

# Dendritic Spine Classification based on Two-Photon Microscopic Images using Sparse Representation

## İki Foton Mikroskopik Görüntülerdeki Dendritik Dikenlerin Seyrek Temsil Kullanarak Sınıflandırılması

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**Abstract**—Dendritic spines, membranous protrusions of neurons, are one of the few prominent characteristics of neurons. Their shapes change with variations in neuron activity. Spine shape analysis plays a significant role in inferring the inherent relationship between neuron activity and spine morphology variations. First step towards integrating rich shape information is to classify spines into four shape classes reported in literature. This analysis is currently performed manually due to the deficiency of fully automated and reliable tools, which is a time intensive task with subjective results. Availability of automated analysis tools can expedite the analysis process. In this paper, we compare  $\ell_1$ -norm-based sparse representation based classification approach to the least squares method, and the  $\ell_2$ -norm method for dendritic spine classification as well as to a morphological feature-based approach. On a dataset of 242 automatically segmented stubby and mushroom spines,  $\ell_1$  representation with non-negativity constraint resulted in classification accuracy of 88.02%, which is the highest performance among the techniques considered here.

**Keywords**—Dendritic Spines, Classification, Sparse Representation,  $\ell_1$ ,  $\ell_2$ , least-squares, Neuroimaging.

**Özetçe** —Sinir hücrelerinin zarlı çıkıntıları olan dendritik dikenler, bu hücrelerin önde gelen yapılarından biridir. Sinirsel aktivitedeki değişiklikler dendritik dikenlerin şeklinin değişmesine neden olur. Bu nedenle diken şekil analizi, sinirsel aktivite ve diken morfolojisi arasındaki doğal ilişkiyi anlamada belirgin bir rol oynar. Şekil bilgisini ele alırken ilk adım, dikenleri literatürdeki dört şekil sınıfına göre sınıflandırmaktır. Tamamıyla otomatik ve güvenilir araçların olmaması nedeniyle bu analiz elle yapılmaktadır. Çok zaman isteyen bu işlem öznel sonuçlar ortaya çıkarmaktadır. Otomatik analiz araçları bu işlemi kolaylaştırabilir. Bu çalışmada, dendritik dikenlerin sınıflandırılmasında  $\ell_1$ -norm temelli seyrek temsile dayalı sınıflandırma yaklaşımı, en küçük kareler yöntemleri ile,  $\ell_2$ -norm yöntemiyle, ve morfolojik özneliğe dayalı yaklaşım ile karşılaştırıldı. Otomatik olarak bölütlenmiş mantar ve güdük dikenleri içeren toplam 242 dikenin bulunduğu veri kümesinde,  $\ell_1$  yaklaşımı %88.02'lik doğruluk oranıyla uygulanan yöntemler arasında en yüksek performansı gösterdi.

**Anahtar Kelimeler**—Dendritik diken, Sınıflandırma, Seyrek Temsil,  $\ell_1$ ,  $\ell_2$ , küçük kareler, Nörogörüntüleme.



Figure 1: Spine Classes: Mushroom, Stubby, Thin, Filopodia.

### I. INTRODUCTION

Dendritic spines were first discovered by Ramon y Cajal in 19th century, and later, the structural changes in dendritic spines were linked to neuron activities [1], [2]. Dendritic spine shape analysis has become significantly important for neurobiological research since it has the potential to enable the neuroscientists to decode the underlying relationship between neuron activity variations and spine morphology changes [1]. Therefore quantitative spine analysis has become an important research topic in contemporary neuroscience. In the literature, dendritic spines are usually grouped into four shape classes: mushroom, stubby, filopodia, and thin [3]. Examples of these four spine shape classes are given in Figure 1.

The motivation behind this paper is the use and wide success of sparsity based algorithms for various image classification problems. Sparse representation attempts to compute the sparse decomposition of signals in a dictionary [4]. Sparse representation has proven to be successful in a wide range of applications; from signal representation to acquisition and compression of high dimensional signals [5]. It has also offered effective solutions to computer vision problems such as face recognition [6] and image classification [7]. It has been claimed that this approach uses the inherent property of most natural images; images from the same class demonstrate degenerate structure [5]. To best of the author's knowledge, this approach has not been applied for spine analysis, therefore, it is an interesting experiment to find out if the sparsity assumption holds for dendritic spines.

Dendritic spine images are acquired in 3D stacks using two photon laser scanning microscopy (2PLSM). From the 3D images, maximum intensity projection (MIP) is calculated

for further analysis. We apply the  $\ell_1$  norm based approach discussed in [6] and compare the classification results with the least squares method (also referred as the orthonormal  $\ell_2$ -norm method in [8]).

