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Hybrid cytological image segmentation method based on competitive neural network and adaptive thresholding

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Abstract

The paper provides a preview of research on the computer system to support breast cancer diagnosis. The approach is based on analysis of microscope images of fine needle biopsy material. The article is devoted mainly to the segmentation problem. Hybrid segmentation algorithm based on competitive learning neural network and adaptive thresholding is presented. The system was tested on a set of real case medical images obtained from patients of the hospital in Zielona Góra with promising results.

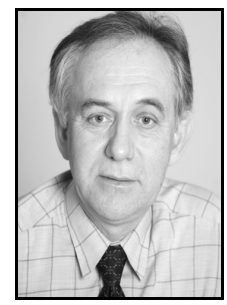
Keywords: image segmentation, neural networks, breast cancer, diagnosis.

Hybrydowa metoda segmentacji obrazów cytologicznych oparta o konkurencyjne sieci neuronowe i adaptacyjne progowanie

Streszczenie

Niniejszy artukuł przedstawia wyniki prac badawczych prowadzonych nad komputerowym systemem wspierającym diagnostykę raka piersi. Zaprezentowane podejscie oparte jest na analizie mikroskopowych obrazów materiału pozyskanego metodą biopsji cienkoigłowej bez aspiracji. Zadaniem systemu jest określenie czy badany przypadek jest zmianą łagodną czy złośliwą. Badania skupione są na dwóch głównych problemach. Pierwszym z nich jest segmentacja obrazów cytologicznych oraz ekstrakcja cech morfometrycznych jąder komórkowych występujących na rozmazach. Drugim problemem jest klasyfikacja raka sutka oraz odpowiedni dobór cech najlepiej opisujących daną klasę. W artykule autorzy położyli główny nacisk na opisie sposobu segmentacji obrazów. Poprawność procesu segmentacji w dużym stopniu decyduje o możliwości wykonania skutecznych pomiarów cech morfometrycznych jąder komórkowych i w konsekwencji dokonania właściwej diagnozy. W artykule przedstawiono hybrydowy algorytm segmentacji oparty o konkurencyjne sieci neuronowe i adaptacyjne progowanie. Jest to metoda alternatywna do zaprezentowanej wcześniej metody bazującej na rozmytym algorytmie c-średnich. Porównanie wyników obydwu metod zamieszczono w artykule. Automatyczny system wspierający diagnostykę raka piersi przetestowano na prawdziwych obrazach medycznych pacjentów regionalnego szpitala w Zielonej Górze. W przeprowadzonych eksperymetach uzyskano obiecujące wyniki.

Prof. Józef KORBICZ



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Słowa kluczowe: segmentacja obrazu, sieci neuronowe, rak piersi, diagnostyka.

1. Introduction

According to the National Cancer Registry breast cancer is the most common cancer among women. In 2008, there were 14,576 diagnosed cases of breast cancer in Polish women. Out of these cases, 5362 deaths were the result. There has also been an increase of breast cancer by 3-4% a year since the 1980's. The effectiveness of treatment largely depends on early detection of the cancer.

Modern medicine does not provide one hundred percent reliable, if possible cheap and at the same time non-invasive, diagnostic methods for the diagnosis of breast pathology. As a result, in practice the important function acting in breast cancer diagnosis is the so-called triple-test, which is based on the summary of results of three medical examinations with different degrees of sensitivity and it allows achieving high confidence of diagnosis. The triple-test includes self examination (palpation), mammography or ultrasonography imaging and fine needle biopsy [1]. Fine needle biopsy is collecting the nucleus material directly from ta umor for microscopic verification. Next, the material (collected cells) is examined using a microscope in order to confirm or exclude the presence of cancerous cells. The present approach requires a deep knowledge and experience of a cytologist responsible for diagnosis. In short, some pathologists can diagnose better than the others. In order to make the decision independent of the arbitrary factor, morphometric analysis can be applied. Objective analysis of microscopic images of cells has been a goal of human pathology and cytology since the middle of the 19th century. Early work in this area consisted of simple manual measurements of the cell and nuclear size. Along with the development of advanced vision systems and computer science, quantitative cytopathology has become a useful method for detection of diseases, infections as well as many other disorders. In the literature one can find approaches to breast cancer classification [2, 3, 4, 5, 6]. The mentioned approaches are concentrated on classifying FNA (Fine Needle Aspiration) or FNB (Fine Needle Biopsy) slides as benign or malignant.

