye, the ray tracing calculation about each triangular patch was done. For an effective semitransparent display, following three factors were introduced; a transmission coefficient of each contour plane to represent multilayeredness, the decrement of light according to the optical path in the imaginary surrounding medium to enhance the feeling of depth of the structures, and the function of the angle between the line of sight and the surface normal on each triangular patch so that the contour of the structures can be understood easily by displaying the peripheral portion of the images darker (Nakamae et al.:1985). After smoothing, the result of calculation was displayed on a graphic display (Graphica, M-508) and photographed. A pair of photos for stereoscopy could be obtained by another series of ray tracing calculation after specifying horizontally a little shifted eye position from the first one.

RESULTS

A macrophoto of a 10.5 day mouse embryo at the same developmentalstage as the reconstructed one is shown in Fig. 4. The right side view of the laminated contour lines by using the relative base points is shown in Fig. 5, in which enveloping outermost contour is in good accordance with the general view of an actual embryo (Fig. 4). A stereopair of the reconstructed whole embryo is shown in Fig. 6. Another stereopair representing the median cut of the reconstructed image is shown in Fig. 7. An example displaying the three-dimensional scale is shown in Fig. 8.

DISCUSSION

Three-dimensional reconstruction of multilayered structures of living organism by observation of serial microscopic sections is an essential step for morphology. It is, however, not so easy especially for beginners to perform this work in their brains. In order to decrease this difficulty, various methods of reconstruction have been attempted. One of the conventional and popular methods is the reconstruction by piling up cardboards, wax plates or some other adequate materials. The elaborate examples are introduced in a textbook of embryology edited by Okamoto (Okamoto et al.:1983). This method has an advantage that no other special equipment than a microscope is needed, but the presentation of multilayered structures is very much restricted. This restriction is recently a little lightened by utilization of transparent plastic plates for the piling-up material (Jong:1984).

Recognition of three-dimensional multilayered structures is greatly helped by drawing also. An interesting method of drawing oblique views was proposed by Claeys and Aerts (1984), in which the correct proportion of respective structures are kept well by successive projection of serial microscopic sections. It is, however, somewhat difficult to present many kinds of structures at a time by this method.

Recently computer graphics have been introduced into the reconstruction of three-dimensional images. One of the remarkable features of the computer-aided reconstruction is that a pair of images for stereoscopy can easily be obtained. Some kinds of software for reconstruction using personal computers are commercially available. Most of them reconstruct images as the piling up of contour lines without smooth contour planes between them. Reconstruction of smooth contour planes of multilayered structures usually requires further calculation which are practically beyond the ability of personal computers. Thus, for example, Baba et al. (1984) reconstructed the images with smooth, but intransparent contour planes using a computer of a larger scale.

It is essentially desirable, however, that reconstructed contour planes are semitransparent in order that multilayered inner structures can be observed thoroughly. At the same time, the data structure must be such one that enables us to deal with multilayered contour lines. Thus, the hierarchical data structure and the argorism for a semitransparent display were proposed by Nakamae et al. (1985).

Another important problem for reconstruction is the base points as a reference for alignment. The most simple and sure way is to put some adequate material or a pair of marks together with a sample material in the embedding medium. By using a sharp laser beam, Thompson et al (1983) drilled a pair of minute holes as the spatial reference through the metacrylate block containing a specimen. Claeys and Aerts (1984) embedded a smoothly cut liver piece as the reference just beside a specimen. Such absolute references are essential if some precise measurements are to be done on the reconstructed images, but the serial sections must be prepared specially with some reference markers.

It sometimes occurs that the reconstruction is desired from conventionally prepared serial microscopic sections without any absolute reference. One solution is that each microphoto is placed on a tablet so that the outermost contour line is in adequate position compared to that of the preceding one. This is an effective way when the structures to be reconstructed is fairly simple without twisting of the major portion. The other solution is to set a pair of relative base points as the reference for reconstruction. The method adopted in this paper (Fig. 3) was proved to be practical and effective to reconstruct good images (Fig. 4). Of course, detailed procedure to set relative base points must be individually considered case by case. Major structures of higher animals, however, have some kinds of symmetry which we can fortunately utilize for setting relative base points. More serious difficulties might arise in dealing with such minute structures as those in electron microscopy, which must be considered differently.

The reconstructed images with smooth, semitransparent and colored contour planes are very attractive and comprehensive. They are very helpful for beginners of morphological study to understand not only three-dimensional structures as a whole, but also their spatial interrelation especially when they are viewed stereoscopically. Moreover, "new" cut surfaces of the reconstructed object, different from the serial microscopic sections, can be made(Fig. 7). For example, median cut which is anatomically significant can be get from cross sections which are also significant anatomically. It is also possible to put the location of eyes "inside" a reconstructed object. Such features are very effective not only in education but also in analyzing abnormal development of living organism. Motion pictures can be made from the photos of successively rotated images of the reconstructed object, or of successively selected elements. The images of reconstructed objects are easily reproducible photographically for many students. These major features, together with other various ones, make the reconstruction method produce excellent materials for teaching anatomy and embryology, which can be effectively utilized in a stereophoto-teaching system (Yasuda and Sato:1981).

