Light Microscopy-Based Reconstruction and Interactive Structural Analysis of Cortical Neural Networks

V.J. Dercksen* Zuse Institut Berlin
M. Oberlaender† Max Planck Florida Institute
B. Sakmann Max Planck Florida Institute
H.C. Hege‡ Zuse Institut Berlin

ABSTRACT

We recently described a pipeline for reverse engineering anatomically realistic cortical networks [3], e.g. representing a cortical column in rat vibrissal cortex [4]. It is important to acquire quantitative data about the structural properties of such reconstructed networks, both for validation of the modeling approach and for investigating biological research questions. Here, we review this reconstruction pipeline and describe current work on a tool for the visual and quantitative analysis of such networks. This tool allows for interactive exploration of the 3D network and provides a query-based interface to quickly obtain quantitative data about selected properties of interest.

Index Terms: I.6.4 [Computing Methodologies]: Simulation and Modeling—Model Validation and Analysis, J.3 [Computer Applications]: Life and Medical Sciences—Biology and Genetics

1 INTRODUCTION

A fundamental question in neurobiology is how sensory information is processed and how this ultimately leads to behavior. A common model system for investigating this question is the whisker-barrel system of the rat. There exists a one-to-one correspondence between the information acquired by a single whisker hair and a segregated area in the primary sensory cortex, its associated cortical column. One approach to increase understanding of column functioning is to reconstruct a 3D model of the network of the constituting neurons including their synaptic wiring and subsequent simulation of signal propagation through the network.

The complete reconstruction of brain volumes at the synapse level, required for determining the electrical circuitry, is currently only possible for small volumes (using electron-microscopic techniques); for volumes having the size of a cortical column, this is technically not yet feasible. We therefore pursue a reverse engineering approach: we acquire all anatomical data using several imaging and reconstruction techniques, register these data into a common coordinate system and subsequently assemble the network and its synaptic wiring. As the data is acquired from different animals, the network does not represent a cortical column in one individual animal, but an "average" column.

Functional responses of neuronal circuits emerge from the morphology and connectivity patterns established among diverse neuronal cell types. Therefore, to create a column model suitable for simulation, accurate neuron numbers and their type-specific spatial distribution as well as dendrite and axon morphologies of all occurring cell types are required. We previously described a pipeline to acquire this anatomical data, and to assemble a neural network model for simulation [3]. Here, we will shortly review this pipeline.

For model validation and investigating biological hypotheses, it is important to quickly obtain quantitative data of the anatomical properties and ultimately relate these to functional results. As a first step we focus on anatomical properties, like correlations between morphological properties or spatial distributions of such properties. This information is derived from the hierarchical geometric representation and semantics of the reconstructed network.

In the past, after network assembly a large set of network properties was pre-computed in a batch process and stored on disk, resulting in thousands of files. The user had to search through the file system to find the data of interest and manually create a visualization. Tabular data, e.g. average number of branching points for neurons of a given cell type, have been displayed with line charts or histograms using some charting software. 3D density fields, e.g. number of synapses between two cell types per volumetric element, have been visualized with iso-surfaces or volume rendering using Amira [7]. 1D profiles, e.g. number of somata in a planar volume perpendicular to the column axis, have been plotted as line or bar charts, sometimes using a 3D scene as context.

For larger networks this approach is impractical. Needed is an interactive visual data exploration environment that gives the user quick access to specified quantities of interest and their visualization. Supported by interaction schemes like linking and brushing, he should be able to drill down to gain insight into the spatial and non-spatial properties of the network anatomy and, ultimately, network function and their relation. In the second part of this abstract we present work-in-progress to achieve this goal.

2 CORTICAL NETWORK RECONSTRUCTION

Our pipeline for creating a 3D neural network model, representing a cortical column, consists of the following steps (see Fig. 1).

First, the number of neurons in the column and their spatial distribution is determined by creating physical sections of the brain volume of interest, applying a stain that reveals all somata and section imaging with a confocal microscope. Soma positions are detected using an automatic method, comprising a segmentation step followed by watershed-based and soma volume model-based cluster splitting [5].

Then dendritic and axonal morphologies of all occurring cell types are obtained by tracing sections containing biocytin-filled neurons, imaged using a transmitted light brightfield microscope. After an automatic tracing step, the sections are interactively post-processed, automatically aligned and finally interactively connected. Using this workflow also complex, wide-ranging axons can be accurately reconstructed. Nine cell types were identified by classifying dendritic morphologies based on geometric properties. The neurons are registered into a common coordinate frame based on manually traced pia and barrel contours as landmarks. Classification and registration allow for determination of the frequency of occurring cell types as a function of cortical depth.

Finally, the column network is assembled by 1) somata placement according to the given soma density, 2) cell type assignment according to the depth-dependent relative cell type frequency, 3) morphology placement by cloning reconstructions and placing them at the soma position of the given type, 4) Specification of connections between pre- and postsynaptic cell type pairs. 5) Precomputation of estimated number of synapses in 3D volumetric grid cells, based on axon-dendrite overlap [3].

