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ONLINE THREE-DIMENSIONAL DENDRITIC SPINES MORPHOLOGICAL CLASSIFICATION BASED ON SEMI- SUPERVISED LEARNING

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Abstract

Recent studies on neuron imaging show that there is a strong relationship between the functional properties of a neuron and its morphology, especially its dendritic spine structures. However, most of the current methods for morphological spine classification only concern features in two-dimensional (2D) space, which consequently decreases the accuracy of dendritic spine analysis. In this paper, we propose a semi-supervised learning (SSL) framework, in which spine phenotypes in three-dimensional (3D) space are considered. With training only on a few pre-classified inputs, the rest of the spines can be identified effectively. We also derived a new scheme using an affinity matrix between features to further improve the accuracy. Our experimental results indicate that a small training dataset is sufficient to classify detected dendritic spines.

Index Terms

dendritic spine; semi-supervised learning; morphological spine classification

1. INTRODUCTION

The detailed 3D shape of neuronal parts can be obtained by fluorescence microscopy techniques, such as confocal laser scanning microscopy (CLSM) and two-photon laser scanning microscopy (2PLSM) to study the fine structure of dendritic spines. Recently, intensive research has been conducted on the quantification of dendritic spine morphology. For example, Cheng *et al.* [1] and Bai *et al.* [2] proposed an algorithm for the automatic analysis of dendritic spines. Koh *et al.* [3] and Rodriguez *et al.* [4] performed spine detection research on 3D image datasets.

Dendritic spines are the post-synaptic part of glutamatergic synapses. Morphological changes of dendritic spines are highly associated with synaptic plasticity and strength. The main aim of morphological classification of dendritic spines is to present an effective way for neuroscientists to classify spines into proper phenotypes and relate them to specific functions. Detected spines can be broadly classified into three categories: mushroom, thin, and stubby based on their “head” and “neck” morphology. Examples of predefined phenotypes for dendritic spines are shown in Fig. 1.

In a previous study [4], a classification method based on the 2D rayburst diameter is proposed. The rayburst diameter in each layer of a spine is calculated, and the head to neck ratio (HNR) is introduced to define the spine shape. HNR is somewhat effective in defining spine morphology but not sufficient, since spines detected in 3D images always have highly complex shapes.

To improve the morphological classification of dendritic spines, we propose a novel algorithm based on a semi-supervised learning approach. First, we detect spines from the dendrite and extract morphological features in 3D. Second, a small portion of detected spines is selected by an expert and labeled as the training set. Finally, the labels of the remaining spines are calculated after the training while all the detected spines are classified by the learning framework.

The rest of this paper is organized as follows. Section 2 describes the detection and morphological classification strategy of dendritic spines of a 3D image dataset. Section 3 shows the semi-supervised learning algorithm in detail, and the semi-supervised learning approach is applied for the first time to the classification of 3D spines. Experimental results are shown in Section 4, and in Section 5, we draw a conclusion and describe future work.

2. MORPHOLOGICAL DETECTION OF DEDRITIC SPINES

Spine detection in 3D space is a challenging task because dendritic spines have complex shapes and cannot be easily segmented from the dendrite. After preprocessing of the 3D image stack, the dendrite together with its spines is segmented from the background through the following steps shown in Fig 2.

To detect the spines, first the centerline is extracted from the dendrite. Then, the 3D surface of the neuron is constructed. On the smooth neuron surface, three geometric features including the distances to the centerline, curvature, and normal variation of every vertex are estimated. A weighted score map is generated based on the features on vertices. Then, surface region growing and watershed are employed to detect and segment the spines. A sample raw image and the segmentation result are displayed in Fig 3.

To classify spine morphology, many features are extracted and calculated from the detected dendritic spines. Since those features have different impacts on the morphological classification, a feature selection strategy will be described in Section 3 to achieve a better classification performance.

3. A SEMI-SUPERVISED LEARNING APPROCH WITH FEATURE SELECTION

In order to improve the accuracy and reduce the size of training dataset, a novel semi-supervised learning approach is proposed to morphologically classify dendritic spines. In this framework, semi-supervised learning is halfway between supervised and unsupervised learning [5]. The approach to semi-supervised learning is to design a classifying function, which is sufficiently smooth with respect to the intrinsic structure collectively revealed by known labeled and unlabeled points [6]. In our approach, detected spines are considered as points with different features. Consider the whole dataset being represented by $\chi = \{x_1, \dots, x_l, x_{l+1}, \dots, x_n\}$, and a label set $\ell = \{1, \dots, c\}$, where n is the number of detected spines, x_i is the i -th spine, and c is the number of classes. Inside the dataset, the first l points have labels $\{y_1, \dots, y_l\} \in \ell$ and the remaining points are unlabeled. The goal is to predict the labels $\{y_{l+1}, \dots, y_n\}$ of the unlabeled data $\{x_{l+1}, \dots, x_n\}$. In this study, the remaining spines are assigned to the different classes, which are represented by the labels.

