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3-D Reconstructions for graphical databases of gene expression

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Modern techniques in molecular biology are providing a great deal of information about the genetic control of embryo development, particularly about the patterns of gene expression. Advances in computing technology now make it feasible and affordable to reconstruct these three-dimensional (3-D) patterns onto 3-D representations of the embryo for detailed comparison and analysis, and their visualization provides considerable insight into the networks of gene activity. Here, we discuss methods for reconstructing such data from serial sections of tissues and discuss approaches to aligning adjacent sections, removing sectioning distortions and delineating the final patterns on their embryo background so as to optimize the quality of the reconstructions.

Key words: 3-D reconstruction / gene-expression / image database / segmentation / visualization

IT IS NOW RECOGNIZED that 3-D reconstruction of embryonic material is an indispensable tool for comparing patterns of gene expression with anatomical and functional information in the developing embryo. The gene-expression data is generally obtained by staining the mRNA or protein products on sectioned material. To maximize the analysis of such data, we must reconstruct the original 3-D pattern from the 2-D expression domains on these sections.

There are many 3-D reconstruction packages that allow the visualization of individual marked structures by contour delineation and displaying the subsequent geometric objects. In these methods, however, the original (grey-level) image data is lost and only the structures selected during the initial phase can be viewed. Furthermore there is no means of displaying the highlighted structures relative to the original histology or cellular structure that was visible on the stained sections. In this paper, we discuss techniques that allow these section images to be digitized and aligned and used as a reference image (e.g. in a database[1-3]).

In practice, the making of such digital representations involves image capture and alignment, structure delineation, 3-D visualization and database integration. These issues are discussed in turn in the light of the experience of the authors as part of the European Science Foundation's Embryological Databases Network. The purpose of this short paper is not to provide a complete survey of 3-D reconstruction techniques, but to discuss practical issues that are important for grey-level reconstructions and delineation in gene-expression databases.

Image capture and alignment

The fundamental problem with 3-D reconstruction from section images is the realignment of the sections; this is because the process of sectioning destroys the 3-D spatial relationship between them. This alignment must therefore be inferred from the visible structures or from some form of fiducial markers (e.g. fixed, corresponding points in each section). Other important considerations for reconstruction include correction for shading (e.g. uneven illumination[4]) and variations in staining density (e.g. from variable section thickness). Although these are important if a 3-D histology image is required, for consistent results from automated methods, and when multiple images of each section are to be 'patched' together, space limitation precludes our discussing them here.

There are two main problems in capturing aligned images. The first is to display the disparity between successive images in good enough 'real-time' to allow easy alignment. The second is to provide a mechanical system that allows the user to control the slide translation and rotation to sufficient accuracy and so realign the images. The first problem can be solved by

