13—2 Segmentation of Neurons Using

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Abstract

This paper deals with segmentation of nerve cells in microscopic images that have in-focus and out-of-focus structures of neurons. Test images are acquired by a CCD camera as a stack of grayscale images, and the dendritic structures are segmented using Mathematical Morphology Tools. Segmentation is decomposed into two stages: first, a filtering scheme is implemented to remove the noise; second, a method to image deblurring is proposed where in-focus and out-of-focus structures are separeted based on Morphological Grayscale Reconstruction. The in-focus parts of the neuron are then passed to the contour detection module. From the results, we can conclude that grayscale shape-size information can be used to sufficiently discriminate between in-focus and out-of-focus dendritic tree structures.

1 Introduction

The present work is concerned with a segmentation approach for three-dimensional reconstruction and measure extraction of nerve cells in microscopic images that have in-focus and out-of-focus structures. These tasks are performed after image segmentation. This paper focuses on the segmentation process developed for contour extraction of the in-focus structures of the neuron.

Neuroscientists have studied the relationship between morphology and function of nerve cells by searching for links between morphology and behaviour, and morphology and disease. Malnutrition in the earliest periods of life can alter brain structure and function, both in laboratory animals and in being human. Recent clinical studies in underweight new-borns and children show that reduced neuro-development has the effect of reducing the visual functions in areas as color vision and contrast sensitiviness, showing that the visual cortex is very sensitive to nutritional changes [3], [5]. To study these effects, quantitative analysis and morphometric characterization of nerve cells must be performed. Detailed measurements, such as number of branch points, branching order, branch lengths, tortuosity, fractal dimension, soma diameter, dendritic tree area, volume and angle between branches, require accurate models of nerve cells. Exact measurement of these parameters is only possible if these tree-like structures are accurately segmented.

Most works found in literature are based on manual tracing of the contours on a white paper sheet and a digitizing tablet, or pixel-by-pixel tracing directly on video. Recently, semi-automatic segmentation methods have been employed to extract nerve cell measurements [2], [6], [9], [11]. Nevertheless, none of the previous works addressed the image deblurring problem produced by optical and confocal image acquisition methods, which affects the intensity and shape measurements. The "halo" effect is reduced with confocal microscopy, but is not removed. Simple threshold is not able to cope with image deblurring [11]. Our approach is based on grayscale shape-size information, which is closer to the way of a natural approach for separation between in-focus and out-of-focus dendrites.

The main contributions of this work are twofold: First, it presents a novel approach to segmentation of nerve cells which deal with the image deblurring problem in neuro images. Second, it improves the efficiency and the accuracy in segmentation process by reduce the subjectivity and human intervention.

In Section II, a brief review on main Grayscale Mathematical Morphology (MM) notions and tools used in this work is presented. In Section III, we present the method for segmentation of nerve cells. In Section IV, we show the results obtained. Finally, Section V presents the conclusions.

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2 Grayscale Mathematical Morphology

Most works on segmentation of neurons are based on photometric criteria. Our aproach is based on Morphological Grayscale Reconstruction [1], [4], [7], [8] using graylevel shape-size informations. The gray-level MM operators deal with functions $f: E \rightarrow [0,255] \in \mathbf{z}$, where E is the usual digital planar grid (gray-level images). The basic geometrical idea behind these operators is to probe the image with a function called *structuring element*, which is defined on a small subset of the image domain of definition. The basic dual grayscale MM operators are dilatation and erosion, defined by

$$\delta_g(f)(x,y) = \max\{f(x',y') + g(x-x', y-y')\}, \quad \forall (x',y') \in (B' + (x,y)) \cap E \tag{1}$$

$$\varepsilon_g(f)(x,y) = \min\{f(x',y') - g(x'-x, y'-y)\}, \quad \forall (x',y') \in (B + (x,y)) \cap E \tag{2}$$

where f is the image, g and B denote respectively the structuring element values and domain of definition $(B \subset E)$. The composition of erosion and dilatation yelds the opening and closing operators. These are morphological filters with good noise-removal properties. The interested reader can find a more thorough description in [4] and [7]. Reconstruction operators have been used to obtain maxima and minima (i.e. peaks, and valleys) in grayscale images. Let I and J be two binary/grayscale images. I is referred to as the *mask* image and J as the *marker* image. In the binary case, the reconstruction operator $R(I,J)$ simply extracts connected components of I which are *marked* by J. In the grayscale case, the grayscale reconstruction operator extracts the *peaks* I which are marked by a function J. For a formal definition of these operators, the reader is referred to [8].

3 Segmentation of Nerve Cell

A problem in 3D reconstruction and measure extraction is to find a suitable segmentation procedure for describing the classes of objects which the system aims to measure. In this wirk we aim to extract measurements of nerve cells found in images as showed in Fig 1. This brings to the segmentation the task of accurately discriminate the objects of interest which have their shape modified by the blurring effect. In our opinion, to be extracted sufficiently reliably, the measuring must be done only on in-focus parts of the neuron, hence the need for remove the out-of-focus ones.