In this paper there is presented a fully automatic method that allows distinguishing malignant cells from benign cells. The classification of the tumor is based on morphometric examination of cell nuclei. In contrast to normal and benign nuclei, which are typically uniform in appearance, cancerous nuclei are characterized by irregular morphology that is reflected in several parameters described in detail further in the paper. It was decided not to use shape features because the previous work showed that the shape factors did not have good discriminative properties [7]. Features were extracted from the segmented images obtained by the hybrid segmentation method based on competitive learning a neural network and adaptive thresholding.

The quality of segmentation and feature extraction was tested by using the set of classifying algorithms. The measure is based on the classification accuracy obtained by leave-on-out cross-validation. In this work four different classification methods were used to rate the feature subsets: k-nearest neighbor, naive Bayes classifier, decision trees and classifiers ensemble [8, 9].

The paper is divided into four sections. Section 1 gives an overview of breast cancer diagnosis techniques. Section 2 describes the process of acquisition of images used for breast cancer diagnosis. Section 3 deals with segmentation algorithm used to separate cells and extract features. Section 4 shows the experimental results obtained with use of the proposed approach. The last part of the work includes conclusions and bibliography.

2. Medical images database

It is necessary to have appropriate amount of real case data to test new developed as well as existing image analysis algorithms. Probably, the most popular database of FNB images and nuclei features is Wisconsin Database of Breast Cancer (WDBC). However, the quality of images delivered in the set is unsatisfactory for the image analysis methods described in the paper. Because of that we decided to use our own data set.

The database contains 500 images of the cytological material obtained by FNB. The material was collected from 50 patients of outpatient clinic ONKOMED in Zielona Góra. It gives 10 images per case which was the recommended amount by specialists from the Regional Hospital in Zielona Góra [2, 7]. This number of images per single case allows correct diagnosis by a pathologist. The set contains 25 benign and 25 malignant lesions cases. Biopsy without aspiration was performed under the control of ultrasonograph with a 0.5 mm diameter needle. Smears from the material were fixed in spray fixative (Cellfix of Shandon company) and dyed with hematoxylin and eosin (h+e). The time between preparation of smears and their preserving in fixative never exceeded three seconds. The images were recorded by a SONY CDD IRIS color video camera mounted atop an AXIOPHOT microscope. The slides were projected into the camera with 40x and 160x objective and a 2.5x ocular. One image was generated for enlargement 100x and nine for enlargement 400x. Images are BMP files, 704x578 pixels, 8 bit/channel RGB. All cancers were histologically confirmed and all patients with benign disease were either biopsied or followed for a year.

3. Segmentation of the Nuclei

Classification of tumor malignancy requires isolating nuclei from the rest of the image. In literature, many different approaches have been already proposed to extract cells from microscope images [10, 11, 12, 2, 3, 4, 5, 7, 13]. This task is usually done automatically, using one of the well known methods of image segmentation [14, 15, 16, 17]. Unfortunately, reliable cell segmentation is a challenging task. Very often cells cluster and overlap together and their boundaries are blurred. Moreover, attempts to generalize segmentation approaches proposed in literature usually fail because such methods work correctly only for specific images. Slides from various sources may vary significantly depending on the method of smear preparation. In order to deal with these problems, we have developed automatic segmentation procedure that integrates results of image segmentation from two different methods. Proposed algorithm uses adaptive thresholding segmentation to distinguish all dark objects (nuclei, red blood cells and others) from bright background. Next, all objects except background are classified using the competitive neural network and further analysis is performed based only on nuclei, which provide crucial diagnostic information [18].

The key idea of thresholding is to separate objects from background based on pixel intensity fluctuations. There are many approaches for computing the optimal threshold [14, 19, 16, 20]. Most of them are based on histogram analysis or examination of pixel intensity. Such global thresholding methods work correctly for images with uniform illumination, but they fail for images with strong illumination differences. Unfortunately, effects of non-uniform lighting conditions can be observed on most microscope images used in this work. In order to solve the problem, the adaptive threshold method was applied. The local threshold is calculated for each pixel using intensities of pixels from its neighborhood. This area was defined as a square window of size 21x21 pixels and the threshold is a mean intensity value of pixels inside the window. Such an approach eliminates the problem with non-uniform illumination but causes that insignificant objects with high local contrast (eg. red blood cells) are also separated from the background.

