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COMPUTER GENERATED THREE DIMENSIONAL RECONSTRUCTIONS FROM SERIAL SECTIONS

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ABSTRACT

A computer program is described which utilizes the X,Y co-ordinates describing outlines traced from serial sections to produce three dimensional reconstructions of the original sectioned specimen. Examples of output from the program are given using data obtained from a stereotaxic atlas of the human brainstem and cerebellar nuclei. The production of stereo pairs allows three dimensional visualization, with or without hidden line removal, of any desired structure or structures making up the original specimen. There is the facility for viewing the reconstructed specimen from different directions.

INTRODUCTION

Three dimensional reconstruction from serial sections is usually undertaken to try and elucidate a particular feature or features of the sectioned specimen which cannot be easily resolved by a study of the individual sections. Many techniques are available for producing both graphical and solid reproductions of sectioned objects (Gaunt and Gaunt,1978), but these are time consuming and yield only limited information.

The three dimensional conception of the anatomy of the human brainstem has always proved difficult because of the large number of nuclei and tracts passing through its length. Afshar et al (1978) have produced a detailed stereotaxic atlas of the human brainstem and cerebellar nuclei which shows a statistically produced composite atlas of transverse sections at 1mm intervals in which are drawn the major nuclei and tracts from the cervico-medullary junction to the upper mid-brain level.

The present paper describes a computer program which converts the essentially two dimensional data available from serial sections into a three dimensional form whereby any structure or structures can be viewed from a variety of directions which is not limited by the original plane of sectioning.

PROBLEMS OF ALIGNING SERIAL SECTIONS

In any three dimensional reconstruction from serial sections, two important criteria have to be satisfied before accurate reconstructions can be contemplated. The first is that there must be at least two reference points in each section which can be used to accurately align each section relative to its neighbour in the plane of the section (the X,Y plane). This has been achieved in the past for soft tissue specimens by incorporating in the blocks external markers such as nerve fibres etc. (see Gaunt and Gaunt,1978). For those specimens which do not have external markers, it is sometimes possible to use internal structures which may not vary much in position in going from one section to the next section. For those serial sections which are not covered by the two examples quoted above, the last and least accurate method for orienting each section is to photograph each section, transfer outlines to tracing paper and then superimpose by 'eye' for the best fit. Two crosses can be then put on each tracing and these can be used as the reference points. For hard tissue specimens which can be sawn, the method described by Sullivan (1972) whereby two parallel and one diagonal groove are milled into one external face of the block is best. On sectioning, the two parallel fiducial markers can be used to align each section relative to its neighbour.

THE Z-PARAMETER PROBLEM

For a three dimensional reconstruction the Z-parameter or distance between sections, is required. For soft tissue sections one usually knows the section thickness and provided no sections have been lost in the series, a Z-parameter can be obtained. Thus, for serial sections which are all 10 microns thick, the Z-parameters for sections 1,2,3 etc. are 0,10,20 etc. For sawn serial sections where there is considerable material lost between sections the method described by Sullivan (1972) is the best possible solution. The position of the diagonal fiducial marker in each section is dependent on where the section was in the original block. Thus, the relative position of this diagonal marker with reference to the parallel markers gives the Z-parameter for each section. For other serial sections which do not have fiducial markers, a knowledge of the amount of material lost between each section (which is dependent on the thickness of the saw) and the thickness of each section, is required before a Z-parameter can be obtained for each section.

X,Y CO-ORDINATE TRACING OF THE BRAINSTEM

The serial sections published by Afshar et al (1978) were ideal for three dimensional reconstructions. They were transverse sections 1mm apart and each section was referenced to fixed reference planes and points. In all, 54 sections were digitised using an X,Y co-ordinate plotting microscope (Afshar and Dykes, in press).

