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Analysis of Neuronal Dendrite Patterns Using Eigenvalues of Graph Laplacians

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Abstract

We report our current effort on extracting morphological features from neuronal dendrite patterns using the eigenvalues of their graph Laplacians and clustering neurons using those features into different functional cell types. Our preliminary results indicate the potential usefulness of such eigenvalue-based features, which we hope to replace the morphological features extracted by methods that require extensive human interactions.

Keywords Pattern Analysis, Graph Laplacian, Eigenvalues of Laplacian Matrices

Research Activity Group Wavelet Analysis

1. Introduction

In recent years, the advent of new sensors and techniques has allowed one to image complicated interconnected structures in biology such as dendrites connected to a single neuron, neuronal axon/fiber tracts in a human brain, and a network of blood vessels in human body. Neuroscientists hope to gain insight into modeling and understanding brain functions by analyzing images of such network structures. The actual analysis of them, however, remains elusive. For example, vision scientists want to understand how the morphological properties of dendrite patterns of retinal ganglion cells (RGCs), such as those shown in Figure 1, relate to the functional types of these cells. Although such classification of neurons should ultimately be done on the basis of molecular or genetic markers of neuronal types, it has not been forthcoming. Hence, neuronal morphology has often been used as a neuronal *signature* that allows one to classify a neuron such as an RGC into different functional cell types [2]. The state of the art procedure is still quite labor intensive and costly: automatic segmentation algorithms to trace dendrites in a given 3D image obtained by a confocal microscope only generate imperfect results due to occlusions and noise; moreover, one has to painstakingly extract many morphological and geometrical parameters (e.g., somal size, dendritic field size, total dendrite length, the number of branches, branch angle, etc.) with the help of an interactive software system. In fact, 14 morphological and geometric parameters were extracted from each cell in [2]. It takes roughly half a day to process a single cell from segmentation to parameter extraction!

In this paper, we examine how to analyze and characterize such neuronal dendrite structures automatically using *computational harmonic analysis* techniques so that we can save human interaction cost in this dendrite pattern analysis.

2. Analysis of Dendrite Structures via Graph Laplacian Eigenvalues

The segmentation and tracing software system used by our collaborator, Prof. Leo Chalupa and his group (Dept. Neurobiology, Physiology & Behavior, UC Davis) provides us with a sequence of 3D coordinates that represent points sampled along dendrite arbors (or paths) of RGCs with the branching information [2]. One of the most natural and simplest ways to model such a

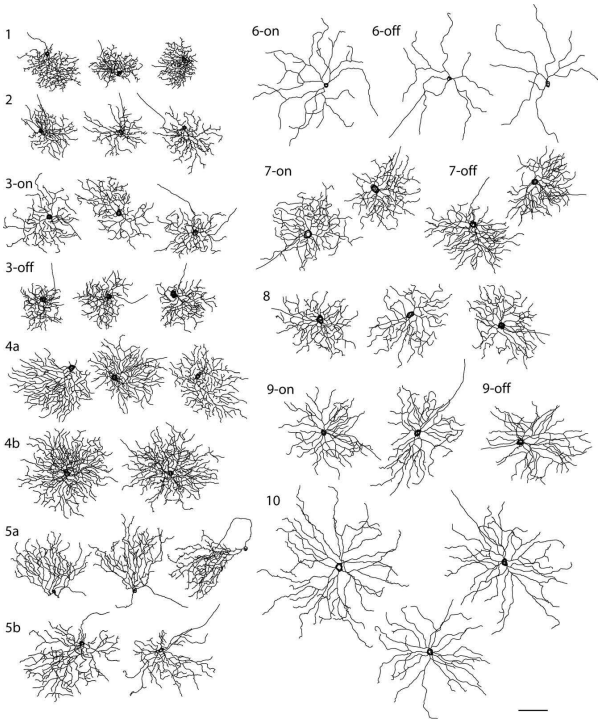


Fig. 1. Dendrites of various types of retinal ganglion cells of a mouse; reprinted from [2] with permission from Elsevier.

