

Three-Dimensional Imaging of the Experimental Spinal Cord Injury

Bradley S. Duerstock^{*}, Chandrajit L. Bajaj[†], Valerio Pascucci[‡], Daniel Schikore[°],
Kwun-Nan Lin[§], and Richard B. Borgens^{*◇}

^{*}Center for Paralysis Research
Department of Basic Medical Sciences
School of Veterinary Medicine
Purdue University
West Lafayette, IN

[†]Department of Computer Sciences
Center for Computational Visualization
The University of Texas at Austin
Austin, TX

[‡]Department of Computer Sciences
Purdue University
West Lafayette, IN

[°]Center for Applied Scientific Computing
Lawrence Livermore National Laboratory
Livermore, CA

[§]Silicon Graphics, Inc.
Mountain View, CA

◇To whom correspondence should be sent:

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Richard B. Borgens, Ph.D.
Center for Paralysis Research
1244 VCPR
School of Veterinary Medicine
Purdue University
West Lafayette, IN 47907-1244
765/494-7600
765/494-7605 fax

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SUMMARY

The pathology of mammalian spinal cord injury (SCI) is usually defined as "central hemorrhagic necrosis", where variable amounts of the peripheral white matter is spared while the central grey matter deteriorates. This condition had traditionally made microinvestigation of the injury site in experimental animal models of SCI only possible through histology. Unfortunately, histological examination is confined to two dimensions, unless the spinal cord is three-dimensionally reconstructed. Three-dimensional (3-D) computer visualization is an ideal tool for the evaluation of the SCI and other soft tissue injuries where multiple pathological features may be embedded within each other. We have evaluated three different algorithms to three-dimensionally visualize spinal cord injuries. These tools are volumetric texturing, surface tiling, and isocontouring. To facilitate similar interdisciplinary efforts, we provide the computer science approaches that form the basis for this software. Our three-dimensional reconstruction techniques are not restricted to imaging alone. For the first time determination of the volume and surface area of spinal cord injuries or other structures embedded within them is possible using the reconstructed 3-D images themselves. During this evaluation we detected characteristic differences in the algorithms between two of the surface reconstruction methods that could influence quantitation. This was not observed, however, when these methods were compared to morphometric data generated by older two-dimensional (2-D) morphometric and geometric best-fitting approaches. We have also demonstrated the use of 3-D imaging technology to dynamically navigate into a three-dimensional spinal cord reconstruction and discuss the value of this and the other capabilities produced by these new methods.

INTRODUCTION

For over 30 years the ability to construct three-dimensional images from biological specimens using the computer has been expanding with technological advances in both hardware and software (Allen and Levinthal, 1990). In recent times, certain areas of biological and medical imaging have been particularly innovative in producing three-dimensional images of acquired two-dimensional data sets because the acquisition techniques are conducive to 3-D reconstruction. Examples would include the reconstruction of Magnetic Resonance Images and Computer Tomography in Medicine, and 3-D visualization of Confocal Laser and Scanning Electron Micrographs in biology (Hashimoto and Kimura, 1988; Salisbury, 1994). These acquisition technologies provide ease in reconstruction since the registration of serial images is automated.

Though such techniques are powerful, they are not as universally utilized in experimental biology and medicine as is light microscopy (LM). The compound light microscope still provides investigators high levels of resolution in concert with many types of specific cell identification. The modern light microscope provides the broadest range of examination, from the subcellular to the tissue level of investigation on one platform. Where once conventional photography was married to light microscopy, the darkroom has been replaced by image digitization using video cameras mounted to the microscope. In spite of these developments, the use of three-dimensional computer managed reconstruction techniques in light microscopy has lagged far behind the other acquisition techniques described above. It is not a common feature of modern LM systems to possess 3-D reconstruction programs as the issues of registration of serial sections, contour tracing techniques, section distortion, and the

completeness and suitability of the data set for 3-D imaging are still daunting problems.

One particular area of need is laboratory-based research in spinal cord injury. These injuries are both intractable and present a growing dilemma to both healthcare and society at large (DeVivo et al., 1995). Experimental treatments to rodent models has traditionally been the means of developing medical interventions such as the acute administration of methylprednisolone (Bracken et al., 1990). Manipulation of such experimental injuries not only requires careful selection of behavioral models (Borgens, 1992), but the means to detect subtle pathological changes in the anatomy of the injury. This is an ideal area of study to apply 3-D based techniques to the morphometry of complicated histological material.

Here we describe and compare three types of three-dimensional visualization techniques. We evaluated 3-D images derived from: 1) volumetric texture imaging algorithms, 2) surface tiling algorithms, and 3) isocontouring algorithms. We compare and contrast approaches for 2-D image registration, contour and object selection, image enhancement and filtering, 3-D image transparency, and the dynamic navigation of the "observer" into these three-dimensional reconstructions.

The capability to quantify the 3-D reconstruction may be equally if not more valuable than revealing the shapes of three-dimensional structures. Past quantitative methods used either a 2-D morphometric approach (Harris and Stevens, 1988; Hashimoto and Kimura, 1988; Halliday et al., 1993; Arndt et al., 1994; Navarro et al., 1994, Salisbury, 1994) to derive volumes and surface areas from the data sets that make up the 3-D reconstruction or through the use of mathematical formulas for geometric shapes that may have a similar morphology to the area of interest (Blight, 1985; Harris and

Stevens, 1988; Bresnahan et al., 1991). We believe this examination of techniques is the first to both evaluate and contrast different mathematical approaches to 3-D reconstruction of a soft tissue injury, in particular the spinal cord, and to calculate the volume and surface area of various structures from the actual 3-D image itself.

MATERIALS AND METHODS

General Procedures

Fully adult (300 g) laboratory rats (Sprague-Dawley) were used in these experiments. Following experimental injury to the spinal cord (see below), they were housed two animals per cage, fed ad libidum, and their health monitored daily. Three animals in this study were sacrificed 19, 20, and 180 days post-surgery by an overdose of Sodium Pentobarbital (0.8 ml of 1 g/ml standard injectable) immediately followed by perfusion/fixation with 6% paraformaldehyde, 0.1% glutaraldehyde in a phosphate buffer. The spinal cords were dissected free and immersion fixed in the above fixative for about 18 hours. One animal that was not injured was sacrificed and the spinal cord was then perfused and fixed in the same manner.