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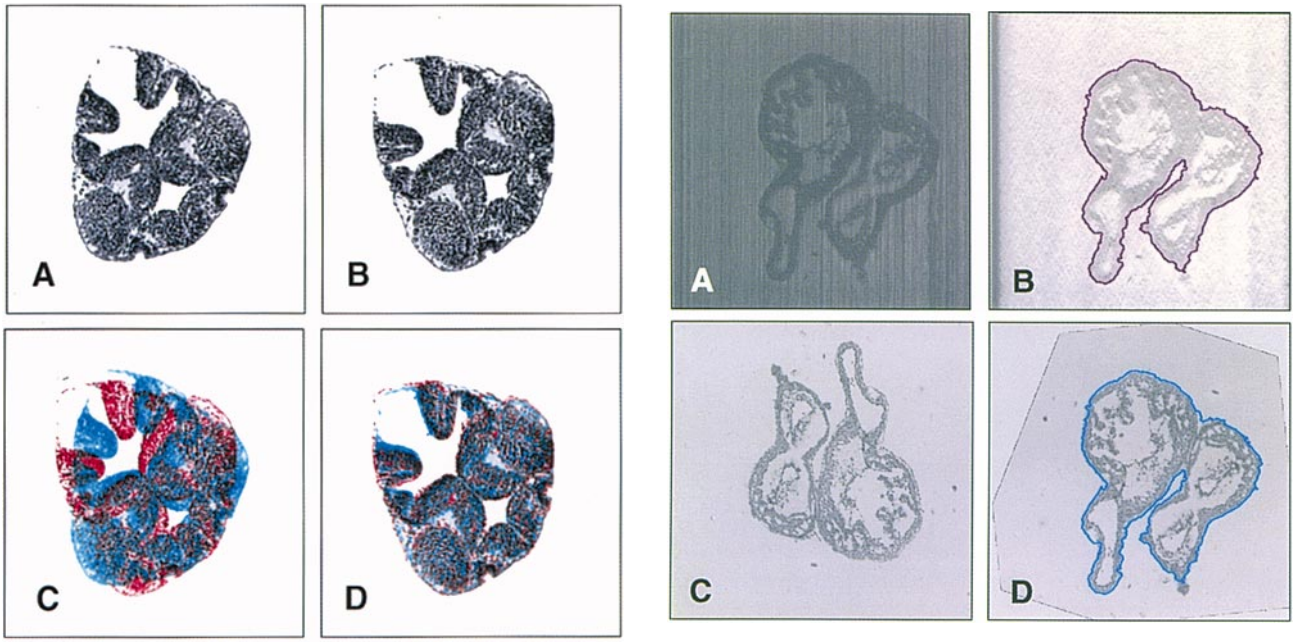


Figure 1. Alignment of grey-value images of the zebrafish embryo. Image A is to be aligned with image B. Image C shows the overlaid images before alignment and image D after alignment. The transformation is calculated by image correlation.
Figure 2. Alignment of the diascopic image (C) to the episcopic image (A) of a section through the heart of an E1 rat. Image A is processed to remove noise from the sectioning burrs and to define the tissue outline shown in image B. The original image (C) is mapped to the correct size shape and orientation using the boundary (D).

displaying a combined image in which the new image is in one colour and the registration image in another. This technique can be implemented in hardware[5,6] or software.[1] Figure 1 shows an example of using an image overlay technique to demonstrate alignment.

One solution to the second problem, that of controlling translation and rotation of the new image, is to use a gliding stage[5] which provides good control for both translation and rotation. Another is to use the translation and rotation controls on a normal stage to bring the section into near registration and then precisely align the image digitally. A motorized stage (translation) and camera (rotation) will allow precise control of the alignment[7] and makes fully automatic alignment possible.

Fiducial markers make alignment more objective. Weninger *et al* [8] describe a technique of drilling the embedding medium on a known grid, then using the images of the holes to align the sections automatically. This is effective for plastic embedding, but cannot be used for wax-embedded material or historical sections. Furthermore the use of a limited number of fiducial points can only correct the systematic distortions arising from the sectioning. If the reconstruction is for resectioning in planes not parallel to the original microtome sections, then alignment by global rotation and translation may not be sufficient because of the random distortions caused by histological processing.

There are two basic approaches to correcting random distortions. The first is to capture an image before sectioning of each section and to use this both

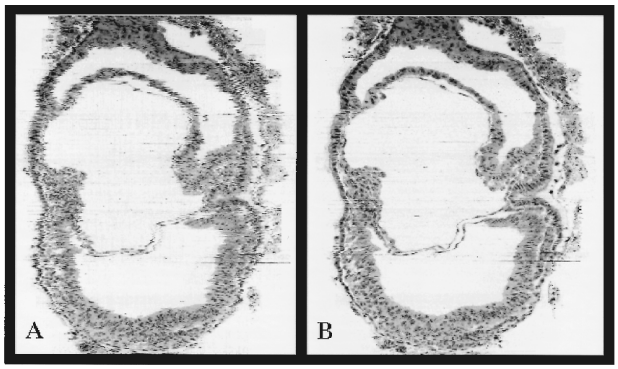


Figure 3. Two images of a vertical section through a reconstruction of the E7.5 mouse embryo. The image on the left is before and the image on the right after automatic distortion correction (warping).

