Mouse Brain Slice Segmentation for Analysis of Physiological Activity

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Abstract: *In the paper we suggest an approach for preprocessing histological images of brain slices. These images are usually received in noisy environment and thus require sophisticated preprocessing. We present two algorithms for brain contour detection and use their results for further nucleus detection that is important for biologists. We test our methods on real mouse brain data and show their applicability.*

Keywords**:** *Image preprocessing, neuroimaging, contour detection, graph cuts.*

1. INTRODUCTION

Analysis of physiological activity is one of the most interesting tasks of microbiological investigation. There are many kinds of such investigation. One example is the investigation of cells activity by image analysis. Such images are represented with immunohistochemical markers. Intensity of these markers corresponds to physiological activity of cells nuclear after whatever physical effects.

Such images may be colored or registered in gray levels. Colored images describe activity of oncology or virus diseases by special chemical preparation which works with separate protein only. Gray images (fig. 1) describe activity by ribonucleic acid concentration. Last case is more convenient for investigation of physiological activity.

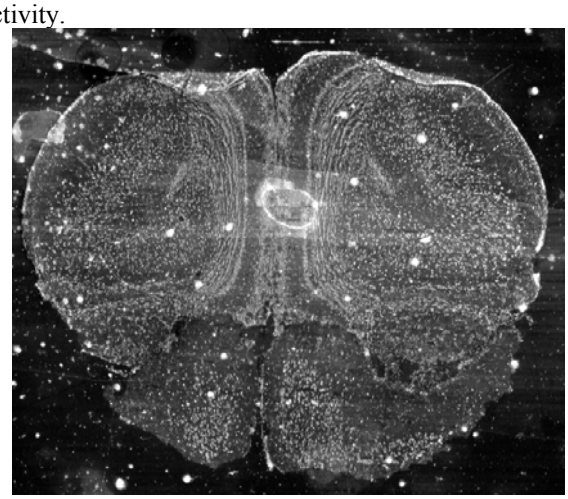

Fig.1 –Gray physiological image of rat's brain

Brain is the most physiologically complex and mysterious organ. Investigations of gene-expression patterns in mammalian brains are one of the most intensive studies in modern neuroscience [2]. In these investigations the key problem is discovering of specific genes that are uniquely expressed in different brain circuits and regions that control behavior. This task is often solved by comparing current brain with some brain atlas – a set of high quality brain slices with marked brain anatomic structures [3]. Allen brain atlas [4,5] is one example of this. Finding correspondence between observed brain and atlas brain allows one to determine physiological activity areas and their correspondence with brain anatomic structures.

It is very difficult to solve these tasks because they include many steps of analysis and processing. However, the first step is brain area extraction and cell nucleus determination. So the main effort of this paper is reliable procedures for image brain segmentation and cell nucleus extractions. In the paper we present automatic methods for pre-processing of noisy brain images. The presence of noise is inevitable and is related to image acquisition procedure.

The rest of the paper is organized as follows. In chapter 2 we consider the peculiarities of brain images. Sections 3 and 4 present two methods for brain area detection from brain slice images in noisy environment. In section 5 we describe an algorithm for nucleus detection which is important for further processing.

2. PHYSIOLOGYCAL BRAIN SLICE IMAGE

Initial data for investigations is a set of brain slice images obtained from adult C57BL6 mouse brain. For each mouse brain about 100 slices were taken. We had data for 6 mice thus having total 600 images for analysis.

The structure of obtained images is: background, brain area, nucleus regions and artifacts. The histogram analysis allows to separate background, brain and nucleus (fig. 2). But still artifacts exist for all range of intensity that give a negative influence.

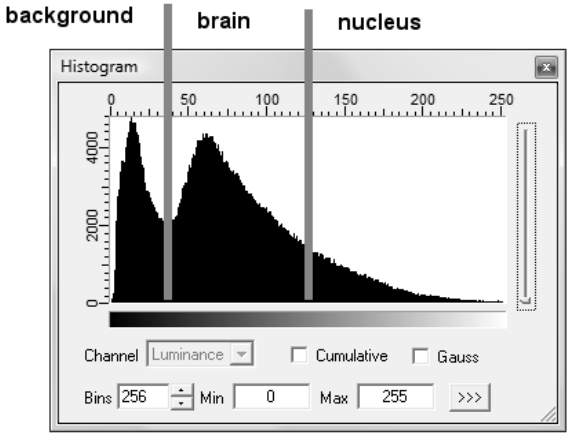

Fig.2 – Thresholding of physiological brain image histogram

While artifacts prevent us to extract real contour of brain, false nuclear-like objects can be observed as well. In such case the result of threshold segmentation has poor

quality and false objects. The specialized segmentation algorithms have been developed to avoid the problems. They consist of two parts: brain extraction and nucleus detection.

