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Extraction of Cell Nuclei using CNN Features

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Abstract

Cytology is one of the decisive factors for the early detection of cancer. It is necessary to have a certain number of years of experience to be able to screen tumor cells for cytodiagnosis. However, this diagnosis has poor objectivity because there are so many parts that the judge's experience and skill is responsible for. In this paper, we present a new method to extract cell nuclei from HE stained images generally used cell staining for Creation of digitized objective indicators in cytology. Our method extracts cell nuclei using combining the input image and the image previously prepared by SVM. Features used in SVM are automatically generated from CNN. Results are demonstrated by experiments using the real images and its ground truths.

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Keywords: CNN; support vector machine; cell nuclei extraction;

1. Introduction

Cancer is still one of the most serious diseases in the world, in spite of the recent progress of its diagnoses and therapies. There is no doubt that quick and accurate pathological cytodiagnosis of materials, which are obtained by surgery, biopsy, or exfoliative cytology, is indispensable for the correct choice of treatment planning[1]. However, the major part of these diagnoses are still dependent on the pathologists' experiences and skills, which can be time-consuming as well as difficult in quantifications. Thus, these strategies sometimes contain the internal risk of human errors and result in different diagnosis among hospitals, leading to patients' unfavorable outcomes. To overcome these drawbacks, a considerable number of trials have been performed to develop more objective and quantitative automated diagnostic system for cancer cells[2], but it has not yet been put to practical use because of the wide diversity of morphology of tumor cells in our bodies. Since cell nuclei, stained with various compounds, are often used as a

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marker for cytodiagnosis, we decided to develop a method to detect these objects from bright field images. Here, this paper proposes a novel approach that detects nuclei from hematoxylin and eosin stained tissue samples.

2. Related Works

Some researches have been done to extract cells. For example, some methods are proposed to extract a cell nucleus using image processing technique such as methods using threshold and edge information[3,4] or using elliptic template matching[5,6].

Methods[3,4] are proposed to segment an image into three kinds of regions (null, cytoplasm and nucleus). The null region is a narrow and stable distribution, but the others fluctuate considerably depending on the specimen. It does not necessarily correspond to the relationship that the pixel value of the cell nucleus region is higher than that of the cytoplasm due to the thickness and staining heterogeneity of the specimen. In methods[3,4], these problems were dealt with by detecting edges by the zero crossing method by the $\nabla^2 G$ operator and variably changing the threshold value. However problem is that accuracy is not high at all when the target is limited to the stomach tissue, or when the edge information is lacked by the cytoplasm.

Method[5] extracts glomerular regions from kidney tissue images using both global and local treatment by model. The objective target extracted by this method is a mass of capillaries called glomeruli. From the viewpoint of the cell nucleus, the cell nucleus also takes a relatively circular or elliptical shape in many cases, but it is not common to take a shape far removed from the ellipse as with the glomerulus. Procedure of this method first binarizes the photographed image by local threshold processing. Next, the internal region is extracted as a boundary region. If the boundary region extracted in this process is not closed as a form surrounding the glomerulus, estimation and correction of the ambiguous boundary region are iteratively performed using density information of the ellipse model and the renal tissue image. Finally, an approximate ellipse is obtained from the detected boundary region, and a region surrounded by the boundary region is approximated with ellipse as an extracted glomerular region. As a merit of this method, it is possible to retrieve an interrupted region even when the boundary region is interrupted because extraction is performed in a global manner. However, problem is that correct extraction cannot be performed when the object to be extracted is far from the ellipse by this method.

In recent years, there has been an attempt to extract cell nuclei using machine learning as well as such image processing based methods. Method[7] is also one of extraction methods using machine learning and color information etc. are used for learning.

