Computer-Based Alignment and Reconstruction of Serial Sections

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BIOGRAPHY

Dr. Fiala received a B.S. in mathematics from Arkansas State University and the M.S. in electrical engineering from Virginia Tech. From 1985 to 1992 he built robot control systems at the National Institute of Standards and Technology. He joined Dr. Harris's lab in 1995, after completing a Ph.D. in computational neuroscience at Boston University.

ABSTRACT

Three-dimensional (3D) reconstruction is an important technique for understanding the structure of sectioned material, such as that obtained by transmission electron microscopy. Free software tools for the personal computer (PC) are available from http://synapses.bu.edu/ for the alignment, reconstruction and analysis of serial sections. The procedures for section alignment and object reconstruction using these tools are described. By recovering 3D structure using the techniques, it is shown that a mitochondrion in the brain can be doughnut-shaped. Reconstructions are also demonstrated for a segment of spiny dendrite, and for a pollen grain imaged by confocal microscopy.

KEYWORDS

brain imaging, confocal microscopy, mitochondria, stereology, TEM.

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INTRODUCTION

Transmission electron microscopy (EM) of ultrathin sections produces high-resolution images of biological tissue, but most of the three-dimensional (3D) structural information is lost. This 3D structure can be recovered from serial sections. Unfortunately, the process of 3D reconstruction is often viewed as prohibitively time-consuming, requiring tools and expertise not available to most laboratories. We have developed techniques that bring reconstruction within the reach of any researcher. Reconstructions can be performed on an ordinary personal computer (PC) running Microsoft Windows, using software tools available at no cost from our website (http://synapses.bu.edu/tools/).

This approach to 3D reconstruction from serial sections is demonstrated on an example series through a mitochondrion. By recovering the 3D structure it is shown that some mitochondria in hippocampal astrocytes are truly doughnut-shaped rather than cup-shaped as is commonly inferred from single section studies [1]. Examples are also given of 3D structure recovered from confocal microscopy of a grain of pollen, and from serial electron microscopy of a dendrite of a spiny neuron.

PREPARATION OF SECTIONS

To recover the structure of a mitochondrion from the rat hippocampus, a series of 16 sections through the mitochondrion was photographed onto 3.25 x 4 inch EM film. The developed negatives were digitized using a high-resolution, large-format film scanner. We use the Polaroid SprintScan45 and the Agfa T-2500, but any large-format transparency scanner capable of 1000 dpi optical resolution should be sufficient for many applications. We have obtained satisfactory results using the transparency adapter on the Epson Perfection 1640SU scanner, for example. Scanning an entire negative at 1000 dpi produces an image size of about 3000 x 4000 pixels, (about 10 megabytes). The resulting resolution will be approximately 400 pixels/μm for negatives photographed at 10,000 times magnification. Higher dpi scanning should be used for lower magnification negatives to obtain a comparable resolution. The image file format is not critical. A conversion program is available from our website to facilitate conversion of image files.

ALIGNMENT OF SECTIONS

Serial sectioning and imaging each section separately induces misalignments between sections. In addition to rotational and translational offsets, misalignments between sections include scaling and/or nonlinear deformation due to mechanical stretching and folding of the tissue, due to temperature changes, and due to optical distortions in the electron microscope [2]. Our alignment software, serial EM (sEM) Align, provides a means to correct these distortions over the entire image. We advocate aligning whole images rather than just individual contours or objects for several reasons. Aligning the whole field constrains

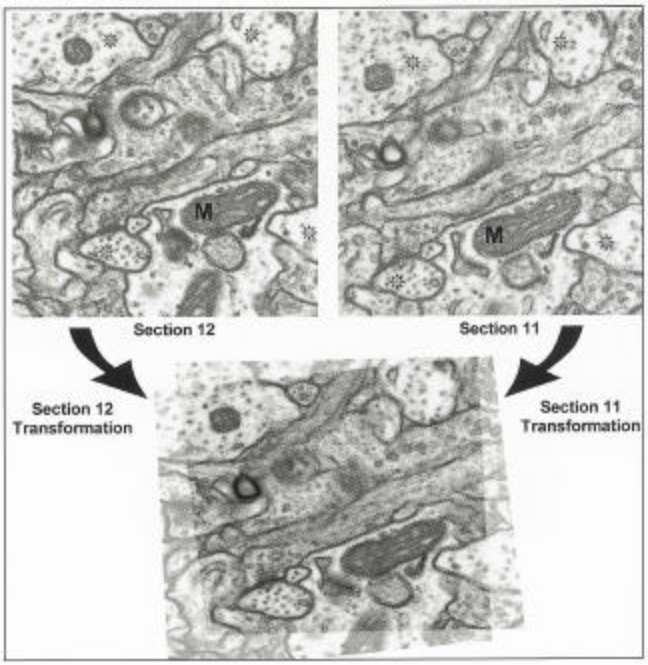

Figure 1:
Two sections (top) through a mitochondrion (M) are aligned by applying a unique transformation to each image. The transformation is determined from the positions of corresponding microtubules (stars) in the sections. A blend of the transformed images (bottom) reveals the quality of the alignment produced.

