

Epidermis area detection for immunofluorescence microscopy

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ABSTRACT

We propose a novel image segmentation method for immunofluorescence microscopy images of skin tissue for the diagnosis of various skin diseases. The segmentation is based on machine learning algorithms. The feature vector is filled by three groups of features: statistical features, Laws' texture energy measures and local binary patterns. The images are preprocessed for better learning. Different machine learning algorithms have been used and the best results have been obtained with random forest algorithm. We use the proposed method to detect the epidermis region as a part of pemphigus diagnosis system.

Keywords: Medical skin images, immunofluorescence microscopy, statistical features, Laws' texture energy measures, multilayer perceptron, pemphigus.

1. INTRODUCTION

Immunofluorescence microscopy is an imaging technique used for dermatological diseases diagnosis. It shows both the localization and endogenous expression levels of proteins of interest. Using this information dermatologist can not only diagnose skin disease, but also predict subsequent illnesses and doses of medicines. This technique is popular for diseases with a complex course like pemphigus vulgaris because of it need very accurate doses of medicines.

Nowadays these dermatological diseases diagnosis technique can be divided into three main groups:

1. Methods based on reflectance confocal microscopy (RCM). This technique is noninvasive but RCM images are more challenging to read than histology. The imaging is in en face orientation (instead of orthogonal), and with only one source of contrast (reflectance). Consequently, the images appear in grayscale contrast. In addition, the contrast and signal-to-noise vary with pigmentation conditions and degrade with depth, especially below the derma-epidermal junction [1].

2. Methods based on high-frequency ultrasonography. It is also noninvasive. The resolution of these methods lies in the range of 16–80 microns, which allows the visualization of the skin and its layers. In addition to the visualization, high-frequency ultrasound imaging allows to obtain quantitative data on the size of the observed objects, such as the thickness of the epidermis and dermis, as well as their acoustic density, which increases the reliability of clinical data. However, major defect of these methods is non-availability of contrast [2].

3. Methods based on fluorescence microscopy. One of these techniques is immunofluorescence. The imaging of immunofluorescence is in orthogonal orientation and can used with more than one sources of contrast. Also its only technique that can specificity of antibodies to their antigen, so it can use for diagnosis some rare but extremely severe immune diseases.

In this work, we propose a texture-based epidermis and dermis segmentation algorithm for immunofluorescence microscopy images. The algorithm includes image preprocessing, feature extracting, feature selection and classification using machine learning technique.

The rest of paper is organized as follows: in Section 2 we describe the segmentation algorithm, Section 3 presents the results, in Section 4 we discuss the possibility of proposed method application for pemphigus diagnosis, Section 5 concludes the paper.

2. SEGMENTATION ALGORITHM

2.1 Preprocessing

Different contrast level, shot noise and dead pixels affect texture classification. We remove them by the following algorithm containing three steps:

1. Luminosity equalization:

$$I_1 = 128 \cdot \frac{I}{I * G_\sigma + \epsilon},$$

where $G_\sigma = \frac{1}{2\pi\sigma^2} \exp(-\frac{x^2+y^2}{2\sigma^2})$, $\sigma = 20$.

2. Median filtering with radius 3.
3. Gaussian filtering with $\sigma = 2$.

2.2 Feature extraction

The preprocessed images are split into 32×32 blocks, which are used for feature extraction. We use the features of three groups: statistical features, Laws' texture energy measures and local binary patterns.

Statistical features

Seven statistical features are calculated: mean value, standard deviation, variance, energy, skewness, kurtosis, and entropy [3]:

1. Mean: $\bar{x} = \frac{1}{N} \sum_{k=1}^N x_k$;
2. Standard deviation: $\sigma = \sqrt{\frac{1}{N} \sum_{k=1}^N (x_k - \bar{x})^2}$ and standard deviation σ^2 ;
3. Energy: $E = \frac{1}{N} \sum_{k=1}^N x_k^2$;
4. Skewness: $\gamma_1 = \frac{1}{N} \sum_{k=1}^N \left(\frac{x_k - \bar{x}}{\sigma}\right)^3$;
5. Kurtosis: $\gamma_2 = \frac{1}{N} \sum_{k=1}^N \left(\frac{x_k - \bar{x}}{\sigma}\right)^4$;
6. Entropy: $H = -\sum_i P(i) \log_2 P(i)$, where $P(i)$ is normalized image histogram.

However these features do not consider spatial distribution of pixel values, they still can represent texture features.