Experimental Spinal Cord Injury

Anesthesia was performed using an intraperitoneal injection of 0.1 ml/100 g body weight of a standardized solution of 10 ml Ketamine HCL (100 mg/ml) and 1.1 ml Xylazine (20 mg/ml). The spinal cord was exposed by a partial laminectomy (10th to 11th thoracic vertebrae; dura left intact) and the dorsal hemisphere crushed using blunted Watchmakers forceps in two cases. In a third animal, a piercing injury to the cord was made with sharpened forceps and a ca. 0.5 mm piece of striated muscle was inserted as an autogenic tissue graft to the cord. All incisions were closed in layers with 3 - 0 proline suture, and the skin closed with wound clips. Immediately post-surgery each animal was subcutaneously injected with 3 ml lactated ringers to prevent dehydration, and the animal placed under a heat lamp for about 24 hours to reduce post-surgical mortality due to shock.

Immunocytochemistry and Staining

The segments of spinal cord (ca. 1 cm in length) containing the area of experimental injury were dehydrated in ascending concentrations of alcohol followed by xylene permitting infiltration and embedding in Paraplast (paraffin) by conventional methods. All of the segment was sectioned on a rotary microtome at approximately 15 microns, and these horizontal longitudinal sections were affixed to microscope slides. Prior to use the slides were dipped in a 0.5% gelatin solution which aids in the adhesion of the sections to the slides during subsequent treatment. Paraffin was partially removed with a 1 hour treatment in a 60°C oven, and completely removed after a 1 hour immersion in 100% xylene. Sections were rehydrated by immersions in descending grades of alcohol to distilled water by conventional methods. Hydrated sections from the crush injuries were incubated in a commercial enzyme and tissue nonspecific antigen blocker (Endo/Blocker M69 and Tissue Blocker, Biomed) for 5 minutes, with a 1 minute rinse in buffer (Automation Buffer, Biomed). The sections were then exposed to the primary antibody for the macrophage, ED1 [MCA - 341, Serotech/Harlan Bioproducts (mouse antirat)] for 10 minutes, and rinsed with buffer. This dominant cell type has been useful in establishing the boundaries of the lesion at the acute and subacute phase of injury (Moriarty et al., 1998; Damoiseaux et al., 1994). The secondary biotinylated antibody [rabbit - antimouse (Lab/Probe, Biomed)] was administered for 10 minutes, rinsed in buffer prior to exposure to the streptavidin peroxidase (10 minutes), rinsed, and exposed to a commercially prepared Diaminobenzidine reagent (Biomed) for 5 minutes, and counter-stained with hematoxylin. Stained sections were rinsed in distilled water and coverslips affixed with a warm glycerol gelatin (Sigma Chemical Co.). The transplant specimen was sacrificed at 180 days post-injury, embedded and sectioned as previously described, but the sections were only stained with hematoxylin. The uninjured spinal cord

segment was immersed in a 10% sucrose solution in a phosphate buffer before being quick frozen in liquid nitrogen, sectioned on a freezing microtome (40 microns), mounted on microscope slides, and stained with hematoxylin and eosin.

Video Frame Grabbing and Image Reproduction

Viewing of spinal cord sections was accomplished with an Olympus Van Ox Universal Microscope. A JVC TK-1070U color video camera mounted on the microscope displayed histological sections on a computer monitor. Images were acquired to a Macintosh Quadra 800 computer with RasterOps[®] MediaGrabber[™] software, and managed on a dual Pentium Pro computer using Adobe Photoshop[®] software. Color plates were produced using Microsoft PowerPoint[®] software and prints were produced on a Epson Stylus Color 800 printer and a Tektronix Phaser 440 dye-sublimation color printer.

Registration of Serial Sections for Three-dimensional Visualization

For 3-D surface reconstruction and volume visualization, the spinal cord segment containing the lesion was acquired to the computer at low magnification (20x). Every serial histological section comprising a spinal cord segment was used for reconstruction. Registration was accomplished by superimposing each successive digitized histological section by optimally positioning and rotating the microscope stage. The boundary of the cord and other fiducial points or objects (such as cysts, central canal, and general features of the lesion) served as aids in serial registration. Further details can be found in Moriarty et al. 1998.

Conversion of Data Set Files

Each of the three visualization methods applied in this study involved a series of image conversions to obtain the correct file type. These algorithms work only with greyscale digital images to reduce the range of pixel values. Both volumetric texture imaging and isocontouring methods convert optical slices to pixel values ranging from 0 to 255. Surface tiling is not dependent on pixel values and requires only uniplanar tracings of areas of interest to produce 3-D images.

Computer Science Approach to Software Development

1. Algorithm: Volumetric Texture Imaging

Volume rendering (Drebin et al., 1988) is a useful technique for visualizing three-dimensional volumetric data sets like CT or MRI scans. In these cases the volumetric data set is obtained by stacking a sequence of 2-D pictures one on top of the other forming a 3-D regular grid of RGB (Red Green Blue) values: one RGB value per voxel. Volume rendering provides a way to see through the entire 3-D data by modeling the level of transparency associated to the RGB values by modulating a so called "transfer function".

Ray casting techniques (Hall and Watt, 1991) render the volume by shooting one ray per display pixel. By integrating the transfer function on the voxels met by the ray while traveling in the 3-D volume, one computes the color value of the corresponding pixel in the image. This technique produces high quality results but is computationally expensive to allow interactive visualization and exploration of the data set.

Advances in graphics hardware for texture mapping allowed the development of a more efficient approach (Cabral et al., 1994). The 3-D volumetric data set is stored in the 3-D Texture Mapping memory of a high end graphics workstation. In this way, the 3-D volume can be sliced by using the capability of the graphics hardware to compute slices of the 3-D texture map. In particular, one first determines the viewing direction and then computes a sequence of planar slices orthogonal to the viewing direction. The slices are rendered as textured polygons and displayed in order from the farthest to the nearest from the observer's viewpoint. In this way, the polygon rendering hardware blends the pictures producing the desired transparency/occlusion effects in the volume to be rendered. One can then take full advantage of the graphics hardware acceleration, obtaining a real-time volume display with interactive speed exploration.

In this report, three kinds of explorations of the volume were allowed by this approach. First, a basic change of view exploration. Second, clipping the volume into multiple planes in any arbitrary position which allowed views into internal regions that were otherwise hidden. Third, the transfer function was modulated which associated different colors and levels of transparency with different ranges of the scalar field defining the 3-D volume.

2. Algorithm: Surface Tiling Reconstruction

The Surface Tiling approach takes as input 2-D polygonal cross-sections in a sequence of parallel planes (Bajaj et al., 1996a; Bajaj et al., 1998). In the present case where the input data was provided as a sequence of histological images, human intervention was required to define the planar polygons (contours) from each image. The three main problems that arose in connecting polygons in parallel slices were: 1)

determination of the pairing between corresponding polygons in the two slices, 2) computation of a tiling between corresponding polygons, and 3) deciding what was the branching of contours when consecutive cross-sections possessed a different number of polygons. Several mathematical approaches have been used to solve these problems (Wang and Aggarwal, 1986; Bresler et al., 1989; Meyers et al., 1992).

