Software for the analysis of immunohistochemical images of spinal cord slices

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Abstract

In this paper supervised implementation of software for immunohistochemical image analysis of cat spinal cord slices, as part of the system for investigation the structure and function of motion control neural networks. Presented implementation of the neurons detection algorithm in the images and shown assessment of the detection quality.

Keywords: *neurons detection; spinal cord slices; image processing; interactive algorithm; NeuN.*

1. INTRODUCTION

Development of means of data analysis in neurophysiological experiments is an urgent auxiliary task, which can improve the efficiency of research in biological motion control systems and information processing. Applied to technical systems, such studies may be aimed at the development of new models of robots behavior control system based on neural networks, and the development of means of rehabilitation of people with reduced mobility due to spinal injuries [1] etc. An important part of this research is building three-dimensional maps of activity of the spinal cord neurons to perform various movement patterns.

Also, one of the important stages in the construction of these cards is neurons detection in image of spinal cord slices, obtained during the physical experiment.

In the neurophysiological research spinal cord divided by cross-sections into segments and represent the structure shown in Figure 1.

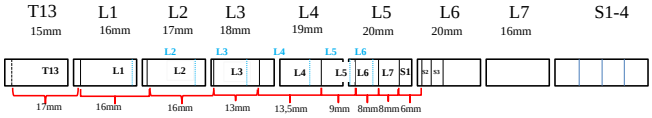

Figure 1 – Spinal cord structure (T13 – S4: spinal cord segments)

Experiments, for identifying structures and functions of motion control neural networks, include:

- invasive registration of neuron electrical activity;
- active neurons detection in the images of spinal cord slices;
- clustering of neurons and isolating specific groups of cells in each segments;
- construction of three-dimensional maps of neuronal activity by segment in a single three-dimensional model of the spinal cord.

The article describes the implementation of the spinal cord image analysis system, allows facilitating search for the neurons in the cats spinal cord slices, allow centralize store the results and general information about the neurophysiological experiment. The software can be used in neurophysiological experiments requiring centralized images processing of spinal cord slices.

2. OBJECT OF RESEARCH

The task is complicated by the several factors, such as some neurons covered by thin layer of tissue, therefore part of neurons have variously transparence, also axon can be marked with the neurons body, it leads to errors in neurons bounding rectangle calculations or axon can be incorrectly recognized as individual neuron. Furthermore images of slice may contain physical tissue defects, such as gaps, indurations etc.

Also important to note that each image may contain neurons with highly different size and the smallest neuron may be several times smaller than the largest neuron, found on the image. In addition, different imaging conditions, such as the light source intensity and transparency of the specimen, can cause variations of the image intensity in each of the color image channels resulting in neurons appearing with different color intensities in different image parts. Image example is shown in Fig. 2.

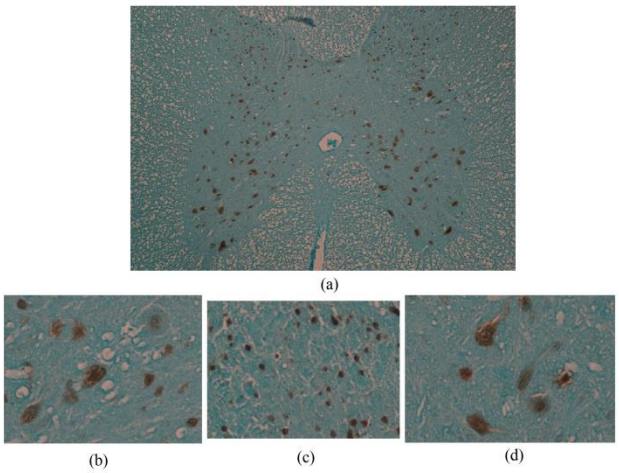

Figure 2 – (a): image of spinal cord slice; (b), (d): image fragments with large neurons; (в): image fragments with small neurons. Important to note that fragments (b) - (d) have same scale relative to the original image. The neurons are represented with dark blobs, the physical defects represented with white fragments.

3. RELATED WORK

Cell detection on immunohistochemical images is quite widely research field of bioinformatics, because each new combination of the neuronal marker, the slice making approach, the resulting image dimension and the type of supervised tissue may generate a new type of immunohistochemical images. We consider two-dimensional images of paraffin NeuN-stained cat spinal cord slices. For this type of images we didn't find any suitable articles.