The next step of image processing is distinguishing nuclei from red blood cells. This task is performed based on color information. It was decided to define three types of objects respect to their color: nuclei, red blood cells and background. In order to classify the defined objects, a competitive neural network with 3 neurons was designed and then learned using Kohonen learning rule [21, 22]. Each neuron has three inputs interpreted as RGB values. Learning samples (RGB values) are selected from segmented images manually as small rectangular parts of the original images. Kohonen learning rule used to tune neurons assumes that only winning neuron weights will be adjusted. Neurons compete to be tuned and the winning neuron with the weight vector closest to the input vector is updated in a single learning pass. The equation shown below describes the update procedure used to tune weights of the winning neuron:

$$w_i^{win}(k+1) = w_i^{win}(k) + \alpha\left(p_i(k) - w_i^{win}(k)\right), \tag{1}$$

where $w_i^{win}(k)$ is i-th weight of winner neuron during k-th learning pass, $p_i(k)$ is the i-th input value and α is the learning rate. After the learning procedure, the network is able to classify pixels to 3 different classes (nuclei, red blood cells, background). Weights of the neurons represent the centers of clusters in the RGB space. Each cluster groups pixels belonging to the predefined objects. Examples of the clusters are presented in Fig. 1. However, in order to segment properly the nuclei, the neuron representing nuclei must be identified first. This task is done automatically by computing and comparing the mean values of weights for each neuron.

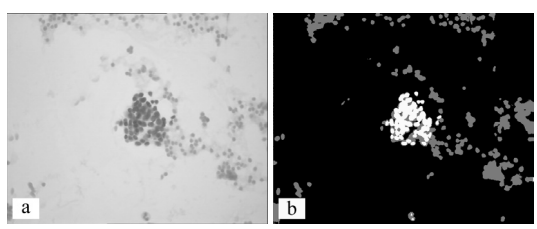

Fig. 1. Competitive neural network based segmentation: a) original image and b) membership of pixels to clusters
Rys. 1. Segmentacja oparta o konkurencyjną sieć neuronową: a) oryginalny obraz, b) przynależność pikseli do klastrów

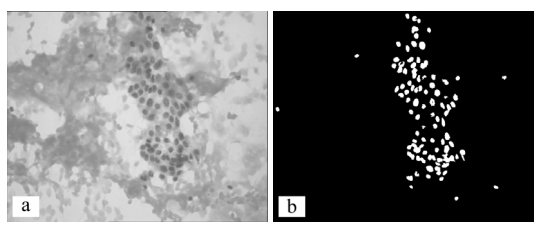

Fig. 2. Hybrid competitive network and adaptive threshold segmentation: a) original image, b) extracted nuclei
Rys. 2. Hybrydowa segmentacja konkurencyjną siecią neuronową i adaptacyjnym progowaniem: a) obraz oryginalny, b) wyodrębnione jądra

The lowest mean value indicates that we are dealing with a neuron representing nuclei. Based on the knowledge which neuron describes nuclei, the network can classify pixels into two different classes: nuclei and not nuclei and, finally, segment the original image (Fig. 2).

Competitive neural network based segmentation is able to correctly distinguish nuclei from the background and red blood cells but behaves worse for clustered and overlapped nuclei. Much better results for separation of the clustered nuclei are obtained using adaptive thresholding segmentation but, as mentioned before, thresholding is not able to distinguish nuclei from red blood cells properly. In order to combine the advantages of both methods, the results of both segmentation algorithms were fused together by multiplying segmented images.

The segmentation results obtained for the developed procedure of nuclei extraction allowed classifying the degree of breast cancer malignancy with relatively high accuracy. However, it must be noted that the proposed method is usually not able to separate strongly clustered nuclei with blurred boundaries and this problem may degrade the results of the classification.