The approach proposed in Bajaj et al., 1996a was used here and gives a more general solution using a Voronoi diagram based scheme with the possibility of introducing new points in-between the slices to better resolve the branching problem. The approach in Bajaj et al., 1998 was extended to provide 3-D tetrahedral meshes of the reconstructed meshes allowing the application of the Finite Element Method for structural properties computation.

The surface tiling reconstruction method that we report here can be summarized in eight main steps:

1. Form closed contours from digitized image slices.
2. Create any augmented contours by inserting new vertices where the projection of that contour would cross another contour. This breaking of contour segments ensures that any intersection between a contour and a contour projection is either a contour vertex or a contour segment.
3. Find correspondences between contours.
4. Form the tiling region of each vertex.
5. Form the Optimal Tiling Vertex (OTV) table where each vertex is associated with the vertex in the adjacent contour connected with the shortest edge.
6. Construct the actual tiling.
7. Collect the boundaries of untiled regions.

8. Form triangles to cover untiled regions based on their Edge Voronoi Diagram (EVD).

Contour Tracing for the Surface Tiling Method

For surface tiling we use a computer program to trace contours on histological images with a mouse on a Sun SPARCstation IPX (Fig. 1). We employed a novel tracing program for contour selection that allows the user see two previous tracings in the same window for comparison. Contours of interest were traced on each histological section of the spinal cord data set. Examples of such contours of interest circumscribed in each section included; the perimeter of the spinal cord segment, the injury site, large cysts, and accumulations of macrophages.

3. Algorithm: Isocontouring Method

A complementary technique to volume rendering is isosurfacing. From the same 3-D volumetric data set one can extract surfaces of constant scalar values. The classic Marching Cubes technique (Lorensen and Cline, 1987) is based on a brute force algorithm that traverse each cell of the volume to determine if it intersects the currently selected isovalue. In such case a look-up table is used to quickly determine the shape of the isosurface contained in the voxel. Typical exploration of a data set are based on continuous change and a repeated rendering of the selected isosurface. Algorithms that provide further advancement to solutions to indexing, scalar field values space, and seed set computation, to both allow and speed isosurface construction can be found in Wilhelms and Van Gelder, 1990; Itoh and Koyamada, 1995; Bajaj et al., 1996b; Livnat et al., 1996; Bajaj et al., 1997; and van Kreveld et al., 1997.

To produce the isocontoured surfaces here, we have used the contour spectrum interface (Fig. 4) combined with the isosurface extraction algorithm (Bajaj et al., 1997). "Contour Spectrum" efficiently precomputes a set of index signatures of a given scalar field. Each signature is a function of the field value space such as the size of the surface area or the volume contained in each isosurface for any specified isovalue. The plot of the signatures provides an interface that aids the user in selecting the desired isosurface (Fig. 4, top). For example, one could select an isovalue with maximum outside volume (Fig. 4, blue line) and a minima of surface area (Fig. 4, red line). The selected isosurfaces at the bottom of Figure 3 represent the 3-D surface reconstruction of the ED1-labeled injury site.

We can also observe that each surface area or volume signature is a low degree spline curve, so exact quantification is provided in real time, by simply evaluating this spline function. Consider a tetrahedron T and a plane P orthogonal to the 'Z' axis (Fig. 2) moving bottom up. The area of intersection of T and P is a continuous function of its 'Z' position. The area signature starts when the P is below T , and increases up to a maximum in the middle of T and finally decreases down to zero when P is above T . The area signature just described is a continuous linear B-spline function.

The B-spline function is an exact representation of the area value. We evaluated the spline polynomial instead of computing the intersection triangle and its area. Since the surface area of an entire isosurface is the sum of the areas inside each cell, we computed the overall area spectrum by summing together the B-splines of all the cells. To determine the volume contained inside each isosurface, we needed to compute the integral of the isosurfaces. Using a piecewise polynomial formula, we integrated each polynomial obtaining a new spline function representing the volume inside the isosurfaces.

Filtering Process for the Isocontouring Method

In order to distinguish features of anatomical importance during isocontouring, a filter was applied to the data set of optical slices (Fig. 3). The histological sections of each spinal cord were convolved to reduce noise. Convolution normalized values of all pixels in a slice by replacing each pixel with a weighted average of the nearby pixels (Fig. 3 B). To diminish artifacts and histological defects, the slices were averaged together. Averaging the optical slices combined overlapping groups of three slices into a single image resulting in the same number of averaged images as original slices (Fig. 3 C). Averaging allowed only biological features that are consistent in two or more slices to be three-dimensionally reconstructed.

Statistical Evaluation

Comparison of three-dimensional visualization measurements provided here used a paired, two-tailed, nonparametric (Wilcoxon or Mann-Whitney) test for significance. Computations were performed using InStat© software.

RESULTS

Each of the three approaches evaluated in this report was based upon different mathematics or algorithms and provided a different means to view the spinal cord and its experimental injury. We first summarize the three methods and their relative differences, and follow this summary with a detailed qualitative and quantitative comparison of the three-dimensional images that were constructed by each of the three approaches.

Volumetric Texture Imaging

This approach was based on volume rendering algorithms that made use of the various differences in pixel values arising from the differences in the staining of cells (or groupings of similar cells) produced by the histological treatment of the tissues. Volume texture imaging provided a three-dimensional image of the biological specimen in which much of its true character was preserved. This result was achieved because the three-dimensional image was constructed from digitized serial sections without any computer-assisted manipulation of the specimen's features. The final volumetric image had a realistic, visual texture reminiscent of the actual spinal cord specimen. This final image could also be manipulated, for example made more transparent or more opaque. This allowed us to emphasize certain features such as pathological cavitations and cysts that in life would likely be filled with fluid (Fig. 5 A and B). The final three-dimensional Volume Textured Image could also be rotated to provide any arbitrary view of the specimen, or cleaved in any arbitrary plane to provide other views of interest embedded within the image (Fig. 5 C and D).