The rest of this paper is organized as follows: a brief summary of studies on dendritic spine analysis and sparse representation is presented in Section II. Section III provides an overview for the methodology of techniques applied. Experimental results are discussed in Section IV. Section V describes the conclusions of this research.

## II. LITERATURE REVIEW

Most of on spine classification compute morphological features and perform classification using rule based algorithms. Rodriguez et al. [9] employed head to neck ratio, aspect ratio, neck length and head diameter; and applied decision tree for classification. They reported intra-operator and inter-operator variability in assigning labels. A recent study on spine analysis [10] considered head diameter, neck length, perimeter, area and other morphological features to classify spines to mushroom and stubby types.

Correct identification of basis for representing the data is essential for sparse representation [5]. The  $\ell_1$ -minimizer based sparse representation has been applied in [6] for face recognition. The main idea of their approach was to train a task-specific dictionary from training images and then represent test images as a sparse combination of training images. Application of sparsity for the face recognition problem is criticized by [8] claiming that face data do not comply with the sparsity assumption. Shi et al. apply the least squares approach for face recognition and claim to achieve more robust performance [8].

To the best of the author's knowledge, sparsity based algorithms have not been used for spine analysis. Main contributions of this paper are application of  $\ell_1$ -norm-based sparse representation for spine classification and its comparison to the least squares method and the  $\ell_2$ -norm method.

## III. METHODOLOGY

### A. Dataset preparation

2PLSM has been used to image mice post natal 7 to 10 days old every 5 minutes<sup>1</sup>. 15 3D image stacks are acquired. 3D images are further projected to 2D using maximum intensity projection (MIP). 242 spines are labeled manually by a human expert. The dataset consists of 182 mushroom and 60 stubby spines.

We applied the disjunctive normal shape models (DNSM) [11] based algorithm to automatically segment the dendritic spines. This algorithm uses DNSM based shape and appearance priors for segmentation [12]. We input a region of interest (ROI) to this algorithm. The ROI is selected in such a way that the head center of spine is located almost in the center of the ROI. Then, we scale the ROI to  $150 \times 150$  pixels. Further, all spine ROIs are aligned in such a way that spine necks are vertically aligned. Several images from the dataset are given in

<sup>1</sup>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).

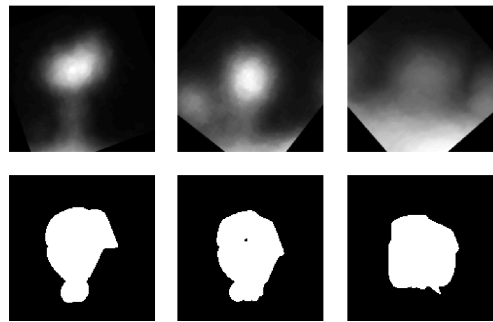


Figure 2: A few images from dataset, input to segmentation algorithm (above) and output of segmentation algorithm (below). Labeled as mushroom, mushroom, and stubby (from left to right).

Figure 2. Finally, we use 10-fold cross validation approach to automatically segment the spines using DNSM. Same training and testing folds are used during classification.

### B. Sparse Representation based Classification

The assumption behind sparsity based classification is that spine shapes from the same class lie on a low-dimensional linear subspace. The idea is to represent incoming test spine image as a linear combination of spines from the training data. The sparse coefficients produced by this representation can then be used for classification [6]. Sparsity requires these coefficients to be dominant for one class and zero for all other classes. This can be achieved using  $\ell_0$  minimization but for many applications that is an NP-hard problem [6]. However, if the  $\ell_0$  solution is fairly sparse, it is equivalent to solving the  $\ell_1$  minimization problem [6].

We construct the matrix  $A_i = [s_{i,1}, s_{i,2}, \dots, s_{i,n_i}] \in \mathbb{R}^{m \times n_i}$  with  $n_i$  training samples from the  $i$ th class, where each training sample represents a column of  $A_i$  by stacking columns of each training image. Hence, each column  $s_i$  has  $m = \text{width} \times \text{height}$  rows. Now, assuming sufficient samples are available for training, any new image ( $t \in \mathbb{R}^m$ ) from the  $i$ th class can be linearly represented in terms of training images of the same class using Equation 1.

$$t = \zeta_{i,1}s_{i,1} + \zeta_{i,2}s_{i,2} + \dots + \zeta_{i,n_i}s_{i,n_i} \quad (1)$$

where,  $\zeta_{i,j} \in \mathbb{R}$  is a scalar  $\forall j$ . For classifying, as the class membership is initially unknown, we construct a new matrix  $A$  containing complete  $n$  training data available for all  $k$  classes, as illustrated in Equation 2.

$$A = [A_1, A_2, \dots, A_k] = [s_{1,1}, s_{1,2}, \dots, s_{k,n_k}] \quad (2)$$

The linear representation would be modified to the form of Equation 3.