3. BRAIN AREA DETECTION

The image histogram analysis shows that histogram distribution has common shape for all brain images. The background area is characterized by the first peak. This property leads to methods of background detection. We use here watershed algorithm on inverse histogram (fig. 3).

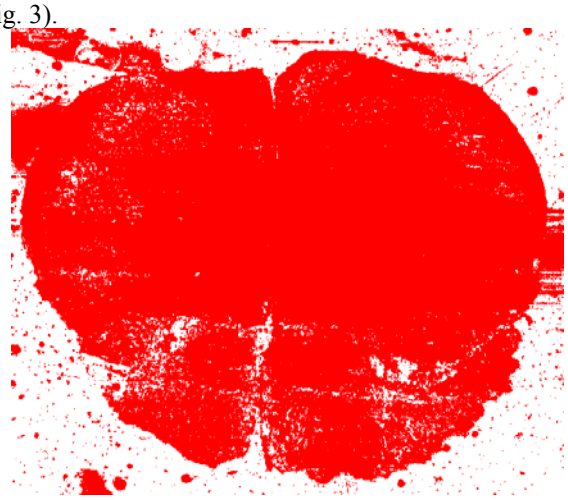

Fig.3 – Simple thresholding of physiological mouse brain by inverse watershed method

After this operation the resulting image has poor quality in sense of extracted objects but it includes important information about brain's border.

The brain on the image is represented by several areas; each of them has individual characteristics and defined by its own method.

The first step is histogram image thresholding. This operation creates a binary image for control operation.

Brain images have two main object characteristics for extraction: border and density of nucleus. Therefore a combination of methods is used here to define a border area: detection of brain's contour and detection by nucleus properties.

3.1. BRAIN CONTOUR DETECTION

Brain's contour is constructed from borders properties. Border should be relatively smoothed but artifacts are usually disrupt it. In order to solve this problem a special algorithm of border detection has been developed.

All small objects around brain are removed by skipping operation to provide further easy processing. The morphological closing operation with very small structure element removes small noise of binarisation. Then the radius distribution of object is collected. The radiuses correspond to lines from the center of mass to the border. This distribution includes many radiuses for every separate angle. For every angle all radiuses are removed except radius of minimal value. Then fitting line is constructed by spline smoothing (fig. 4).

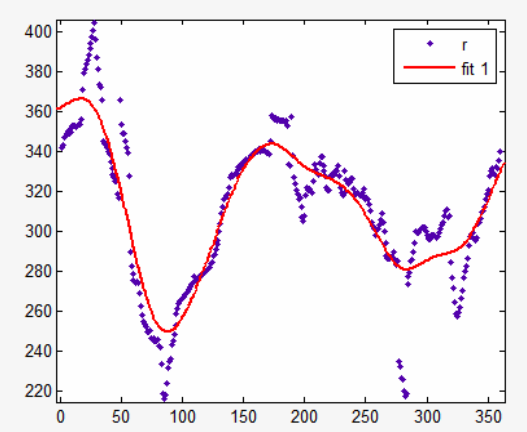

Fig.4 – The distribution of radial radius from centre of mass to border (r) and fitting this data by spline smoothing (fit 1)