Surface Tiling Reconstruction

Surface Tiling algorithms required that the investigator scribe the boundaries of regions of interest within each two-dimensional histological image captured to the computer. This generated sets of two-dimensional contours. The space between the contours was then converted into a series of triangles (called primitives) in order to construct a three-dimensional image by the amalgamation of all of the contours derived from all histological sections comprising the data set. These surfaces were then revealed as a tiled mesh or "wireframe", or as a smooth surface (Figs. 6 and 7 A and C). These images were not "lifelike" when compared to volume texture imaging. Rather, they were accurate models of the biological specimen that could be used to emphasize particular features of surfaces or embedded surfaces that were a part of the final three-dimensional image (Figs. 9, 10, 11 A and C). Surface Tiled Images could also be interrogated to provide quantitative measurements of surface area and volume of any structure that was a component of the final 3-D image. Finally, the algorithms used to construct surface tiled images allowed dynamic navigation, that is, placing the observer inside the three-dimensional image to move about at an arbitrarily chosen rate of speed and direction and arbitrarily viewing chosen structures from any angle.

Isocontouring Surface Reconstruction

This was the most novel set of mathematics applied to three-dimensional reconstruction of two-dimensional spinal cord injury data sets. The algorithms underlying this method reconstructed three-dimensional surfaces from pixels that had the same intensity or color value—a so called "isovalue" (Fig. 4). Isocontours derived from shared specific sets of isovalues were converted to boundaries, i.e. bounded contours. Sets of boundaries were then derived within each two-dimensional data set

and were analogous to the boundaries produced by the manual methods employed during Surface Tiling, except that these boundaries were not manually traced by the investigator (Fig. 7 B and D). The limits of the pixel values sampled to form isocontours was predetermined by the investigator, and the isosurfaces were then rendered by the computer without human interaction with the data set. This also reduced the time required to produce the 3-D image, since manual tracing of contours on each captured two-dimensional histological view was not needed. Isocontouring allowed quantitative querying of the surface area and volume of the various components of interest present within the final three-dimensional reconstruction and dynamic navigation of the observer "into" the image (see below).

Registration of Serial Histological Sections

The steps taken prior to three-dimensional rendering were the most arduous and time-consuming aspects of the entire visualization process for all three methods. Unlike CT, MRI, and confocal microscopy, registration of histological sections is not automatic. Alignment and ordering of sections within the data set must be done by the investigator. Registration involves the positioning of each histological section on top of the next (translation), the degree of X-Y rotation of each section, and the warping or stretching of individual sections. We elected to register histological sections during capturing of the video image observed with the light microscope. We matched each consecutive section with its neighbor by superimposing an overlay of each slice on the next adjacent slice. Figure 6 shows the surface of the spinal cord lesion embedded within one of the histological sections used to three-dimensionally reconstruct it. The image illustrates proper registration of slices and its impact on the subsequent 3-D surfaces.

Filtering of Optical Slices for Isocontouring Reconstruction

The manner in which the surface tiling approach selected structures of interest was different from volume texturing and isocontouring. For both volume texturing and isocontouring methods, the ability to discriminate objects of biological importance from 2-D slices was dependent upon pixel value differences. Therefore, the challenge in structural identification and selection during surface tiling was different from the other two methods. Difficulties that arise during 3-D reconstruction with pixel-based algorithms rested in the problems characteristic of histological sections including: 1) artifacts, 2) noise, and 3) histological defects.

1) Artifacts are extraneous materials captured along with the spinal cord segment. Artifacts appear under the coverslip but are not part of the tissue to be imaged, for example torn parts of dura mater or spinal roots (Fig. 3 A).

2) Noise is extraneous pixels on an optical slice that have the same value as the pixels that composed the cells or structures to be visualized. For both volume texturing and isocontouring methods a single specified isovalue displayed not only the object of interest but also nonessential points with the same pixel value in other structures (Fig. 8).

3) It is common to have defects like tears, shredding, or folds in histological preparations, which are exacerbated as histological section thickness is reduced for higher resolution light microscopy. These flaws rarely affected the overall morphology of structures within the entire data set, but they could be incorporated into the final 3-D image. Small differences in the value or intensity of staining were not often observed and were adjusted by altering the brightness levels of a captured optical slice. During

the contour tracing process used in surface tiling, these pixel value faults can be ignored. However, to discern anatomical objects of interest during isocontouring, we applied our filter to each optical slice in each data set.

Qualitative and Quantitative Comparison of 3-D Surface Reconstructions

Only isocontouring and surface tiling permitted quantitative interrogation of the 3-D reconstruction. Both algorithms developed triangulated wireframes as the framework for their 3-D surfaces. The calculation of volume and surface area was accomplished from these closed, 3-D tiled meshes (Fig. 7). Therefore, we were able to qualitatively and quantitatively compare the same 3-D biological objects reconstructed by both visualization methods.

We chose to compare these two methods using a sample of three spinal cord segments, two of which had been injured. Figure 9 shows a qualitative and quantitative comparison between these methods for a normal, uninjured spinal cord. Both surface tiling and isocontouring algorithms produced 3-D surfaces of this spinal cord that were morphological similar. The normal spinal cord was included in our evaluations as the shape and character of this anatomy was familiar. This is in contrast to 3-D reconstructions of injuries which are highly variable in their anatomical character.

Figures 10 and 11 show a qualitative and quantitative comparison of injured spinal cords. Both surface tiling and isocontouring methods produce similarly shaped 3-D structures. Quantitatively, the isocontouring volume and surface area measurements were consistently lower than those values derived from surface tiling reconstruction (Figs. 9 C, 10 E, and 11 C).

There was a significant difference in volume and surface area for the same selected structures when surface tiling and isocontouring methods were compared ($n = 8$, $P = 0.008$). The mean percentage difference between surface tiling measurements and isocontouring measurements was approximately 26% with a range from as high as 38% to as low as 8%.

We applied other three-dimensional quantitative approaches used to calculate volume and surface area of the spinal cord injury (Blight, 1985; Bresnahan et al., 1991) for comparison to our 3-D techniques. Past quantitative approaches have been based upon the principles of 2-D morphometry and best-fit geometric equations. Two-dimensional morphometry applied a stereological approach of multiplying the unit area of a region of interest by the section thickness for each serial histological section, the sum of which equaled the volume of the three-dimensional structure. The best-fit principle compared the three-dimensional structure of interest to a known geometric shape (an elliptical cylinder or a sphere). The surface area and volume formulas of the geometric shape were then calculated using the measurements of the histological tissue. Volume and surface area measurements computed by the isocontouring method were not significantly different from 2-D morphometric and geometric best-fitting approaches ($n = 8$, $P = 0.5$, Wilcoxon). Quantitation by surface tiling was not significantly different from these approaches as well ($n = 8$, $P = 0.7$, Wilcoxon).

Three-Dimensional Dynamic Navigation

The value of three-dimensional imaging was enhanced when a 3-D model was animated. Motion added depth and texture to a 3-D reconstructed spinal cord, not often evident when still images were viewed. When pathological cavities or cysts

within the lesion were viewed following 3-D volumetric texture imaging, certain rotations of the 3-D image allowed the observer to look dorsal/ventrally through the cavitations of the spinal cord injury (Fig. 5).

