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Exhibits

EXTRACTION OF NEURONS FROM IMAGES OF MOUSE BRAIN SECTIONS ON THE BASE OF AUTOMATED SELECTION OF CONNECTED MORPHOLOGICAL FILTERS

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This paper reports on an ongoing work on the development of the algorithm for automated selection of connected morphological filters for image segmentation purposes. A measure of similarity between two image partitions is proposed. It is used to select exact filters from given classes of connected morphological filters in such a way, that the partition, derived by a watershed-based segmentation algorithm, has a maximal similarity measure with the given partition. This technique is used to extract neurons from images of mouse brain sections. Experimental investigations confirmed that the developed technique supports automated processing and analysis of section images.

Introduction

Now and in foreseeable future an image is one of the main tools to represent information in scientific researches, particularly in medicine and biology. Thus development and application of modern mathematical apparatus for automation of image mining becomes one of the breakthrough challenges for theoretical computer science. Paper authors developed theoretical basis [5] and elements of information technology [3] for automated morphological analysis of lymphoid cell nuclei of diseased hemoblastoses, which were fundamentals for creation of system for automated diagnostics of oncological blood diseases. This paper is devoted to development of mathematical tools that supports automatic extraction of experimental data for filling a model of preclinical stage of Parkinson's disease (PD) [1].

The disease is characterized by a progressive degeneration of dopaminergic (DA-ergic) neurons [9, 15] in the substantianigra pars compacta (SN) leading to a dopamine (DA) depletion in the striatum. As a result, parkinsonian patients lose the ability to control their movements. Construction of the experimental models is crucial for the research of neurodegenerative disease pathogenesis.

Application of the developed method allows one to estimate quantitatively a) a degeneration of DA-ergic neurons in the substantianigra and their axons in the striatum after specific neurotoxin administrationion and also b) a functional condition of remained dopaminergic neurons and axons. The model shows differences of DA-ergic neurons features between experimental (the group of animals that were injected by the toxin) and control (the group of animals that were not injected by the toxin) groups.

The initial data is digital images of the immunostained sections of various brain areas. DA-ergic neurons were labeled on serial sections (a thickness of 20 microns) of the substantianigra (Fig. 1), and their fibers (axons) on sections of the striatum by immunohysto-chemestry for tyrosine-hydroxylase (TH) (TH is the specific enzyme of DA synthesis). Experimental data has been received from digital images of neurons. An approach to the analysis of digital image of distal parts of axons (terminals) is proposed in [4].

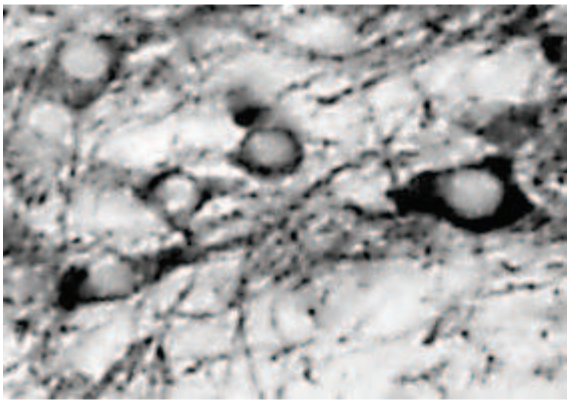

Fig. 1. Neurons in SN

The proposed method provides automated analysis of digital images of DA-ergic neurons and allows one to estimate their number and significant features.

The proposed similarity measure is described below in Sec. 2. Section 3 reviews the results of the method application.

Similarity measure of image partitions

We use the following common definition of image partition [13].

Definition 1 (Partition).Let E be an arbitrary set. A partition D of E is a mapping $x \longrightarrow D(x)$ from E into 2^E such that

1. for all $x \in E : x \in D(x)$
2. for all $x, y \in E \times E :$

$$D(x) = D(y) or\ D(x) \cap D(y) = \emptyset$$

$D(x)$is called the class of the partition of the origin x.

To measure the similarity between two given partitions D_1 and D_2 we propose to calculate the average value of the information gain criteria $IGain(D_1(x), D_2(x))$ [2], used in information theory and machine learning to measure the difference between two probability distributions, in the following way.

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 the partition of the origin x will be:

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

And the final measure will be the average throughout all pointы of the image definition domain.

As an example we provide a similarity measures between different image partitions from the Berkeley segmentation dataset [6]. See fig. 2 and table 1.