In the study of cell nucleus extraction, cell nuclei extraction is performed locally and it is rare to extract cell nucleus globally in general. However, it is essential to extensively extract the cell nuclei in a pathological diagnosis system in order to actually diagnose by looking at the cell at a medical site in practice. In addition, cell images become various forms due to the way of staining and the part to collect. Therefore, it is hoped to develop a method which can stably extract cell nucleus from any image. Based on the above information, we attempt to extract the cell nucleus from a single image in a globally using the basis of this idea.[7]

3. Pre-processing

In this paper, we use SVM (Support Vector Machine)[8] to create a score image showing the region where the cell nucleus exists in advance to cope with the difficult part in image processing similar to the method.[7] In this section, we describe the creation of classifiers and generation procedure to create such an image.

3.1. Create Classifier

In order to generate a score image showing a region with cell nucleus using SVM, a classifier is necessary to judge a cell or not. Therefore we first make classifiers. As a learning dataset necessary for creating classifiers, we use the image of the cell nucleus and background cut from the HE(Hematoxylin and Eosin) stained image created in advance. Features used for learning SVM are automatically generated by convolution and pooling of CNN (Convolution Neural Network) are used. Actually, CNN used in our research is alex-net, and the feature extracted from the seventh layer of the net is used.

Then, proposed method is developed in this research to improve the accuracy of cell nucleus extraction as the accuracy of the score image created is higher. Thus, we compared it with those appropriately combining HOG (Histograms of Oriented Gradients) feature and color histogram frequently used for general object recognition. Here, classifiers are evaluated to verify whether the CNN feature used for creating the score image is valid or not. Data set used for the experiment evaluation are 600 cell images of human (300 cell nuclei, 300 background) and 400 cell images of mouse (200 cell nuclei, 200 background). The evaluation method is a 10-fold cross validation test where 70% of the data set is used for testing and 30% is used for learning. The average value is adopted as an evaluation under the conditions that cross validation is performed five times for each feature. Table 1 shows the results of comparative experiments.

Table 1: Result of comparative experiment by 10-fold cross validation test

	Dataset	
	human	mouse
HOG&colors	0.892	0.886
CNN	0.967	0.941

It is shown from Table 1 that HOG and colors evaluation value takes 90% before, while the CNN feature one takes very high score of around 95%. Therefore, it is proved that CNN features are effective in the cell nucleus detected.

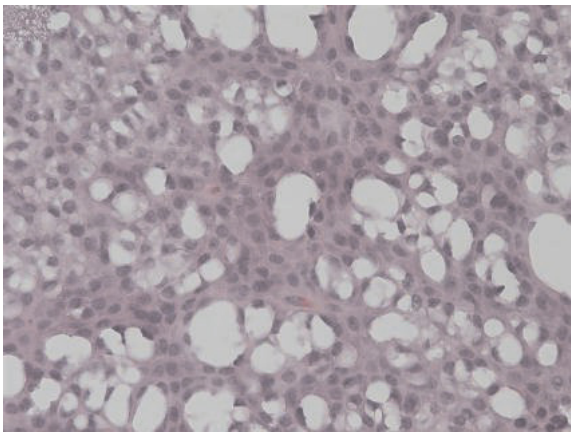
3.2. Generate score image

Using the classifier above, create a grayscale image showing where the cell nucleus is located. Actually, several hundreds of cell nuclei are scattered in the bright field image of cells stained with HE. In the reference method[7], a grayscale image showing the SVM score for each pixel is created from the pathological image using the learning result. However, since the pathological image of the cell is generally photographed with a high magnification microscope, the resolution is very high , there is a problem that when a score is calculated for each pixel, it takes a huge amount of time to create a score image. Therefore, we attempted to detect multiple cell nucleus areas and shorten the time by exhaustively raster scanning the detection windows in our research. It was possible to shorten the time more than double than searching for each pixel by using the detection window.

