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**SEGMENTATION ALGORITHMS OF BIOMEDICAL IMAGES:
DEVELOPMENT AND QUANTITATIVE EVALUATION***О. Березький, Ю. Батько, Г. Мельник, С. Вербовий, О. Піцун*

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**АЛГОРИТМИ СЕГМЕНТАЦІЇ БІОМЕДИЧНИХ ЗОБРАЖЕНЬ:
РОЗРОБКА ТА КІЛЬКІСНА ОЦІНКА**

The article presents the comparative analysis of the biomedical image segmentation methods. The work discusses segmentation methods on the basis of previous labeling and spatial moments. The experimental results show that the developed methods have higher accuracy by signal-noise ratio compared to the nowadays known. Moreover the authors have developed the quantitative evaluation of the segmentation algorithms based on the metrical approach.

Key words: Biomedical image, segmentation; labeling; spatial moments, evaluation.

У статті представлений порівняльний аналіз методів сегментації біомедичних зображень. У роботі досліджуються методи сегментації на основі попередньої розмітки та просторових моментів. Експериментальні результати показують, що розроблені методи мають більш високу точність за співвідношенням сигнал-шум у порівнянні з відомими. Крім того, автори розробили алгоритм кількісної оцінки алгоритмів сегментації на основі метричного підходу.

Ключові слова: біомедичні зображення, сегментація, розмітка, просторові моменти, оцінка.

Introduction

Biomedical images are used for diagnostics and treatment. The images of normal and abnormal cells and tissues are obtained from light microscopes. Those images are modern histology and cytology research objects. The tasks of microscopic image analysis automation are solved with the help of automated microscopy systems (AMSs). AMSs consist of hardware and software systems for digital processing of the microscopic images [1]. One of the most important stages of optical and geometrical parameter automation measurement is the selection of microobjects on histological images [2, 3]. The biomedical image analysis appears to be difficult because of the high variability of parameters and the weak contrast of most microobjects.

The microobjects of histological images are sections of certain organs' tissues. The tissue consists of rounded cells, which are placed in layers. Cells dimensions range from several micrometers with the smallest of them being from 0.5 to 1.2 microns. Microobjects on cytological images are the individual cells that are placed randomly.

The histological image analysis, performed with the help of AMSs, consists of the following stages: imagery, manual and automatic selections of the microobjects (cells, nuclei, segments of different colour or brightness, etc.), size measurement, shape, position and optical parameters of the selected microobjects or their parts, their classification and statistical processing of the measurement results.

Images segmentation leads to the division of images into regions with similar characteristics. Some of the main image attributes for segmentation are brightness for monochrome images and colour component for colour images. Edges and textures are also

used for segmentation. The segmentation process divides only the image and doesn't identify individual segments and their relationship [6].

Currently there are no universal methods of the segmentation process. They often use a set of specialized methods that are the most common for this class of problems. In the work [2] are proposed the characteristics for the segments after segmentation.

Image segmentation method based on previous labeling

Several approaches to the segmentation algorithm classification are known, namely: Fu and Mui [8], Pal and Pal [9], Skarbek and Koshana [10], Lucchese and Mitra [11], Jipkate [12]. The approaches are based on the following criteria: the properties of points, regions, region edge, a priori knowledge about microobjects, etc. Let's see the other criteria for the algorithms segmentation separation. They are: image type (colour, grayscale, binary), nature of the segmentation process (parallel or sequential processing) [13, 14, 15]. However, these characteristics are ambiguous. For example, threshold segmentation can occur in parallel or in sequential modes and handle both binary and grayscale images. It leads to ambiguity in the classification algorithms for segmentation. In our opinion, the further discussed criteria allow more complete classification of segmentation algorithms.

Algorithms based on texture properties. The decision to include a point into the segment is taken on the basis of texture features similarity at that point. This type of algorithms is recommended to use for images with repetitive regions.

Task definition. The analysis of the segmentation algorithms and biomedical image features shows that segmentation methods development on the basis of the relations between points and texture features of regions is a vital task [18].

As can be seen from the above review, there is no universal method of segmentation and each algorithm has its advantages and disadvantages. The proposed approach uses the characteristics of individual image points and the relationship between them.