network-like structure is to construct a *graph*. Hence, our first task is to convert such a sequence of 3D points to a connected graph G consisting of the vertex set V and edge set E. To fix our notation, let G be a graph representing dendrite patterns of an RGC, $V = V(G) = \{v_1, v_2, \ldots, v_n\}$ where each $v_k \in \mathbb{R}^3$ is a 3D sample point along dendrite arbors of this RGC, and $E = E(G) = \{e_1, e_2, \ldots, e_m\}$ where e_k connects two vertices v_i, v_j for some $1 \le i, j \le n$, and write $e_k = (v_i, v_j)$. Let d_{v_k} be the degree (or valency) of the vertex v_k. In fact, dendrite patterns of each RGC in our dataset can be converted to a *tree* rather than a general graph since it is connected and contains no cycles. We also note that we only deal with *un*weighted graphs in this paper. In other words, we essentially examine the connectivities and complexity of the dendrite graphs, which may not reflect the physical lengths of the dendrite arbors. We will defer our investigation of models that reflect such physical realities as our future project, which includes the weighted graphs where each edge $e \in E$ has weight $w_e := \|v_i - v_j\|^{-1}$, i.e., the inverse of the physical distance between two vertices of e.

Once we construct a graph per RGC, we proceed as follows:

Step 1: Construct the *Laplacian matrix* (often called the combinatorial Laplacian matrix) $L(G) := D(G) - A(G)$ where $D(G) := \mathrm{diag}(d_{v_1}, \ldots, d_{v_n})$ is the diagonal matrix of vertex degrees and $A(G) = (a_{i,j})$ is the adjacency matrix of G, i.e., $a_{i,j} = 1$ if v_i and v_j are adjacent; otherwise it is 0.

Step 2: Compute the eigenvalues of $L(G)$;

Step 3: Construct features using these eigenvalues;

Step 4: Repeat the above steps for all the RGCs and feed these feature vectors to clustering algorithms.

Our rationale behind using the Laplacian eigenvalues is the following: They reflect various *intrinsic* geometric information about the graph e.g., connectivity (or the number of separated components), diameter (the maximum distance over all pairs of vertices), mean distance, etc.; see, e.g., [1, 3] for the details on the graph Laplacian eigenvalues. In fact, we view the dendrites connected to a neuron as a musical instrument, try to "listen" to its sounds, and check if those can be used to characterize the dendrite patterns. We know that it is not possible to uniquely identify a graph from its Laplacian eigenvalues in general. In particular, "almost all trees are cospectral"; see, e.g., [3]. In practice, however, it is often possible to obtain good approximation of a graph from them. Hence, we believe that the features based on the Laplacian eigenvalues of a graph will be useful for various recognition and clustering purposes.

Before stating the facts or theorems in [3, 4] (see also [1]) that are used to construct our features, let us fix our notation and define several key quantities. Let $|\cdot|$ denote a size of a set. Let $|V| = n$, and let $0 = \lambda_0 \le \lambda_1 \cdots \le \lambda_{n-1}$ be the sorted eigenvalues of $L(G)$. Let $m_G(\lambda)$ denote the multiplicity of λ as an eigenvalue of $L(G)$, and let $m_G(I)$ be the number of eigenvalues of $L(G)$, multiplicities included, that belong to I, an interval of the real line. A vertex of degree 1 is called a *pendant* vertex, and a vertex adjacent to a pendant vertex is called *pendant neighbor*. Let $p(G)$ and $q(G)$ be the number of pendant vertices and the number of pendant neighbors of G, respectively. For a nonempty subset of vertices $S \subset V(G)$, let ∂S be the *boundary* of S defined as $\partial S := \{e = (u, v) \in E(G) \,|\, u \in S, v \notin S\}$. Let $i(G)$ be the *isoperimetric number* of G:

$$i(G) := \inf \left\{ \frac{|\partial S|}{|S|} \,\middle|\, \emptyset \neq S \subset V, |S| \le n/2 \right\}. \quad (1)$$

The isoperimetric number is closely related to the *conductance* of a graph, i.e., how fast a random walk on G converges to a stationary distribution. The *Wiener index* $W(G)$ of a graph G is the sum of the entries in the upper triangular part of the distance matrix $\Delta(G)$ of G, where $(\Delta(G))_{i,j}$ is the number of edges in a shortest path from vertex v_i to vertex v_j. The Wiener index of a molecular graph has been used in chemical applications because it may exhibit a good correlation with physical and chemical properties of the corresponding molecule.

We now list several theorems we use in this paper.

