Extraction of Neurons from Images of Mouse Brain Slices Based on Automated Selection of Linked Morphological Filters[1]

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Abstract—We describe the results of a study on creating an algorithm for automated selection of linked morphological filters to solve image segmentation problems. We propose a closeness measure for image division. It is used to select the best filters from given families of linked morphological filters in such a way that division obtained by applying the watershed-segmentation algorithm to a filtered image has the maximum closeness value with the given division. This method is used to extract neurons from images of mouse brain slices. Experimental research has confirmed that this method is applicable for automated processing and analysis of slice images.

DOI: 10.1134/S1054661811020404

INTRODUCTION

Now and in the near future, an image is and will continue to be the main method of representing information in scientific research, especially in medicine and biology. Therefore, the development and application of a timely mathematical apparatus for automating image understanding is becoming one of the breakthrough directions in theoretical informatics.

The authors have developed theoretical bases [5] and IT elements [3] for automated morphological analysis of lymphoid cell nuclei in patients via hemoblasts. These bases and elements have become the foundation for a system of automated diagnoses of oncological diseases. The study is devoted to the development of mathematical methods supporting automated extraction of experimental data to supplement the model of preclinical-stage Parkinson's disease (PD) [1].

This disease is characterized by progressive degeneration of dopaminergic neurons [9, 15] in the substantia nigra. As a result, the afflicted lose motor control ability. To understand the pathogenesis and to develop methods to diagnose and treat PD, it is very important to study experimentally the degeneration of dopaminergic neurons caused by specific neurotoxins.

Application of the developed method makes it possible to quantitatively evaluate (a) the degree of generation of dopaminergic neurons and their axons resulting from the action of a toxin and (b) the functional state of surviving neurons and axons. The model represents the differences in neuronal features between the experimental group (the animals administered a toxin) and control group (the animals not subjected to a toxin).

As the source of experimental data for constructing the PD, we use digital images of dopaminergic neurons and fibers of brain slices from the experimental animals. Images were obtained at the Laboratory of Hormonal Regulation, Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences.

Dopaminergic neurons are exposed on 20-μm-thick serial slices of substantia nigra (Fig. 1), and the terminals of their axons, on 12-μm-thick serial slides of the striatum. The resolution of the initial images is 0.0117 $\mu m^2/pixel^2$.

The experimental data were obtained on digital images of neurons. The approach applied for image analysis of axons (terminals) is described in [4].

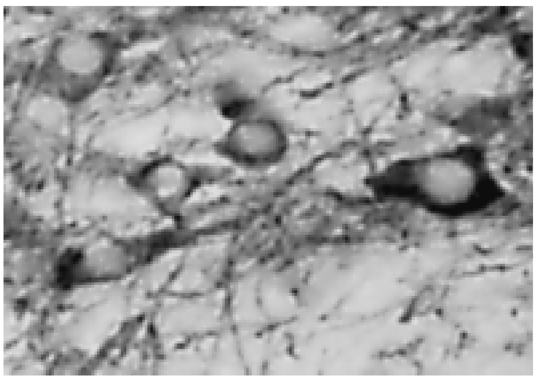

Fig. 1. Neurons in substantia nigra.

[1]The study was partially supported by the Russian Foundation for Basic Research (project nos. 09-07-13595 and 10-01-90013) and the project of the RAS Presidium program "Basic Sciences in Medicine 2011."

Received January 28, 2011

The proposed method automates digital image analysis of dopaminergic neurons and makes it possible to estimate their number and important features.

The proposed closeness measure is described in paragraph 2. In paragraph 3, the results of applying this method are briefly described.

CLOSENESS MEASURE FOR IMAGE DIVISION

We use the following commonly accepted definition of image segmentation [13].

Opr. 1 (segmentation). Let E be a random set. Division D of set E is the image $x \longrightarrow D(x)$ from E at 2^E such that

1. for all $x \in E$: $x \in D(x)$;
2. for all $x, y \in E \times E$: $D(x) = D(y)$ or $D(x) \cap D(y) = \varnothing$.

$D(x)$ is called the class of division of point x.

To measure the closeness between two divisions D_1 and D_2, we propose calculating the mean value of the information gain criterion IGain($D_1(x)$, $D_2(x)$) [2] used in information and machine-learning theory to evaluate the closeness of probability distributions.

Let $P(x) = |D_1(x)|$, $N(x) = |(D_1 \vee D_2)(x)| - |D_1(x)|$, $p(x) = |(D_1 \wedge D_2)(x)|$, $n(x) = |D_2(x)| - |(D_1 \wedge D_2)(x)|$.

Let also $H(P, N) = -\frac{P}{P+N}\log_2\frac{P}{P+N} - \frac{N}{P+N}\log_2\frac{N}{P+N}$.

Then, the information gain for the given class of division x is expressed as follows:

$$\mathrm{IGain}(D_1(x), D_2(x)) = H(P(x), N(x)) - \frac{p+n}{P+N}H(p(x), n(x)) - \frac{P+N-p-n}{P+N} \times H(P(x)-p(x), N(x)-n(x)).$$

The value for the entire image is calculated as the average over all points of its determination region.

As an example, we present closeness estimations between different divisions of images from Berkley Institute's segmentation dataset [6] (Fig. 2 and table).