DATA REQUIRED FROM SERIAL SECTIONS

Figure 1 gives the flow chart for the data required for the computer program. At this point, as described above, it is assumed that all X,Y co-ordinates from each serial section have been scaled and rotated to a common origin and magnification, and that a Z-parameter for each section is known. The standard distance (Fig.1) is the absolute value of 1000 of the units used for X and Y. Each section traced is allocated a section number (an integer) and any structure traced within that section is known by a particular line number (another integer). All the outlines traced in each section must be closed outlines (i.e. the first X,Y co-ordinate is the same as the last X,Y co-ordinate) and can be traced in either a clockwise or anti-clockwise direction, provided the sense of tracing is consistent for all outlines and all sections. Outlines should be traced so that there is a small gap between the first X,Y co-ordinate and the last one, as the program automatically adds an extra X,Y co-ordinate at the end which is identical to the first one. One extra X,Y co-ordinate (X=400.0, Y=0.0) is required at the end of a traced outline so that when this co-ordinate is detected the program will go on to read the next line number followed by the co-ordinates defining that particular outline. Should there be no more outlines to trace in that particular section, a line number equal to - 700 is allocated to signify that the data from a new section is to be read. If the new section number is equal to -800 then this signifies that there is no more data to follow.

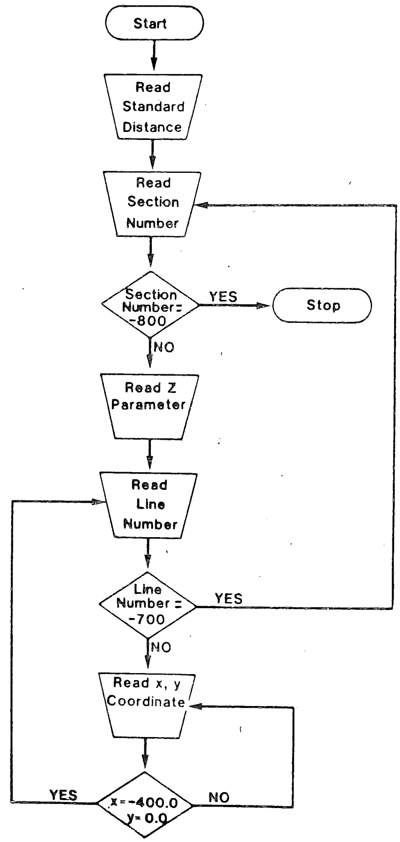


Fig.1 Flow chart giving the order of the digitised co-ordinate data. This data is called for in the main three dimensional reconstruction program.

Figure 2 gives an example of a computer generated output of what was actually traced from one of the serial sections given by Afshar et al (1978). Each structure in the section has been allocated a different line number. Since the sections given were from one side of the brain-stem, we computer 'generated' the other half (Fig.3) as a mirror image of the left hand side.

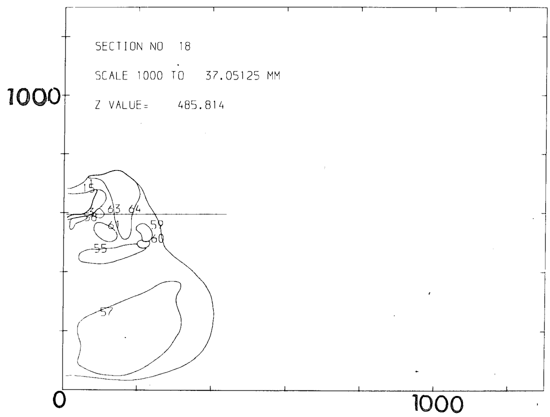


Fig.2 Computer generated drawing of section number 18. This has been drawn using the data digitised from the original (see Afshar & Dykes, in press).

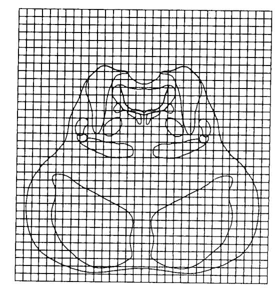


Fig.3 Computer generated drawing of the same section as shown in Fig.2 after the missing half has been computer generated.