$$s = Ax_0 \in \mathbb{R}^m \quad (3)$$

Where,  $x_0 = [0, \dots, \zeta_{i,1}, \zeta_{i,2}, \dots, \zeta_{i,n_i}, 0, \dots, 0]^T \in \mathbb{R}^n$  is the sparse coefficients vector, ideally with all zero elements except the ones associated with the  $i$ th class. Here, class information is encoded in entries of vector  $x_0$ , which can be easily exploited to perform classification. As discussed earlier,  $\ell_0$  minimization problem is NP-hard and it is equivalent to  $\ell_1$  solution assuming that it is sufficiently sparse. The solution

for this problem can be achieved in polynomial time and there are several solutions reported in the literature.

In this paper, we use an  $\ell_1$  regularized least squares problem (LSP) solution proposed by Kim et al. [13], as presented in Equation 4.

$$\text{minimize } \|Ax - t\|^2 + \lambda \|x\|_1 \quad (4)$$

where  $x \in \mathbb{R}^n$  is the variable,  $\lambda$  is the regularization parameter and  $t \in \mathbb{R}^m$ . We also used another  $\ell_1$  regularized LSP solution proposed by Kim et al. [13] with additional non-negativity constraint, as given in Equation 5.

$$\begin{aligned} \text{minimize } & \|Ax - t\|^2 + \lambda \sum_{i=1}^m x_i \\ \text{subject to } & x_i \geq 0, i = 1, 2, \dots, m \end{aligned} \quad (5)$$

Optimizing regularization parameter  $\lambda$  is important to achieve sufficiently sparse solutions using Equation 4 and 5. For this purpose, we identify a sparsity measure and optimize sparsity for both of these techniques. Hurley and Rickard [14] compared different sparsity measures and declared Gini index (GI) to be performing best based on several intuitive attributes. GI is widely used as a sparsity measure; have various advantages over other methods, GI is normalized, an index value of 0 means least sparse solution and 1 means the sparsest solution. We use bisection method (bracket method) to optimize the regularization parameter  $\lambda$ . Optimized value of  $\lambda$  for  $\ell_1$ -based solution (computed using Equation 4) is found to be 425 with average GI value of 0.956. Similar optimization problem has been solved for  $\ell_1$  with non-negativity constraint (using Equation 5), optimized value of  $\lambda$  for this problem is found to be 0.001 that results in 0.963 GI value, which is slightly better than  $\ell_1$ -based approach.

In order to perform classification, the sparse representation-based classification (SRC) algorithm proposed by Wright et al. [6] is applied. It performs classification based on minimum residuals (as illustrated in Equation 6). Where,  $\delta_i(\hat{x}_i)$  represent residuals for the  $i$ th class.

$$\text{Class}(t) = \text{argmin}_i \|t - A\delta_i(\hat{x}_i)\|_2 \quad (6)$$

For an overdetermined case,  $m > n$ , the solution of linear system of equations,  $x_0$ , is mostly unique. Here,  $n$  is the number of training images and  $m$  is the size of image ( $\text{width} \times \text{height}$ ). Hence, uniqueness of solution depends upon number of training images and their dimensions. For spine classification problem,  $m = 242$  and  $n = 22500$ , therefore it is an overdetermined system.

#### C. The least squares method method for classification

The idea behind the least squares method is similar to  $\ell_1$  approach, i.e., represent test image as a linear combination of training images. However, in comparison to the  $\ell_1$  case,  $\zeta$  are estimated by applying the least squares method using Equation 7 [8].

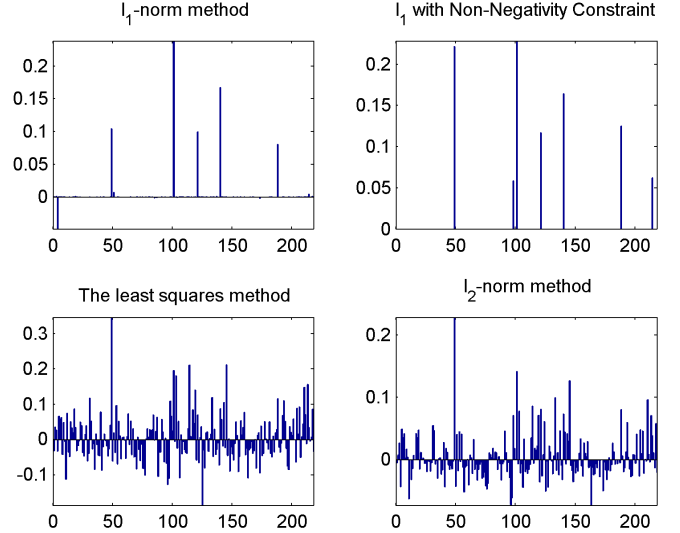


Figure 3: Linear representation coefficients using different minimization algorithms.

