DENDRITIC SPINE SHAPE ANALYSIS USING DISJUNCTIVE NORMAL SHAPE MODELS

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ABSTRACT

Analysis of dendritic spines is an essential task to understand the functional behavior of neurons. Their shape variations are known to be closely linked with neuronal activities. Spine shape analysis in particular, can assist neuroscientists to identify this relationship. A novel shape representation has been proposed recently, called Disjunctive Normal Shape Models (DNSM). DNSM is a parametric shape representation and has proven to be successful in several segmentation problems. In this paper, we apply this parametric shape representation as a feature extraction algorithm. Further, we propose a kernel density estimation (KDE) based classification approach for dendritic spine classification. We evaluate our proposed approach on a data set of 242 spines, and observe that it outperforms the classical morphological feature based approach for spine classification. Our probabilistic framework also provides a way to examine the separability of spine shape classes in the likelihood ratio space, which leads to further insights about the nature of the shape analysis problem in this context.

Index Terms— Disjunctive Normal Shape Model, Spine Classification, Shape analysis, Kernel density estimation, microscopy, neuroimaging

1. INTRODUCTION

Dendritic spines are small membranous protrusions of the dendritic shaft, and are strongly related to the functional characteristics of a neuron. Dendritic spine morphology changes are associated with neuron activities, which make the spine shape analysis an important subject for neuroscientists. The ability to automatically analyze the spine shapes would assist neuroscientists in identifying the underlying connection between spine shape and neuronal function.

In this paper, we propose an automated approach for dendritic spine shape analysis. We use recently proposed Disjunctive Normal Shape Models (DNSM) for feature extraction. DNSM [1] is an implicit parametric shape representation that represents a shape as a union of convex polytopes, which are formed by intersections of half-spaces. DNSM based shape and appearance priors have been recently introduced and have been successfully applied to various segmentation problems [2]. We apply the DNSM shape and appearance priors based approach to segment the dendritic spines from intensity images, and further use this parametric representation as our feature vector.

Dendritic spine shapes are usually classified into four groups: filopodia, thin, stubby and mushroom [3]. However, whether to view spines as belonging to distinct shape classes or whether to model them through a continuum of shape variations is still an open question. Peters and Kaiserman-Abramof pointed out that some spines were difficult to classify into traditional classes [4]. Spacek and Hartmann introduced two new shape classes lying between mushroom and stubby; and mushroom and thin [5]. Wallace and Bear argued that spine dimension measurements from their data do not agree with the idea of distinct shape classes [6].

As we will discuss later in this paper, spine classes are distributed very closely, which makes the classification problem challenging. For classification purposes, we decided to apply kernel density estimation (KDE). This nonparametric approach naturally provides the likelihood of a spine being member of each spine class compared to other approaches which use techniques to produce scores which can be interpreted as probabilities. Hence, our KDE-based approach has the potential to represent complicated shape distributions well. In addition, it also provides a natural framework to examine the distribution of shapes, including the question of whether the spine shapes constitute a continuum across classes. This work is based on two-photon laser scanning microscopy (2PLSM) images.

Major contributions of this paper are; use of the DNSM based implicit parametric shape representation for feature extraction, and development of a KDE based spine shape classification approach. Exploring the separability of spine shape classes in likelihood ratio space is another contribution of this research.

The rest of this paper is organized as follows: an overview of related studies is given in section 2. Detailed methodology is described in section 3. Experimental results are discussed in section 4. Section 5 presents the conclusion of this study and future work suggestions.

2. LITERATURE REVIEW

While there are several automated approaches proposed for dendritic spine segmentation, only a few studies address the classification problem. Rodriguez et al. [7] applied morphological features based approach on 3D confocal laser scanning microscopy (CLSM) images, and classified spines using a decision tree. To validate the performance of their algorithm, they compared their results with labels provided by human experts. In this context, they reported intra-operator and inter-operator variability. Rule based classifiers based on morphological features are commonly used in the literature to identify the shapes of spines from CLSM images (Son et al. [8] and Shi et al. [9]) and 2PLSM images (Koh et al [10]). Ghani et al. [11] also developed a morphological features based approach but applied state-of-the-art classification techniques, and also reported that head diameter and neck length are the most distinguishing features for the classification of mushroom and stubby classes.