for alignment and for distortion correction. An effective way of doing this[6] is to use a horizontal microtome on which a camera is mounted. The system captures an episcopic image from the end of the block and, because this will always be in a known position and orientation, it can be used to correct both the alignment and random distortions in the diascopic or section image. This process is illustrated in Figure 2. (A variation on this method is to use fiducial markers[8] to obtain the approximate alignment and then make small local corrections using image congruencing.)

The second approach assumes that the tissue boundaries formed smooth 3-D surfaces before sectioning and a program then digitally warps the images to satisfy this criterion. This approach[8] uses a fully-automatic matching and warping technique. The basic idea is to model each section as a thin elastic sheet and to use image analysis to match each image with the two adjacent images in the stack. This matching process defines a set of 'forces' which acts to align structures in the image, with the required distortions being calculated using the Finite Element Method. Figure 3 shows the result of this methodology. While this method can be applied to any sectioned material, it cannot recover systematic distortions and may introduce reconstruction artefacts. Nevertheless, if the required result is a representative embryo, then this may be sufficient.

Delineating structure

Once the 3-D reconstruction has been made, its separate tissues can be digitally identified, the embryo is thus segmented into recognizable regions or domains. These regions can be labelled and visualized, stored in a database and used for comparison and analysis. The delineation process, referred to as painting, can be very time-consuming, unless special staining is possible.[8] Here, we describe manual and semi-automatic methods that have been developed in the Netherlands (TDR-3Dbase), Edinburgh (paint) and Strasbourg (nsurfx). The delineation process can be done using a computer mouse or trackball, or via a graphics tablet using a pen or puck. The tablet has the advantage of a high sample resolution and gives a more natural feel to the drawing process.

TDR-3Dbase

This system generates a contour model using a digitizer tablet, with the contours being stored in a geometrical database that can convert to other geometrical representations.[6,15] TDR-3Dbase consists of an input module for rudimentary work in the contour representation and a module to manipulate the other geometrical representations. Structural and geometrical information is defined in a database that is set up at the beginning of an input session and is extended as needed. Contours are drawn using the tablet for each section (e.g. on a screen image or a photograph) and displayed as overlays on a background of the section image and appropriately labelled. Contours from non-aligned images can be registered on the basis of two reference points.

Paint

This program has the particular advantage that delineation is possible within a section plane of arbitrary orientation within a 3-D reference image (Figure 4), so allowing a painted region to be immediately displayed in any orientation. In addition to drawing (regions defined by contours) and painting (region defined by a 'paintbrush'), the program includes tools designed to facilitate the delineation process.

- Geometry object. This allows interactive placement of geometric shapes (e.g. marking cells).
- Threshold. When an image pixel is selected, the grey-value at that point defines a set of connected pixels that all lie within a range of grey-values close to the selected value. This range can be extended interactively and the region added to or subtracted from the current domain.
- Fill. After several tissues have been delineated, the image may have a number of disconnected regions. Fill allows the user to flood-fill these regions.
- Affine transform. Selected painted regions can be translated, rotated or re-scaled.
- Morphological operations. Image-processing operations, based on erosion and dilation, can be used to smooth the boundaries and to remove small holes or small regions.
- Image tracking. This uses the image data from a region defined on an adjacent section to automatically match the boundary on the current section. The contour from the adjacent section

Figure 4. Screen shot of the paint program. Two views are visible with the plane orientations indicated by the yellow lines in the 3D feedback display. A number of domains have been defined and are also displayed in 3D. The left-hand window shows how the wash overlay regions allow easy review of boundaries for correction.

is used as a starting point to search for a best match boundary. After the new contour has been automatically generated, the user is prompted to confirm and possibly edit the match.
- Edge tracking. This is similar to image tracking, except that the matched feature is edge-strength (in image processing, this is defined to be the grey-level gradient or slope) and the contour will be matched to regions of high edge-strength.
- Domain-review. This tool allows a user to rapidly review and assign independently defined domains. It is useful for reviewing the output from automatic segmentation methods.