*e-mail: dercksen@zib.de, correspondence author
†e-mail:marcel.oberlaender@mpfi.org
‡e-mail:hege@zib.de

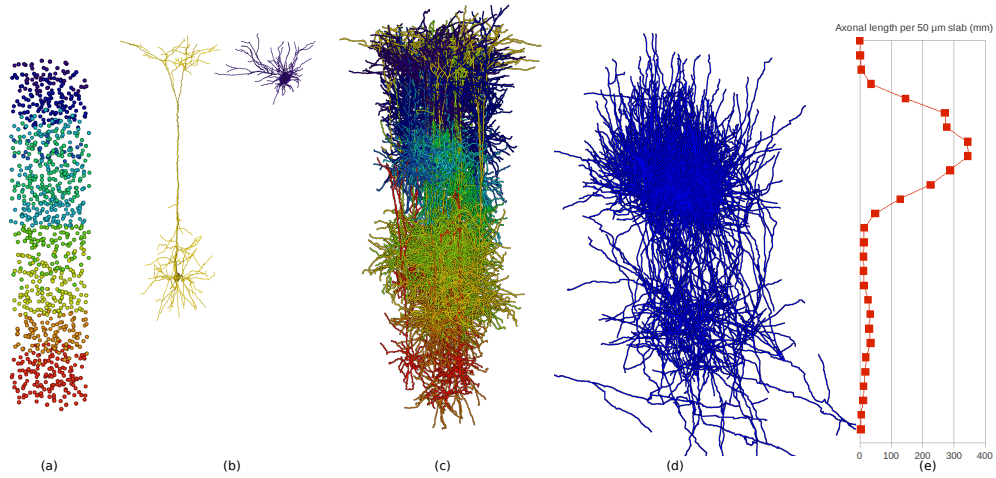

Figure 1: Illustration of column network assembly and analysis. (a) Soma column, (b) typical dendrite morphologies of two different cell types, (c) dendrite column, (d) axons projecting into column, (e) axon density profile.

3 Query-based structural analysis

We propose a query-based analysis tool for visually based quantitative analysis of large neural networks, implemented as an Amira module.

The input of the analysis tool, the network model, consists of a set of geometric objects, the neuron morphologies, positioned in 3D space. These objects carry attribute information on different levels. Each neuron has a 3D position (soma center coordinates) and a cell type label. Each cell type has a spine and bouton density (number of spines/boutons per μm dendrite/axon). Neuron substructures (sections) have semantic labels (*Soma*, *Dendrite*, *Axon*) and a radius is defined at the polyline vertices defining the sections.

As a first step the user shall be able to extract information of interest from this hierarchical, heterogeneous data and interactively create 3D and/or chart views. Later also time-dependent functional data is to be analyzed. Here we focus on the first step.

We use the following approach. First, a subset of the geometry for which quantitative data is to be computed is selected, based on geometric and/or attribute properties. Second, a function is applied that computes the requested data from the selection and results in an intermediate data set, that has one of a small number of predefined types: currently a table, a 1D profile or a 3D voxel volume. This data is then displayed with common techniques; tables are visualized using histograms, scatter plots, line graphs; spatial data is displayed using DVR, MIP, isosurfaces, and slicing, using coordinated multiple views [6].

During iterative tool development and enhancement in cooperation with neurobiologists, a domain-specific language (DSL) [1, 2] is used for specifying selections, computations and visualizations. This enables fast prototyping and addition of user-requested features, as one can quickly add new selection predicates and data mapping functions without having to develop a GUI. Also, session state is captured in text form, allowing session management: saving/restoring, provenance tracking and version control. After analysis workflows have been established a GUI could be added.

For example, to visualize the total axon length of all L5B neurons as a function of cortical depth (here represented by the z-axis), one first selects all axonal branches of L5B cells:

```
  SELECTION VPM-Axon = Axon WHERE
CellType("VPM")
```

Then the total axon length of the selection in 50 μm bins along the z-axis is computed and plotted (see Figure 1(e)):

```
  PROFILE1D Axon-Profile =
BranchLengthProfile(VPM-Axon, Z, 50)

  PROFILEPLOT Axon-plot = Plot(Axon-Profile)
```

4 Conclusion

We reviewed a pipeline for the reconstruction of cortical neural networks, based on anatomical data acquired by light-microscopic techniques. To aid the neurobiologist in understanding network structure, we presented work-in-progress on an interactive tool for visually based quantitative analysis. By allowing specifically targeted queries the user has fine-grained access to heterogenous input and derived quantities in an exploratory manner. As size and complexity of neural network models increase such a tool becomes indispensable, even more when time-dependent data from numerical simulation are included.

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