Three-Dimensional Structure of Synapses in the Brain and on the Web

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Abstract - The anatomical database that describes the neural networks of the brain will eventually contain sufficient detail to identify all neurons and the individual synapses between them. One approach to obtaining this level of detail is to reconstruct brain volumes from serial electron microscopic sections. Current techniques allow the creation of relatively small ultrastructural volumes, but these contain an enormous wealth of data. Many different kinds of data, including neuron membrane structure, synaptic connectivity, organelle distributions, etc., can be extracted from a single database using this representation. The ultimate size of the ultrastructural database of the brain depends on the advancement of techniques for data creation as well as the development of informatics tools for ultrastructural data mining.

I. INTRODUCTION

The development of artificial neural networks as useful computational devices has historically relied on intuition and reasoning rather than on complete biological details. Advances in neuroinformatics may allow engineering to use neurobiology for more than just inspiration. Sufficiently detailed neuroanatomy and neurophysiology could be a resource for circuit discovery in much the same way that bioinformatics serves as a resource for gene and protein discovery. Such a neuroinformatics database would need to incorporate details of neuroanatomy sufficient to determine neuronal connectivity. In addition to the position and three-dimensional (3D) structure of neurons, this database would include the locations, sizes and numbers of synaptic connections between neurons.

Due to the small size of synapses and their dense packing in the brain (Fig. 1), electron microscopy is currently the only viable method for their visualization. Electron microscopy (EM) is inherently a source of two-dimensional (2D) information, whether it be the intensity pattern of electron transmission through a section, or the reflection of a scanned electron beam from the specimen surface. Additional techniques must be utilized to recover the full 3D structure of brain tissue. For transmission EM this means either serial sectioning and reconstruction or computed tomography, or a combination of the two [8][11]. Serial sectioning can also be used with scanning EM to recover 3D information [6][7].

In the serial-sectioning approach, a small block of tissue is embedded in plastic and shaved into a series of very thin sections, making a long ribbon of tissue sections. Ribbons are transferred to specially prepared holders and stained with heavy metals. Then each section, or a portion of each section, is imaged at very high magnification onto photographic film. Ultrathin (40-60 nm) serial sections imaged in this way offer excellent resolution of membranes, synapses, and intracellular components (Fig. 1). Consequently, this approach has been widely used in neuroscience research. The entire nervous system of the nematode *C. elegans* including the pattern of synaptic connections was determined from 8000 EM sections [17]. The synaptic connectivity of the mammalian basal ganglia [10], retina [2], and other brain regions has also been investigated using large-scale serial EM.

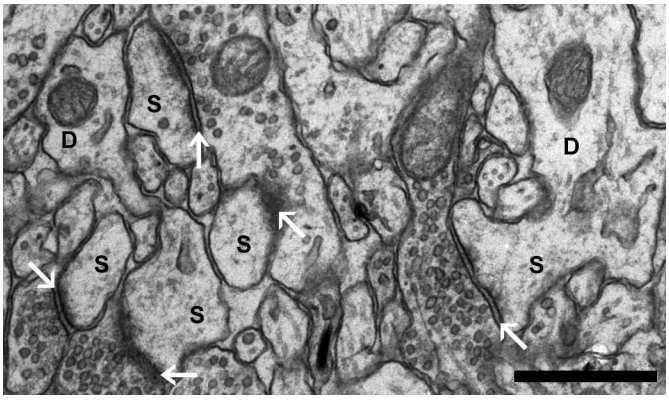


Figure 1: Transmission electron micrograph of an ultrathin section from a region of high synaptic density in the rat hippocampus. Dendrites (D) extend small protrusions called spines (S) that make synapses (arrows) with passing axons. The axons contain numerous round vesicles that are filled with neurotransmitter. From a digitization of 366 pixels/μm. Scale: 0.5 μm.

The potential for serial EM to serve as a basis for neuroinformatic anatomy is only just beginning to be explored. One project underway is the entire anatomy of *C. elegans* [1]. A small amount of neuroinformatics anatomy is also available for the rat hippocampus [13]. Large EM studies of brain are not yet available. Creation of large databases has been hindered by the limited informatics tools available for digital volume reconstruction and data extraction, and by the absence of an efficient means of dissemination.

II. VOLUME RECONSTRUCTION

Serial sectioning and 3D reconstruction have a long history [16]. Techniques first developed in the late 1800's were quickly adapted to EM in the 1950's. But a persistent problem of the approach was the realignment of sections. Since each section is cut and imaged separately, it is subject to independent amounts of scaling and/or nonlinear deformation due to cutting, folding, drying, temperature changes, and optical distortions in the imaging system [16][12]. Most reconstruction efforts relied on manual realignment of traced contours of a few objects of interest. Complete alignment and reconstruction of whole section images simply was not feasible.