Most of the studies reported in the literature are focused on CLSM images, only a few of them analyzed 2PLSM images. Analyzing 2PLSM images is comparatively more challenging due to low signal to noise characteristics. The reason behind using 2PLSM is that it allows imaging of living cells.

3. METHODOLOGY

Data description and details of the proposed approach are provided in this section. Mice post natal 7 to 10 days old animals are imaged using 2PLSM.[1] 15 stacks of 3D images are acquired, which are further projected to 2D using Maximum Intensity Projection (MIP) for this study. MIP is a standard procedure used in neuroscience studies. Dataset prepared for this research consists of 242 spines extracted from 15 dendrite branches. Out of 242 spines, 182 are mushroom and 60 are stubby. Mushroom spines have long necks and large heads, while stubby spines have very short necks (as illustrated in Figure 1. The spines are manually classified by an expert using MIP images, and classification algorithm outputs are compared with those labels. Our approach uses disjunctive normal shape models (DNSM) based algorithm for automated segmentation of spines and feature extraction. Shape classification is performed using kernel density estimation (KDE) based classification algorithm.

3.1. Segmentation and Feature Extraction

We define a region of interest (ROI) as an input to the segmentation algorithm. ROI is selected such that spine head is placed approximately in the center. Selected ROI is scaled to 150 pixels in the horizontal and vertical direction. Further, ROIs are aligned in such a way that spine necks are in vertical position with respect to the horizontal axis in the ROI (as illustrated in Figure 1). Currently, this process is performed manually. However, the spine head center can be automatically located by employing the spine detection approach described in [12] and then using Hough Circle Transform (HCT) to fit a circle in the spine. The alignment process can be automated as well by locating spine neck with respect to the dendrite extracted using the algorithm in [11].

DNSM approximates the characteristic function of a shape as a union of convex polytopes which themselves are represented as intersections of discriminants (half-spaces). The DNSM approximation to the shape characteristic function is given in Equation 1.

$$f(\mathbf{x}) = 1 - \prod_{i=1}^{N} \left(1 - \prod_{j=1}^{M} \frac{1}{1 + e^{\sum_{k=1}^{D+1} w_{ijk} x_k}} \right) \qquad (1)$$

Where, $D = 2$ for 2-dimensional (2D) shapes, $\mathbf{x} = \{x, y, 1\}$, M is the number of discriminants, and N is the number of polytopes. The only free parameters of the model are w_{ijk} which determine the orientation and location of the discriminants that define the half-spaces. Further details of the DNSM can be found in [1].

For this study, we use the DNSM based segmentation approach discussed in [2] that exploits the parametric nature of DNSM. It applies the DNSM based shape and appearance priors. The segmentation of spines using the DNSM shape and appearance priors has two stages: training and testing. During the training stage: first, the training spine shapes are represented by their DNSM parameters. Then, the local shape and appearance priors are constructed from the training samples. The use of local shape prior results in good segmentation even when limited training shapes are available, because the method generates a rich set of shape variations by locally combining training samples. In addition, by studying the intensity statistics around each discriminant of the DNSM model, the local appearance priors with better expressive capability are constructed from the training samples. Since segmentation of spines require differentiating between the spine regions and the dendritic part (both of which have similar intensity levels in 2PLSM images), the use of a local appearance prior is crucial. During the testing stage, the spines are segmented by minimizing the weighted average of the shape and appearance prior energy terms using the gradient decent. Further details about training and testing process can be found in [2]. This approach has several parameters which must be adjusted for different applications: number of polytopes N, number of half spaces M; and level of contribution from appearance and shape priors, γ and α. Values for these parameters has been found by empirical analysis, $M = 16$, $N = 8$, $\gamma = 0.5$ and $\alpha = 0.05$. Segmentation results for a few spines are given in Figure 1.