Four domain management features that have proved especially helpful are allowing:

- the user to control the resolution of drawn overlaps of adjacent structures so that common boundaries are only drawn once;
- contours on one section to be propagated to the next for minor editing, rather than complete redrawing;
- temporary 'lifting' of the paint to inspect the structure underneath;
- the paint to be made translucent.

Nsurfx

Nsurfx has similar features to paint and includes tools for data analysis and measurement. Additional techniques that have proved useful for delineation are the following.

- Normalization. This allows the user to modify image contrast and so make structures easier to recognize.
- Image enhancement. The program provides a mechanism for controllable edge enhancement to highlight boundaries.
- Colour LUTs. The look-up table (mapping from image values to display colour) facility enables colour to be used to enhance image structures.

Both nsurfx and paint provide windows of arbitrary magnification. Users have found these helpful for accurate positioning of boundaries. Paint provides 'magnify-on-demand' in which the user creates a magnify window as required for a drawing task. Nsurfx uses the notion of a single permanent magnified view which is always centred over the region of interest. Actions in one window are propagated to the other. Experience suggests that this latter mode is more efficient and easier to use.

Visualisation

After a 3-D structure has been defined, it can be visualized by sectioning, by contours and surfaces, and by direct volume rendering. The choice depends on the purpose, application and hardware available. A key constraint is that the software should run fast enough to be used interactively unless high-resolution images can be generated 'off-line' for later review. There are many visualization packages available, e.g. IRIS-Explorer, AVS, Voxelview, VolVis and PHIGS.

Sections

If grey-level images have been retained in the 3-D reconstruction, the user can browse interactively through the volume examining successive histological sections at any orientation. Simply panning through the volume in any direction allows the viewer a rapid and detailed view of the spatial relationships between complex structures that cannot be done under the microscope. This is different to the 3-D visualization discussed below because it presents the data in the more familiar mode of histological sections, without the difficulties of analysing successive sections under the microscope. The anatomical or gene-expression domains that had previously been delineated can now be displayed either as transparent or solid overlays on the histology (e.g. Figure 4).

Contours and surfaces

The most common form of 3-D visualization displays the structures as geometric objects with the appropriate 3-D perspective; with hardware support this is a fast procedure. The objects can be viewed from any position to reveal the 3-D structure and spatial relationships, and displayed in many ways (e.g. as contours, filled planes, triangulated surfaces or simply a scatter of points[2,5,7,8,10-12]). There are many visualization packages available for this type of display. Figure 5 shows the range of display techniques available with the 3DBase serial section database system. Figure 6 shows a surface display of the developing tooth[10] using SunVision (Sun Microsystems, CA, USA) and Figure 7 shows surface