Besides rotation and translation of the 3-D object, panning and zooming were features that enhanced an understanding of the injury through visual inspection. Particularly with isocontouring and surface tiling methods, the user's vantage point of the 3-D object did not have to be static. A structure could be viewed from any position, even from the inside peering outwards. This was useful when exploring internal structures such as the cystic cavities that occurred at the injury site (Fig. 12 A, B, and C). By combining zoom capability with object positioning, we were able to navigate through any structure within the 3-D image. In Rows D, E, and F of Figure 12 the vantage point of the user moved through the opening of the central canal as it bifurcated dorsally into a spherical cyst and ventrally into a protruding tube-like structure that appeared in cross-sections to be continuous with the central canal and other cysts. Dynamic navigation showed the "tube" not to be continuous with other nearby structures.

DISCUSSION

Many areas in experimental biology would greatly profit from 3-D reconstruction or visualization techniques. This is especially true in the study of spinal cord injury. Full understanding of the pathology of CNS injury has eluded us in part because of the numerous cellular and chemical interactions which occur simultaneously over time, resulting in complex pathological anatomies which are embedded within each other. For example, the injury site within the spinal cord segment is approximately the length of one vertebral segment of the spinal cord, and contains cysts and cavitations which may also contain islands or aggregations of cells such as macrophages. These structures are all visible at low to medium ranges of magnification with the light microscope.

It is also equally important to quantify the volumes and surface areas of these various structures. This capability could determine of the relative success of an experimental treatment aimed at modifying or eliminating these pathological features. Some of the methods explored here allowed us to quantitatively interrogate the actual three-dimensional images following 3-D reconstruction from histological sections. To our knowledge, this is the first such demonstration of this ability in any 3-D application to light microscopy.

Pathology of the 3-D Spinal Cord Injury

The uninjured spinal cord served as a control since its histology is familiar to the neurobiologist (Fig. 9). The 3-D character of a SCI is less familiar because of the variety of injury models used (i.e. compression, weight-drop, and transection injuries) and the numerous methods in which the lesion is examined (i.e. stains and planes of

sectioning). Only through training and experience does an investigator usually derive a mental construct of the three-dimensional shape of the injury.

Our observations of the gross necrosis of 3-D reconstructed SCIs corroborated earlier descriptions that were obtained using conventional histological methods (Hughes, 1992). We also found a relationship between the severity of a SCI and the size of the spinal cord segment. This supported the observation made in Noble and Wrathall, 1985, using a cross-sectional area measurement of the lesion epicenter, that the greater the SCI, the more reduced the diameter of the spinal cord. This was evident by the large difference in spinal cord segment volumes between severe and mild SCIs compared to the relatively small difference in cord segment surface areas (Figs. 10 E and 11 C). Additionally, the severely-injured spinal cord (Fig. 10) contained 50 fewer longitudinal sections than the mildly-injured cord (Fig. 11) though both data sets comprised almost the entire spinal cord segment.

Histological Consideration

Every section of the spinal cord was used in the three-dimensional surface reconstructions and volume visualizations reported here (though in some cases small amounts of tissue were lost as the microtome advanced into and out of the specimen). This was necessary in order to produce 3-D images with as much detail as possible. If every third or every tenth section is used, a smoother 3-D object may be produced at the expense of querying precise 3-D measurements. Interpolating large gaps between consecutive sections during 3-D reconstruction would invariably lead to inaccuracies in 3-D visualization and subsequent quantitation. The necessity for precise representation of the 3-D visualization must be weighed against the effort required to produce the 3-D image. We used longitudinal histological sections instead

of transverse sections, in part because larger numbers of transverse sections would be required to reconstruct a spinal cord segment of equal length. Moreover, at the chosen magnification the entire unit area of the lesion was contained within each longitudinal histological section.

Registration of Serial Sections

The registration of consecutive histological sections during or after image capturing is problematic. Image registration problems include; position and rotation of serial sections, shredding and folding of individual sections, shrinkage from fixation and dehydration, and compression and stretching introduced during sectioning (Hibbard et al., 1993; Ross et al., 1994). Registration can be further complicated if more than one field of view is required per histological section. Then individual optical slices are created from a mosaic or montage of optical images. In such cases, the use of motorized microscope stages makes tracking adjacent fields of view more manageable (Allen and Levinthal, 1990; Montgomery and Ross, 1993).

Many registration methods require fiducial markers of some sort. Fiducial markers maybe inherent to the histological tissue; such as the boundaries of anatomical structures, blood vessels, or populations of identifiable cells (Young et al., 1987; Harris and Stevens, 1988; Allen and Levinthal, 1990; Martone et al., 1993; Liss, 1995). Man-made fiducial points can also be made by introducing holes in the tissue (Bron et al., 1990), dye-filled holes external to the tissue (Vuillemin et al., 1992), notches (Hashimoto and Kimura, 1988), externally embedded guideposts (Chawla et al., 1981; Prothero and Prothero, 1986), or externally embedded collars (Williams and Doyle, 1996).

We did not use internal, artificial fiducial markers because they would have disrupted the tissue contained within the captured area of interest. Unwanted internal fiducials would also appear in the final 3-D image during volume rendering and isocontouring if performed. Unfortunately, external fiducial markers could also fail if they moved or are lost during sectioning, or become deformed during histological preparation (Vuillemin et al., 1992). Our method of superimposing consecutive slices based on intrinsic tissue features was conceptually similar to other techniques (Allen and Levinthal, 1990; Martone et al., 1993; Roesch et al., 1996). To correct optical slices for normal stretch that incurred during histological preparation, presupposes that compression or distortion of the tissue is constant throughout the entire section. Most likely some tissue types (or absence of tissue, such as cysts) deform more easily than others and compression would not be constant throughout the plane of sectioning (Brändle, 1989; Williams and Doyle, 1996).

Automated, computational techniques have also been attempted to improve registration. These methods apply algorithms to: 1) superimpose the centroids and/or principal axes of sequential images (Bron et al., 1990; Bergognoni et al., 1991; Montgomery and Ross, 1993), 2) register the images from analyses of their auto- and cross-correlation functions (Hibbard et al., 1993), 3) match consecutive contours using a best-fit method (Toga, 1990), or 4) align successive sections using an exclusive-or index that is based upon image grey level distribution and tissue morphology (Honghui and Qunsheng, 1996). These registration methods also do not adequately resolve non-linear tissue distortions like section warping. Registration methods based upon the shape of the images, such as the center of mass and principal axes approaches, could cause misalignment when sections are naturally asymmetric (Toga, 1990). Additionally, if contour tracing was required for serial section registration, then

another step would have to be added to the isocontouring and volumetric texture imaging approaches.