Laws' texture energy measures

Another approach to generating texture features is to use local masks to detect various types of texture. Laws developed a texture-energy approach that measures the amount of variation within a fixed-size window [4]. He proposed 3×3 , 5×5 , and 7×7 convolution masks for texture discrimination. The mask size of 5×5 is used in our work. The Laws masks, 2D kernels, are constructed out of a set of one-dimensional kernels of 5 elements based on primitive geometric features:

L5 (Level) = [1 4 6 4 1], E5 (Edge) = [-1 -2 0 2 1], S5 (Spot) = [-1 0 2 0 -1], R5 (Ripple) = [1 -4 6 -4 1].

Using combinations of these four kernels, 16 masks of 5×5 sizes are formed. For example,

$$L5S5 = \begin{bmatrix} 1 \\ 4 \\ 6 \\ 4 \\ 1 \end{bmatrix} \times [-1 \ 0 \ 2 \ 0 \ -1] = \begin{bmatrix} -1 & 0 & 2 & 0 & -1 \\ -4 & 0 & 8 & 0 & -4 \\ -6 & 0 & 12 & 0 & -6 \\ -4 & 0 & 8 & 0 & -4 \\ -1 & 0 & 2 & 0 & -1 \end{bmatrix}.$$

Based on the 16 generated Laws masks, nine masks are derived, where some of the similar masks are averaged to obtain a single mask. They are: $L5E5/E5L5 = (L5E5 + E5L5) / 2$, $L5S5/S5L5$, $L5R5/R5L5$, $E5E5$, $E5S5/S5E5$, $E5R5/R5E5$, $S5S5$, $S5R5/R5S5$, and $R5R5$, respectively [5].

The image is convolved with these masks and the average values over the block form nine feature values. We also downsample the image with factors of 2, 4 and 8 to calculate feature values for different scales, so 36 values are used for the feature vector.

Local binary patterns

We also tried to use Local Binary Patterns (LBP) [6] to describe textures, but at feature selection stage, all LBP features were excluded.

2.3 Feature selection and classification

We use open source software Weka for relevant feature selection and classification using machine learning algorithms [7]. We use the following classifiers: support vector machines (SVM), Multilayer Perceptron, Random Forest (RF), J48 decision tree, k-nearest neighbor (KNN) with different parameters in combination with Ranker + Correlation feature selection method [8], [9].

3. TESTING AND EXPERIMENTAL SEGMENTATION RESULTS

3.1 Training and testing data sets

We use the database containing immunofluorescence microscopy and reflectance confocal microscopy images of 72 patients from Moscow Regional Research and Clinical Institute. For every patient we have 10 to 12 images. In addition, there is an expert segmentation of 3–4 images for 12 patients with different skin diseases. All images have the resolution of 2561×1781 pixels. The database is split into training and testing sets with 2:1 ratio. The dataset is imbalanced. Therefore, we have used SMOTE algorithm [10] to balance the training set by oversampling the minority class.

3.2 Results

The best results have been obtained with Random Forest classifier. Figure 1 show the ROC curves for the considered classification methods for testing data set. Table 1 compares result of immunofluorescence microscopy technique and reflectance confocal microscopy technique in the form of confusion matrices, for the two layers (epidermis and dermis) for Random Forest classifier.

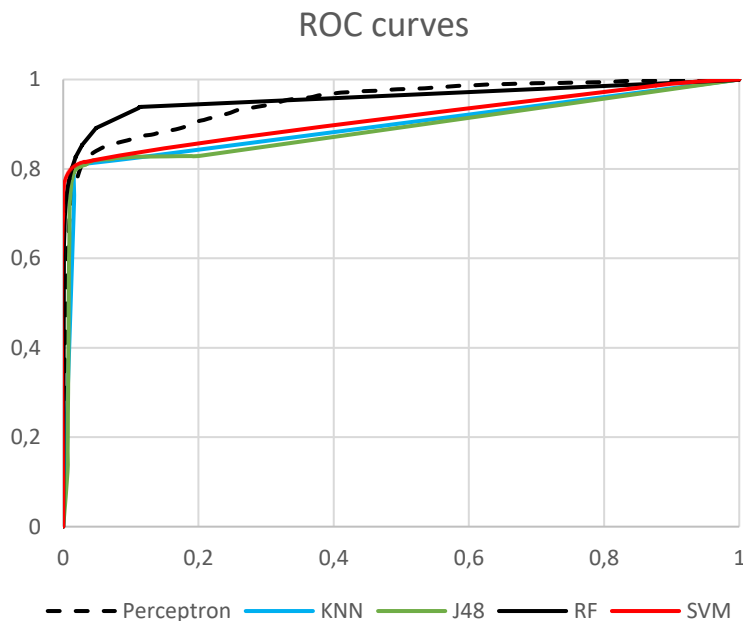


Figure 1. ROC curves for the considered classification methods.