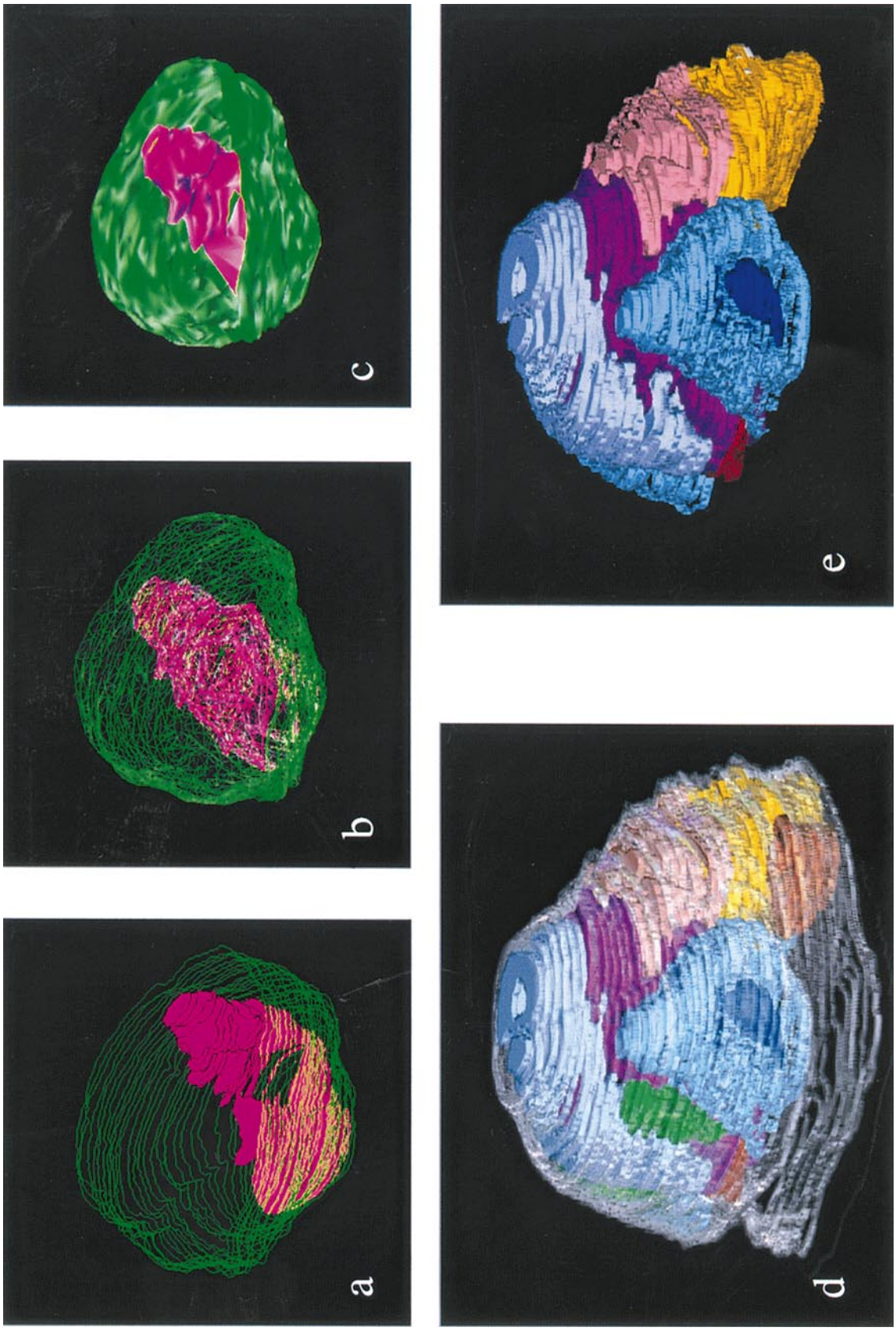


Figure 5 (see caption on facing page).

rendered structures using AVS (Advanced Visual System Inc., MA 02154, USA).

In each case, the colours, lighting conditions, shading and surface properties can all be adjusted and this not only allows the user to control visualization, but also provides visual cues to give an apparent 3-D effect. This is more realistic with motion cues, and movement under interactive control is one of the most powerful methods for understanding 3-D structure. If the workstation cannot render the scene quickly enough, then a good solution is to record a series of views and play them back as a movie, although the user can no longer interact with the sequence.

Another important means of providing 3-D visualization is through stereo. This can be achieved with red-green offset images viewed with red-green glasses or as two adjacent images to be merged using stereo viewers. The best technique, however, uses special glasses (CrystalEyes, StereoGraphics Corp, San Rafael, CA 94901, USA) synchronized with the screen display to provide left–right views of the 3-D structure to each eye.