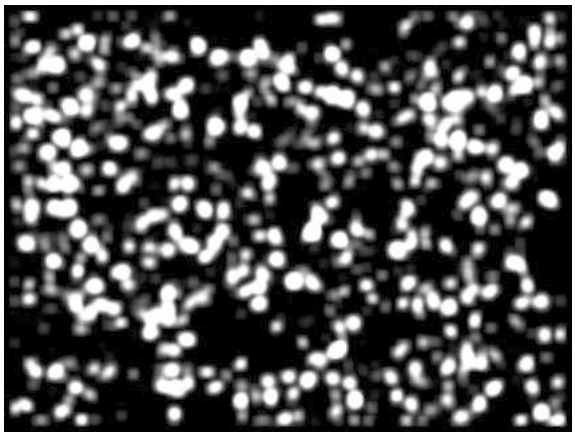
The size of general cell nucleus rarely exceeds 70 px by 70 px. Thus, the size of the detection window is 70 px by 70 px. We derive the cell nucleus likelihood by using SVM by extracting features with the same CNN as above for all detection windows obtained by raster scan. It is considered that the higher the likelihood, the higher the probability that the cell nucleus appears in the detection window, and the lower it is, the lower it is reflected since the SVM used here is classified by two classes (cell nucleus, background). The dataset of the cell nucleus that we created beforehand has a cell nucleus near the center of the image. Then, it is considered that the cell nucleus exists with high probability near the center of the detection window showing high likelihood. From the above, we created a grayscale image showing the score which is the probability the cell nucleus exists according to two parameters such as the obtained likelihood and the distance from the center. The input image and the generated score image are shown in the following Fig. 1.

4. Extract cell nuclei

The score image created in the previous section shows an area where the probability of the existence of the cell nucleus to the input image is high. It is understood that extraction of cell nucleus is insufficient by itself as you can see Fig. 1(b). In this section, attempt to extract the cell nucleus by combining the score image and the input image.



(a) Input image



(b) Score Image

Fig. 1: Create score image

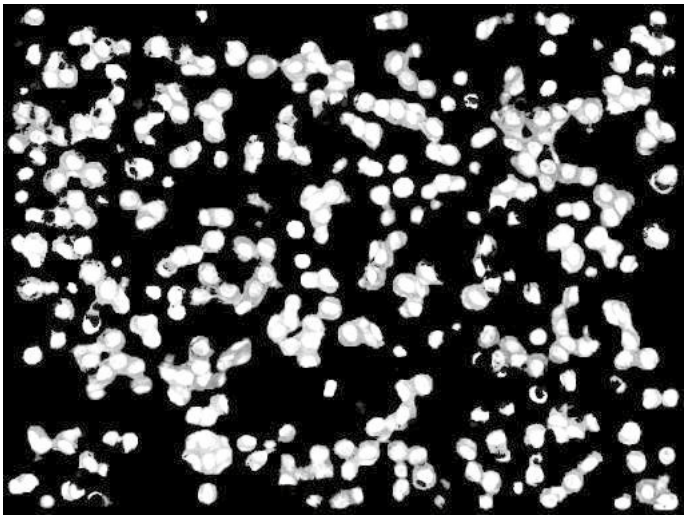


Fig. 2: Provisional extracted image of a cell nuclei

4.1. Finding cell nuclei candidates

First, a provisional extracted image of a cell nuclei is created from an input image and a score image. Provisional extracted image P is calculated by the following equation

$$P = 2\,(S - red(I)) \tag{1}$$

where S means score image and $red(I)$ means red color of input image I. We conducted a simple cell detection as a preliminary experiment for cell nuclear extraction. In experiment, we used simple grayscale image of the input image and color information of each of RGB as comparison targets. As a result, we use red color of input image for cell nuclei extraction because it was the highest detection accuracy. An example of provisional extraction by our method is shown in Fig. 2.