the alignment of individual objects, ensuring that they are not artificially straightened or distorted. Aligning the whole field often allows several objects to be reconstructed and analyzed at once. Aligned images also facilitate identification and tracing of objects. A prerequisite to any stereological analysis is the ability to correctly identify objects by their sectioned profiles [3,4]. Difficulties in identifying a structure on a single section can be resolved by following the structure on adjacent sections. This is much easier to do with aligned section images.

The sEM Align software assists the user in finding a set of transformations, one for each section, that will place the series of images into alignment [4]. Each section may be translated, rotated, skewed, scaled, stretched, and bent, either by manual adjustments of the user, or by computation from a set of point correspondences entered by the user. The speed of the image movement is independent of image size and fast enough for smooth user interaction. This allows alignment of large images to be done on an ordinary PC without special-purpose hardware.

The 16 sections containing the mitochondrion of interest were aligned as follows. The central section, section 8, was not transformed. All other sections were aligned to it starting with section 9. Section 9 was aligned to section 8 using the point correspondence method. This involves selecting 3-6 pairs of corresponding points in the two images. In many systems that use point correspondences for alignment, artificial fiducials are introduced prior to sectioning to support alignment [5]. Fortunately, in serial EM of the brain, there are many intrinsic fiducials in the form of cross-sectioned microtubules and other cylindrical organelles. Corresponding microtubules were selected from the cross-sectioned dendrites in the corners of the sections. Four microtubule correspondences were sufficient for the software to automatically compute the transformation that brought two sections into alignment (Fig 1). Different microtubules were selected in each pair of sections to avoid biasing the alignment with the spatial relationships of any single set of fiducials.

To evaluate the quality of alignment on-screen, section images were compared by blending and flickering. Blending displays two overlaid sections, each with half the luminance of the original image (Fig 1, bottom). Provided sections are sufficiently thin, blended images become sharper as they align, blurrier as they go out of registration. Flickering between section images produces apparent motion in the direction of offset that can also guide further adjustments [6].

Once section 9 was satisfactorily aligned to section 8, this transformation was saved and section 10 was aligned to 9. Then section 11 was aligned to 10, and so on, until the end of the series was reached. This process was repeated for the lower half of the series, aligning 7 to 8, 6 to 7, etc., until the entire set of 16 sections was aligned. The alignment was completed in less than an hour. Both the unaligned and aligned series may be downloaded from an online tutorial (http://synapses.bu.edu/lab/howto/mito.htm), along with the set of transformations that produced the final alignment.

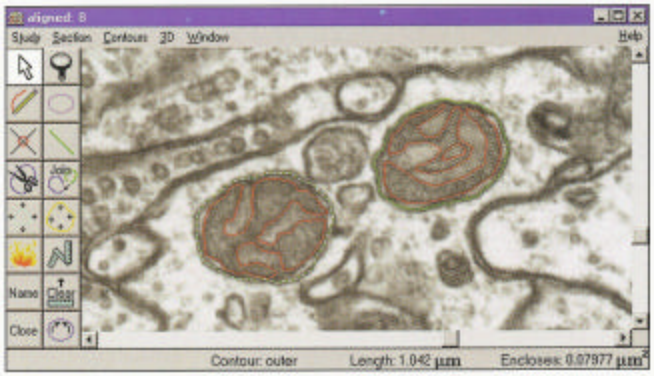

Figure 2:
A screen shot of the IGL Trace software showing the contours for the outer (green) and inner (red) membranes of the mitochondrion. Information about the highlighted contour (dashed green) is shown in the status bar at the bottom of the window.