Volume rendering

Solid and rounded 3-D structures can easily be visualized as geometric surfaces, but other patterns are more difficult to visualize and for these direct volume rendering can provide a good alternative. Most methods start with the technique of ray-tracing and can result in a rendering that ranges from an 'X-ray' absorption view to a mixture of cloud-like structure and sharp surfaces.[13]

Although volume rendering is often slower than visualizing geometric structures, it does allow visualization of structure without the need for segmentation, i.e. the time-consuming stage of delineation can be avoided. A second benefit for gene-expression data is the possibility of producing 'whole-mount' style images, perhaps with some selected structures highlighted for comparison with an observed whole-mount staining pattern (see Figure 7).

Figure 5. 3D reconstruction of the head region of a 48-hr. zebrafish embryo (52, 7 μm paraffin sections) in three different geometrical representations. Images of Fig. 1 are used to generate the models using TDR-3Dbase, i.e. contour (a), surface (b,c) and volume (d,e). The identified structures are based on sections oblique to the AP axis of the embryo. a) Contour model with ectoderm (green) and diencephalon (magenta); effective use of hidden lines for diencephalon simulates transparency. b,c) Surface model of the same selection as 5a, but without and with Gouraud Shading resp. d,e) Volume model, with ectoderm (transp.) telencephalon (lb), diencephalon (magenta), metencephalon (salm.), myelencephalon (y), otic vesicle (brn), eye (cyan) and lens (bl), nose (g) and stomodeum (r). e) A subset of 5d.

Databases

The purpose of the reconstruction techniques described here is to provide the framework for analysing and comparing information from many experiments, either on the same embryonic material or mapped onto a common spatial framework from other embryos. To manage this information and make it searchable, it is necessary to introduce a database management system (DBMS). This is the approach taken by Verbeek *et al* [2] for managing a database of the section material of an individual embryo, and underlies the design of the mouse atlas and gene-expression database[1,3,14] being implemented at Edinburgh, UK, and the Jackson Laboratory, USA, to provide an Internet-accessible database of mouse embryo anatomy and mapped gene-expression data.

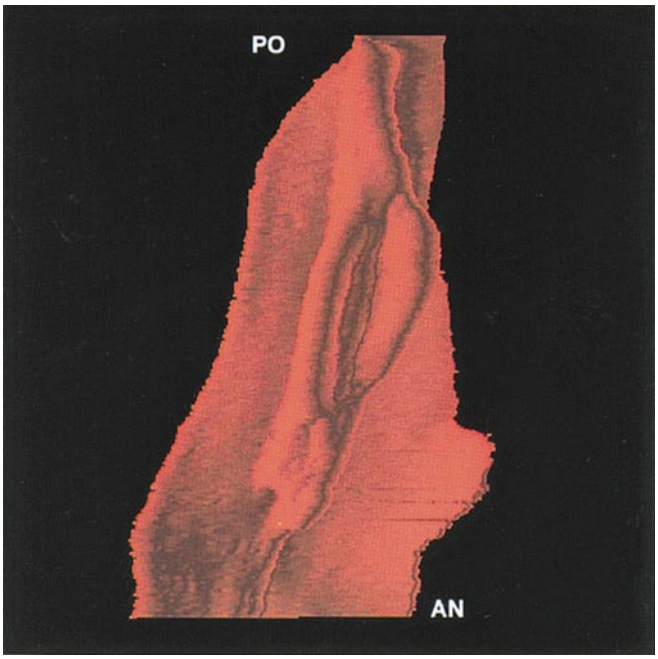
As the underlying spatial framework on which all image information is based is a regular array of image sample points, a voxel model is the natural format for the data and also represents the basic resolution; as Verbeek *et al*[2] point out, however, the voxel representation is inappropriate for all database uses (especially visualization) and other representations, such as contour and surface models (see Table 1), are needed. Clearly an important requirement of the system is the ability to convert from one representation to another. Algorithms exist for each transformation, but their use may lead to a loss of information.