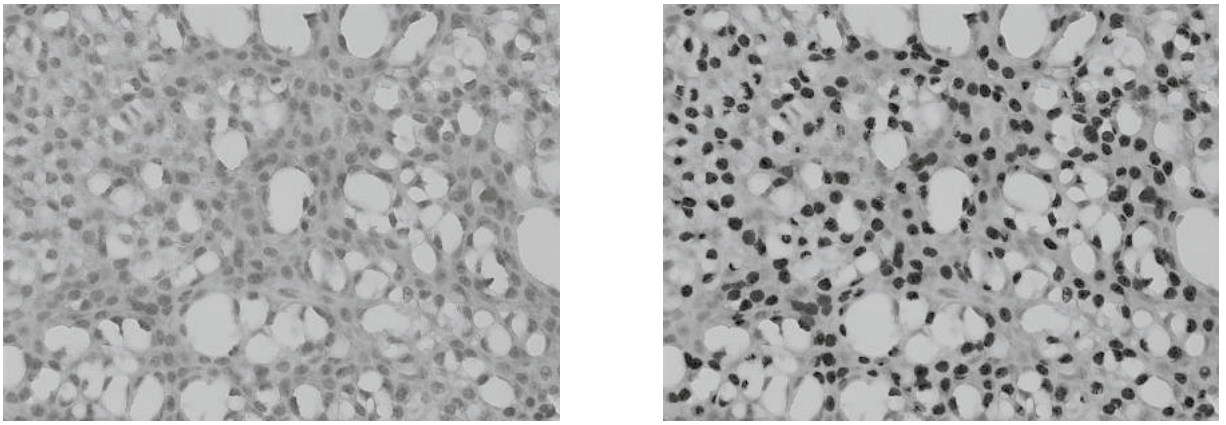
4.2. Emphasizing cell nuclei area

In the above extraction method, not only the luminance of the score image created by the classifier but also the luminance of the cell nucleus of the input image itself depends on the extraction accuracy. Therefore, it may be insufficient to detect cell nuclei with low staining density. Our method deal with this problem by using the labeling process.

First, binarize the provisional extracted image P with a low threshold to derive a binarized image B. Next, we perform to labeling process and we get label regions $B(R_k)$ from binarized image B. Then we extract the minimum luminance value $red(I)B(R_k)_{min}$ in the label region from the red color of the input image $red(I)$. We make emphasized image E by the following equation

$$E = \sum_{k=1}^{n} red(I)B(R_k) - red(I)B(R_k)_{min} \tag{2}$$

where n means total number of labels and $red(I)B(R_k)$ means areas corresponding to the label areas $B(R_k)$ in the red color of input image . An example of emphasizing cell nuclei area by our method is shown in Fig. 3.



(a) Before emphasis (b) After emphasis

Fig. 3: example of emphasizing cell nuclei area

4.3. Detecting cell nuclei

The luminance of the cell nucleus enhanced grayscale image was subtracted from the luminance of the score image to generate a new temporary extracted image. Next, we extracted specific contours of cell nuclei by binarizing the image with a high threshold value. There is a possibility that hollow defects may appear at the time of temporary extraction because staining unevenness and hollow defects may be mixed in HE staining images. Therefore, we carried out filling process on the image to fill the hollow defects. Since noise is existence in the image, we deletted the noise in the image using labeling processing. We deleted the area with area of 100 or less as noise since the area of the cell nucleus is around 3000. Finally, our method extracted cell nuclei using smoothing processing.

5. Experiments

Experiments using real HE staining images were done for the evaluation of the proposed method. Input images used for experiment have many various cell nuclei and the size of the image is 1920 px × 1440 px. The input images are shown in Fig. 4. The methods were applied to the areas obtained from the ground truths. The ground truths of the input images are shown in Fig. 5. Green pixels in the figure denote cell nuclei pixels and other color ones denote

pixels that clearly show the overlapping cell nuclei. The results of cell nuclei extraction of our method are shown in Fig. 6.

Next, the results were evaluated quantitatively. Thus, we use two types of evaluations: quantitative evaluation for pixel and quantitative evaluation for cell nucleus. The evaluations of pixel are calculated by