As discussed earlier, DNSM is a parametric shape model. Once segmentation of the given image has been performed, it gives $M \times N \times 3$ parameters,w_{ijk}, representing the segmented image. These parameters can also be used as feature vector to train a classifier and perform classification. In this study, we test the potential of DNSM based feature vector for classification of dendritic spines. We also use this parameter space for studying the shape statistics.

[1]All animal experiments are carried out in accordance with European Union regulations on animal care and use, and with the approval of the Portuguese Veterinary Authority (DGV).

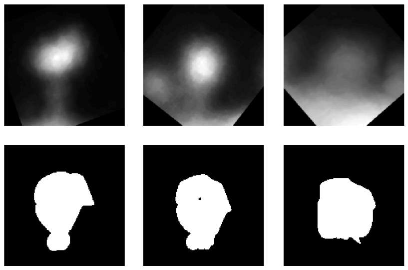

Fig. 1. A few images from dataset, without segmentation (above) and segmented images (below). First 2 spines are labeled as Mushroom and 3rd spine as Stubby.

3.2. Classification

For classification, we perform non-parametric density estimation and apply a likelihood ratio test (LRT). Our non-parametric density estimation approach is similar to [13]. Suppose that we have m features $x_1, x_2, \ldots, x_m$ sampled from an n-dimensional density function $p(x)$. The Parzen density can be estimated using Equation 2.

$$\hat{p}(x) = \frac{1}{m} \sum_{i=1}^{m} k(x - x_i, \Sigma) \tag{2}$$

Where, $k(x, \Sigma) = \mathcal{N}(x; 0, \Sigma^T \Sigma)$ is an n-dimensional kernel, which can be simplified using the assumption that kernel is spherical, i.e.,$\Sigma = \sigma I$. Applying this assumption Equation 2 can be simplified, as given in Equation 3.

$$\hat{p}(x) = \frac{1}{m} \sum_{i=1}^{m} k(d(x, x_i), \sigma) \tag{3}$$

Where $d(x, x_i)$ is the ℓ_2 distance between x and x_i in $\mathbb{R}^n$ and $k(x, \sigma) = \mathcal{N}(x; 0, \sigma^2)$ is the 1D Gaussian kernel. Kernel size (σ) is estimated by the bracket method (also known as the bisection method). First, we compute 1D kernel size from each feature vector and use this m dimensional kernel size vector to compute minimum (σ_{min}) and maximum kernel size (σ_{max}). Finally, we apply the bracket method to compute the optimal kernel size in $[\sigma_{min}, \sigma_{max}]$ range by iteratively bisecting the interval and selecting the subinterval that contains the optimal kernel size.

Once we have estimated the likelihood of an image belonging to Mushroom (l_m) and Stubby (l_s) classes, we can perform classification using the LRT, as depicted in Equation 4, here M denotes mushroom and S denotes stubby class. This approach simplifies the classification process by mapping an n-dimensional classification problem to 1D problem, specifying the problem in terms of likelihood ratios.

$$\frac{l_m}{l_s} \underset{\text{Decide S}}{\overset{\text{Decide M}}{\gtrless}} 1 \tag{4}$$

4. RESULTS

We compare the performance of this new feature extraction technique with a morphological features based approach we have published earlier [11]. For this purpose, we use the automated segmented images and apply the morphological feature extraction algorithm.

Table 1. Classification Results, comparison of feature extraction and classification approach

Classifier	**Features**	**Accuracy**
KDE	Ghani et al. [11]	79.34%
	DNSM	**85.54%**
SVM	Ghani et al. [11]	80.17%
	DNSM	**84.30%**
Neural Network	Ghani et al. [11]	77.69%
	DNSM	**85.54%**

In order to test the potential of our classification approach, we compare its performance with two state-of-the-art classification algorithms, support vector machines (SVM) and Neural Networks (NN). We used the linear kernel for SVM and 2-layer network with 193 nodes in each layer for Neural Network. We compared both feature extraction schemes for all three classifiers using 10 fold cross validation; results are presented in Table 1.