Modern computer technology has the ability to handle large section images at high resolution, making digital whole

volume reconstruction possible. To deal with the complex misalignments between sections, we developed software that utilizes a remapping of image coordinates using bivariate polynomials [3]. Adjacent sections are aligned by computing the polynomial coefficients that minimize the error between user-selected correspondence points. While not fully automatic, this method of aligning serial sections is more general than correlation based methods since it allows correction for scaling, skew, and deformation errors, in addition to the usual rotation and translation [5][14]. The method is also faster, since the alignment computation does not depend on image size.

The Windows software for reconstructing volumes from serial sections is freely available online [13]. Alignment of 100 serial sections using this software can be done in about 8 hours of work on an ordinary personal computer [3]. Small brain volumes of up to 100 μm^3 have been reconstructed in this manner. Larger volumes are possible, but how large a volume is reasonable given current techniques?

III. VOLUME SIZE

There are a number of practical issues with reconstructing large volumes from ultrathin sections. One problem is section size. Grids for holding specimens in conventional EM can accommodate sections no wider than 1-2 mm. A reasonable maximum section area given this technology may be on the order of 1 mm^2. Another difficulty is cutting long series of ultrathin sections. Long series of up to 2,000 sections have been obtained for several recent reconstructions [4][15][10]. In practical terms, large section area limits series length due to the large number of grids required.

Given these practical issues, the reconstruction of single tissue blocks as big as 0.1 mm^3, about the size of a small insect's brain (Table I), seem within the state of the art. Larger tissue blocks would need to be dealt with piecemeal or by constructing special instruments for holding and scanning larger sections [8]. Refinements in technology might allow sectioning and imaging to be done within the microscope [6][7], thereby eliminating the need for handling individual sections.

Even small volumes contain an enormous amount of data. At a nominal resolution of 400 pixels/μm and 50 nm section thickness, a volume of 0.1 mm^3 would be composed of 3.2×10^{14} pixels. Assuming a compression factor of 4 can be utilized without loss of information, a volume of 0.1 mm^3 would contain 73 terabytes (Tb) of raw data. If this volume was gray matter of the mammalian brain it would contain 10^7 to 10^8 synapses.

Volume reconstruction and dissemination of a column of cerebral cortex is well within current technology (Table I), but a project of this magnitude has not yet been undertaken. The most time consuming part of such a project would appear to be the manual handling and imaging of as many as 2,000 sections. Even volume reconstruction of the entire brain of a small insect seems reasonably approachable, although the amount of labor involved given current technology would be enormous. The entire brain of a mammal cannot be reconstructed without advances in automation, but key processing circuits could be gleaned from much smaller reconstructions of parts of the retina, visual cortex, and other regions.

TABLE I. Model Volumes for Neuroinformatics

Model Volume	~mm^3	storage (Tb)	neurons	www
Nematode	10^{-3}	1	302	[1]
Cortical column	10^{-2}	10	10^4	
Fly brain	10^{-1}	100	10^5	
Visual hypercolumn	10^1	10,000	10^6	
Frog brain	10^2	100,000	10^7	
Rat brain	10^3	1,000,000	10^8	
Human brain	10^6	1,000,000,000	10^{11}	

IV. VOLUME DISSEMINATION

The world wide web (www) provides a ready medium for dissemination of reconstructed volumes. The conventional approach is to create an interactive browser that indexes into the volume from a simpler graphical representation and returns a detailed image only for the requested region. This provides both a user-friendly interface and reduces the bandwidth requirements for data communication. Such an

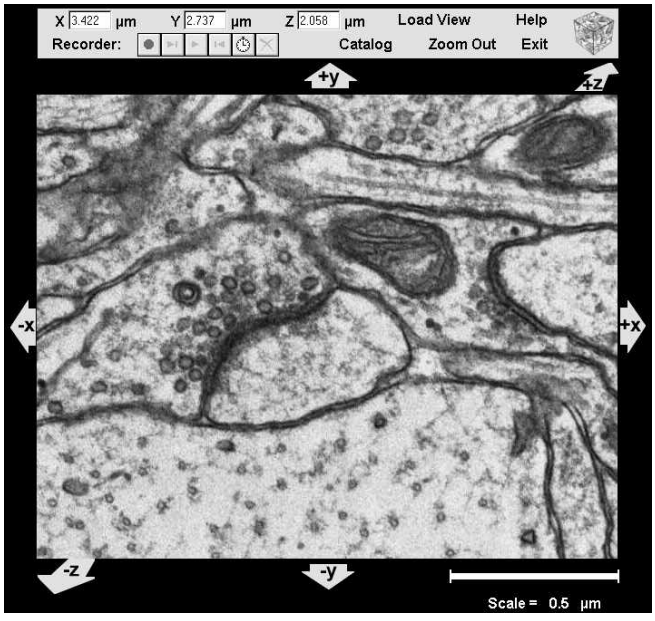


Figure 2: Brain Volume Explorer (BVE) interface for browsing a volume with an adjustable magnification of up to 400 pixels/μm. This magnification allows easy identification of synaptic components on a computer screen.

approach is used for the National Library of Medicine's Visible Human [9], and for the C. elegans EM anatomy [1].