- $m_G(0)$ is equal to the number of connected components of G.
- The number of pendant neighbors of G is bounded as:
$$p(G) - m_G(1) \le q(G) \le m_G(2, n], \quad (2)$$
where the second inequality holds if G is connected and satisfies $2q(G) < n$.
- For $n \ge 4$, the isoperimetric number $i(G)$ satisfies
$$i(G) < \sqrt{\left(2 \max_{v \in V(G)} d_v - \lambda_1(G)\right) \lambda_1(G)}. \quad (3)$$
- Let G be a tree. Then
$$W(G) = \sum_{k=1}^{n-1} \frac{n}{\lambda_k}. \quad (4)$$

3. Numerical Experiments and Preliminary Results

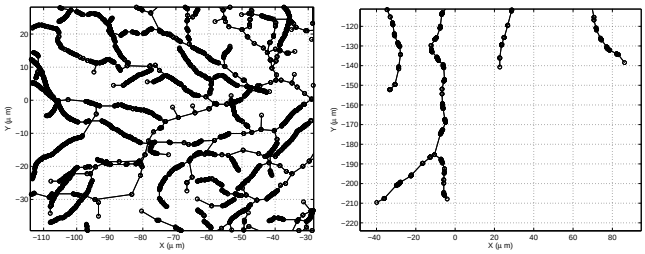
In this section, we report our preliminary results we obtained very recently. We only use the dendrite patterns categorized into the so-called "monostratified" RGCs, meaning the dendrites of those RGCs are confined to either the On or the Off sublaminae of the inner plexiform layer (a layer immediately below the RGCs toward rods and cones) [2], which should be contrasted with "bistratified" RGCs whose dendrites span the On *and* Off sublaminae).

The following features were used to characterize the dendrite patterns of 130 monostratified RGCs.

Feature 1: $(p(G) - m_G(1))/|V(G)|$ as a lower bound of the number of the pendant neighbors $q(G)$ as shown in (2) with the normalization by $|V(G)|$;

Feature 2: The normalized Wiener index $W(G)/|V(G)|$ via (4) ;

Feature 3: $m_G(4, \infty)/|V(G)|$, i.e., the number of eigenvalues of $L(G)$ larger than 4 (normalized) ;



(a) RGC #60 (b) RGC #100

Fig. 2. Zoom up of a part of two RGCs belonging to Cluster 1 (a) and Cluster 6 (b). One can see some "spines" in (a).

Feature 4: The upper bound of the isoperimetric number $i(G)$ shown in (3) .

We normalized Features 1, 2, 3, by the number of vertices in the graph because we wanted to make features less dependent on the number of samples or how the dendrite arbors are sampled. Of course, the number of vertices itself could be a feature although it may not be a decisive one. On the other hand, Feature 4 was not explicitly normalized because the isoperimetric number (1) itself is a normalized quantity in terms of number of vertices.

Feature 1 was used because the number of pendant neighbors seems to be strongly related to the so-called "spines," short protrusions from the dendrite arbors. Figure 2(a) shows several spines as the edges of length 1 each of which is attached to a terminal vertex of degree 1. Hence, we expect that the larger this lower bound $p(G) - m_G(1)$ is, the more likely for the RGC to have spines. In contrast to Figure 2(a), Figure 2(b) shows an example of the RGC whose Feature 1 value is small. Apparently, there is no spine in this figure and each of the pendant neighbors has exactly one pendant (or terminal) vertex. The reason why we used Feature 3, the normalized version of $m_G(4, \infty)$, is based on our following observations. The Laplacian eigenvalue distribution of each RGC dendrite graph typically looks like that in Figure 3. It consists of a smooth bell-shaped curve that ranges over $[0, 4]$ and the sudden burst above the value 4. We have observed that this value 4 is critical since the eigenfunctions corresponding to the eigenvalues below 4 are semi-global oscillations (like Fourier cosines/sines) over the entire dendrites or one of the dendrite arbors whereas those corresponding to the eigenvalues above 4 are much more localized (like wavelets) in branching regions. Figures 4 and 5 demonstrate our observation.