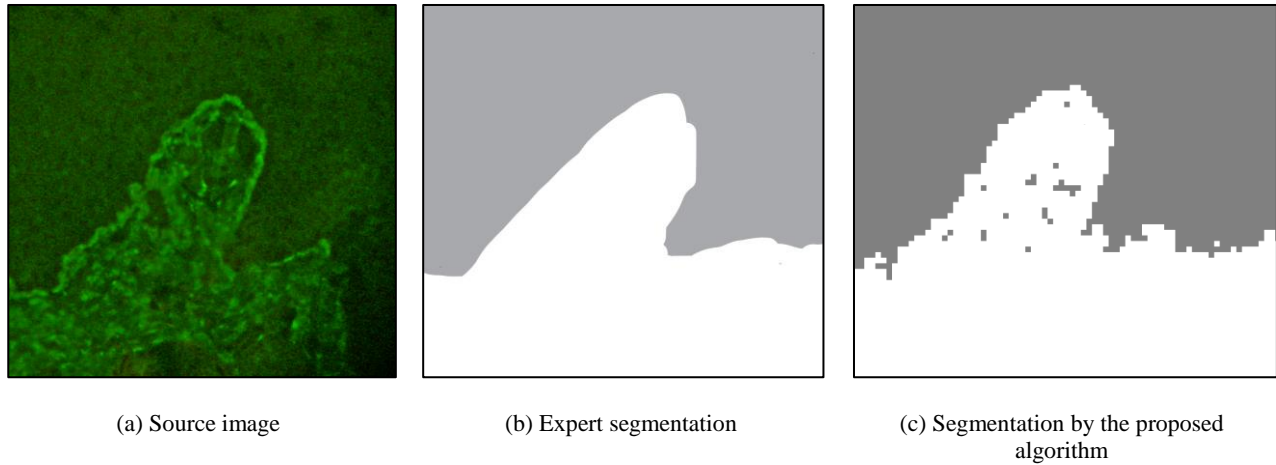


Figure 2. The results of the proposed segmentation algorithm.

	Immunofluorescence microscopy		Reflectance confocal microscopy	
	Epidermis	Dermis	Epidermis	Dermis
Epidermis (%)	87	13	89	11
Dermis (%)	9	91	13	87

Table 1. Confusion matrices for immunofluorescence microscopy technique and reflectance confocal microscopy technique.

4. PEMPHIGUS DIAGNOSIS

We have included the segmentation method into the automatic pemphigus diagnosis system [11]. The system performs the analysis of cell structures in the given image and calculates the severity value. For better diagnostic results, only the cells in the epidermis area should be processed because the structures from other layers may influence the result. The proposed method solves this problem. The combination of the proposed segmentation method and intermediate preprocessing results of the pemphigus diagnosis system are shown in Figure 3.

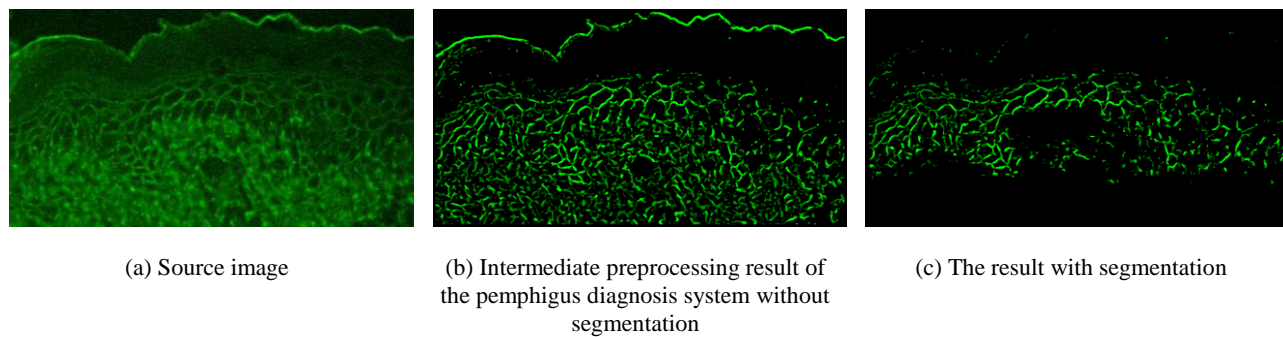


Figure 3. Application of the proposed segmentation method for pemphigus diagnosis system.

5. CONCLUSION

A novel segmentation method for immunofluorescence microscopy images has been proposed. This method can be used in various dermatological diagnostic systems. Experimental results show its effectiveness and demonstrate its application for the pemphigus diagnosis system.

In future work we expect to improve the classification results by enhancing preprocessing as well as using more texture features. We also plan to enlarge the image dataset to make the results more representative.

As a conclusion we can also say that immunofluorescence microscopy, despite the fact that it is invasive, for many severe skin diseases is almost only diagnosis technique, so research in this area is extremely important.

The work was supported by Russian Science Foundation grant 17-11-01279.

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