Before learning, the N detected spines with d features are considered as the dataset χ with N rows and d columns. An expert for neurobiology prepared a training dataset $\{x_1, \dots, x_l\}$, including several samples for each class. The label matrix Y is pre-labeled with the training samples, where $Y_{ic} = l$, if data i is pre-labeled in class c , l is the class to which the spine belongs; and $Y_{ic} = 0$ otherwise.

Since features in one column have the same meaning in morphology, the input dataset is normalized in each column, and the similarity between features of different data can be calculated by considering the detected spines as a graph. The semi-supervised learning algorithm is described as follows:

1. Calculate the affinity matrix W , which is defined as:

$$W_{ij} = \exp(-c_k \cdot \|x_i / x_j\|^2 / 2\sigma^2) \\ = \exp(-\sum_{k=1,d} c_k \cdot |x_{ik} / x_{jk}|^2 / 2\sigma^2) \quad (1)$$

where i, j are different points, k means the k -th feature of one point, and σ is a constant. A coefficient c_k is introduced to represent the weight of the k -th feature. Feature selection is applied to make the coefficients more reliable, meaning that they reflect the impact of different features more accurately. c_k is normalized with

$$\sum_{k=1}^d c_k = 1.$$

2. Construct the matrix $S = D^{-1/2} W D^{1/2}$, where D is a diagonal matrix with the (i, i) -

element equal to the sum of the i -th row of W , with $d_{ii} = \sum_{j=1}^n w_{ij}$.

3. Iterate the similarity matrix F until it converges, where

$$F(t+1) = \alpha S F(t) + (1 - \alpha) Y \quad (2)$$

and α is a predefined constant in our approach.

4. Let F^* denote the limit of the sequence $\{F(t)\}$, where

$$y_i = \arg \max_{j \leq c} F_{ij}^* \quad (3)$$

5. Since $0 < \alpha < 1$, and the eigenvalues of S is in $[-1, 1]$,

$$F^* = \lim_{t \rightarrow \infty} F(t) = \lim_{t \rightarrow \infty} (\alpha S)^{t-1} Y + (1 - \alpha) \lim_{t \rightarrow \infty} \sum_{i=0}^{t-1} (\alpha S)^i Y \quad (4)$$

then the classification matrix can be calculated as:

$$F^* = (I - \alpha S)^{-1} Y \quad (5)$$

Therefore, F^* can be calculated without iteration as described in [7]. After these procedures, the labels of the remaining dataset $\{x_{l+1}, \dots, x\}$ can be obtained.

To get higher performance, two main improvements are applied in this semi-supervised framework. First, in the application of the morphological classification of spines, different 3D features are obtained from the spine detection, such as the head and neck diameter, spine length, and volume, etc. To improve the results, features selection should be performed before the learning process. For each of the features, the best set of c_k can be calculated. The most effective features for the classification according to the experimental results, will be described in Section 4.

Second, the affinity matrix W plays an important role in the learning algorithm, where $\{w_{ij}\}$ reflects the similarity between the points x_i and x_j . Since even after normalization, most features still exhibit different orders of magnitude, the distance between x_{ik} and x_{jk} is not appropriate to reflect the similarity. Therefore, the value of (x_{ik}/x_{jk}) is introduced to replace the distance $(x_{ik} - x_{jk})$. The training performance is highly improved by this new definition.

Generally, after feature selection and semi-supervised learning, the rest of the dendritic spines are assigned to different classes. Since there is no iteration in this framework, the time consuming part of the semi-supervised framework depends only on the number of features being used.

4. EXPERIMENTAL RESULTS

4.1. Image Acquisition

To validate our proposed algorithm, we use a dataset that includes eleven 3D image stacks of medium spiny neurons from striatum, obtained from the Greengard Lab at The Rockefeller University. The 3D image stacks are acquired by a confocal laser scanning microscope with a resolution of $0.064 \times 0.064 \times 0.12 \mu\text{m}/\text{pixel}$, which each image in the stack having 512×512 pixels. Since the average spine length is about $1 \mu\text{m}$, the resolution of these images is high enough to get clear geometrical features of the spines during for detection. All 3D images stacks are 16-bit gray-scale images. All the dendrite spines are manually marked by an expert to produce a total of 876 spines in 11 image stacks for the classification experiments.