Finally, Figures 6 and 7 show the scatter plots of these four features of 130 RGCs (we only show two such plots here out of six possible scatter plots). The numbers in the plots are the cluster numbers obtained by Coombs et al. [2] using the hierarchical clustering algorithm on the 14 morphological features. From these figures, we can observe that Cluster 6 RGCs separate themselves quite well from the other RGC clusters. In fact, the sparse and distributed dendrite patterns such as those in Clusters 6 and 10 are located below the major axis of the point clouds in Figure 6 and above the major axis of the point clouds in Figure 7. These imply that the dendrite patterns belonging to Cluster 6 and 10 have smaller number

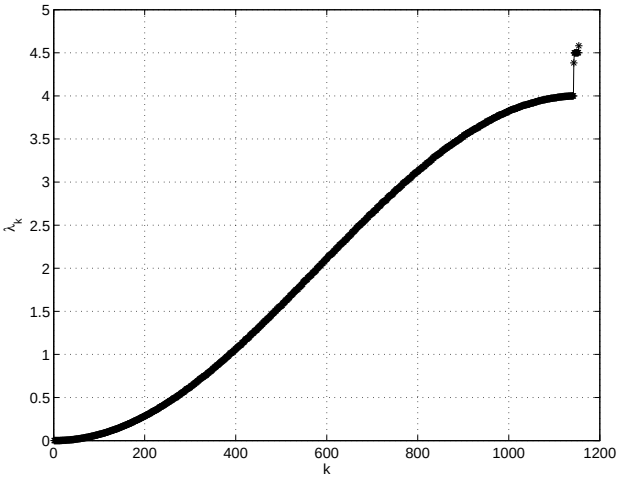


Fig. 3. A typical distribution of the Laplacian eigenvalues. RGC #100 in Cluster 6 was used for this figure.

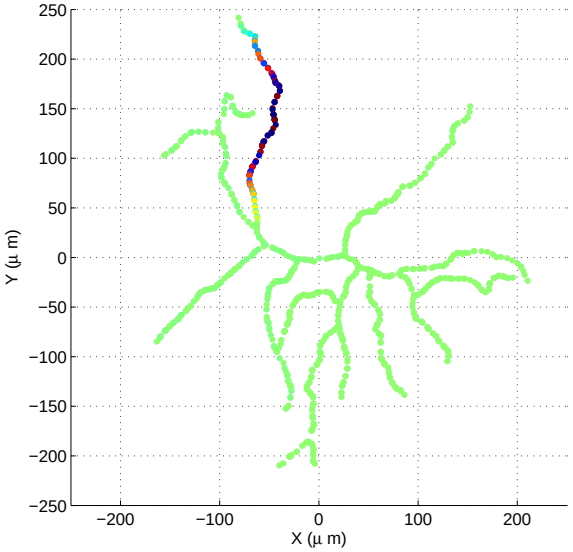


Fig. 4. The Laplacian eigenfunction of RGC #100 corresponding to the eigenvalue $\lambda_{1141} = 3.9994$, immediately below the value 4. Note that the support of this eigenfunction is semi-global, i.e., covers one whole dendrite arbor.

of spines and smaller Wiener indices compared to the other denser dendrite patterns such as Clusters 1 to 5. Also we observe that the feature variability of RGCs in Clusters 7 and 8 are higher than the other clusters.

4. Discussion

Our results reported here are still preliminary. There are many things to be done. Among them, the most urgent is to answer the following natural questions: 1) Among the features derivable by directly analyzing a graph (e.g., those 14 features used in [2]), which ones can be derived from the Laplacian eigenvalues and which ones cannot? 2) Among the features derivable by both methods, which ones can be derived more easily using the Laplacian eigenvalues than the direct graph analysis? For example, computing the isoperimetric number $i(G)$ of a given graph G is an NP problem in terms of

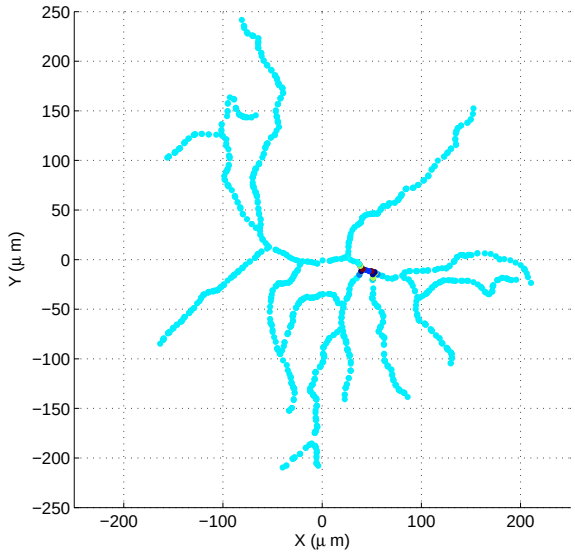


Fig. 5. The Laplacian eigenfunction of RGC #100 corresponding to the eigenvalue $\lambda_{1142} = 4.3829$, immediately above the value 4. Note that the support of this eigenfunction is localized around the branching point.