We introduce the notation:

I – input image;

I_{s_i} – input image marked by i - type labeling;

V_{ij} – j homogeneous region in the input image marked by i type labeling;

$M_k(x, y, z), x=1..l, y=1..m, z=1..8$ – the array of coefficients for the relationships k labeling, l – the width of the input image, m – the height of the input image, z – the number of the neighboring pixels.

The array of total interconnections coefficients M_{sum} equals (1):

$$M_{sum} = \sum_{k=1}^n M_k, \quad (1)$$

where n – the number of previous labeling used in the process of segmentation.

Definition 1. Labeling is the process of splitting the input image I into an array of homogeneous regions V_j based on the criterion of homogeneity KO . Homogeneity criterion is defined previously by the analysis of the input image $I \xrightarrow{KO} \{V_j\}$

Definition 2. If two neighboring points $I(x_1, y_1)$ and $I(x_2, y_2)$ are in homogeneous areas, the relationship between them equals 1: $R=1, P(I(x_1, y_1)) = P(I(x_2, y_2))$. Here $I(x_1, y_1)$ and

$I(x_2, y_2)$ is two neighboring pixels;

$P(I(x_1, y_1))$ – identification of a homogeneous region to which the pixel represents $I(x_1, y_1)$;

$P(I(x_2, y_2))$ – identification of a homogeneous region to which the pixel represents $I(x_2, y_2)$;

R – the coefficient of the correlation between two pixels.

Definition 3. If two neighboring points $I(x_1, y_1)$ and $I(x_2, y_2)$ are in different homogeneous regions then the correlation between them equals 0: $R=0, P(I(x_1, y_1)) \neq P(I(x_2, y_2))$,

Definition 4. The total coefficient of the relationship between two pixels $I(x_1, y_1)$ and $I(x_2, y_2)$ is defined as the amount of bonds at n labeling is (2):

$$R_{sum} = \sum R_i, i = 1..n, \quad (2)$$

where R – coefficient of the relationship between two neighboring pixels $I(x_1, y_1)$ and $I(x_2, y_2)$;

This approach analyzes previous labeling of the image and sets anchor points not only to a specific area, but also to the neighboring points. Algorithms of the previous labeling can be selected depending on the input image. The image of stable relationships will be recognized as homogeneous.

The segmentation algorithm is the following:

- 1) We provide previous labeling input image I via n labeling;
- 2) We form the array of factors relationships M_k between neighboring points for each one with n labeling of the input image;
- 3) We form the total array of factors relationships M_{sum} between neighboring points for each one with n labeling of the input image;
- 4) We provide the group input image points I in the homogeneous region based on the relationships of the total interconnection coefficients M_{sum} .

The previous labeling can be carried out in three ways.

Manual. Labeling of the image on the homogeneous region is carried out manually by n independent users. This way is time-consuming and subjective because the previous labeling is influenced by a human factor. The advantage of this approach is that the number of previous labeling can be minimal.

Automated. The process of the previous labeling uses the known methods of segmentation, but a user sets the input parameters. The advantages of this approach are high accuracy and speed with increasing objectivity of the previous labeling.

Automatic. Previous labeling is based on an automatic analysis of the input image, such as the histogram analysis of brightness distribution and definition of thresholds for labeling.

Since this algorithm was developed for the segmentation of colour images during the previous auto-labeling images, it offers the transition from a three-dimensional representation of colour to a one-dimensional. The representation of images in one-dimensional space allows the automatic analysis of colour distribution histograms of the algorithms to determine thresholds.

Previous labeling can be made in different colour bases.

We use the following rules for the complete segmentation process to classify the input image points in the homogeneous region based on the relationships between the neighboring points:

1) if the relationship between two neighboring points $I(x_1, y_1)$ and $I(x_2, y_2)$ is $\max M_{sum} \rightarrow \max$ for the input image, then the data points are combined into a homogeneous region V_j (Fig. 1,a).

