Journal of Neuroscience Methods 190 (2010) 299–309

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

A computational framework for studying neuron morphology from *in vitro* high content neuron-based screening

Yue Huang[a,b], Xiaobo Zhou[a,*], Benchun Miao[c], Marta Lipinski[d], Yong Zhang[a], Fuhai Li[a], Alexei Degterev[c], Junying Yuan[d], Guangshu Hu[b], Stephen T.C. Wong[a]

[a] *Methodist Hospital Research Institute, Radiology Department, 6565 Fannin Street, B5-022, Houston, TX 77030, United States*
[b] *Biomedical Engineering Department, Medical School, Tsinghua University, Beijing 100084, People's Republic of China*
[c] *Department of Biochemistry, Tufts University School of Medicine, Boston, MA 02111, United States*
[d] *Department of Cell Biology, Harvard Medical School, Boston, MA 02115, United States*

ARTICLE INFO

Article history:
Received 23 June 2009
Received in revised form 11 May 2010
Accepted 16 May 2010

Keywords:
High content screening
Microscopy image
Nuclei segmentation
Neurite outgrowth
Line-pixel detection
Branch area

ABSTRACT

High content neuron image processing is considered as an important method for quantitative neurobiological studies. The main goal of analysis in this paper is to provide automatic image processing approaches to process neuron images for studying neuron mechanism in high content screening. In the nuclei channel, all nuclei are segmented and detected by applying the gradient vector field based watershed. Then the neuronal nuclei are selected based on the soma region detected in neurite channel. In neurite images, we propose a novel neurite centerline extraction approach using the improved line-pixel detection technique. The proposed neurite tracing method can detect the curvilinear structure more accurately compared with the current existing methods. An interface called NeuriteIQ based on the proposed algorithms is developed finally for better application in high content screening.

1. Introduction

With the use of advanced high resolution fluorescence microscopy imaging techniques, high content screening (HCS) has made it possible to study the intricate nervous system, and discover candidates for drug targets (Zhou and Wong, 2006). However, HCS generates volumes of microscopy images, each of which contains millions of pixels. Thus, instead of manual analysis which is tedious and time consuming, fully automated methods are required to extract and analyze phenotypic change information in large amounts of microscopy image data. The main goal of this paper is to propose an image processing system to analyze neuron images for studying neuron mechanism. This comprehensive system is developed to analyze two types of neuron images viewed from in vitro microscopy in high content screening experiments. This system includes (1) segmenting and counting neuronal nuclei in nuclei image; (2) labeling neurite in neurite image and (3) calculating some neuron morphology features.

There have been a number of nuclei segmentation methods reported in the literature, including thresholding, edge based methods, watershed algorithm, active contours, and other pattern analysis algorithms (Chen et al., 2007; Ge and Parvin, 1999; Li et al., 2005; Nandy et al., 2007). While these methods are popular and effective, they suffer from certain limitations. Thresholding cannot effectively deal with clustering nuclei (Li et al., 2007b). Edge based methods always leads to misidentification of noisy edges and discontinuous boundaries (Lin et al., 2003). Active contours require the contours' initialization, which can be a challenging work (Li et al., 2007a). The performance of morphological approaches is not good in nuclei detection if the nuclei vary significantly in size or shape. Watershed-based methods are widely used in nuclei segmentation, especially when clustered nuclei are present (Malpica et al., 1997). However, classical watershed often results in over-segmentation. In this paper, a modified watershed algorithm is proposed to address nuclei detection and segmentation.

Neurites can be considered as bright elongated line-like structures surrounded by a dark background. Therefore, the problem of labeling neurites is equivalent to that of detecting curvilinear structures in microscopy images. Many efforts have been devoted to detect curvilinear structures in images. Some researchers proposed a tracing technique called exploratory algorithm or vector tracking, which starts with detecting a set of seed points followed by tracing the centerlines from these initial points recursively until certain pre-defined stop conditions are satisfied (Al-Kofahi et al., 2002; Zhang et al., 2007a). This method is computationally efficient since

* Corresponding author. Tel.: +1 617 525 6242.
E-mail addresses: XZhou@tmhs.org, zhou@crystal.harvard.edu (X. Zhou).

0165-0270/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2010.05.012

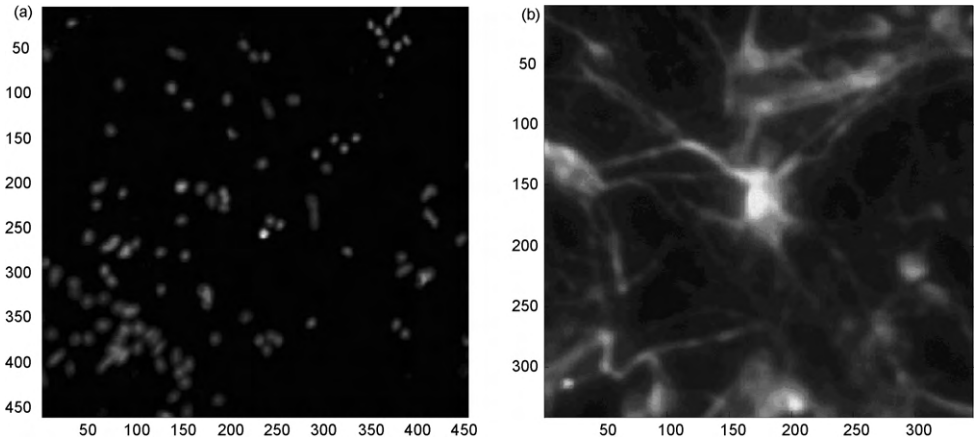

Fig. 1. Sample neuron images from (a) nuclei channel and (b) neuite channel. The number in *x* and *y* axes shows the size of the image. The units of *x* and *y* axes are both 'pixel'. The resolution of the image is 1.23 μm/pixel. Figs. 3, 5, 6 and 7 have the same measurement.

