Nuclei Detection Using Deep Learning

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***Abstract*— An important objective in the analysis of Pap smear images is automatic detection of a cell's nucleus. Various methods that automatically detect the nuclei of cervical cells have been proposed to improve the analysis of screening test images. In this paper, we propose a Convolutional Neural Networks-based method that automatically detects the nuclei of cervical cells. Following training using a public dataset provided by the Overlapping Cervical Cytology Image Segmentation Challenge - ISBI 2014, the network's fully connected layers are converted to convolutional layers to enable processing of images of any size. Our results were then compared with those achieved by other participants who successfully submitted their work to ISBI 2014 and other studies that used the same dataset. Our experimental results indicate that the methodology provides fast nuclei detection with precision and recall that are comparable with the state-of-the-art methods used to detect the nuclei of cervical cells.**

***Keywords*— Nucleus detection, Convolutional Neural Networks.**

I. Introduction

Cervical cancer is one of the most common causes of cancer death for women worldwide. It is the fourth most common cancer type among women in the world and the second in Latin America [1]. The chances of being cured increase significantly if diagnosis occurs at an early stage of the disease. Fortunately, the Papanicolaou test (also known as the Pap test), introduced by Dr. Georges Papanicolaou in the 1940s, can prevent most cervical cancers by finding abnormal cervical cell changes (pre-cancers) such that they can be treated before they have a chance to turn into cervical cancer.

The Pap test is a screening test, performed by a specialist, in which cervical cells are examined under a microscope to ascertain if abnormalities are present. However, one of the drawbacks of this method is that because of misinterpretations, inaccuracy, or inexperience, the test may produce inaccurate results that can have a significant impact on patients' treatment. To avoid this possibility, researchers have been actively developing new algorithms to automate the analysis of Pap smear images in order to achieve reliable and precise test results.

An important objective in the analysis of Pap smear images is automatic detection of a cell's nucleus. This is important because the nucleus presents significant changes when the cell is affected by cancer [10]. In the literature, many algorithms have already been proposed to automatically detect the nuclei of cervical cells. A Viterbi search-based dual active contour was used to segment the nuclei of cells by Bamford and Lovell [2]. Plissiti et al. [9] used watershed transform followed by Fuzzy C-means clustering to detect and segment cell nuclei. Watershed transform was also combined with hierarchical trees by Genctav et al. [3]. Morphological reconstruction and clustering was used by Plissiti et al. [10].

Guan et al. [19], Li et al. [21], and Saha et al. [24] proposed techniques based on clustering algorithms. In the techniques by Guan et al. [19] and Li et al. [21], first a non-linear filter is used to eliminate most dark small contaminations, and then K-means clustering is applied to perform nuclei detection. The technique proposed by Saha et al. [24] introduces a circular shape function (CSF) to impose a shape constraint over the clusters formed by the c-means clustering algorithm.

Superpixel is also a very popular nucleus detection approach. Lee and Kim [12] used superpixel and adaptive thresholding, whereas Tareef et al. [25] extracted feature vectors based on the shape, texture, and boundaries of the superpixels and trained an SVM classifier using the feature vectors to perform nucleus detection. Song et al. [13] and Tareef et al. [14] used a combination of superpixels and Convolutional Neural Network (CNN). They first used a clustering algorithm to generate the superpixels, and then used them to obtain the patches utilized to train the CNN. In their case, the learning objective was to make the CNN correctly classify the patch containing the superpixels into one of three classes: background, cytoplasm, or nucleus.

Other methods are based on algorithms such as Phansalkar's local search from low contrast images [5], and Maximally Stable Extremal Regions (MSER) [4], [6]. Lu et al. [4] used MSER in conjunction with an optimization function to minimize the energy function constrained by some known properties of the cells. Nosrati and Hamarneh [6] used MSER combined with Random Decision Forest. Further, a framework for detecting the nuclei of cells based on Markov Random Field (MRF) was proposed by Zhao et al. [11].