$$\eta_p = \frac{TP_p}{TP_p + FP_p} \tag{3}$$

$$\xi_p = \frac{TP_p}{TP_p + FN_p} \tag{4}$$

$$F_p = \frac{2\eta_p\xi_p}{\eta_p + \xi_p} \tag{5}$$

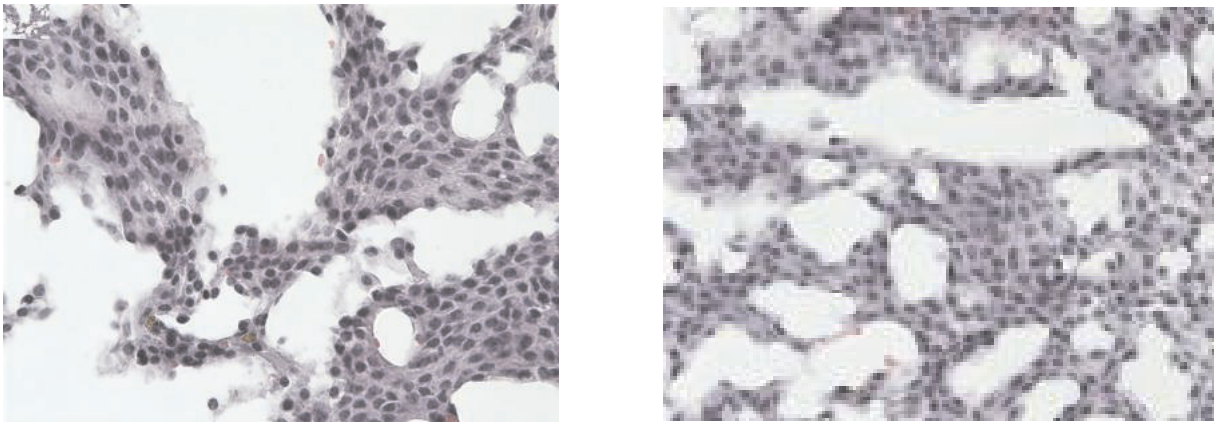
The evaluations of cell nucleus are derived similarly:

$$\eta_c = \frac{TP_c}{TP_c + FP_c} \tag{6}$$

$$\xi_c = \frac{TP_c}{TP_c + FN_c} \tag{7}$$

$$F_c = \frac{2\eta_c\xi_c}{\eta_c + \xi_c} \tag{8}$$

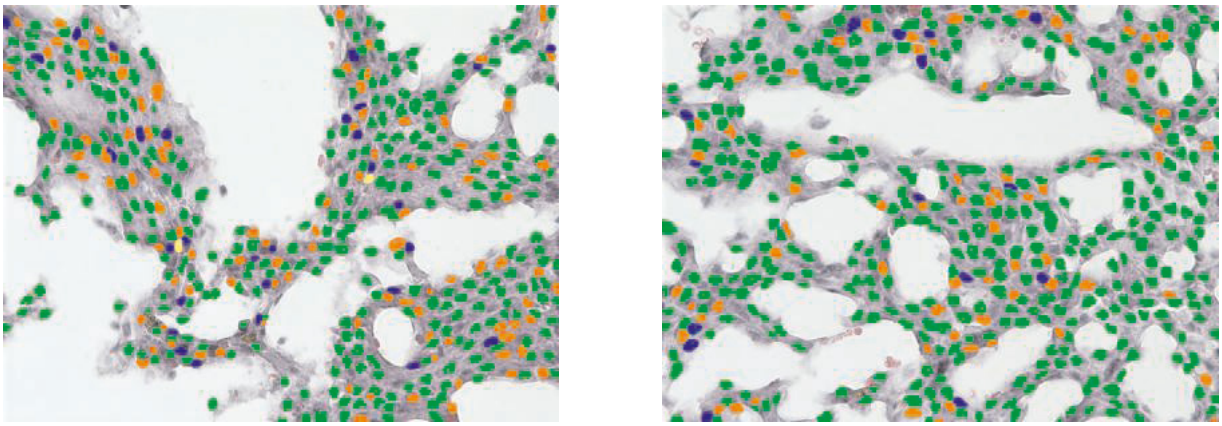
where η and ξ mean the extraction rate and the discrimination rate, F signifies F-measure respectively, TP is the number of true positives, FN is the number of false negatives, FP is the number of false positives, subscript p denotes pixel, subscript c denotes cell nuclear. TP_c is added when the extracted cell nucleus occupies more than 80% of the cell nucleus of the ground truth as seen from the ground truth, FP_c is added in other case. FN_c is added when the extracted cell nucleus is less than 80% of the ground truth as seen from the extracted cell nucleus. Table 2 shows η_p and ξ_p of input images and Table 3 shows η_c and ξ_c of them.