2) If the point of interconnection $I(x_1, y_1)$ with the neighboring point $I(x_2, y_2)$ is bigger than the relationship with the other neighboring points, these points are combined into a homogeneous region V_j ;

3) If the point $I(x_1, y_1)$ has the same relationship with two (or more) neighboring points $M_{sum}(x_1, y_1, z) = M_{sum}(x_2, y_2, z) = M_{sum}(x_3, y_3, z)$, which are combined in a homogeneous region $I(x_2, y_2) \in V_j, I(x_3, y_3) \in V_j$, then this point is connected to the corresponding homogeneous region $I(x_1, y_1) \in V_j$ (Fig. 1,b);

4) If the point $I(x_1, y_1)$ has the same relationship with two (or more) neighboring points $M_{sum}(x_1, y_1, z) = M_{sum}(x_2, y_2, z) = M_{sum}(x_3, y_3, z)$, which do not belong to one homogeneous region $I(x_2, y_2) \in V_i, I(x_3, y_3) \in V_j, i \neq j$, the point is connected to the area with more neighbors (fig. 1,c).

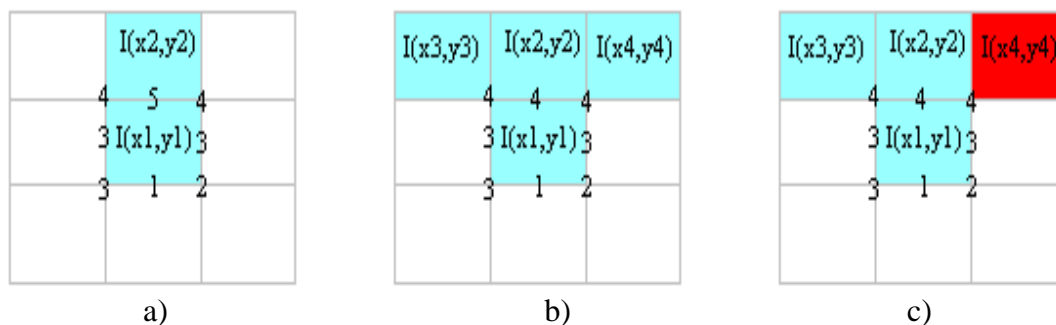


Fig. 1 Example of points integration

The result of the algorithm is a set of homogeneous regions. Because microobjects in the image usually consist of groups of homogeneous regions, we use the procedure for an additional association of homogeneous regions.

Texture segmentation algorithm

Texture segmentation algorithm consists of the following steps [15]

- a) calculation of the texture features for each image point within the sliding window size $W \times W$,
- b) the constructed texture field segmentation.

We use textural features based on spatial moments of the field and the distribution of gray levels matrix.

The texture image can be quantitatively described by simple statistical characteristics, such as mathematical expectation, dispersion and moments of higher order [10]. The term spatial moments (SMs) comes from mechanics. When SMs are being applied to the images, it reflects the distribution of gray levels in the image along its axis. On their basis we can calculate the features of the region that are invariant to rotation, translation and scale [11]. Spatial moments of the region in the point with the coordinates (x, y) and function value of

the gray level $f(x, y)$ are calculated as (3):

$$m_{p,q} = \iint x^p y^q f(x, y) dx dy \quad (3)$$

We consider the image as a function of two variables $f(x, y)$ and calculating the number of lower-order moments for each pixel in the image for $(p+q) \leq 2$. The moments are calculated within the local window size $W \times W$ around each pixel.

In the discrete version of the SMs, within the window boundaries, with the center pixel being (i, j) , moments are calculated as the sum with normalized coordinates (x_m, y_n) :

$$m_{p,q} = \sum_{-W/2}^{W/2} \sum_{-W/2}^{W/2} f(m, n) x_m^p y_n^q \quad (4)$$

where m, n – the coordinates of the point related to the window.

In our algorithm we use moment of inertia $m_{1,1}$.

In gray levels of the distribution matrix (GLDM) [18] P_d for translating vector $d=(dx, dy)$, value $p_{i,j}$ is the number of gray level of pair value occurrences, where i and j are placed at a distance d . Thus, for each image point $f(x, y)$ the matrix P_d can be associated, which characterizes the distribution of brightness in the window size $W \times W$ centered at coordinates (x, i) . The elements of the matrix P_d are defined as the following:

$$P_d(i, j) = \sum_{(m,n) \in D} f_{i,j}(x_{m,n}; x_{m+d,n+d}) \quad (5)$$

where D – window with $W \times W$ dimensions (W - odd),

$i, j = 0, 255$ – the brightness value of the point,

$x_{m,n}$ – the brightness value of the point with coordinates (m, n) .