DESCRIPTION OF PROGRAM AND EXAMPLES OF OUTPUT

The program to produce three dimensional reconstructions from the outlines traced from serial sections has been written in Fortran and runs successfully on a CDC 6600 machine at the University of London Computer Centre. It uses several microfilm plotting sub-rountines available from the library of descriptive instructions for microfilm plotting (DIMFILM) currently available at ULCC. In addition to providing views on microfilm, the program allows views to be written on 8 inch disks so that the data can be used by micro-computers to enable colour graphics to be used.

Each three dimensional structure characterized in a section by a line number can be drawn with or without hidden line removal. All examples to be given here have hidden line removal applied to each separate structure.

There are two selectable modes of operation for the program. The first (Fig.4) allows one structure to 'hide' an overlapped structure. The second mode of operation

(Fig.5) allows the hidden or overlapped structures to be viewed through an overlapping structure.

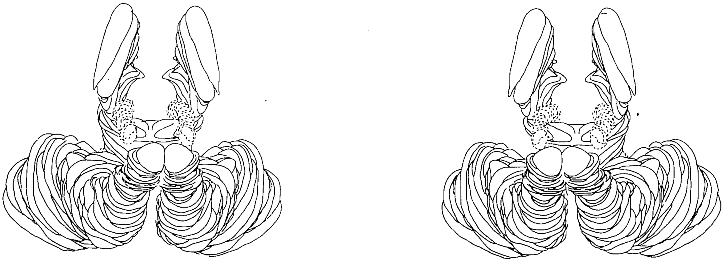


Fig.4 The red nuclei, superior cerebellar peduncles, central tegmental tracts and corticospinal tracts have been drawn. This is a stereo pair with an interocular angle of 6°. One structure 'hides' any structure it overlaps.

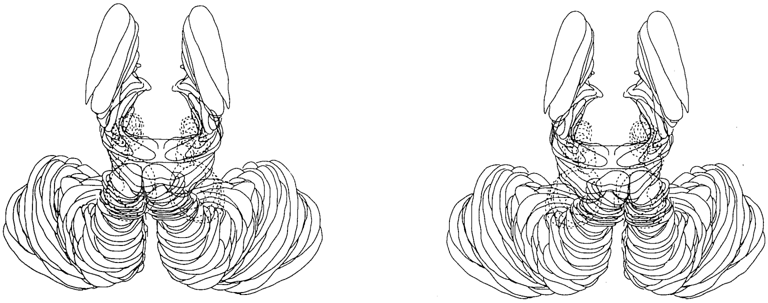
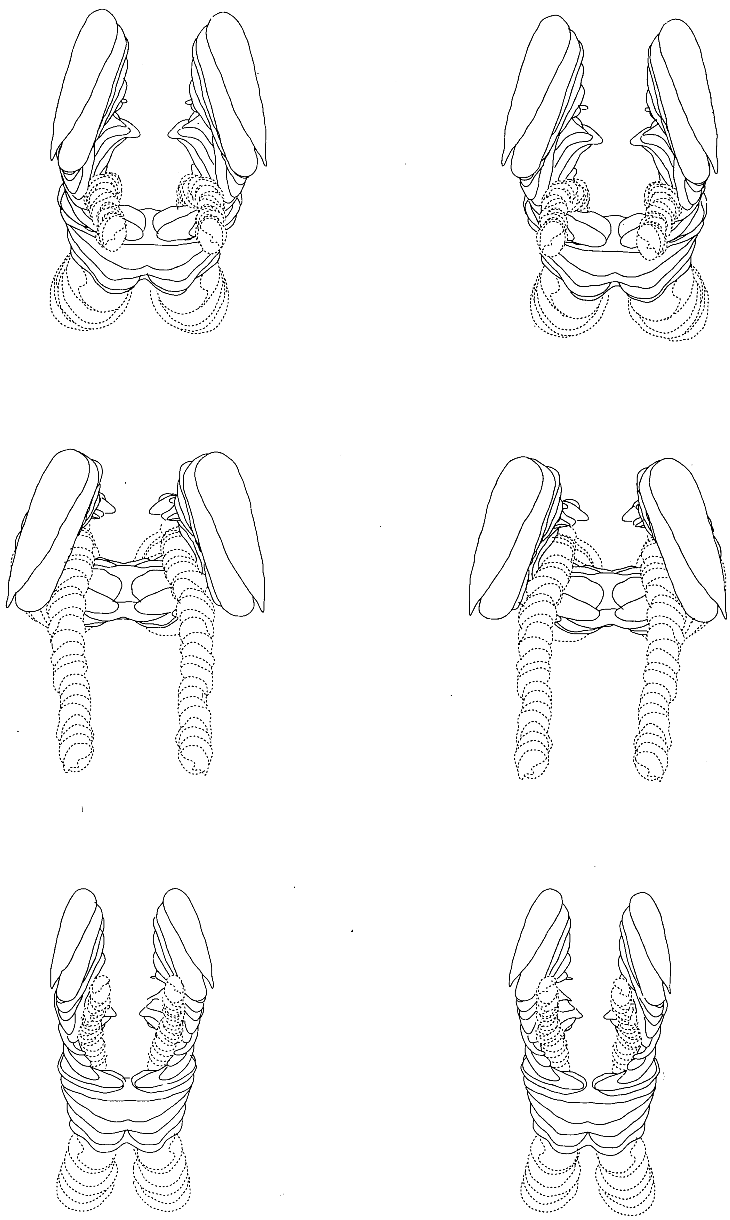