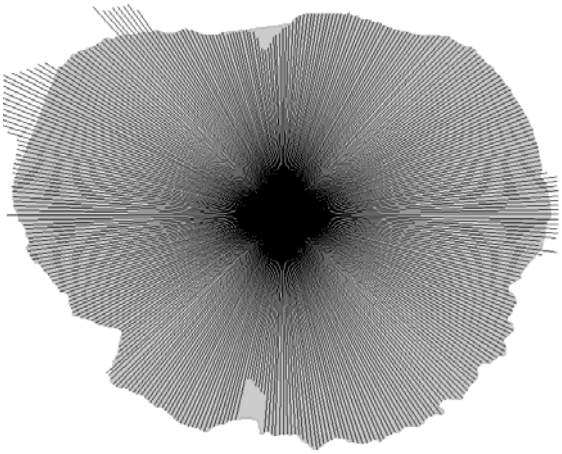

Fig.5 – The Binary image of brain after border smoothing and distribution of minimal radius

This is a piecewise polynomial that varies from linear to cubic. Level of smoothing is adjusted with smoothing parameter. The default parameter value depends on the data and often produces the smoothest fit. The predictor data is centered at zero mean and scaled to unit standard deviation.

This smoothing removes border noise from artifacts but, however, partially changes the true border (fig. 5).

For further correction current border result (fig. 5) is united with border threshold segmentation (fig. 3) by logical conjunction (fig. 6).

Fig.6 – The result of consolidation threshold segmentation and border smoothing operation

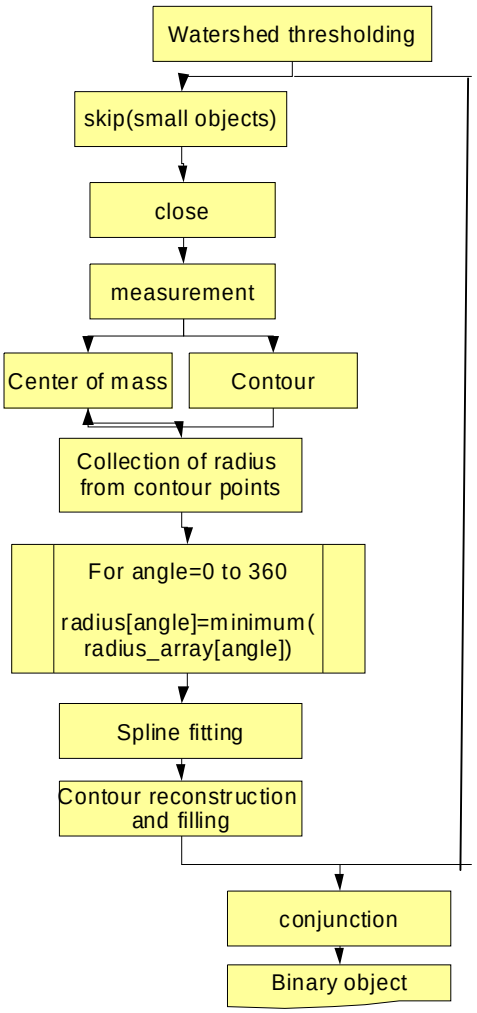

Fig.7 – The algorithm of detection of smooth brain contour

Summarizing all mentioned above steps the algorithm of brain contour detection can be represented by scheme (fig. 7).

Although we got here smooth brain contour without artifacts sometimes the constructed brain pattern is less than real object and few nucleus can be found outside of this pattern. In order to compensate such effects an additional correction procedure by nucleus properties is necessary here.

3.2. BRAIN DETECTION BY NUCLEUS PROPERTIES

The following algorithm is based on conglomerate cells features. Nucleus and sharp border on gray brain image have hat-like intensity profile. It gives us an idea that the best extraction method would be a morphological "top-hat" operation (fig.8).

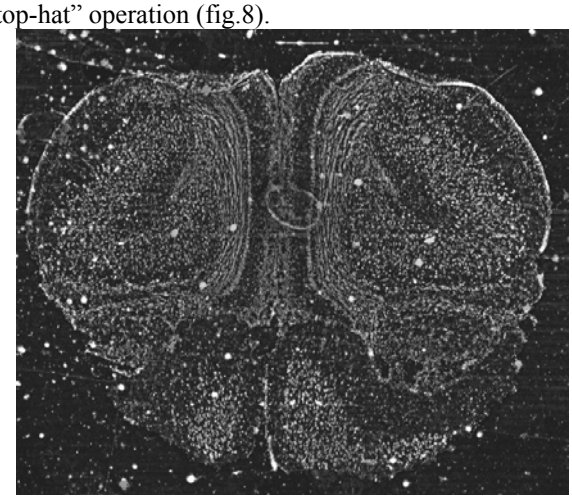

Fig.8 – The result of top-hat operation

Then thresholding of brightness derivate histogram has been performed to obtain a binary pattern (fig. 9).