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Explanation of figures.

Fig. 4. Right lateral view of a 10.5 day mouse embryo (bar: 1 mm).

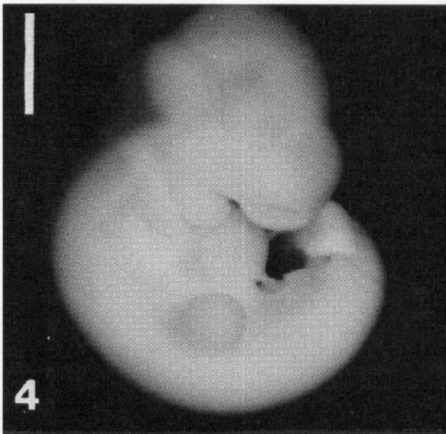
Fig. 5. Right lateral view of the reconstructed image by piling up every 20 contour lines according to the relative base points. The outermost enveloping contouris in good accordance with that of Fig. 4.

Fig. 6. Stereopair of the reconstructed whole embryo in right anterior oblique view.

Fig. 7. Stereopair of the "new" median cut half of the reconstructed embryo (see text).

Fig. 8. Reconstructed image displayed together with the scale indicating the level of every hundredth section from below.

(Fig. 6 is printed on Plate III at the opening of this volume)



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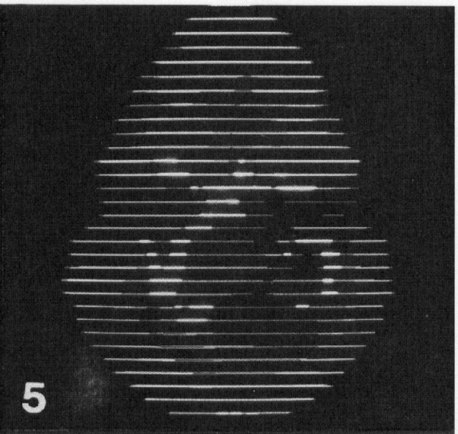

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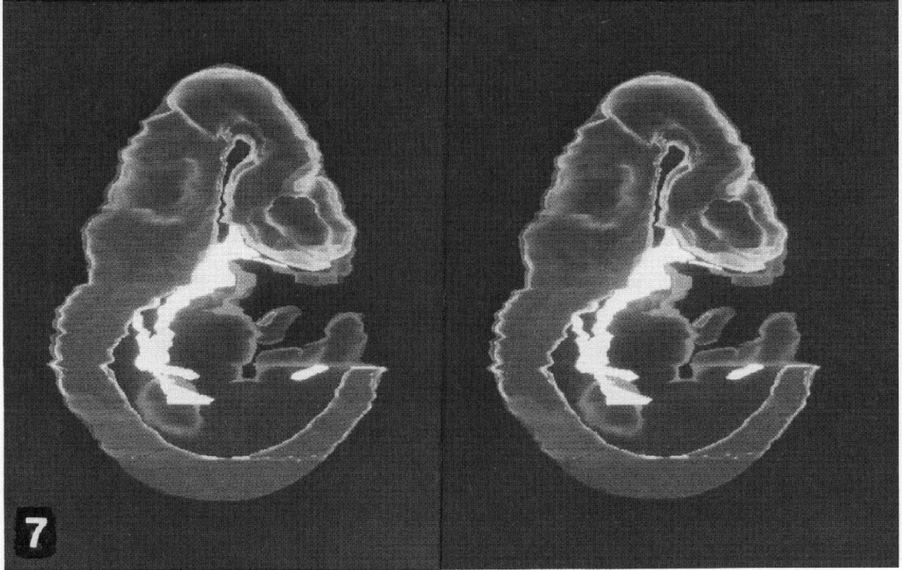

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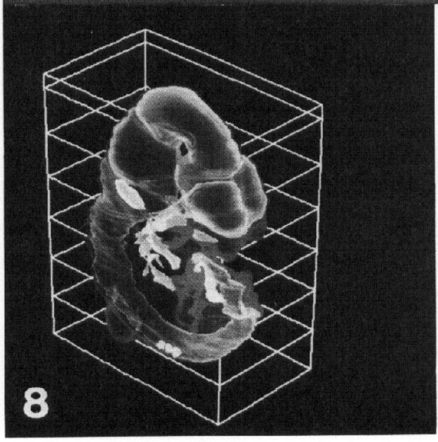

8