The function $f_{i,j}(x_{m,n}; x_{m+d,n+d})$ is defined as:

$$f_{i,j}(x_{m,n}; x_{m+d,n+d}) = \begin{cases} 1, & (x_{m,n} = i \text{ and } x_{m+d,n+d} = j) \\ & \text{or } (x_{m,n} = j \text{ and } x_{m+d,n+d} = i) \\ 0, & \text{else} \end{cases} \quad (6)$$

The function is an indicator of the fact, that points that are located at a given distance, have certain levels of brightness. The parameter d determines the distance at which neighboring points are analyzed. On the basis of GLDM the textural features are determined: energy, entropy, contrast, homogeneity and correlation.

Texture features are calculated on the matrix $P_d(i, j)$, that describes the distribution of brightness within the region with its center in the point (x, y) . After processing the entire image for each feature of the matrix, the field of texture features is formed. The matrix contains the values of features in all processed points. To describe the features, we use the auxiliary values:

$$m_j = \sum_j j P(i, j), \quad p_j = \sum_j P(i, j), \quad (7)$$

mathematical expectation $Mx = \sum_i i p_i$;

The following formulas, that enable to calculate the characteristics, are presented below.

1. Total mean value:

$$F_1 = \sum_i m_i p_i \quad (8)$$

2. Inertia:

$$F_2 = \sum_i \sum_j (i - j)^2 P(i, j) \quad (9)$$

The proposed algorithm of image texture segmentation consists of the following steps:

1. Construction of the texture field G , every point of which is $g \in G$ according to (1)

$$g(i, j) = m1, 1 = \sum_{-W/2}^{W/2} \sum_{-W/2}^{W/2} f(m, n) x_m y_n \quad (10)$$

2. Normalization G , $g \in [0, 255]$.

3. Search for the thresholds t_1, t_2, \dots, t_n using the following steps:

a) Setting the interval $R=[a, b]$; $a=0$ and $b=255$.

b) Calculation the mathematical expectation μ and the standard deviation σ of all pixels from the interval R .

c) Calculation of the thresholds t_1 i t_2 as $t_1 = \mu - k\sigma$ and $t_2 = \mu + k\sigma$;

d) Calculation of the intervals $a = t_1 + 1$, $b = t_2 - 1$;

e) Repeat steps $a-d$, $n/2$ times (n – number of thresholds) setting new limits of the interval $a = t_1 + 1$, $b = \mu$ and $a = \mu + 1$, $b = t_2 - 1$.

4. Segmentation of the texture field G and as a result we obtain $n+1$ binary masks s_i , $i=\{1, \dots, n+1\}$:

$$s_i(x, y) = \begin{cases} 1 & \text{if } t_{i-1} \leq g(x, y) \leq t_i \\ 0 & \text{else} \end{cases} \quad (11)$$

where $t_0 = 0$, $t_{n+1} = 255$.

5. Segmentation of the input image aiming to obtain $n+1$ images \hat{s}_i , $i=\{1, \dots, n+1\}$

$$\hat{s}_i(x, y) = \begin{cases} f(x, y) & \text{if } s_i(x, y) = 1 \\ 0 & \text{else} \end{cases} \quad (12)$$

To test the GLDM (5) as a texture feature we must perform this algorithm constructing $g(i, j) = F5$ in step 1. The parameter k serves to control the spacing between the lowest and highest thresholds.

The optimal number of thresholds n (the number of algorithm iterations respectively) may be set a priori based on the application. The number of thresholds can also be chosen on the basis of signal/noise ratio changes ρ . The value of ρ can be calculated between the original and segmented image of the average values of pixels inside the segments.

The quantitative evaluation algorithms

The quantitative evaluation of the segmentation algorithms quality is based on the following algorithms [23].

The algorithm for determining the discrete Frechet distance.

Consider the algorithm for determining the discrete Frechet distance, in case of two contours (Fig. 2).

1. Let the contour of each segment C and R be presented in the form of the polygonal curves (11).

$$\sigma(C) = (v_1, \dots, v_r), \quad \sigma(R) = (w_1, \dots, w_s), \quad (11)$$

where r, s – the number of linearly approximated segments.