Otherwise, we should base on the experience of neurons detection in other types of two-dimensional immunohistochemical images [2, 3], but these methods don't work in case of processing images with physical defects. Furthermore, most of currently studying approaches work on 3D confocal images [4-7]. Furthermore most of the automated cell detection methods are not suitable replacement for manual cell detection [8]. It is useful to note some articles [9, 10] considering processing images with inhomogeneous background and analysis object, part of the information from these articles may serve as a help for further development. From existing software should to note ImageJ [11] – public domain, Java-based image processing program, however, in order to improve performance decided to develop new application.

4. DEVELOPMENT

Process of neurons detection is presented on Fig. 3.

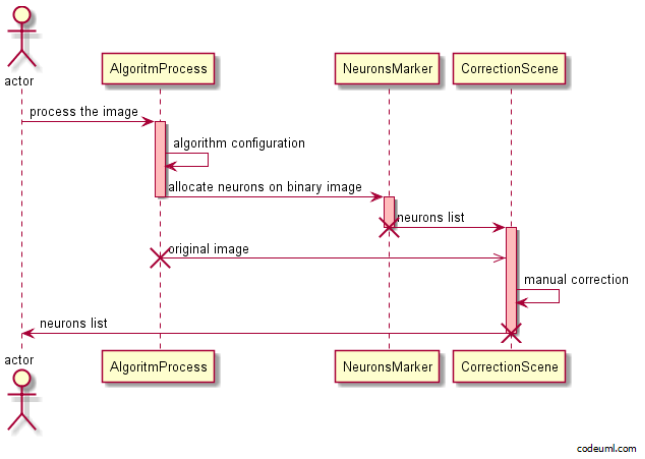

Fig. 3 – Sequence diagram for the neurons detection process

Module "AlgoritmProcess" in this diagram is a combination of methods, configured manually or automatically according to precedents. The general scheme on Fig. 4 shows the operation of the algorithm for binary image producing

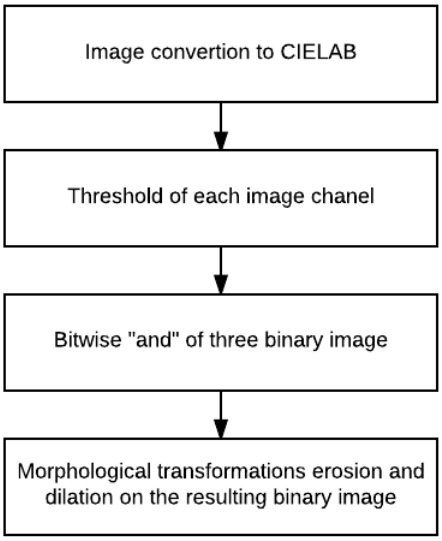

Fig. 4 – Algorithm for binary image producing

Module "NeuronsMarker" allocates connected regions in the binary image; connectivity value is 8 that mean that the eight nearest neighbor pixels will be considered together. Next, rectangles describing the connected regions that have passed the binary limits, draw on the vector graphics scene "CorrectionScene" over the original slice images, on this scene the user has the ability to manually add or remove the neurons outlining rectangles from the scene.

Proposed work method allows objects detecting, without any information about the size, shape and number of detecting objects. The disadvantage of this model could be the noise appearance caused by low-quality images, as well as the presence of outside elements in the image. However, this solution has a high fault-tolerance and can handle with any image.

For database development used "Entity – Relationship" model (ER), ER diagram shown in Figure 5

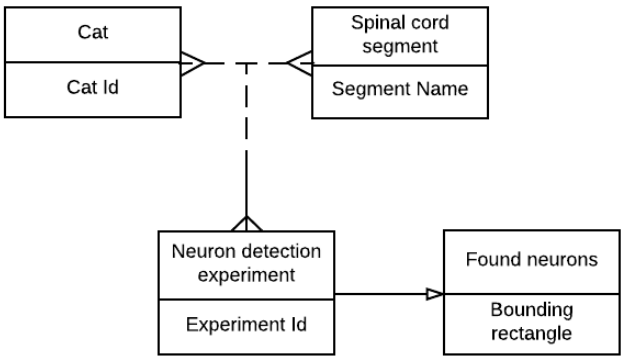

Figure 5 – ER - diagram

As a result of the database design, received five relations:

- Cat(CatId, …);
- Segment(SegmentName, …);
- Experiment(ExperimentId, …);
- Neuron(ExperimentId, bondingRectangle, …);
- Slice(CatId, SegmentName, SliceNumber, …);

In the obtained relations, shown the key columns, other additional columns must be easily complemented.