it only processes the pixels near the centerline. Zhang et al. (Sofka and Stewart, 2006; Zhang et al., 2007b) used this tracing technique to find the starting points and end points first in each neurite, then a dynamic programming approaches is applied to link each pair of points. Ali et al. (Ali et al., 1999) proposed a multi-scale matched filter to define the tracing direction and calculate 'vesselness' of each traced pixel using a 6-dimensional measurement.

A line-pixel detection algorithm has been proposed in several works (Steger, 1998; Xiong et al., 2006; Zhang et al., 2007b). This method uses a model to find the local geometric properties of the lines, and examines each pixel followed by a linking process which connects the detected centerline points into connected centerlines. Meijering (Meijering et al., 2004) proposed a semi-automated method that links consecutive ridge pixels derived from the line-pixel detection algorithm by selecting starting and ending points manually and calculating the global minimum cumulative cost function. Xiong et al. (Xiong et al., 2006) also detected the branch points and end points of neurites based on line-pixel method. The performance of these methods is also determined by user specified parameters selection such as the maximum of neurite width and a threshold for the strength of line.

However, the aforementioned algorithms discussed above cannot directly extract the centerlines in branched areas. In most tracing methods, each pixel has only one direction because the direction of each point in the centerline is defined as the same as its boundary whose response is largest with the template across directions (Al-Kofahi et al., 2002). Most line-pixel detection algorithms use the Hessian matrix to determine the normal direction of each pixel. However in bifurcation areas, the orientation is unclear. Xiong et al. in (Xiong et al., 2006) used a circle with constant radius to find the centerline at each end point, and then branch points could be easily detected. However, some branch points will be missed because it is hard to select an appropriate radius for all conditions. Al-Kofahi et al. presented a method which is formulated as a generalized likelihood for branch detection by checking each ending point after the tracing phase (Al-Kofahi et al., 2007). Tsai et al. (2004) described an approach a so-called exclusion region and position refinement to improve the accuracy of estimating the location of branch structure at the end of tracing process. Most of these methods cannot detect the branch points and the single line simultaneously, but only consider it as a post-processing step. In this paper, we shall propose a modified line-pixel method to detect neurite branch and single line simultaneously.

The rest of the paper is organized as follows. Section 2 describes the animal model in material part and illustrates our approach step by step. Validation is presented in Section 3. Section 4 presents the development and application of interface NeuriteIQ and we conclude the paper in Section 5.

2. Materials and methods

In this section, we describe neuron images acquisition and the proposed method for neuron image processing and analysis.

2.1. Neuron images acquisition

Normal E15 C57BL/6 mouse in the cortical hemisphere, including the hippocampus, are obtained from the Department of Biochemistry at Tufts University. Neurons are derived from C57BI/6 mouse using a standard protocol: E15 C57BI/6 pregnant females are euthanized by overdose of isoflurane, cortical hemispheres are dissected from embryos in HBSS- (Ca^{2+} and Mg^{2+} free) and treated with 0.1% trypsin for 10° at RT. After washing with HBSS-, they are triturated with fire-polished Pasteur pipettes in 0.025% DNAse I, 12 mM MgSO4, spun down 5 min at 900 rpm and washed in HBSS-. Another 5 min spin at 900 rpm the neuronal pellet will be resuspend in DMEM supplemented with 10% fetal calf serum, penicillin/streptomycin and glutamine to 0.2-1X106/mL and plated in poly-D-lysine coated plates. The cells will be incubated at 37 °C for 24 h, where the media will be changed to Neurobasal with B27 supplement, penicillin/streptomycin and glutamine, and the neurons will be incubated at 37 °C for additional 4 days to allow full neuronal differentiation and neurite extension. Then, neurons were blocked in 0.4% Triton X-100 buffer with 10% normal donkey serum for 30 min at RT, followed by incubation with neurite stain TUJ1 (Type III neuronal specific tubulin beta) antibody at a 1:250 dilution in 0.1% Triton solution overnight at 4 °C. After two more washes with PBS, neurons were incubated with nuclear stain Sytox Green (Molecular Probes, 1:5000 dilution) for 30 min at 37 C. Laser confocal fluorescence microscopy is used for capturing neuron images. To acquire different subcellular structures, e.g. neurite and nuclei, green (excitation 480 nm/emission 535 nm) and red (560 nm/630 nm) fluorescent filters were used to control contrast in the final image captured with a CCD (charge coupled device) digital camera system. The quality of these standard wide field microscopy images is good if the images are not captured out of focus. Finally, two types of *in vitro* data, nuclei images and neurite images, were generated, as shown in Fig. 1. In Figs. 1, 3, 5, 6 and 7, the number in *x*, *y* axes shows the size of the image with the unit 'pixel'. The image resolution is 1.23 μm/pixel. In the following, we search for an efficient method to process and analyze these images.