2. Let's form a sequence L between the curves C and R

$$L = (v_{a_1}, w_{b_1}), (v_{a_2}, w_{b_2}), \dots, (v_{a_m}, w_{b_m}), \quad a_1 = 1, b_1 = 1, a_m = r, b_m = s.$$

3. We obtain the Euclidean norm of the sequence $\|L\| = \max_{i,j=1,\dots,m} d(v_{a_i}, w_{b_j})$,

using the following steps.

- 3.1. If $i=1$ and $j=1$, then the distance is given as the Euclidean distance between the points (Formula 12).

$$d = \sqrt{(w_{b_j} - v_{a_i})^2}; \quad (12)$$

- 3.2. If $i>1$ and $j=1$, then the distance is given by the formula (13).

$$\max\{d(v_{a_{i-1}}, w_{b_1}), d(v_{a_i}, w_{b_1})\} \quad (13)$$

- 3.3. If $i=1$ and $j>1$, then the distance is:

$$\max\{d(v_{a_1}, w_{b_{j-1}}), d(v_{a_1}, w_{b_j})\} \quad (14)$$

- 3.4. If $i>1$ and $j>1$, then the distance is given by the formula 15.

$$\max\{\min(d(v_{a_{i-1}}, w_{b_i}), d(v_{a_{i-1}}, w_{b_{i-1}}), d(v_{a_i}, w_{b_{i-1}}), d(v_{a_i}, w_{b_i}))\} \quad (15)$$

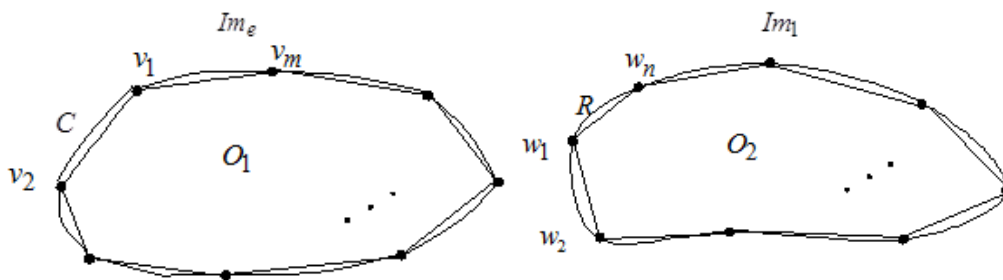


Fig. 2 Determining the discrete Frechet distance

The algorithm for determining the Hausdorff distance

Let's use the results of the regions contours representation. Let's present the regions O_1 and O_2 in the form of the convex polygons $O_1 = (v_1, v_2, \dots, v_m)$ and $O_2 = (w_1, w_2, \dots, w_n)$, where v_i ($i = \overline{1, m}$), w_i ($i = \overline{1, n}$) – the sequences of vertices linearly approximated sections of the external borders of the regions. Then the Hausdorff distance between the convex regions O_1 and O_2 is calculated according to formula 16:

$$d_H(O_1, O_2) = \max\left\{ \max_{i=1, \dots, m} d_{O_2}(a_i^1, b_i^1), \max_{i=1, \dots, n} d_{O_1}(a_i^2, b_i^2) \right\}, \quad (16)$$

where $d_{O_2}(a_i^1, b_i^1)$ – the projections of the vertex region O_1 to the region O_2 , $d_{O_1}(a_i^2, b_i^2)$ – the projections of the vertex region O_2 to the region O_1 [24].

The projections d_{O_l} ($l = \overline{1, 2}$) are calculated according to the expression:

$$d_{O_l}(v, w) = \begin{cases} \|(v, w) - Proj_{O_l}(v, w)\|, & (v, w) \notin Interid(O_l), \\ 0 & \text{in other cases} \end{cases} \quad \text{where } Proj_{O_l}(v, w) \text{ – the point at}$$

which a minimum Euclidean distance is implemented from a point $P(v, w)$ to the region O_l [23].