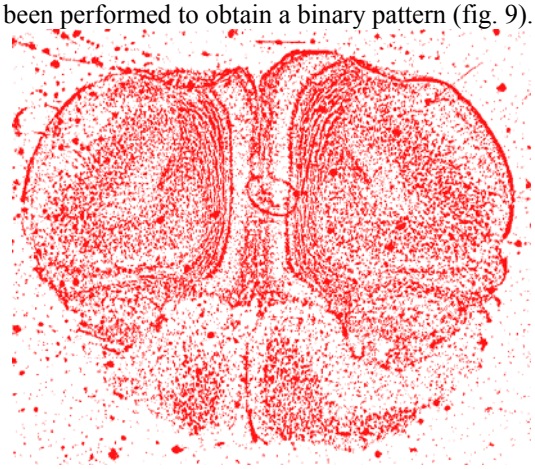

Fig.9 – The resulting image after thresholding of derivate histogram

The pattern includes impulse noise artifacts, nucleus and fragments of brain border. The major portion of nucleus stands near other nucleus. Therefore it is possible to connect nucleus and fragments of brain's border by one iteration of morphological closing with 25-pixels round structure element. Then filling operation followed by "skipping" removes connected holes from image and small binary objects, respectively (fig. 10).

Morphological smoothing (open-close operation) cuts "hairiness" of border that exists by artifacts. The resulting binary pattern consists of regions with high geometrical density of nucleuses. Combining this result together with the result of brain contour detection (fig. 5) by disjunction allows taking the full brain area (fig 11).

After all we obtain the final result of brain segmentation (fig. 12) using a logical image conjunction of threshold segmentation pattern (fig. 3) and the result we had on the previous step (fig. 11).

Fig.10 –Morphological processing after skipping of small objects

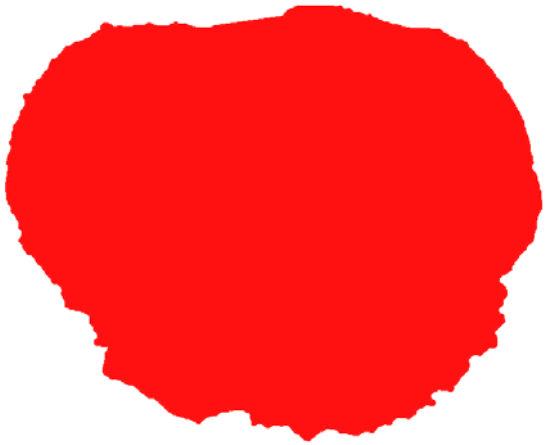

Fig.11 – Aggregate consisted of contour detection and detection by nucleus properties

Fig.12 – The result of brain segmentation

4. GRAPHCUT SEGMENTATION METHOD

In order to make segmentation more robust we have developed another segmentation method based on graph cut algorithm [1].

4.1. GENERAL GRAPH CUT ALGORITHM

Suppose we are given a graph $G = \{V, E, W\}$, where V is a set of nodes, E is a set of edges, W is affinity matrix, which associates a weight for each edge in E. A cut of a graph is a partition of V into two disjoint subsets (A and B). The min-cut of a graph is such a cut that the sum of weights associated with edges between different segments is minimal:

$$C_{\min}(A,B) = \sum_{u \in A, v \in B} W_{u,v} . \quad (1)$$

However, min-cut determination problem is NP-hard. Adding the requirement that two predefined nodes, denoted terminal nodes or source and sink nodes, in G must be in separate sets, significantly reduces the problem complexity. Finding the min-cut separating the source and the sink nodes, the s-t cut, can be done in polynomial time. If we associate the weight of each edge with flow capacity it can be shown that the maximal amount of flow from the source to the sink is equal to capacity of a minimal cut. That's why the min-cut problem is also known as the max-flow problem.