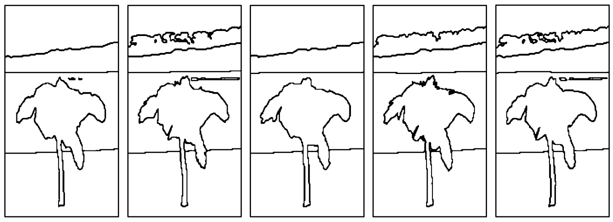

Fig. 2. Image segmentations (from left to right $D_1...D_5$).

EXPERIMENTAL

In conducting experiments, we used classes of linked morphological filters [10, 11, 12], which were equalizations [7, 8]. We further introduce the main concepts. A linked operator acting on a function [10, 11] is a transformation that enlarges division, which corresponds to plane regions of functions (regions with constant values of the function). As a result, when applying a linked operator to an image, all contours represented in the image are either suppressed or remain unchanged. The best known examples of such filters are openings and closings with the aid of reconstruction [10, 16]. Meyer proposed expanding this class of filters so that they would act simultaneously both on "peaks" (maximums) and on "dips" (minimums). They were called equalizations [7]. The main properties and examples of equalizations can be found in [7, 8].

We used parameterized families of equalizations obtained from families of function markers:

• erosion and dilation of the initial image with the use of a structurizing element—a flat disk with a given radius;

• feature (area, height, volume) filters of the initial image with a given parameter.

On the basis of these families, we constructed two-parameter families of linked filters determined by two parameters as the difference between two equalizations in a given family. After filtration of the initial image, object markers are found as regional extrema (maximums and minimums) and background markers are calculated using distance transformation [14, 16]. Then, the watershed-segmentation algorithm [14, 17] is applied to the image of the gradient modulus modified with allowance for markers [16]. As a result, division of the initial image is obtained.

As an example, we present the result of parameter selection using the suggested closeness measure. Figure 3 depicts the manually obtained neuron body mask. Figure 4 shows the internal and external object markers using the area fefature filter at the maximum value of the closeness measure with the given division.

Closeness measure values

	$IGain(D_1, \ldots)$	$IGain(\ldots, D_1)$
D_2	0.071227	0.011381
D_3	0.009677	0.007626
D_4	0.069097	0.011868
D_5	0.069372	0.009748

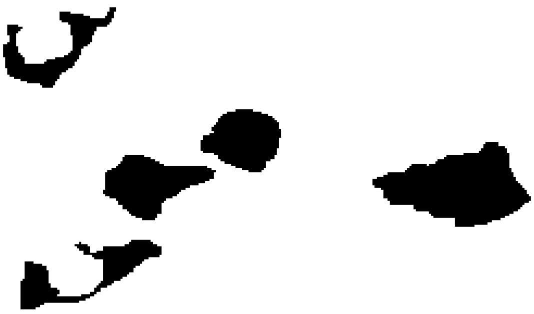

Fig. 3. Neuron body mask.

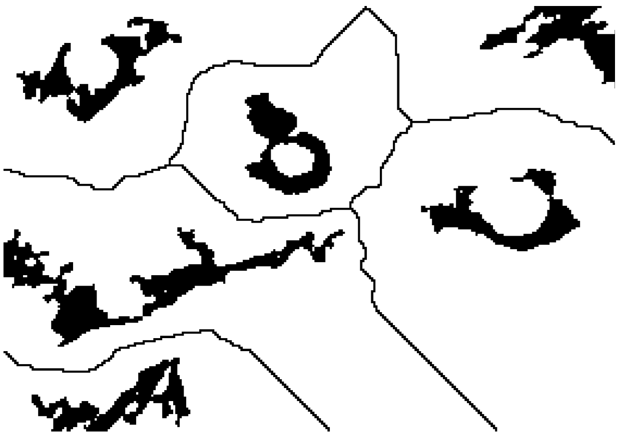

Fig. 4. Internal and external object markers.

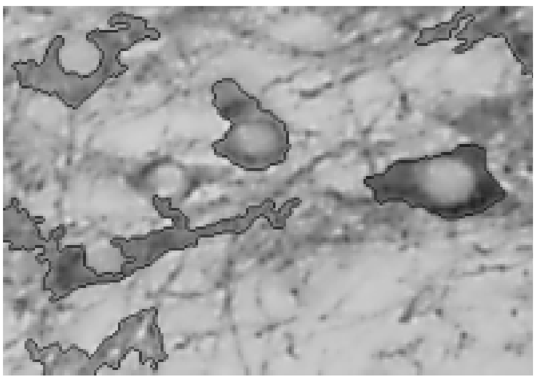

Fig. 5. Watershed lines in initial image.

And finally, Fig. 5 shows the corresponding watershed lines.

CONCLUSIONS

We propose a closeness measure for image division. It was used to select the best filters from given families of linked morphological filters such that division obtained as a result of applying the watershed-segmentation algorithm to the filtered image has the maximum closeness value with the given division. This method is used for extracting neurons from images of mouse brain slices.

Experimental research has confirmed that the developed method makes it possible to automate the development and analysis of slice images of substantia nigra and helps to determine the characteristics important for constructing a model of preclinical-stage PD.

The software implementation of the developed mathematical methods makes it possible to substantially accelerate PD research because the model is augmented with experimental data. The results are an important step in studying these substantia nigra system as PD develops.

Future studies should include a search for ways to combine automatically selected filters to construct stable image segmentation procedures.

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