Let's present the Hausdorff determining distance algorithm by the following steps:

1. Let's set up the polygonal regions with the sequences vertices $O_1 = (v_1, v_2, \dots, v_m)$ and $O_2 = (w_1, w_2, \dots, w_n)$ that are obtained from the previous algorithm. We find the distances d_{O_l} ($l = \overline{1, 2}$) for all vertices of regions O_1 and O_2 according to the expression (3).

2. According to the expression (2) we obtain d_H .

Experimental results

In order to carry out computer experiments the software module has been developed in Java programming language using the OpenCV computer vision library. This module is designed for pre-processing & image segmentation and the evaluation segmentation results. It implements segmentation algorithms developed by the authors. The segmentation algorithms are evaluated, based on the metric approach [16]. The preprocessing algorithm is presented in details [19].

Fig. 7 shows the fragment of a histological image of a breast tissue slice. Histological images contain such complex micro-objects as parietes of glands and ducts. The texture analysis is calculating of textural features space based on PM (Fig. 8b) and its threshold processing.

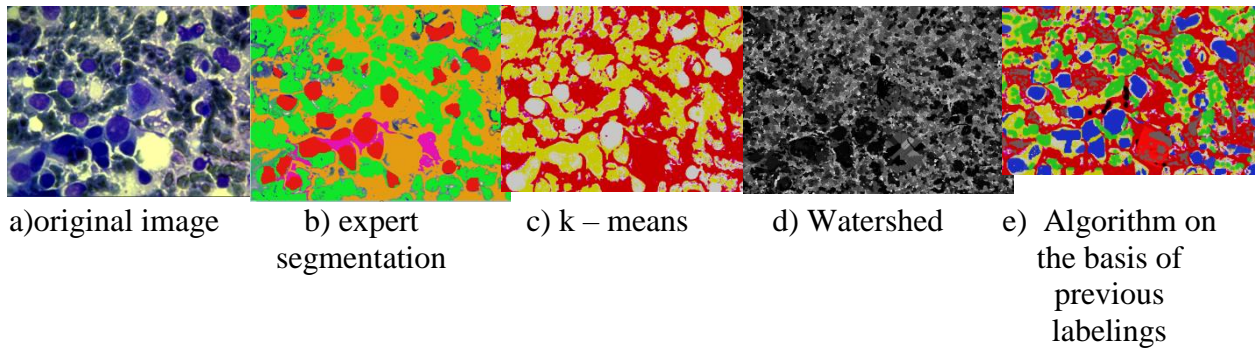


Fig. 7 Previous labeling images by different algorithms

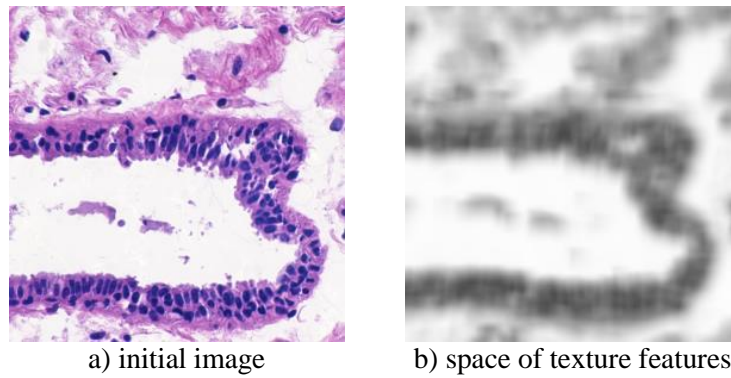


Fig. 8 A fragment of the initial image and the image space of texture features

As a result of threshold processing we have obtained labeling (Fig. 9,a) as well as have identified the breast duct paries (Fig. 9,b).