If a database is required to hold information from sections cut at arbitrary orientations then it is clear from Table 1 that an underlying voxel representation is required. If the sections can be guaranteed to be at

Table 1. Qualitative assessment of the suitability of the different 3-D shape representations for a range of analysis and visualization requirements

	Contour	Volume	Surface
Data input	++	++	– –
Boolean algebra	+/–	++	+/–
Numerical analysis	+/–	++	+
Fast rendering	++	–	+
Realistic rendering	– –	++	++
Electronic knife	– –	++	+

++, Excellent; +, good; +/–, moderate; –, poor; – –, bad.



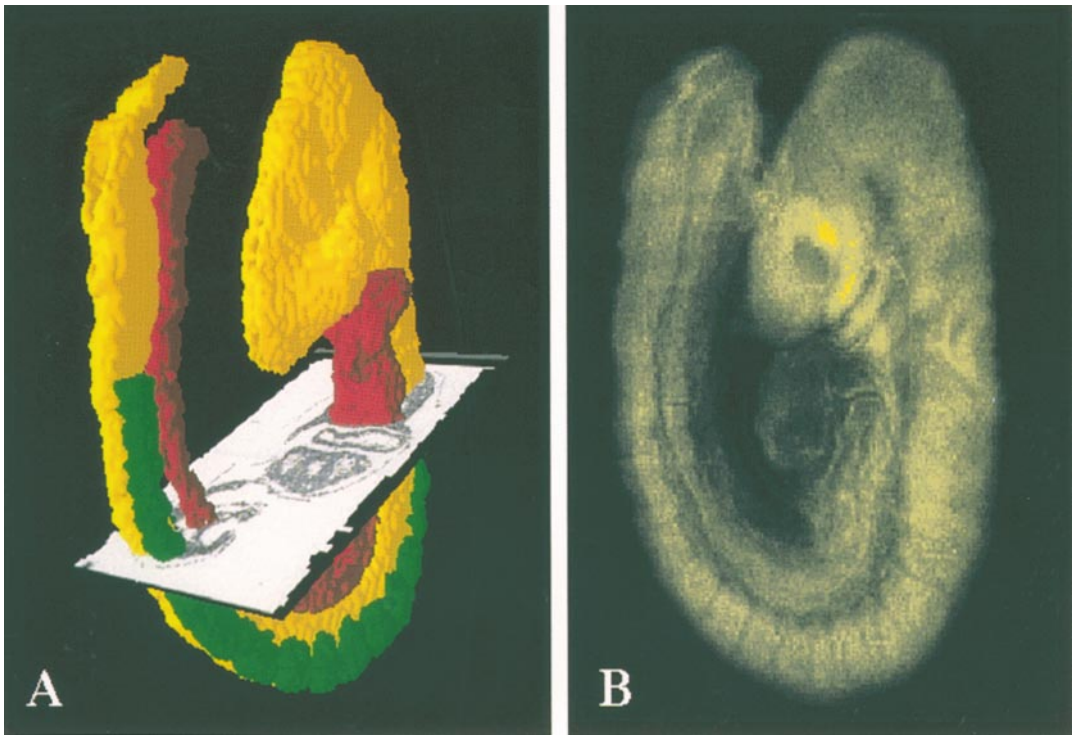


Figure 6. Aerial view of a 3-D reconstruction of the dental epithelium of the first upper molar at 15.0 embryonic day. (po, posterior; an, anterior.)
Figure 7. Two views of a reconstruction of the E9 mouse embryo. The left view shows the surface display of selected anatomical structures (neural tube, yellow; gut, brown; somites, green) with an arbitrary section showing the histology. The right view is a volume rendered image of the same embryo in which the same structures are clearly visible.

standard orientations and positions then a contour representation is sufficient with the benefit of a lower demand on computing resource and complexity. While the choice must depend on the application, it should be clear that tools are now available for most aspects of 3D reconstruction to solve many of the problems that the user is likely to meet. People interested in more details of the programs discussed here should email the authors.

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