Fig.5 The same as shown in Fig.4 but this time one structure can be viewed through an overlapping structure.

The original X,Y co-ordinates can be rotated about three orthogonal axes by specifying the angles of rotation required (Fig.6). However, angles approaching 90° are not recommended as this is equivalent to viewing the section on edge.



(a)

(b)

(c)

Fig.6 The same structures as shown in Figs. 4 and 5 minus the corticospinal tract. (a) shows the original data while (b) and (c) are after a +30° and -30° rotation about the X-axis.

At present there is no upper limit to the number of separate structures that can be drawn (Fig.7) but difficulty in distinguishing structures becomes more apparent the larger the number of structures. At present only black and white microfilm is available at the University of London Computer Centre, but the ability to transfer the data to micro-computers with colour graphics is an added advantage as colour coding helps in the interpretation of views with large numbers of structures.

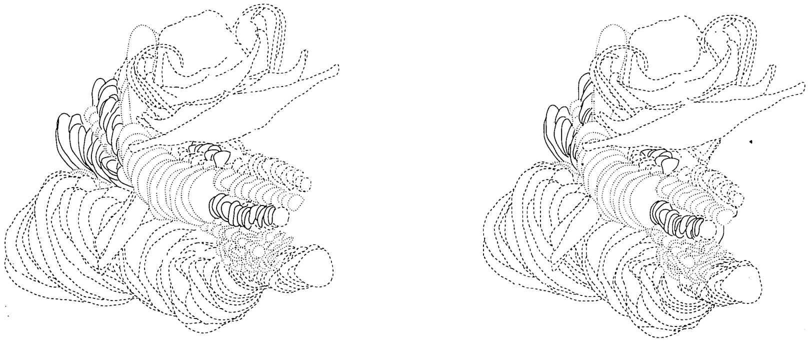


Fig.7 Stereo pair of 30 structures traced from the data supplied by Afshar et al (1978). The tracts and nuclei from mid-line and one side of the brainstem have only been drawn with hidden line removal between structures being applied. This view is after a 15° rotation about the X-axis followed by a 15° rotation about the Y-axis and finally a 15° rotation about the Z-axis.

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