Tab. 1. Results of classification for individual features using competitive network based segmentation (cn), fuzzy c-means based segmentation (fcm) and manual segmentation (m)
Tab. 1. Wyniki klasyfikacji dla pojedynczych cech z wykorzystaniem segmentacji opartej o konkurencyjną sieć neuronową (cn), segmentacji opartej o rozmyty algorytm c-średnich (fcm) oraz segmentacji manualnej (m)

Feature	Statistic	kNN			Naive Bayes		
		cn	fcm	m	cn	fcm	m
area	mean	64.7	59.0	81.9	67.5	62.4	84.7
	variance	66.4	68.1	86.4	67.2	71.2	84.4
perimeter	mean	65.2	68.9	84.4	68.9	71.5	84.4
	variance	70.4	76.6	83.3	69.5	77.5	83.3
eccentricity	mean	70.4	65.8	52.5	72.6	70.1	56.1
	variance	70.7	68.9	52.8	74.4	73.5	57.2
major axis length	mean	69.8	61.5	81.4	71.2	68.9	83.6
	variance	61.8	67.8	79.7	69.8	69.2	80.6
minor axis length	mean	64.7	59.0	83.9	73.5	65.5	83.6
	variance	64.7	67.8	83.9	67.8	65.0	83.9
luminance mean	mean	70.9	71.2	67.8	74.9	73.5	67.5
	variance	61.0	63.2	54.2	64.4	65.8	56.4
luminance variance	mean	72.9	71.8	64.2	76.1	71.8	67.5
	variance	70.1	68.9	55.0	73.8	71.5	54.7
distance from centroid	mean	75.5	74.4	78.3	76.6	78.3	79.4
	variance	68.7	66.4	75.3	71.5	70.1	76.7

Feature	Statistic	Decision trees			Ensemble class.		
		cn	fcm	m	cn	fcm	m
area	mean	63.5	61.5	82.5	65.8	59.8	83.9
	variance	60.4	69.5	82.8	67.0	71.5	86.4
perimeter	mean	65.8	67.2	81.4	67.2	69.5	83.6
	variance	68.1	76.6	80.0	72.6	76.9	83.3
eccentricity	mean	67.2	62.7	50.6	72.9	67.2	50.0
	variance	70.7	66.7	54.4	72.4	68.9	54.7
major axis length	mean	66.1	60.4	80.3	68.1	62.7	81.9
	variance	63.0	68.7	77.2	65.5	69.8	78.9
minor axis length	mean	72.4	56.7	79.4	72.4	60.7	83.6
	variance	65.8	68.4	83.6	67.2	65.8	84.2
luminance mean	mean	72.9	68.9	66.1	72.9	72.9	69.2
	variance	63.0	61.5	54.2	60.7	64.4	56.9
luminance variance	mean	71.8	72.9	61.4	75.8	72.9	65.8
	variance	69.5	65.0	56.4	71.8	68.1	56.4
distance from centroid	mean	78.1	70.9	76.7	78.3	74.6	76.7
	variance	69.2	67.8	70.8	69.5	69.2	75.0

4. Experimental results

The FNB images were segmented with the aforementioned method. Then a set of features was extracted from the result images. Finally, classification was performed. The same procedure of extracting and classifying was applied to manual segmentation and the FCM algorithm in order to measure the effectiveness of the segmentation and compare it to the previous approach.

For each cell the following features were extracted: area, perimeter, eccentricity, major axis length, minor axis length, luminance mean, luminance variance and distance from the centroid of all nuclei on the image. For each slide the mean and variance of certain features were computed. Finally, all input variables were normalized.

Tab. 2. Results of classification for selected sets of features using competitive network based segmentation (cn), fuzzy c-means based segmentation (fcm) and manual segmentation (m)
Tab. 2. Wyniki klasyfikacji dla wybranych zbiorów cech z wykorzystaniem segmentacji opartej o konkurencyjną sieć neuronową (cn), segmentacji opartej o rozmyty algorytm c-średnich (fcm) oraz segmentacji manualnej (m)

Features set	kNN			Naive Bayes		
	cn	fcm	m	cn	fcm	m
area (m), area (v), perimeter (m), luminance mean (m), luminance variance (m), major axis length (v), minor axis length (v), dist. from centroid (m), dist. from centroid (v)	84.3	84.0	93.7	82.9	82.6	92.3
area (m), perimeter (m), perimeter (v), luminance mean (v), dist. from centroid (m)	84.6	80.3	91.5	80.3	79.5	91.5
area (v), dist. from centroid (v)	74.4	67.0	74.4	76.4	67.8	90.0
area (m) luminance mean (m), major axis length (v), dist. from centroid (v)	83.8	82.1	93.2	82.1	79.5	92.9
perimeter (m), perimeter (v), luminance mean (v), dist. from centroid (m)	86.6	82.9	91.5	83.2	83.8	92.3