VOLUME CALIBRATION

After alignment, the series of aligned sections forms a reconstructed 3D volume representation of the original tissue block. But before this can be used for analysis, the magnification factors of the dimensions of the volume must be determined. The section images were calibrated by photographing and digitizing the image of a diffraction grating replica with the series. This calibration grid was combined with the series (as section 0) and imported into our tracing program (IGL Trace). By drawing calibration lines on the grid, the dimensions in pixels per micrometer of the sections were determined [4]. Calibration of section thickness was done by the method of cylindrical diameters [7], wherein the measured diameter of a mitochondrion within a section was divided by the number of sections in which it appeared. Using this technique on a super-set of the example series, the mean section thickness was determined to be 49 nm.

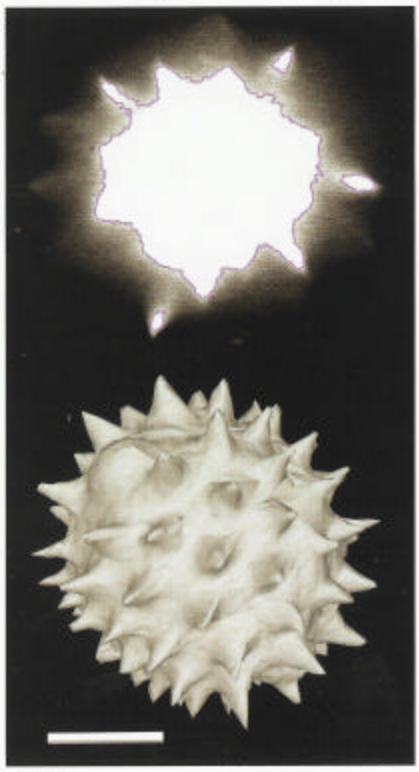

Figure 3:
Visualization of the 3D structure of a fluorescently-labeled pollen grain. The top image shows a confocal section with the traces automatically generated by IGL Trace. The reconstruction (bottom) was generated by IGL Trace and rendered in 3D Studio MAX. Scale bar = 10 µm.

IDENTIFICATION, TRACING AND ANALYSIS

IGL Trace aids the user in producing polygonal overlays on the aligned sections that define the boundaries of objects (Fig 2). Both interior and exterior boundaries can be defined, allowing the delineation of objects with holes and indentations. These boundary contours can be generated by manual tracing with the mouse, or by an automatic tracing feature that uses boundary growing from a user designated interior point. By defining the contours of an object on each section, the object's shape is described. The program can then report the dimensions of the object, or generate a 3D representation of the object.

For the mitochondrion, the boundaries of the outer and inner membranes were traced manually using the cursor (Fig 2). For images that are more binary, such as those obtained with confocal fluorescent microscopy (Fig 3, top), automatic tracing can be used successfully. For example, a 30 µm pollen grain was imaged using the Noran OZ confocal microscope. Z-spacing was selected to produce a section thickness of 0.75 µm, resulting in 46 sections to image the entire object. The gray-scale intensity images were imported into IGL Trace. The boundaries of the in-focus part of the object in each section were outlined using the automatic tracing feature, with a section-by-section manual adjustment of the intensity threshold to compensate for uneven bleaching during acquisition. The result is an excellent 3D representation of the pollen grain (Fig 3, bottom).

In addition to reconstruction work, IGL Trace is also useful for stereological studies in aligned volumes [4]. Sampling frames and other stereological grids [8] can be created as contours and copied into each section on which an analysis is to be performed. Profiles can be marked with contours and counts of these contours easily obtained in a text report file.

OBJECT RECONSTRUCTION

One of the more useful parts of the program is the ability to directly generate a 3D repre-

sentation. IGL Trace generates 3D objects in VRML format to facilitate the viewing of these objects with standard tools such as web browsers. The type of 3D objects generated include individual points or contour centroids, contour lines, planar slabs, and fully-surfaced 3D objects. The surfacing of 3D objects is achieved by a Delaunay triangulation based on the work of Boissonnat [9]. This produces a surface consisting of triangular surface patches (Fig 4).

The contours from IGL Trace can also be exported to other programs through a variety of formats including Steve Young's HVEM format, universal contour file format, ROSS contour file format, AutoCAD DXF, and NUAGES contour format. These contours can then be surfaced by external programs [10].

IMAGE RENDERING

The surfaced inner and outer membranes were imported into 3D Studio MAX to produce a final image of the reconstructed mitochondrion. This program was used to smooth the surface representations, add color and transparency, and render a final image (Fig 5). There are many advantages to using a commercial 3D graphics program for final image production. Not least of these are the endless possibilites for showing off your hard-earned data by generating dramatic images. As an example, we reconstructed a segment of a spiny dendrite from the stratum radiatum of hippocampal area CA1 using sEM Align and IGL Trace. The final image (Fig 6) was produced in 3D Studio MAX by rendering the dendrite over a light micrograph of Golgi-impregnated dendrites.