In many of the methods presented above, cell nucleus detection is one step in a process aimed at performing a more general automatic analysis. Therefore, a precise and reliable method to perform nuclei detection is very important. This method should also be fast, as other methods have to be used in the process to perform the cell segmentation.

In this paper, we propose a method based on CNN for detection of the nuclei of cervical cells in images with overlapped cells. In contrast to Song et al. [13] and Tareef et al. [14], the CNN is trained from patches extracted directly from the training images, in which the central pixel of each training patch belongs to one of three classes: background, cytoplasm, or nucleus. This is done with the aim of making the network learn how to correctly classify the central pixel of the patch.

The remainder of this paper is organized as follows. Section II describes the materials and methodology applied in the experiments. Section III presents and discusses the experimental results obtained. Section IV concludes this paper.

(a) (b) (c) (d)

Fig. 1. Example of the images available in the dataset: (a) original image; (b) nuclei annotation; (c) annotation of the first cytoplasm; (d) annotation of the second cytoplasm.

II. Materials and Method

A. Dataset

In 2014, the International Symposium on Biomedical Imaging took place in Beijing, China. One of the events held in this Symposium was the Overlapping Cervical Cytology Image Segmentation Challenge (ISBI 2014), in which the goals were to perform automatic nuclei detection, and automatic extraction of the boundaries of individual cytoplasm from overlapping cervical cytology images. ISBI 2014 provided a public dataset from which it is possible to download the images and their respective annotations. This dataset is described in [4], [7], and [8].

The dataset consists of 16 Extended Depth Field real cervical cytology images and 945 synthetic images. The 945 synthetic images are divided as follows:

- 45 images for training
- 90 images for testing
- 810 images for evaluation.

All 45 training images and the 90 testing images are annotated. The annotation consists of the ground truth for nuclei, individual cytoplasm, the number of cells in each image, and the cytoplasm overlap ratio. The 810 remaining images can be used by the evaluation code to measure cytoplasm segmentation. The 16 real images are also annotated; however, in this case, only the ground truths for nuclei segmentation are available. A script to generate more synthetic images is also available. An example of the images comprising the dataset is given in Figure 1.

As this dataset is used in several of the studies published in the literature [4], [5], [6], [14], [24], [25], comparison of study results is relatively easy.

B. Methodology

A CNN [17] is a type of feed-forward neural network that is based on the structure of the visual cortex in animals. CNNs are highly suited to work with image processing and have been applied to solve various problems related to image processing. They have also been used with success to analyze medical images in applications such as neuronal segmentation [15], mitosis detection [16], and skull extraction [20].

We used a lightweight library called Lasagne to construct the CNN. Lasagne is written in Python and is used to build and train neural networks in Theano. We trained the network using an approach similar to that employed by Ciresan et al. [15]. In the approach, square patches with size 95×95 pixels are extracted from the training image in which their central pixel belongs to one of the target classes. The training objective is to make the network learn how to classify this central pixel. In case a pixel is located near the image border, the image is mirrored to facilitate extraction of the training patch.

The network is trained to classify each pixel as belonging to one of the following classes: nucleus, cytoplasm, or background. The network is trained with three classes instead of only two–nucleus and non-nucleus–for two reasons. First, because the number of available samples with nuclei is much less than the number of non-nuclei samples, a three-class approach is used to somewhat balance the number of samples. Second, both cytoplasm and background information are important to differentiate nuclei area from those dark areas of overlapped cytoplasm that generally occur near cytoplasm borders.

All pixels belonging to nuclei present in the training set are used. Further, the same number of background pixels is randomly selected because the background is the easiest region to correctly classify. Then, it is not necessary to retrieve more samples from it. In the case of cytoplasm samples, we calculated the frequency of the pixel levels and chose those in the 60th percentile because we noticed that the major problem was to differentiate nuclei from darker parts of the cytoplasm. After some experimentation, the 60th percentile was chosen because it provided a good compromise between acquisition of meaningful cytoplasm samples and class imbalance. It was observed that retrieving more samples, by increasing the percentile, did not produce better results. The final training set was still slightly imbalanced as there were more cytoplasm samples then nucleus and background samples. However, this imbalance was not large enough to affect the final result.