Suppose we are given an image of mouse brain slice. We build an undirected graph G from this image in the following way. Set of nodes V includes all pixels of the image and two additional nodes: source (s) and sink (t). Each pixel node is connected with its neighbors (we used 8-connectivity in our approach). Source and sink are connected with all pixel nodes and are not connected with each other. Fig. 13 illustrates construction of graph G.

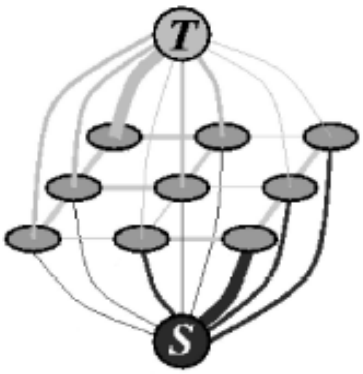

Fig. 13 – Constructing a graph from an image

We associate source with object and sink with background. So pixel nodes, which are in source group after graph cut, are pixels of object (set A) and pixels from sink group are pixels of background (set B).

Graph cut algorithm applied to such a graph minimizes the following energy:

$$E = \sum_{u \in A, v \in B} W_{u,v} + \sum_{u \in A} W_{u,t} + \sum_{v \in B} W_{v,s} . \quad (2)$$

Edges between non-terminal nodes correspond to how alike two pixels are. We use the following measure:

$$W_{u,v} = \exp\left(-\frac{(I_u - I_v)^2}{2\sigma^2}\right) \cdot \frac{1}{\mathrm{dist}(u,v)}, \quad (3)$$

where $\mathrm{dist}(u,v)$ – distance between two pixels, I_u, I_v – intensities of pixels, σ – algorithm parameter.

In our approach we use some prior information about

the image. Mouse brain slice must be placed in the center of the image. We call a group of pixels in the center of the image as *object seed pixels*. Also pixels at image border must be referred to background. We call border pixels as *background seed pixels*. Graph cut algorithm must refer object seeds to object and background seeds to background. We achieve that by making $W_{u,s} = \infty, W_{u,t} = 0$ for object seeds and $W_{u,s} = 0, W_{u,t} = \infty$ for background seeds.

We restore object and background gray color models from seed pixels, supposing that both object colors and background are normally distributed. According to this assumption we set last weights on image graph as follows:

$$W_{u,s} = \lambda \cdot (-N(I_u \mid \mu_{Obj}, \Sigma_{Obj})); \quad (4)$$
$$W_{v,t} = \lambda \cdot (-N(I_v \mid \mu_{Bkg}, \Sigma_{Bkg})).$$

Here $N(x \mid \mu, \Sigma)$ – probability density function of normal distribution, λ is method parameter responsible for importance of color information in segmentation.
We use Kolmogorov-Boykov algorithm [1] as a graph cut algorithm.

4.2. APPLYING GRAPH CUT METHOD TO MOUSE BRAIN SLICE

Consider we are given a mouse brain image (Fig. 1). At first, we apply a standard median filter with small radius to make the image smoother. After that we apply graph cut segmentation method described above with parameters $\lambda = 0.001, \sigma = 0.03$. Such parameter values were found experimentally and showed good performance for all similar brain images.

After applying graph cut we may have several unconnected fragments. We choose the largest component as a mouse brain. The result of this method is shown on Fig. 14.

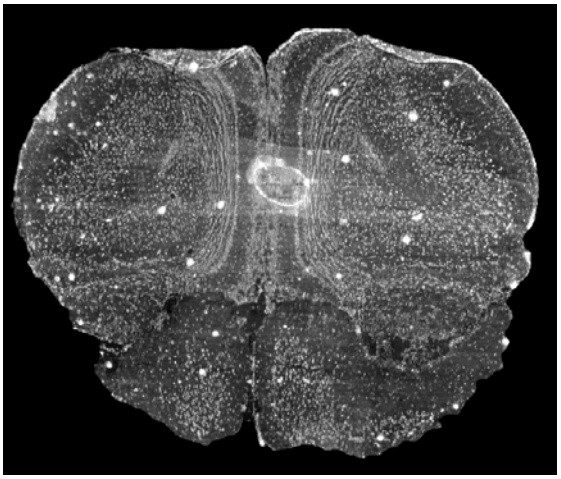

Fig.14 – Graph cut algorithm result for mouse brain.