5. REALISATION AND RESULTS

An important requirement of the system design is able to handle large images (>24 millions pixels). For this reason, program is implemented on C++, GUI was developed using Qt [12]. Images are processed with OpenCV [13] – library of programming functions at computer vision. Also was created database to store data, which includes information about slices, spinal cords segments, algorithm configurations, binary and original images characteristics, detected neurons.

Because the threshold values configured manually, an important feature of the implementation is a momentary binary image upgrade, when threshold value changed, on images with resolution 6*4 thousand pixels, time for changing value of threshold, upgrading binary image and display them in the window approximately 130 milliseconds. Time for allocation connected regions in the binary image, performed after configuring of the main algorithm, may vary from 80 milliseconds to 1 second. Measurements were performed on a personal computer with a Core i7-2.8GHz, 4 GB memory and 64-bit operating system.

Algorithm, based on interactive setting threshold values, provides the expected error in the manifestations of tissue defects Fig. 6(a), otherwise allows detecting complex shapeless neurons, hiding deep in tissue Fig. 6(b), however, in some cases, the threshold filter separates hiding neurons Fig. 6(c). The axon of a neuron can also mistakenly release as a single neuron, which causes an increase of false positive, or type 1, detection errors, shown in Fig. 6(e). Example of successful detection of large neurons is shown in Fig. 6(d). One of the problems of large neurons detection using threshold approach is the problem of coupled neurons detection Fig. 6(f), on this stage such error correction is performed only in the manual mode. Problems with the detection of small neurons in general are similar to the problems described for large neurons, but are less common. The main difficulty in the detection of small neuronal is selection of binary limits, because occur extremely small neurons, which increase the number of false negative, or type 2 detection errors, example of a successful small neurons detection is shown in Fig. 6(g).

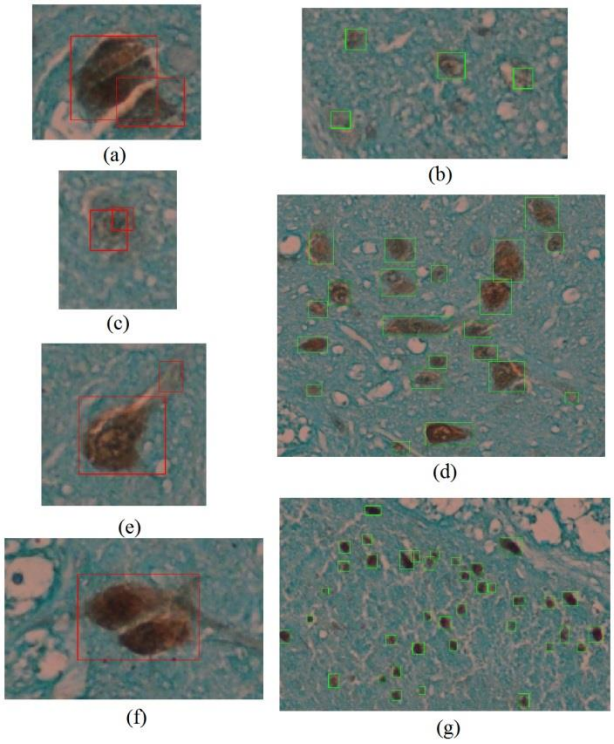

Figure 6 – Examples of the neuron detection algorithm process

Excluding manual correction, detection errors, on average are above 8,12%, from which 4,95% - false positive and 3,17% - false negative. Clearly that the number of false positive detection errors more than half bigger than number of false negative errors, it is related to the fact that a greater number of problems occur in the processing of large neurons, because them more susceptible to tissue defects. On average, one image contains 500 neurons, of which about 40 neurons require the user's attention.

6. CONCLUSION

In this paper proposed method for detecting neurons in the cat spinal cord slice images, also described used means and technologies. The results show that most of the detection errors are directly related to the quality of the input image.

In the future planned to create a module for calculating the neurons metrics, which will enabled to improve the quality of detection by special attention to places of potential errors. In this case it will be possible to classify neurons using the transparency and form. That will help to apply the appropriate algorithms to different classes of neurons.

In addition, to solve presented problem, plans to use the convolution neural networks. In this case developed software will be used to generate training and test samples.

One of the directions for further work is the construction of three-dimensional maps of the spinal cord neurons distribution, based on several processed images, which are processing results are stored in the database.

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