Features set	Decision trees			Ensemble class.		
	cn	Fcm	m	cn	fcm	m
area (m), area (v), perimeter (m), luminance mean (m), luminance variance (m), major axis length (v), minor axis length (v), dist. from centroid (m), dist. from centroid (v)	85.8	82.6	90.3	85.5	84.0	93.4
area (m), perimeter (m), perimeter (v), luminance mean (v), dist. from centroid (m)	84.3	79.2	91.7	83.8	81.8	93.4
area (v), dist. from centroid (v)	73.8	66.1	88.0	75.5	68.7	88.6
area (m) luminance mean (m), major axis length (v), dist. from centroid (v)	80.6	80.6	89.7	82.9	81.8	93.4
perimeter (m), perimeter (v), luminance mean (v), dist. from centroid (m)	79.5	81.5	90.0	84.6	84.9	91.7

It was decided to use four well known classification algorithms such as k-nearest neighbor (with k = 9), naive Bayes classifier (with normal kernel distribution), decision trees (with GINI criterion) and classifiers ensemble [8, 9]. However, it must be mentioned that ensemble of classifiers is not a separate classification technique and its classification procedure is based on the results of others classifiers used in the experiments. Simply,

the answer of classifiers ensemble is determined by voting procedure and class that gathers majority vote wins and represents the answer of the classifiers ensemble.

The prospective accuracy of the resulting classifiers was tested using the leave-one-out validation technique [9]. Since the number of samples is relatively small, using chosen classification algorithms with leave-one-out is computationally tractable and allows for accurate estimation of the error.

The quality of the segmentation is not perfect. Many cells are joined or the centers of the cells are removed. However, classification using features such as area, luminance statistics or general distribution of cells on image gave very promising results. The discriminative power of individual features was estimated with the previously indicated classifiers and the results in form of the recognition rates are presented in Table 1. The recognition rate is defined as a percentage of successfully recognized cases to the total number of all cases. The results obtained with the aforementioned segmentation method was compared with the FCM based algorithm and manual segmentation results. Some features, specially describing the shape of cells such as the area or major axis length were significantly worse for automatic segmentation. The reason is low quality of the segmentation process. Surprisingly, some other features, mainly characterizing the luminance and texture, the mean luminance for instance, gave better recognition rate for automatic than manual segmentation.

To find a set of features the best discriminative benign and malignant cases sequential forward selection was applied. Taking into account the fact that different subsets can be optimal for different classifiers, two approaches were applied to forward selection. First the same subset of features for each classifier and classifiers ensemble was used to assess the final quality of subset. The second approach assumes that the classifiers have different optimal subsets of features. In Table 2 the most interesting results are presented. The competitive network approach is better than the FCM method in most cases. The best classification rate was 86.6%. Such a result is very promising according to the fact that the quality of the segmentation method might be considerably improved by detecting overlapping and joined objects. Also, it must be mentioned that in the experiment each image was treated as a separate case.

5. Conclusions

The method presented in the paper is an alternative to our previous approach to cytological image segmentation for breast cancer diagnosis. Despite the similarities in the modus operandi of both methods, one might see that competitive networks work better for the malignancy classification problem. The features describing luminance and texture can be extracted from images segmented with the method on a satisfactory level. However, acquisition of information about the shape of nuclei still needs some improvements. It might be achieved by detecting overlapped cells on images and splitting or excluding them from the classification process. Also, the recognition rate should be improved by adding more sophisticated features not tested during current investigations. The accuracy of the segmentation process obtained in the developed approaches is very promising, despite the fact that the current methods are not able to handle properly overlapped nuclei yet. Another challenge will be applying the whole segmentation and classification system for virtual slides generated by virtual scopes which are able to produce images with extremely high resolutions reaching 9 gigapixels and more. Such huge slides require completely new way of analysis. It will be crucial to develop algorithms to find interesting parts of the slides containing valuable information for further processing and diagnosis.

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otrzymano / received: 03.09.2011
przyjęto do druku / accepted: 03.10.2011 *artykuł recenzowany*