Our implementation of this basic approach is a javascript-based interface compatible with most internet browsers [13]. The Brain Volume Explorer (BVE) interface (Fig. 2) allows any web client to control the selection and downloading of data by 3D geometrical navigation through the ultrastructural volume. A user request in microns is translated into a server volume query. The server indexes into the data volume, extracts the part that satisfies the query and returns a compressed image to the client.

V. DATA MINING POSSIBILITIES

For a reconstructed volume of brain to be truly useful, the neuroinformatics interface must provide for much more than simply browsing the volume. Researchers must be able to extract and investigate the specific types of data in which they are interested (Table II). For a volume reconstructed from EM, the amount of data that can be mined is enormous. The shapes and locations of neurons can be determined by identifying and tracing their bounding membranes in each section. The dendritic and axonal arbors can be completely reconstructed and the location of each synapse identified. The 3D relationships of neurons can be determined (Fig. 3), including their proximity through the extracellular spaces to determine whether volume transmission effects are possible. Densities of synapses within a volume or along a dendrite or axon can be quantified. Cell types can be distinguished and quantified, including glia and their relationship to blood vessels and neurons. Finally, intracellular organelles can be quantified, such as the dendritic distributions of endosomes and polyribosomes that have important implications for synaptic plasticity.

Since the data is already in digital form, the appropriate informatics tools should be able to automatically extract this information, at least in theory. To this point all reconstructions have been done by human identification and hand tracing of the objects of interest. For a field of dendritic segments such as that of Fig. 3, as many as 20 hours of labor is required for identification and tracing. And the dendrites are only a fraction of the volume. I estimate that the cellular membranes in gray matter can be identified and hand-traced at a rate of 1-2 hours per μm^3 using our tools [3]. Thus, a large volume of 0.01 mm^3 would take thousands of person-years to trace all cells manually.

TABLE II. Data Available from Reconstructed Volumes

Data Type	Informatics Tools Required
Neuron structure/location	Tracing of cell membranes
Connectivity	Identification of synapses
Synapse distributions	Identification and tracing of synapses
Organelle distributions	Identification and tracing of organelles
Cell type distributions	Identification of cell types
Brain circulatory system	Tracing of blood vessels