Volumetric Texture Imaging

Volumetric texture imaging is a commercially widespread method of 3-D visualization. Since the final 3-D image is produced by virtually stacking the optical sections, no additional 3-D graphics are rendered (i.e. tessellation of wire meshes). Thus, the 3-D image looks like the sum of its component optical slices. Biological features are then distinguished by the characteristic pixel intensities of the slices.

Animation of volume textured images increased conspicuity of embedded structures such as cysts. These 3-D images maintained the original character of the tissue, in contrast to three-dimensional surface reconstructions (surface tiling and isocontouring) which are representations or models of the structures being imaged (Fig. 5).

We could not quantitatively query volume visualizations or their subcomponents because during volumetric texturing, vague anatomical boundaries that are defined solely by color value make morphometry infeasible (Toga, 1990). Unlike with surface tiling and isocontouring, the computer cannot distinguish the biological structures within the spinal cord injury as separate entities without the generation of closed 3-D surfaces. Theoretically, volume could be approximated by counting the voxels from a range of intensity values that compose a particular structure(s) of interest (Bron et al., 1990), but the noise of the sections does not permit structures to maintain a single density or voxel intensity. Additionally, we have not found a method of calculating surface area measurements from volume visualizations because wireframe surfaces are not generated to demarcate anatomical boundaries for the computer.

Surface Tiling Method

Stacking manually-traced contours is by far the most pervasive method for building three-dimensional surfaces. Generally, this has been accomplished by scanning or digitizing camera lucida drawings, acetate overlays, or tracings from photographic prints (Young et al., 1987; Hashimoto and Kimura, 1988; Bresnahan et al., 1991; Halliday et al., 1993; Martone et al. 1993; Navarro et al., 1994; Liss, 1995; Beattie et al., 1997). Now image acquisition, registration, contour selection, 3-D surface reconstruction, and finally structural quantification can all be accomplished via a keyboard and mouse.

Circumscribing contours by hand or a pointing device is the most tedious process in the surface reconstruction method. Thus, some are motivated to trace contours from every other or every tenth histological section (Bresnahan et al., 1991; Ross et al., 1994; Liss, 1995; Beattie et al., 1997). Manually-scribing contours around biological features can also introduce human subjectivity in the identification of pathological features. Clearly, no two humans can trace a complex histological structure within a data set of sections for 3-D reconstruction identically.

Computer-automated extraction of contours from biological objects of interest has been attempted by others to reduce the labor and subjectivity of manual contour tracing. Popular methods of selecting areas of interest use segmentation or edge detection algorithms (Toga, 1990; Montgomery and Ross, 1993; Durikovic et al., 1998). These algorithms attempt to select objects of interest based on their specific and uniform pixel intensity or by their distinct boundaries from the surrounding tissue. Since these physical qualities are hard to find consistently in each histological section, automated contour extraction is difficult.

Isocontouring Method

In many regards, isocontouring is a combination of both volumetric texture imaging and surface tiling. Isocontouring selects objects of interest based upon pixel intensity (isovalue) and then constructs a wireframe surface around that 3-D object or objects. The isocontouring method was the newest visualization technique employed and by far, less labor intensive than surface tiling. Isocontouring also eliminates the bias of contour selection during 3-D surface reconstruction.

Like surface tiling, isocontouring algorithms produced well-defined 3-D surfaces which could be automatically quantified. This is especially important in spinal cord injury and soft tissue pathology in general. Unlike surface tiling however, the quality of the histological sections used for 3-D reconstruction had to be more complete and as free of technical faults as possible. The problems encountered during isocontouring were shared with those of volumetric texturing as described above. Physical defects inherent to the tissue and a lack of contrast in tissue labeling also make isocontouring more difficult.

Comparison Between Surface Reconstruction Methods

Qualitatively, all three spinal cord segments and subcomponents of the injury reconstructed by either surface tiling or isocontouring algorithms were very similar (Figs. 9, 10, and 11). Quantitatively, the measurements for the biological features we queried by the two different 3-D surface reconstruction methods were significantly different. However, there was no significant difference between values derived from our 3-D techniques compared to those from standard quantitative approaches. Thus, older 2-D morphometric and geometric best-fitting approaches have shown that

quantitation by surface tiling and isocontouring can be reliably achieved. We conclude that quantitative differences between surface tiling and isocontouring stemmed from differences in their algorithmic paradigms.

We made a statistical comparison of surface area and volume measurements combined from our spinal cords to determine similarity in quantitation between both our 3-D surface reconstruction methods. Because of algorithmic differences between surface tiling and isocontouring, we were unable to compare measurements for every anatomical feature of the spinal cords we examined. Occasionally, when an isovalue was chosen during isocontouring, two or more distinct structures were generated concurrently. In Figure 10 B both the entire spinal cord segment and the cysts within were rendered at the same time from the same isovalue. However, with an additional software utility we were able to separate multiple, biological structures from each other. We were then able to calculate surface area measurements for each of these separated structures (Fig. 10 E, asterisks). During this study volume quantitation of concomitant sets of isosurfaces was not possible.

The surface tiled lesion in Figure 10 C included the labeled macrophages, pathological cysts, and the decomposed spinal cord parenchyma. Subsequently, the larger cysts were selected and their volumes were subtracted from the total lesion volume. In contrast, the isosurfaces of the injury site in Figure 10 D enveloped only the areas of the set of histological sections that shared a common pixel value, specifically the isovalue for ED1-labeled macrophages. Thus, isocontouring produced a more intricate lesion surface with a smaller surface area and volume than produced by the surface tiling method. Therefore, a quantitative comparison of injury sites between both methods was inappropriate.

Morphometric differences between the two 3-D visualization methods reported here were due to contrasting algorithmic approaches in: 1) how these methods produce 3-D surface reconstructions and 2) how these methods quantitatively query these 3-D images. Both methods produced 3-D wireframes, however they are based upon different reconstruction principles. The spinal cord measurements calculated by both methods were significantly different, in part because of different tessellation approaches. Typically soft tissues are smooth or rounded, so planar triangle primitives must conform to produce as natural a surface as possible. As illustrated in Figure 7 the isocontouring method built 3-D tiled meshes with smaller and more numerous triangles than did the surface tiling method. Therefore, isosurfaces were smoother than surface tiled 3-D surfaces which could cause discrepancies in surface area and volume measurements. Evident in Figures 9, 10, and 11, this did not effect qualitative similarities between the spinal cords to any marked degree. However, slight differences in surface topologies generated by each technique would translate into quantitative differences, especially for surface area.