CONCLUSIONS

Although it is commonly thought that the rare ring-shaped profiles of mitochondria encountered in EM are sections through cup-shaped structures [1], serial section EM with 3D reconstruction clearly reveals that mitochondria form closed toroids (Figs 4 and 5). Therefore, the ring-shaped profiles seen in single sections likely come from doughnut-shaped mitochondria. In this and many other studies, our approach to serial section analysis and 3D reconstruction has proven practical and cost-effective. Planned improvements to the software include the ability to montage and align multiple images per section and enhancements in the correction of complex distortions such as folds.

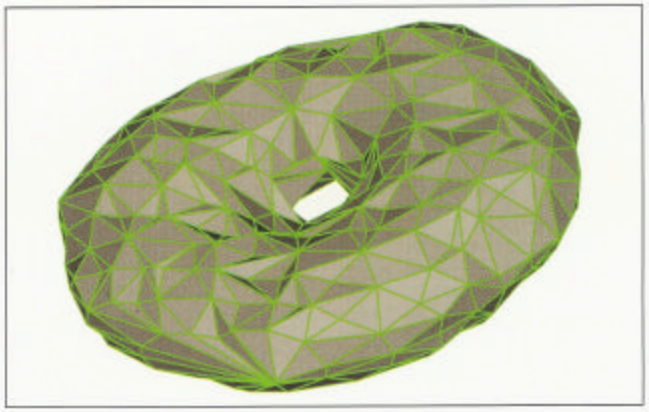

Figure 4:
The 3D surface generated by IGL Trace from the contours of the outer membrane of the mitochondrion. The surface consists of a set of triangular surface patches.

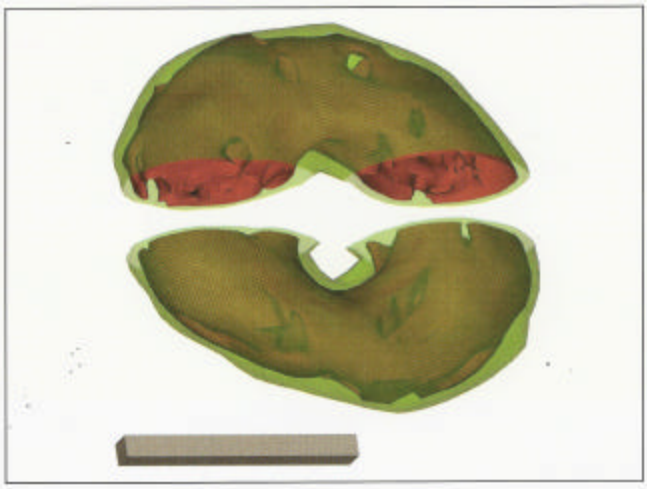

Figure 5:
A final rendering of the mitochondrion reveals the doughnut shape. The outer membrane (green) is depicted as semitransparent and the mitochondrion is transected in the middle to show the structure of the inner membrane (red). Scale bar = 0.5 µm.

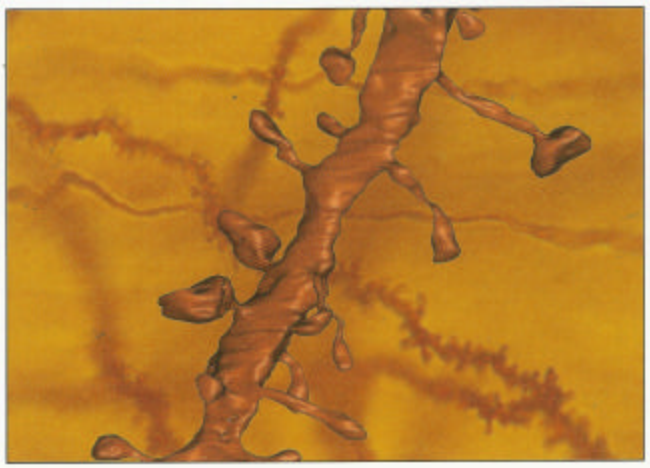

Figure 6:
A segment of a spiny dendrite from the hippocampus of a 21-day-old rat. The surface of the dendrite was reconstructed from 109 serial sections by manually tracing contours and generating the surface using IGL Trace. The result was smoothed and colored in 3D Studio MAX and rendered over a Golgi image from the hippocampus of another 21-day-old rat.

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