We applied Rectified Linear Units [23] to all convolutional and fully connected layers. In the beginning, the network started training with a learning rate of 0.005 and momentum of 0.9. The learning rate subsequently decreased and the momentum increased after every 10 epochs. In order to avoid overfitting, a dropout of 0.5 [18] was applied to all fully connected layers. In addition, an early stopping approach, in which the training was terminated if the validation loss had not improved following five consecutive epochs, was used. The architecture of the network used in this study is shown in Figure 2.

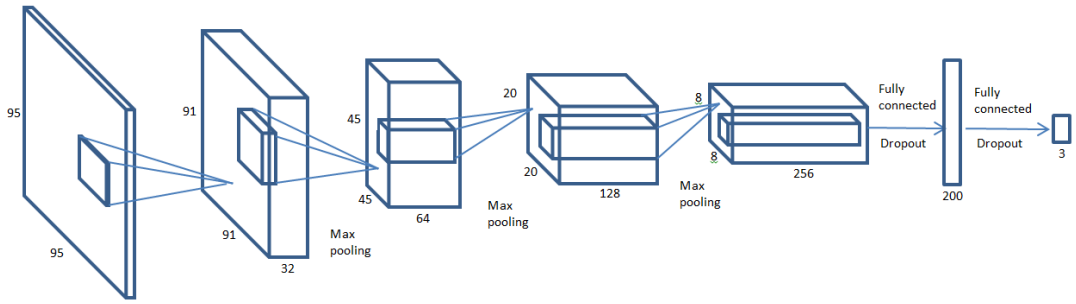
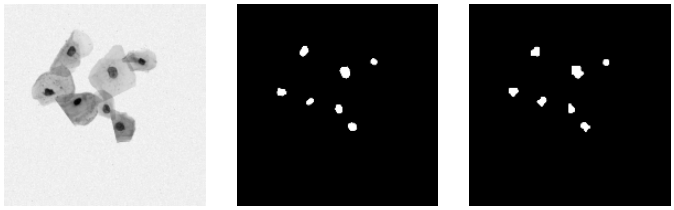


Fig. 2. Architecture of the network used for training.



(a) (b) (c)

Fig. 3. Test image, its ground truth and method result: (a) original image; (b) nuclei annotation; (c) result achieved by the method.

C. Method evaluation

Once the network was trained, all fully connected layers were changed to convolutional layers [22]. This modification made it possible to use images of any size as network input. This also resulted in test results being produced at a faster pace as the whole test image could be processed at once instead of having to utilize a sliding window approach. The result is a pixel density map whose size is smaller than the input image. As we are interested in object-level detection, we simply upscaled the density map using linear interpolation to have the network's output the same size as the testing image in order to assess the metrics. The loss in precision is compensated by results being produced quickly and a network that is much easier to train.

We used the nuclei detection metric proposed by Genctav et al. [3] as our evaluation metric. The precision and recall of nuclei detection is computed by considering the detection region A and (ground truth) annotation B, in which a correct detection is represented as follows:

$$\frac{(A \cap B)}{A} > 0.6 \; and \; \frac{A \cap B}{B} > 0.6 \tag{1}$$

Then, precision and recall are calculated as follows:

$$precision = \frac{\#\ of\ correctly\ detected\ objects}{\#\ of\ all\ detected\ objects} \tag{2}$$

$$recall = \frac{\#\ of\ correctly\ detected\ objects}{\#\ of\ all\ objects\ in\ the\ ground\ truth} \tag{3}$$

III. Results

To evaluate our method, we measured the precision and recall for nuclei-object detection as [3]. Table I compares our results to some state-of-the-art methods found in the literature. Fair comparison is guaranteed because all the studies used the same dataset. From the table, it is clear that our method achieved significant results. The precision is equivalent to that of the other methods and the recall achieved is superior. This is especially interesting considering that there is an error associated with the resizing of the image and the metrics are based on having a 60% match of the areas of the ground truth and obtained result for each nucleus.