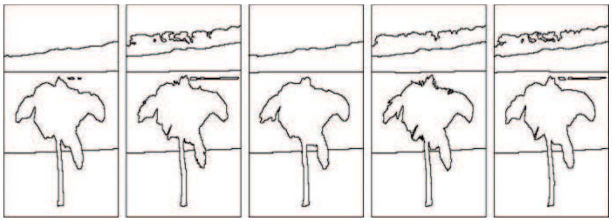

Fig. 2. Image partitions
(from left to right $D_1 \dots D_5$)

Table 1. Similarity measure values

	$IGain(D_1, \dots)$	$IGain(\dots, D_1)$
D_2	0,071227	0,011381
D_3	0,009677	0,007626
D_4	0,069097	0,011868
D_5	0,069372	0,009748

Experiments

In our experiments we used the classes of connected morphological filters [10, 11, 12] derived from levelings [7, 8]. Let us remind these notions. A connected operator acting on a function [10, 11] is atransformation that enlarges the partition of the space created bythe flat zones of the functions (zones with constant values). As the consequence all the contours presented in an image are either suppressed or remained the same, while applying the connected operator. The most known examples of such filters are openings and closings by reconstruction [10, 16]. F. Meyer proposed to extend last filters to operate simultaneously on image peaks (maxima) and valleys (minima). They are called leveling [7]. Basic properties and extensive examples of leveling can be found in [7, 8].

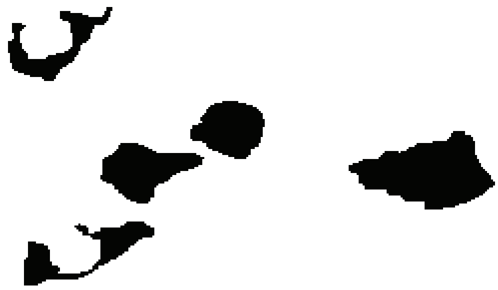

Fig. 3. Neuron somas mask image

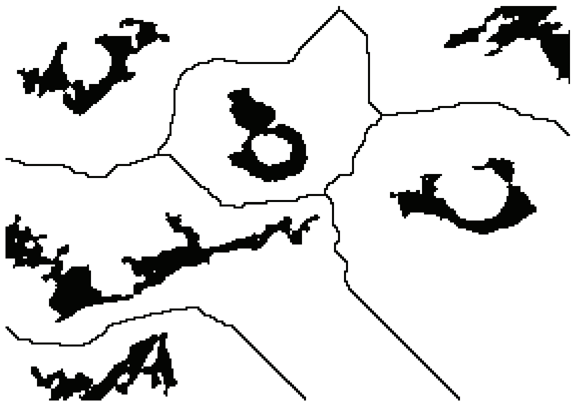

Fig. 4.Inner and outer markers of objects

We used parameterized families of extensive leveling which are constructed from different marker functions:

- erosions and dilations of the initial image with a structured element – flat disk of a given size;
- attribute (area, height, volume) filters of the initial image with a given parameter.

Then, we derive families of connected filters, which are defined by two parameters, as the difference between two leveling of a given family. After filtering of the initial image we find markers of objects as regional extremes (maxima and minima) and calculate background markers using distance transform[14, 16]. Then the watershed transformation [14, 17] is applied to the modulus of gradient of the filtered image modified with respect of markers [16].Φs a result we get the partition of the initial image.
We provide an example of parameters selection by means of the proposed similarity measure. In fig. 3 there is the mask of neuron somas, obtained manually. Fig. 4.presents object inner and outer markers, when using area attribute filter (described above) with the highest similarity to the given partition. And finally, corresponding watershed lines are shown in fig. 5.

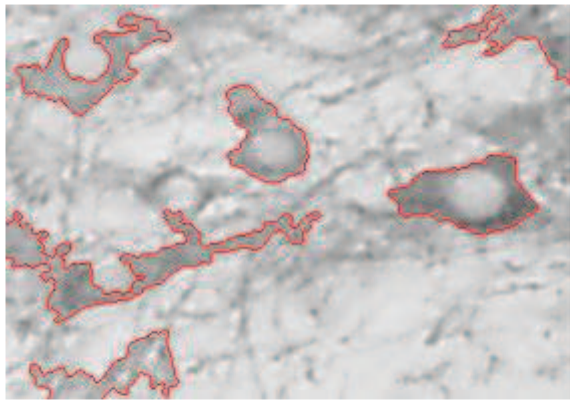

Fig. 5.Watershed lines superposed on the initial image from fig. 1

Conclusion

We proposed a measure of similarity between two image partitions. It was used to select exact filters from given classes of connected morphological filters in such a way, that the partition, derived by a watershed-based segmentation algorithm, has a maximal similarity measure with the given partition
Experimental investigations confirmed, that the developed method supports automated processing and analysis of SN section images and helps to define characteristics, which are essential for preclinical stage PD model construction.
Software implementation of the developed mathematical methods allows one to speed up significantly the research of PD at the cost of automation of experimental data model filling and automation of model investigation by means of computer experiments. The obtained results constitute the important stage of researches of dopaminergic nigrostriatal system at developing PD, which will allow research of compensatory mechanisms. Hereafter it will allow compensatory mechanisms studying with the purposes of its management.
The future investigations include finding the ways to combine automatically selected filters to construct robust segmentation procedures.

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