Two important issues in the design of the segmentation process are efficiency and robustness. The first is concerned with finding the contours of the neurons as quickly as possible, and the second is concerned with error management where false contour detection is to be dealt with. Typical images of neurons presents variations in contrast, shape and size of the neurons, which introduces complexity to segmentation. To obtain a final image with only in-focus structures, we perform image filtering and image deblurring steps. We also perform image inversion to get the rigth topographic image model, where the objects of interest (in-focus structures of the neuron) become "peaks". Image filtering is accomplished by using the opening by reconstruction operator $R_{ope}(I)$ [8]. The noise to be removed is characterized by small white points around the neuron. These points and the in-focus structures of interest have similar intensities. The $R_{ope}(I)$ operator recquires two images as input: the mask image I (inverted image in our case) and the marker image. The marker is obtained by a serie of opening transformations on I ($Ope(I)$). The reconstruction is obtained by iterating grayscale conditional dilatations of

$$J = Ope(I) \tag{3}$$

"under" I until stability is reached. To deal with image deblurring, we also performan opening by reconstruction. The mask and marker images are the same as for image filtering, but the number of iterations is only 1. A grayscale subtraction

$$S = \textit{original image - output from image deblurring} \tag{4}$$

is performed and the result is thresholded (T=128). The image resulting from image filtering is combined with the thresholded image to perform the extraction of the in-focus structures of the neuron. The reconstruction operator is used with the filtered image (mask) and the thresholded image (marker). Grayscale conditional dilatations are performed until stability is reached (Fig. 2). The Morphological Gradient operator is used to detect the contours. We can compare the detected contours with original image in Fig. 3.

4 Experiments and Results

This section presents results on applying our segmentation procedure on images of a neuron. Wistar rats were sedated and perfused. The brains were removed and cut on a vibratome at a thickness of 200 μm. The tissue slices with neurons from the visual cortex were stained with NADPH-diaphorase and biocytin. The stained sections were imaged directly from a optical microscope using a coupled CCD camera. A Nikon N.A. 1.0 x 40 objective lens was used The segmentation method was applied on 79 test images (optical sections recorded at 0.25 μm, 0.5 μm and

1 μm intervals through the entire slice, resulting in 3 stacks of images). Each image was 640 x 480 pixels. The procedure was applyed on each image and comparison of detected contours with manual segmentation by experts showed similar results.

5 Conclusions

This paper has presented a method to perform segmentation of nerve cells from microscopic images. There has not been much work in segmentation of nerve cells based on shape-size informations. Previous works have addressed mainly the reconstruction and measuring procedures in a manual or semi automated way without treat the image deblurring prior these tasks. The segmantation technique presented in this paper has different features addressing issues of image deblurring and being a natural approach to handle the segmentation. Of particular significance is the fact that the segmentation process was capable of both identifying and removing noise and out-of-focus parts of the cell, which have similar features in intensity, shape and size to in-focus structures. The method divides the segmentation process into two stages: an image filtering stage and a image deblurring stage. This method improves the robustness and efficiency in segmentation and contour detection by reduce the subjectiveness and human intervention when analysis of large samples are necessary for statistically useful conclusions.

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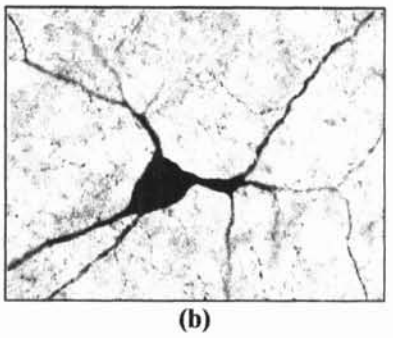
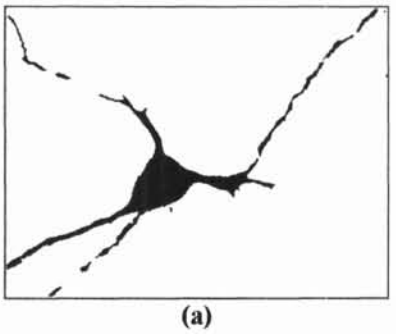
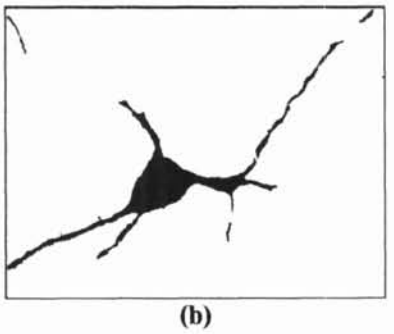


(b)

Fig. 1. Two typical images (a) and (b) (optical sections) of nerve cell.

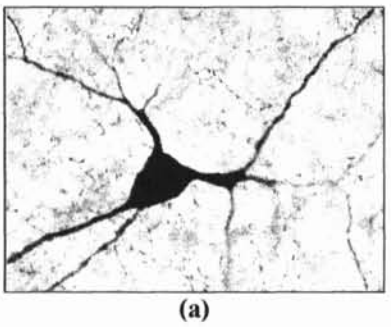


(a)

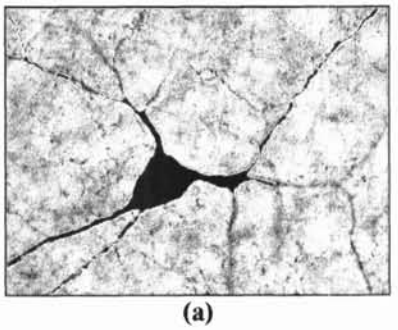


(b)

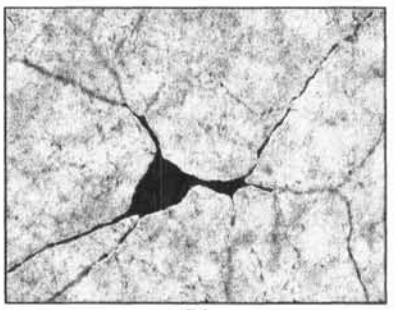
Fig. 2. The thresholded images.



(a)



(a)



(b)

Fig. 3. Contour images superimposed to original images.

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