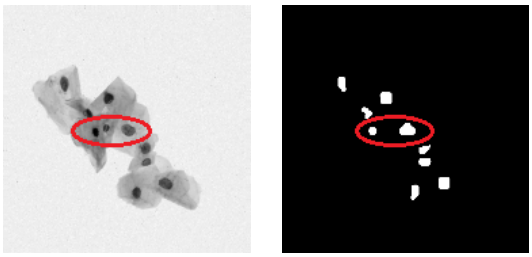
TABLE I
Object-level results for nuclei detection

Method	Precision	Recall
Lu et al. [4]	0.977	0.883
Ushizima et al. [5]	0.959	0.895
Nosrati et al. [6]	0.903	0.893
Saha et al. [24]	0.918	0.915
Tareef et al. [25]	0.99	0.94
Tareef et al. [14]	0.994	0.911
Our method	0.929	0.917

Figure 3 shows a test image, its nuclei annotation, and the output of our method. In this figure, it can be seen that all nuclei were correctly detected and, although we carried out linear interpolation to make the output the same size as the test image, the shape, size, and position of all nuclei are very similar to those of the annotation image.

The average running time on the synthetic dataset by the method by Ushizima et al. [5] is approximately 2 s per cell segmentation using an un-optimized Fiji script on a Cray XC30 supercomputer with a 12-core Intel "Ivy Bridge" processor at 2.4 GHz and 64 GB RAM. In the case of Nosrati et al. [6], the proposed method segments each cell in approximately 4 s using un-optimized MATLAB code running on a 3.4 GHz CPU with 16 GB RAM. In [4], the running time is approximately 50 s per cell segmentation using un-optimized MATLAB code on a PC with a 2.66 GHz Intel Core 2 Duo processor and 8 GB RAM. In the method proposed by Tareef et al. [25], the average time per cell for nuclei segmentation was 18.25 s using non-optimized MATLAB code on a PC with a 3.2 GHz Intel Core i5 processor and 8 GB RAM.

We ran all 90 test images in the network described in



(a) (b)

Fig. 4. Example of a result with a missing nucleus: (a) original image; (b) network output.

(a) (b) (c)

Fig. 5. Example of a detected nucleus that does not meet the conditions in Equation 1 (a) original image; (b) nuclei annotation; (c) network output.

Section II-A in a total execution time of 335.16 s, with average execution time per image of 3.72 s using a PC with an Intel i7-3632QM 2.20 GHz CPU with 8 GB of RAM. The execution time per image was not affected by the number of cells present in the image. This suggests that it is possible to use the proposed method for nuclei detection in the methods above without much impact, if any, on their overall execution time.

There are two situations in which some improvement can be achieved. In the first situation, if there are two or more nuclei very close to each other, the network may not detect one of the nuclei. This situation is depicted in Figure 4. It is clear that in the central part of the result image–marked with a red circle–one nucleus is missing. The second case occurs when a nucleus is detected but it does not achieve the condition established in Equation 1. This situation is shown in Figure 5–the problem is highlighted by the yellow circles.

IV. Conclusions

Nuclei detection is an important step towards segmentation of cells in an image. Over the years, many methods have been developed that rely on nuclei detection to perform segmentation. Therefore, a fast, accurate, and reliable method to perform nuclei detection is very desirable. In this study, we developed a method that detects nuclei in overlapped cells using the convolutional neural network deep learning technique. We showed that once the convolutional neural network is trained, with a simple modification a fast and accurate method to perform nuclei detection is obtained that achieves results comparable with those achieved by the state-of-the-art methods. The proposed method processes images very quickly and, in the future, it may be used as an intermediate step in methods aimed at cellular structure segmentation and analysis.

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