Both methods compute volume and surface area measurements from their 3-D surfaces uniquely. Overall, the method of quantitation for isocontouring is more sophisticated than surface tiling, although both methods query tiled wire meshes. Surface area measurements from both methods were calculated by summing the areas of all the triangle primitives in a wireframe surface. However, isocontouring and surface tiling use different approaches to calculate volume measurements from their reconstructed wireframes. The volume and surface area computations for isocontours are exactly correct for the approximation we have selected (triangulated surfaces). Isocontouring uses a B-spline function to calculate the area of each triangle primitive in the wireframe surface to compute surface area. For volume measurements the wireframe is broken down into tetrahedron subcomponents. These tetrahedral

subvolumes are automatically measured then summed (Bajaj et al., 1997). Volume computation by surface tiling is accomplished in another manner. Volume measurements are automatically calculated from the wireframe model by applying a prismatoid formula to each pair of contours in the data set (Bajaj et al., 1996a). Under certain circumstances reported in Bajaj et al., 1996a and Moriarty et al., 1998 when consecutive contours are not connected properly, estimation of volume measurements during surface tiling occurs. The quantitative strategies are sound for both methods, but these algorithmic differences probably contributed to the variance in measurements.

The filter used during isocontouring may also affect the overall size of the 3-D isosurfaces since filtered slices are less resolved than the original histological sections. When using surface tiling methods, it is possible that manually tracing contours on each section could overestimate areas of interest to produce tiled surfaces that are more over-smoothed than those constructed using the isocontouring method.

We plan to verify the accuracy of our quantitation methods by comparing measurements produced by our 3-D reconstruction techniques to individual measurements calculated by precise stereological procedures. If 3-D quantitative querying techniques are imprecise then we could determine whether this is a constant inaccuracy or if there are certain circumstances in which 3-D quantification may fail. The latter was the observation of Arndt et al., 1994 in which pixel counting (2-D morphometry) was compared to tessellation measurements of MRI 3-D models of known volume.

Navigation within Biological Structures

Dynamic navigation allows the observer to explore in more detail the relationship between anatomical features in the reconstructed 3-D image. Instead of observing cell populations, nerve tracts, and cysts as separate pathological entities, one can visualize them in a more lifelike way as connected parts of a system. In particular we have provided visual evidence to the postulate that there may be a confluence of the central canal with the formulation of pathological cysts (Beattie et al., 1997). Thus, some cysts may be CSF-filled and the surrounding parenchyma is subject to unique forces of hydrostatic pressure.

Moreover, animation of a 3-D object allows the eye to track features of interest based on motion, yielding powers of perception and discrimination unavailable to two-dimensional plates of three-dimensional visualizations. These features are particularly apparent during the rotation of the spinal cord images shown in Figure 4 but are not as apparent when these images are still. This ability to pass on new and important features of a reconstructed image to others may only be allowed by electronic publishing, where the reader can animate these objects.

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FIGURE LEGENDS

Fig. 1. Defining the Boundaries of Regions of Interest for Three-Dimensional Reconstruction by Surface Tiling Algorithms.

This photomicrograph of one longitudinal/horizontal section of an injured spinal cord reveals several external and internal features for 3-D reconstruction. The external surface of the section (to lead to a spinal cord perimeter), the surface of the internal and central region of injury, and the largest cystic cavitations within this region of injury have been circumscribed. The injury zone has been stained by ED1 monoclonal antibody immunocytochemistry (refer to the methods). The large pathological cavities were apparent against the background staining of this tissue. A similar set of boundaries was produced for every histological section comprising the entire spinal cord segment for subsequent reconstruction as a data set. This spinal cord histological section is approximately 15 μm in thickness and 2.6 mm wide at the widest point shown. The left margin of the spinal cord is rostral and the right, caudal.

Fig. 2. B-Spline Representation of the Area of Intersection of a Horizontal Plane with a Tetrahedron as a Function of its Z Position.

T represents a tetrahedral primitive that is the subcomponent of all 3-D isosurfaces and P , the plane orthogonal to the Z axis moving bottom up. The area of intersection of T and P is a continuous function of its Z position. The area signature starts when the P is below T , and increases up to a maximum in the middle of T and finally decreases down to zero when P is above T .

Fig. 3. Filtering the Image Prior to Three-Dimensional Reconstruction with Isocontouring Algorithms.

In all three images, an identical longitudinal/horizontal histological section of a region of undamaged spinal cord is shown. This section is 40 μm thick, and the spinal cord was approximately 2.9 mm wide. In A, an unadulterated histological image stained with hematoxylin and eosin was captured to the computer (refer to methods). In B, this same image was first converted to a greyscale image and then convolved by the averaging of every pixel value to its adjacent pixel values. This results in an image, in which a more restricted (i.e. less extended) set of pixel values can be used to produce the final three-dimensional reconstruction that does not depend on the prior defining of physical boundaries as in the tiling procedure (Fig. 1). In C, the section shown in A and B is sandwiched between the two adjacent serial histological sections producing an amalgamation of three separate convolved sections. This procedure averages various aspects of the image, reducing or eliminating irrelevant or minor anatomical details such as small tears in one of the histological sections or extraneous spinal cord material projecting from the main image produced during histological sectioning (large arrow). C = central canal, G = gray matter, W = white matter. Rostral to the top, caudal to the bottom.

Fig. 4. Defining Isocontours with Pixel Values and a Graphical Interface.

This view shows the two windows in which the reconstructed image is evaluated and above it, the isocontour pixel values used to produce it. The range of isovalues (59 - 255) are displayed for the investigator. The vertical (red) index line (set at 95.5556) is able to be moved along this gradient to arbitrarily increase or decrease the range of values used to produce the image displayed in the lower window. In this procedure the observer can subjectively define both the overall character of the image through topological changes. The surface area and volume measurements of the image are simultaneously changed as the index line is moved. This allows a real time evaluation of these quantitative factors while the three-dimensional image is managed. The image shown in this example is of a 3 week old spinal cord injury defined by the staining of phagocytic cells which occupy this region in extreme numbers. This image was embedded within a segment of injured spinal cord and as well contained other pathological features such as large cysts and smaller cavitations. These features are displayed at other chosen isovalues. For example, an isovalue of approximately 146 displayed the cysts but were not shown in this view (Fig. 10 B). The image was altered in three-dimensional space by the observer in the lower window, where the angle of view, amount of rotation, and panning of the image surface was accomplished.