$$\hat{\zeta} = \text{argmin}_{\alpha \in \mathbb{R}^n} \|t - A\zeta\|_2 \quad (7)$$

Solution of Equation 7 can be found by re-formulating the pseudo-inverse. We can perform QR factorization, since our input data is real  $\mathbf{Q}$  would form an orthonormal basis, and  $\mathbf{R}$  an upper triangle matrix. Using this approach we can estimate representation coefficients  $\hat{\zeta}$ , as given in Equation 8.

$$\begin{aligned} \text{Compute } \mathbf{QR} &= \mathbf{A} \\ \hat{\zeta} &= \mathbf{R}^{-1} \mathbf{Q}^T \mathbf{t} \end{aligned} \quad (8)$$

Once  $\hat{\zeta}$  are computed, classification is performed using Equation 9. This is similar to the SRC algorithm described by [6].

$$\text{Class}(t) = \text{argmin}_k \|t - A_k \hat{\zeta}_k\|_2 \quad (9)$$

#### D. The $\ell_2$ -norm method for classification

Using this approach, we represent the test image as a linear combination of training images, however, we use Tikhonov regularization to achieve this representation. We estimate the representation coefficients,  $\hat{\zeta}$ , by applying  $\ell_2$ -norm constraint on coefficients, as illustrated in Equation 10. We optimized value of regularization parameter,  $\lambda$ , using GI. This method resulted in an average GI value of 0.48 for optimized  $\lambda$ . After computing the representation coefficients, we use Equation 6 to perform classification.

$$\text{minimize } \|Ax - t\|^2 + \lambda^2 \|x\|_2 \quad (10)$$

## IV. RESULTS

Experiments have been conducted to compare the performance of the four algorithms (including two versions of the  $\ell_1$ -norm-based approach). The  $\ell_1$  minimization algorithms provided in the  $\ell_1$  minimization toolbox [15], the least squares method (referred as  $\ell_2$ -method in [8]) and the  $\ell_2$ -norm method

Table I: Classification results

Representation algorithm	Accuracy
$\ell_1$ -norm	87.60%
$\ell_1$ with non-negativity constraint	<b>88.02%</b>
The least squares method	81.41%
$\ell_2$ -norm	85.12%

Table II: Classification results using the morphological feature-based approach.

Classifier	Accuracy
SVM	78.51%
KNN	80.17%
RF	81.41%

are applied to compute the sparse representation. Linear representation coefficients achieved for a single spine image using different algorithms are given in Figure 3. It is evident from achieved results that  $\ell_1$  minimization algorithm with additional non-negativity constraint gives the sparsest solution and least squares approach gives least sparse solution.

Sparse coefficients computed using  $\ell_1$ ,  $\ell_1$  with non-negativity constraint, the least squares method, and  $\ell_2$ -norm method have been used to perform classification. Classification results are computed using 10-fold cross validation. Training and testing folds are the same as those used for DNSM based segmentation. Classification results are given in Table I. As discussed earlier,  $\ell_1$  with non-negativity approach result in most sparse solutions, and least squares method provides least sparse solutions, classification results also support these observations. The least squares method provides 81.41% accuracy; classification accuracy is slightly improved using Tikhonov regularization, which classifies to 85.12% spines correctly. It slightly improves with the  $\ell_1$ -norm approach and classifies 87.60% spines correctly. We can achieve a slightly better performance, i.e., 88.02% accuracy, using SRC algorithm proposed by Wright et. al. [6], when sparse coefficients are computed using the  $\ell_1$ -norm approach with additional non-negativity constraint proposed by Kim et al. [13].

In order to compare the classification results of the sparsity based approach with a standard morphological feature-based technique, we implemented the algorithm described in [10] and computed classification results, given in Table II. It is established that sparsity based technique performs better than the morphological feature-based technique.

## V. CONCLUSION

Dendritic spine shape analysis is important for neurobiological research due to functional coupling of spine morphology with neuron activity. This analysis is currently performed manually due to unavailability of reliable automated analysis tools. This paper aims to contribute to the effort of filling this gap. We compared three state of the art classification techniques for dendritic spine classification. It is found that the  $\ell_1$  minimization approach with additional non-negativity constraint gives the sparsest solutions. The least squares method (also called orthonormal  $\ell_2$ -norm approach in [8]) gives 81.41% accuracy for spine classification which is similar to the performance achieved using morphological feature-based approach. The  $\ell_2$ -norm method performs slightly better

than least squares method and correctly classifies 85.12% test images. The  $\ell_1$ -norm approach with additional non-negativity constraint results in 88.02% accuracy which is better as compared to all other techniques considered in this paper.

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