As we can see from Table 1, proposed feature extraction technique results in higher classification accuracy for all classifiers with respect to commonly used morphological features. We suggest that the potential of DNSM based feature extraction approach for dendritic spine classification needs to be further explored. Confusion matrix for DNSM features with KDE and Neural Network classifier is given in Table 2, which shows that KDE classifier gives relatively fair weight to both classes. Another conclusion to draw from Table 1 is that, DNSM features combined with Neural Network or KDE based Likelihood Ratio classification outperforms other methods. Given the statistical description in terms of the DNSM features, our approach works using the likelihood ratio, which is the sufficient statistic for the classification problem.

As discussed earlier, whether to view spines as belonging to distinct shape classes or to model them through a continuum of shape variations is still an open question. Since our KDE based approach gives the likelihood of a spine being member of Mushroom (l_m) and Stubby (l_s) classes, it can be used to examine this question in a principled manner. We computed the histogram of likelihood ratios, as given in Figure 2 and analyzed whether we see two distinct modes or a continuum of shapes. It is evident from the presented histogram that we do not see two clearly separable distributions but a mixture of distributions, which are closely inter-

Table 2. Confusion Matrix for DNSM features with KDE and Neural Network classifier

	Neural Network		KDE	
Classified as →	M	S	M	S
M	170	12	159	23
S	23	37	12	48

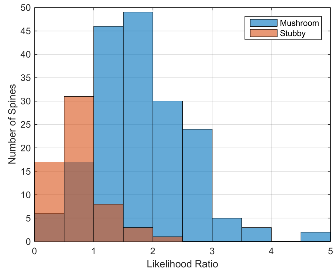

Fig. 2. Histogram of likelihood ratios

linked. One can observe two classes, Mushroom and Stubby, but there is a significant overlap between their distributions. For analyzing the statistical significance of this framework, we performed two-sample t-test with null hypothesis to have same mean for both class distributions. Two-tailed test gives us an insight that both class distributions have different mean, meaning that there exist two distinct classes. It rejects the null hypothesis with p-value of 1.77×10^{-29} that strongly supports the significance of our analysis.

If we treat this problem as a classification task, best performance (assuming equal priors and the probability of error as the decision criterion) can be achieved by thresholding likelihood ratio space at "1" to perform classification. However, classifying the spines lying around this threshold is a difficult decision since values of l_m and l_s are very close in these case. Our KDE based framework provides a principled approach to handle such spines, if values of l_m and l_s are not very different, one might use the help of neuroscientists to manually look at spines and make a decision. Further analysis may also include 3D image evaluation of the spines whose likelihoods for different classes are very close.

5. CONCLUSION

Since dendritic spine shape analysis can lead to a better understanding of the connection between spine shape changes and neuronal activities, it is an attractive research aspect for neuroscientists. Availability of dependable automated analysis tools can expedite this research. Methods proposed in this paper aim to develop such tools. We propose a new spine shape analysis framework that classifies the spines into the groups suggested in literature. We apply the DNSM based approach to segment the intensity images of spines and provide an interesting shape representation. Further, we use this shape representation to estimate the density for each spine class and apply LRT to perform classification. Experimental results confirm that DNSM based representation performs better as compared to morphological features based analysis. We also observed that neural network and KDE based classification algorithm combined with DNSM features outperform other state of the methods. Our suggested framework also enables the analysis of the continuum of shapes in a principled manner. Preliminary analysis reveal that two distinct classes exist but they are close,i.e.,signal to noise ratio (SNR) is low. In future, we will investigate continuum of shapes in detail and attempt to answer the question whether spine analysis problem should be addressed as a classification problem or not.

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