4.2. Experiments for the Morphological Classification of Dendritic Spines

Prior to the classification of the dendritic spines, typical phenotypes of each class are predefined by an expert neurobiologist. The proposed learning framework allows more features to be added into the training process. The feature selection procedure returns the most effective features, as shown in Table 1 for the three pre-defined spine phenotypes. Since these features are essential for the differentiation between the three phenotypes, larger weights of c_k are applied in the calculation of the affinity matrix.

In practice, the phenotype of stubby spines is similar to that of dendritic protrusions, which are actually a part of dendrite but functionally different from spines. Therefore, the detected spines can be classified using two different strategies. The first is to classify all the detected spines into three classes: mushroom, thin, and stubby. The second classification strategy classifies stubby as protrusion separately and considers mushroom and thin as spines in one category. Afterwards, we validate the classification performance by comparing the results obtained with our automated approach with those by manual analysis.

As shown in Fig. 4, an increase of the training dataset gradually leads to a higher classification accuracy of the algorithm. However, the error rate decreases slowly. For each

class, if at least eight samples are retained in training dataset, the classification accuracy is stable. At the same time, the total number of the training dataset only accounts for a very small portion of the whole dataset. Other semi-supervised algorithms, such as SVM [5], need a larger portion of training dataset. In comparison, our approach can also generate good performance with a training dataset consisting only of a few samples for each class.

In order to further compare the results with those of the algorithm introduced in [4], another experiment is conducted on the sample datasets using NeuronStudio, an existing software for dendritic spine detection developed by Rodriguez et al [4]. The dataset consists of two image stacks with size of 512×512 pixels in each slice and resolution of $0.05 \times 0.05 \times 0.10$ $\mu\text{m}/\text{pixel}$. In this experiment, a total of 147 dendritic spines are detected by NeuronStudio, and five samples for each class are selected for the morphological classification. We also use the features generated from NeuronStudio to validate both algorithms.

Table 2 shows that our learning algorithm has similar accuracy for the classification of the spines into three categories with NeuronStudio and has a higher accuracy in distinguishing protrusions from spines. The results also show that the distance between features in protrusions and normal spines are much larger than that of the mushroom and thin classes, which helps in the spine detection. Moreover, our approach for spine classification provides additional information for the performing functional analysis of spines.

5. CONCLUSION

In this work, we proposed a novel semi-supervised learning framework for the classification of three dendritic spine phenotypes detected in 3D images. We applied a feature selection strategy and an improved affinity matrix for the classification of spines. Varying weights for different features can further improve the accuracy over conventional methods of assigning equal weights. Experimental results also show that the new definition of the affinity matrix results in a better performance. Given a small training dataset, our algorithm can achieve a high accuracy for the differentiation between protrusions and spines and also shows good results for the classification of the three phenotypes, i.e., mushroom, thin, and stubby.

Future work will improve two key steps in the morphological classification of dendritic spines. First of all, spine detection has to be enhanced for dealing with spines with highly complex shapes. The improvement in detection will subsequently increase the classification accuracy. Second, more features in 3D space should be included in the training framework. The semi-supervised approach will obtain more reliable results for the morphological classification by introducing new efficient 3D features extracted from neurite images.

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Figure 1.
Examples of the predefined phenotypes, mushroom, thin, and stubby.

Figure 2.
Flowchart of dendritic spine detection

Figure 3. Examples of detected spine in 3D image stack. (a) 2D projection map of the dendrite, and (b) detected spines are marked along the dendrite.

Figure 4.

Error rates for morphological classification of dendritic spines. The X coordinate shows the error rate for classification, and the Y coordinate denotes the number of training samples in each class. The line marked with circles shows the results obtained by classification of the phenotypes into two categories, and the line with triangles shows the results of classification into three categories

Table 1

Examples of the most effective features in morphological classification of dendritic spines. The unit of length is micron (μm), and the unit of angle is degree.

	Mushroom	Thin	Stubby
HNR	15.63	16.59	None
HLR	0.059	0.085	0.076
Head Diameter	0.156	0.165	0.220
Neck Diameter	0.01	0.01	None
Max Distance	2.72	1.61	1.92
Angle to XY Plane	21.24	-16.86	1.45
Length	3.07	1.95	2.90

Table 2

Classification performance for the sample datasets of [4]. The size for the training dataset is set to five samples in each class

	NeuronStudio	Our Approach
Error Rate in 2 Classes	9.5%	6.0%
Error Rate in 3 Classes	17.1%	19.9%