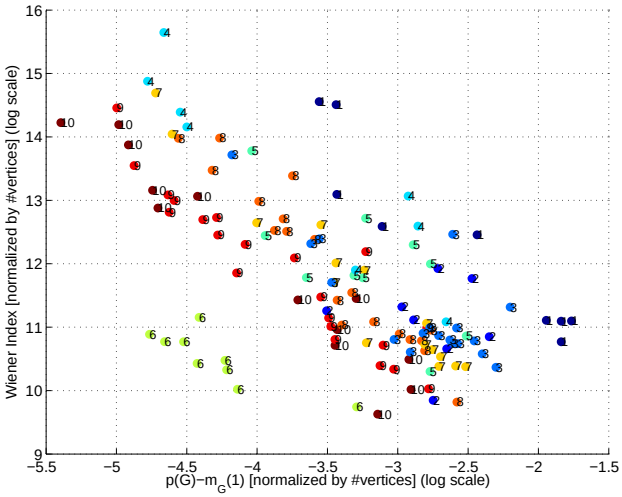


Fig. 6. A scatter plot of the normalized lower bounds of the number of the pendant neighbors vs the normalized Wiener indices.

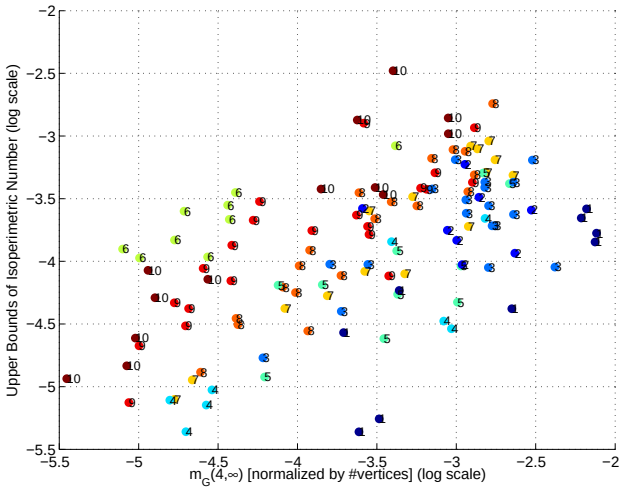


Fig. 7. A scatter plot of the normalized number of the eigenvalues larger than 4 vs the upper bounds of the isoperimetric numbers.

number of the vertices [4], and yet we can estimate its upper bound easily using the Laplacian eigenvalue as shown in (3).

Next, we also need to deepen our theoretical understanding of the sudden behavior change (like a phase transition) of the Laplacian eigenfunctions corresponding to the eigenvalues below and above 4 as demonstrated in Figures 3, 4, and 5. Note that this phenomenon occurs in each cell.

Another interesting thing we need to investigate is to "resample" the dendrite patterns so that each tree has the same number of vertices. If we can do so, then there is no need to normalize the above features by $|V(G)|$, and we can really examine whether those features are reflecting topological information of the dendrite patterns rather than the number of vertices. This resampling, however, must be done very carefully (e.g., not skipping the vertices with degree other than 2) so that we do not change the topology of the patterns.

Yet another investigation should be to consider the Dirichlet-Laplacian eigenvalue problems by explicitly imposing the Dirichlet boundary condition on the terminal nodes of the trees, and then compare the eigenvalues with those of the combinatorial Laplacians; see [1, 4] for more about the Dirichlet-Laplacian eigenvalues.

Finally, analysis using the weighted graphs, as briefly mentioned in the beginning of Section 2, should be carefully done. On one hand, the weighted graphs reflect more physical reality of the RGCs, hence we can expect more accurate results. On the other hand, analysis of such graphs are expected to be tougher than combinatorial Laplacian used in this paper because for example, $m_G(1)$ among the different RGCs does not have the same meaning anymore.

This is quite an interdisciplinary research project that taps into extremely rich mathematical ideas. We hope to report more results in the near future.

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