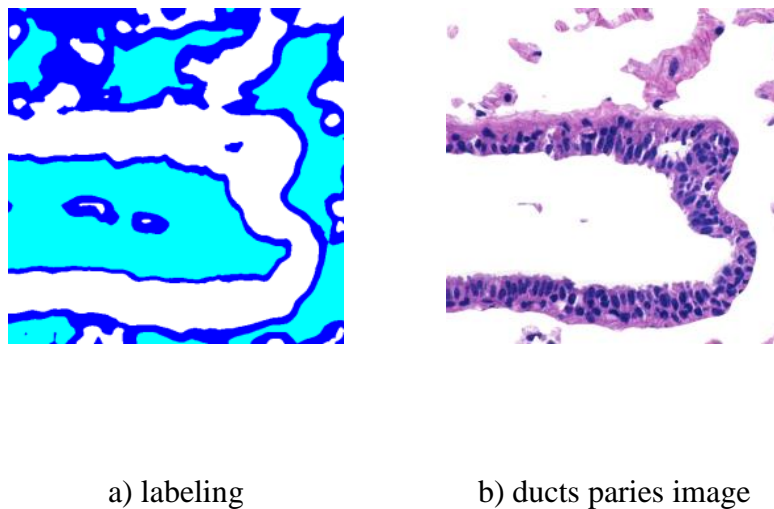


Fig. 9 Labeling image and the ducts parietes identification





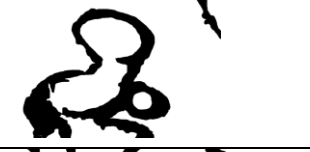
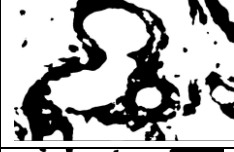


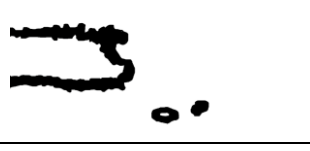
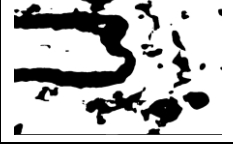
Table 1 shows the comparison of image segmentation algorithms. The Hausdorff, Frechet, Gromov – Hausdorff, Gromov – Frechet metrics are used for the comparison.

Table 1. Comparison of segmentation algorithms

metric algorithm	Hausdorff metric	Gromov-Hausdorff metric	Frechet metric	Gromov-Frechet metric
k-means	67.89	64.63	67.89	64.63
watershed	130.9	125.7	132,76	126.1
The algorithm is based on the previous labeling	64,38	63,27	64,38	63,27

Table 2 shows the results of the evaluation of the texture segmentation algorithm.

Table 2. Evaluation of texture segmentation algorithms

Standard image	Texture segmentation	1	Hausdorff metric
		2	Gromov-Hausdorff metric
		3	Frechet metric
		4	Gromov-Frechet metric
		1	124.14
		2	121.64
		3	260.93
		4	165.92
		1	342.06
		2	333.95
		3	342.06
		4	333.95
		1	113
		2	108.17
		3	412.95
		4	393.07
		1	431.18
		2	430.72
		3	432.65
		4	430.75
		1	190.06
		2	190.06
		3	224.61
		4	224.61

Conclusion

1. Using of connection points for the segmentation of biomedical images has a number of advantages compared to the characteristics of the individual points:

- the ability to process images of any type;
- the increased resistance to image segmentation when the micro-objects are close to each other.

- reducing of the input image noise and distortion effect on the overall result by analyzing of images with different segmentation algorithms.

2. The texture segmentation application, based on spatial moments, allows identifying complex micro-objects such as cell layers, the parietes of blood vessels and ducts.

3. Using of the Hausdorff, Fréchet, Gromov – Hausdorff, Gromov - Fréchet metrics provides quantifying of the segmentation algorithms quality in automatic mode.

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RESUME

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Segmentation algorithms of biomedical images: development and quantitative evaluation

The article presents the comparative analysis of the biomedical image segmentation methods. The work discusses segmentation methods on the basis of previous labeling and spatial moments. The experimental results show that the developed methods have higher accuracy by signal-noise ratio compared to the nowadays known.

This paper showing that the using of connection points for the segmentation of biomedical images has a number of advantages compared to the characteristics of the individual points such as: the ability to process images of any type; the increased resistance to image segmentation when the micro-objects are close to each other, reducing distortion effect on the overall result by analyzing of images with different segmentation algorithms.

The texture segmentation application, based on spatial moments, allows identifying complex micro-objects such as cell layers, the parietes of blood vessels and ducts.

Moreover the authors have developed the quantitative evaluation of the segmentation algorithms based on the metrical approach. Using of the Hausdorff, Fréchet, Gromov – Hausdorff, Gromov - Fréchet metrics provides quantifying of the segmentation algorithms quality in automatic mode.

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