Fig. 5. Volumetric Texture Imaging of a Segment of a Spinal Cord Injury.

All views presented here are rotations and/or sectioning of a three-dimensional visualization of a spinal cord segment containing a compression produced lesion. The bright green area is the lesion embedded within the semitransparent brown of the undamaged spinal cord tissue surrounding it. In A, the image opacity was managed to show the twin horns of spinal cord grey matter (arrow) and in B, to more clearly show cavitations within this zone of injury (arrow). This longitudinal/horizontal series of histological sections produced the longitudinal views of A and B, which were additionally sliced by management of the image to produce the oblique transverse plane shown in C, and the cross-sectional view shown in D. Thus, the ability to view any "slice" or plane of the volume texture three-dimensional image is possible as is the ability to vary its opacity displaying features of interest that may be embedded within the image. Quantitative querying of these types of images was not possible however.

Fig. 6. Surface Tiling Reconstruction of the Spinal Cord and its Embedded Injury.

In A, the three-dimensional reconstruction of only the injured spinal cord tissue is shown (in blue). This is embedded within one of the longitudinal/horizontal histological sections used to produce the overall 3-D image. This plane of section can be dynamically raised or lowered through the stationary 3-D image to demonstrate the registration of the injury within the whole spinal cord segment data set. This segment of spinal cord was about half of the vertebral segment containing the lesion and was histologically sectioned in the horizontal plane (~80 fifteen μm sections produced this view). Following reconstruction, an authentic transverse plane may be generated through this spinal cord segment as shown in the upper right plane - though this was not the plane of original sectioning. This is even more apparent in the tiled reconstruction shown in B. This was another injured spinal cord segment, 3 weeks post-injury, where the injured spinal tissue was also shown in blue pseudocolor. The horizontal/longitudinal section containing the injury is real - while the transverse histological section is computer-generated (~130 longitudinal/horizontal histological sections at 15 μm thickness). In contrast to the volumetric texture image shown in Fig. 5, tiled images can be interrogated as to their surface areas and volumes (refer to Figs. 10 E and 11 C). In both of these views the ventral (anterior) surface of the spinal cord is facing upwards, in A the rostral end forward and B the caudal end forward.

Fig. 7. Wireframe Images Derived with Different Surface Reconstruction Algorithms.

All images shown were reconstructed from a segment of undamaged spinal cord (approximately 1/2 vertebral segment in length, 42 longitudinal/horizontal histological sections of 40 μm thickness comprised the data set). The 3-D images in A and B show this entire data set (note the ventral fissure of the spinal cord segment in both A and B), while only the gray matter is shown following extraction from the data set in C and D. In C and D, note the characteristic "butterfly" shape of the gray matter due to the upward projecting dorsal horns and the ventral horns at the bottom of the image. In A and C, the images were produced by surface tiling algorithms, while the reconstructions in B and D were generated by isocontouring algorithms. Both algorithms produced images based on polygonal primitives. The triangulations generated to form the 3-D image are larger and more apparent in A and C, but less conspicuous and providing more surface detail in B and D. Note that both types of algorithms provide very similar three-dimensional images from an identical data set. We also point out that while the injured regions of spinal cord are pathological - producing reconstructed images foreign or less familiar - data sets such as these demonstrate that lifelike and familiar forms are generated by the same approaches. Quantitative evaluation of this data set is provided in Fig. 9 C.

Fig. 8. Refinement of the Isocontoured Three-dimensional Image Through Filtering.

In A, an isocontoured image of the spinal cord gray matter extracted from the data set shown in Figure 1 and 6. Note that isocontour values numerically close to those comprising largely the gray matter embedded within the spinal cord segment data set have not been convolved and filtered from the reconstructed image. Convolution and the averaging of three adjacent convolved slices as described in the Methods for the entire data set allows the gray matter to be more clearly discerned in B.

Fig. 9. Quantitative and Qualitative Comparison of Surface Tiling and Isocontouring Approaches to Building the Three-Dimensional Image.

In A, an uninjured spinal cord data set is shown by surface tiling reconstruction, in B, the same data set by isocontouring (refer to Figs. 7 and 8). In C the surface areas and volumes of select characteristic features are provided. Note that isocontouring algorithms produce characteristically lower values for any queried region (refer to the text).

Fig. 10. Quantitative and Qualitative Comparison of Surface Tiling and Isocontouring Approaches to Building the Three-Dimensional Image.

In A and C, a 3 week-old spinal cord injury data set is shown rendered by surface tiling reconstruction and in B and D by isocontouring algorithms (refer to Figs. 4 and 6 A). In A, the embedded cysts are shown in red pseudocolor. In B and D, the cysts and lesion were not colored, but easily revealed for comparison against background. In E, the surface areas and volumes of select characteristic features are provided. Note that isocontouring algorithms produce characteristically lower values for any queried region (refer to the text).

Fig. 11. Quantitative and Qualitative Comparison of Surface Tiling and Isocontouring Approaches to Building the Three-Dimensional Image.

In A, a second 3 week-old spinal cord injury data set is shown by surface tiling reconstruction, in B by isocontouring algorithms. This spinal cord was not as severely injured as the example presented in the proceeding figure. In C, the surface areas and volumes of select characteristic features are provided. Note that isocontouring algorithms produce characteristically lower values for any queried region (refer to the text).

Fig. 12. Dynamic Navigation into a Spinal Cord Injury Reconstructed by Isocontouring Algorithms.

This reconstructed data set shows a segment of rat spinal cord containing an injury produced by piercing its surface with a sharp probe during the transplantation of a piece of striated muscle taken from the same animal (refer to Methods). The reconstructed 3-D image was managed to clearly show the outside perimeter of the spinal cord and particularly three large cysts (numbered 1 - 3) that formed within it in a longitudinal array. The injured spinal cord is presented with its dorsal (posterior) surface up and the rostral end to the left. The thin arrow points to the central canal of the spinal cord, easily viewed in A and B (a rotated view of A). The wide arrow in A points to a longitudinally extended component of the most rostral cyst. This pathological fluid-filled "tube" is also marked with the black arrow in view C. m = inserted piece of striated muscle graft.

Rows D, E, and F demonstrate a navigation into the caudal opening of the central canal marked in A and B. The rows as read from left to right probe deeper into the canal opening into the large cyst (final view of row D). Row E shows internal features of the cyst after entering its rear opening, its roof (second view), and its opening into the small caudally extending tube (third view) in that order. In Row F, the observer continues to probe deeper entering the small tube extending off the cyst caudally to its endpoint. This algorithm approach allowed such a navigation to be dynamic, that is moving at a rate of speed and with panning ability controlled and directed by the user.