5. NUCLEUS DETECTION

Using the results of brain segmentation we may limit the region for nucleus search. Common brightness distribution for nucleuses corresponds to Gaussian-like shape and most artifacts have brightness on wing of the histogram (fig.15).

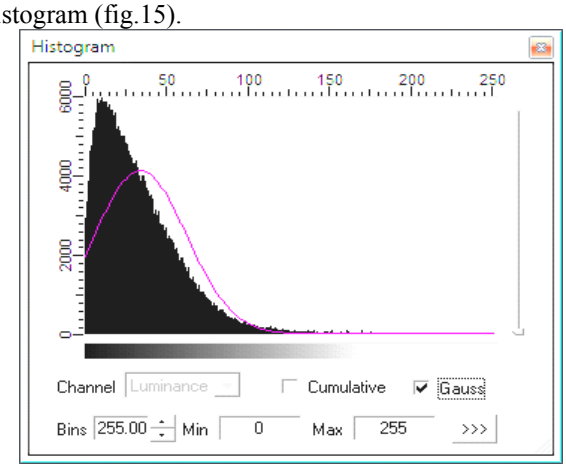

Fig.15 – The histogram of brightness inside region of brain and Gauss-like curve

Therefore artifacts have been detected by thresholding of brightness histogram wing and then removing of small objects (fig. 16) since small objects correspond to nucleuses here.

Fig.16 – Binary patterns of artifacts

Before nucleus detection gray image is averaged by low-pass filter to remove small impulse noise and smooth brightness surface of image. In topological sense nucleuses are represented as hills. So one of the best operations to detect them is watershed of inverted image. The watershed was done for 10-20 levels of brightness. Then resulting image was subtracted from averaging image and binarized by simple thresholding with further removing of artifacts regions. Finally binary image of nucleus is obtained (Fig. 17).

Every nucleus has individual intensity of brightness. It can describe physiological activity of nuclear. Conjunction of binary and gray image allows to take distribution of brightness for nucleus only. It corresponds to the distribution of physiological activity (Fig. 18).

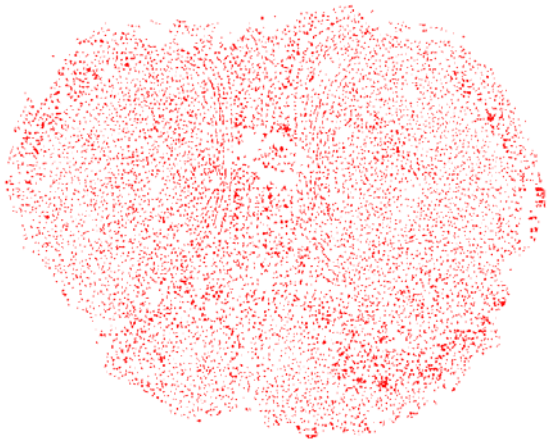

Fig.17 – Binary image of nucleuses

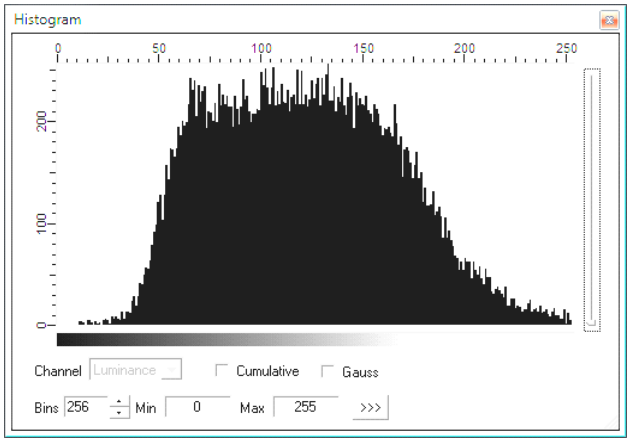

Fig.18 – Distribution activity of nucleus that is calculated from physiological image

6. CONCLUSION

The analysis of physiological activity of brain can be carried out by image analysis procedures. In this paper we proposed algorithms for brain contour detection and nucleus extraction. These algorithms were tested on real histological images of mouse brain slices. The results allowed us to conclude that the proposed approach can be effectively applied even to noisy images.

7. ACKNOWLEDGEMENT

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