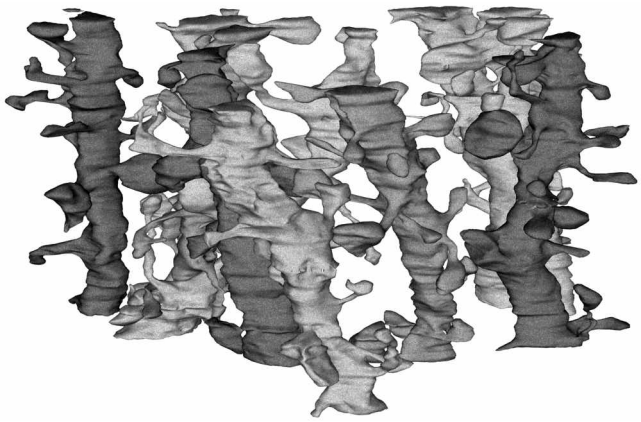


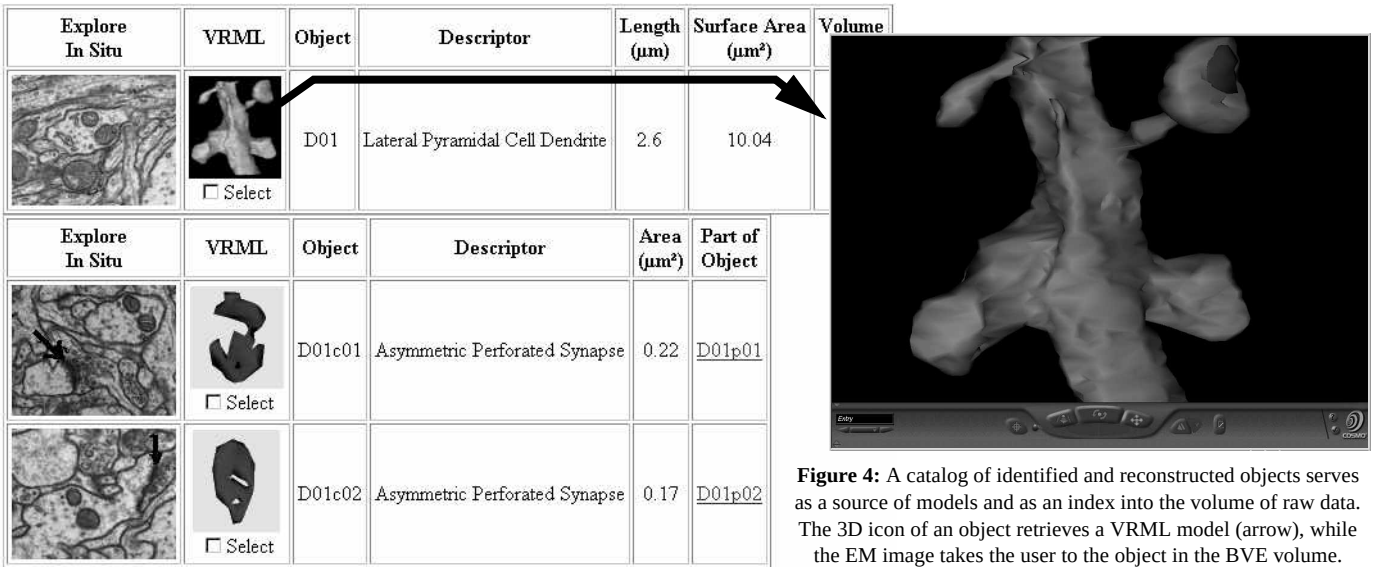
Figure 3: Segments of spiny dendrites automatically surfaced by computer from hand-traced profiles in a reconstructed volume of rat hippocampus.

Table II lists the informatics tools needed to extract various types of data from EM brain volumes. To determine neuron structure it will be necessary to devise programs capable of automatically tracing the membrane profiles of dendrites, axons, and cell bodies through serial sections. Likewise, to locate and quantify synapses and organelles, programs capable of unsupervised identification of objects in single and multiple sections are required. Although trained investigators can carry out these tasks reasonably reliably, no tools are currently available that can do them. However, progress is being made on some fronts. For example, the surface of objects can be automatically computed from hand-traced contours on sections (Fig. 3). Additional tools capable of unsupervised identification and tracing would greatly enhance access to brain volumes by providing the ability to generate an object-oriented index to the volume.

VI. OBJECT-ORIENTED INDEX

After identification and tracing of the components of a volume, an object-oriented index can be built to facilitate database queries. This would allow users to search on particular anatomical elements and quickly quantify them or locate them within the volume. I have implemented a prototypical index for a sample volume reconstructed at Synapse Web [13]. This index is accessible from the Catalog button of the BVE (Fig. 2).

The BVE Catalog consists of object entries organized by anatomy, e.g. axon, dendrite, synapse, etc. Each object entry contains a brief object description, measurement values, and links to other representations (Fig. 4). A graphical representation of the object *in situ* links to the appropriate region and magnification of the BVE. The catalog provides a Virtual Reality Modeling Language (VRML) model of each object that can be quickly downloaded and examined (i.e. rotated and magnified) with a web browser's VRML plug-in. The VRML



Explore In Situ	VRML	Object	Descriptor	Length (µm)	Surface Area (µm²)	Volume
	☐ Select	D01	Lateral Pyramidal Cell Dendrite	2.6	10.04	

Explore In Situ	VRML	Object	Descriptor	Area (µm²)	Part of Object
	☐ Select	D01c01	Asymmetric Perforated Synapse	0.22	D01p01
	☐ Select	D01c02	Asymmetric Perforated Synapse	0.17	D01p02

Figure 4: A catalog of identified and reconstructed objects serves as a source of models and as an index into the volume of raw data. The 3D icon of an object retrieves a VRML model (arrow), while the EM image takes the user to the object in the BVE volume.

model is a representation of the surface of the object appropriate for model-based simulations or other analyses. VRML representations of objects in the catalog can be arbitrarily composed into groups. These composite models can be similarly examined to study the 3D relationships of objects.

VII. CONCLUSIONS

A neuroinformatics database based on serial EM of the synaptic anatomy of a column of cerebral cortex or other representative brain regions would be of great scientific and engineering value. A database consisting of only reconstructed volumes could be readily created with current technology. However, the mining of data from a volume remains a largely labor-intensive endeavour. One short-term solution to this problem would be to develop informatics tools that aid in online data mining by interested researchers. A searchable database of models and quantitative information could then be community-built as various elements were investigated in the raw data. Ultimately, informatics tools may improve enough to allow automatic extraction of cellular and subcellular structure and synaptic networks. If technology for the sectioning and imaging of really large volumes also becomes available, then one day the entire human brain may be available online and anatomically searchable.

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