(a) First HE staining image (b) Second staining image

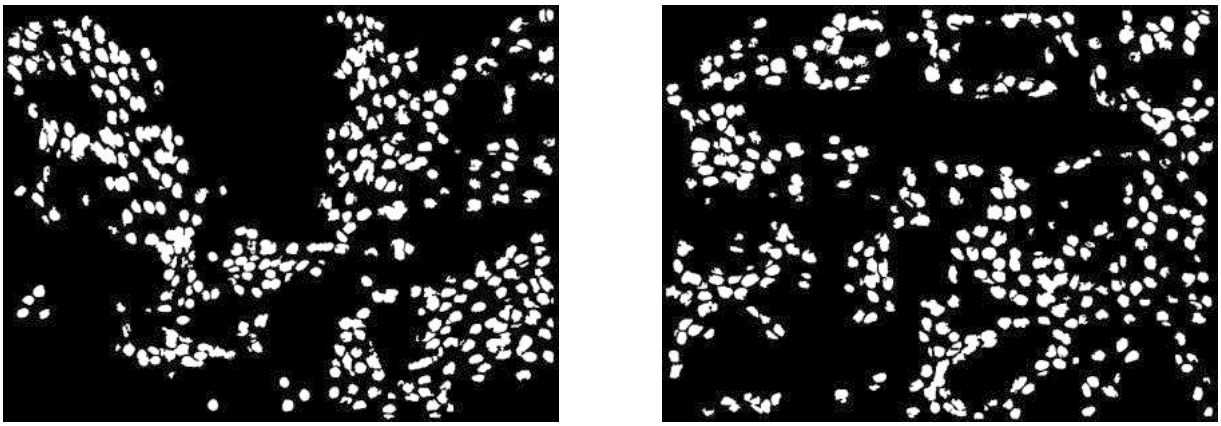
Fig. 4: Input images

The results show that a certain number of cell nuclei can be extracted. In addition looking at the Table 3, the discrimination rate is higher than the extraction rate in cell nucleus. This result shows that false detection of cell nucleus is extremely small but there are some cell nuclei that can not be extracted yet. However, there is a mixture of a plurality of cell nuclei overlapped in the extracted cell nucleus, and it can not be separated well. The reasons are the following: first, it is difficult to extract contour using difference of brightness due to superimposition of cell nuclei. Second, there is a lack of learning of classifiers such as not using images of overlapping cell nuclei at all when creating learning data sets.



(a) First HE staining image (b) Second HE staining image

Fig. 5: Ground truths



(a) First HE staining image (b) Second HE staining image

Fig. 6: Extraction result

Table 2: Evaluations of pixel

	First	Second
η_p	0.7846	0.8360
ξ_p	0.7687	0.6496
F_p	0.7766	0.7311

6. Conclution

In this paper, we extracted cell nuclei from HE stained images frequently used in actual pathological diagnosis as a preliminary step of cancer diagnostic system. It is difficult to deal with things such as color unevenness of cell nucleus alone by image processing technology alone. In order to deal with such problems, we generated a score image showing the probability of existence of cell nucleus by using SVM using feature amount feature generated from CNN. Then we tried to create a cell nucleus extracted image by combining the generated score image and the input image. As a result, it was possible to extract the cell nucleus by extracting the cell nucleus from the two kinds of HE stained images using the proposed method and obtain the extraction high precision result in the experiment.

Table 3: Evaluations of cell nucleus

	First	Second
η_c	0.7466	0.6878
ξ_c	0.8794	0.9198
F_c	0.8076	0.7673

Future tasks include improving the extraction accuracy by increasing the number of data sets, and separating the overlapping portions between the nuclei. In the cancer diagnostic program, information that is actually used for cytodiagnosis such as difference in gradient of brightness in cell nucleus, circularity, ratio of cell nucleus to cytoplasm, for example, is extracted using the information of the cell nucleus extracted this time.

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