Neural network based segmentation of cell nuclei and lymphocyte detection in whole slide histology images

Elzbieta Budginaitė

Institute of Data Science and Digital Technologies, Vilnius University, Akademijos str. 4, LT-08663 Vilnius, Lithuania

E-mail: elzbieta.budginaite@mf.stud.vu.lt

Abstract. Visual examination of cancer tissue is regarded as the golden standard diagnostic method for experienced pathologist. Assessment of cell morphology and tissue distribution are the main diagnostic criteria for cancer staging and prognosis, with tumor immune infiltrate being one of the main diagnostic features. Such manual approach, however, lacks objective quantitative characteristics and thus is susceptible to inter-observer variability. This raises a demand for computer-aided diagnosis tools. In this article we propose a convolutional neural network-based algorithm for automated cell nuclei segmentation and lymphocyte identification in H&E stained colorectal and breast cancer tissues.

Keywords: digital pathology, deep learning, convolutional neural networks, whole slide images, cancer

1 Introduction

The emergence of whole slide imaging (WSI) enabled vast digitalization of routine histopathology. The aim of WSI technique is to transform physical glass slides into digital form, thus superseding regular microscopy analysis in multiple aspects: automatized slide scanning enables high-throughput, high-resolution workflow and superior precision, WSI allows convenient sample archiving as well as possibility for remote tissue sample analysis [1], [2]. Recent FDA approval for WSI usage in clinical practice further accelerates clinical implementation of WSI technology in daily pathologist`s practice [3]. Due to rapid accumulation of WSI data resources and large scale of digital whole slide, manual annotation of the tissue becomes especially time-consuming task for pathology experts. With the lack of universal standardized threshold values, tissue annotations may suffer from insufficient reproducibility, high intra- and inter- observer variability. Therefore, automatized tissue pattern recognition in whole slides is of paramount importance.

Cell counting by cell type is especially meticulous tissue analysis task, which is merely applicable for whole slide images without automated workflow. Quantification of immune infiltrate by counting tumor-infiltrating lymphocytes (TILs) along tumor margins in tumor microenvironment has gathered researchers` attention as a reliable prognostic measure for colorectal and breast cancer outcome [4], [5]. Usual quantification of TILs include manual counting of lymphocytes through microscope in H&E stained tissue slide considering the total count or density per square area unit as the final TIL density measurement. Manual TIL counting is difficult to scale, time-inefficient and prone to subjectivity, thus could be replaced with a standardized automated lymphocyte detection workflow.

Due to superior performance in computer vision tasks, deep learning-based algorithms have predominated computer-aided diagnosis area over recent years. Convolutional neural networks have proven to outperform previously-state-of-the-art medical imaging techniques. Encoder-decoder based model architectures have been successfully applied in various medical image segmentation tasks such as nuclei segmentation, rendering the skip connections approach proposed in U-Net model by Ronneberger et al [6]. The deep, semantic feature maps from U-Net decoder are combined with shallow, low-level feature maps from encoder part of the model via skip connections, thus maintaining the fine-grained features of the input image – this renders U-Net applicable in medical image segmentation, where precise detail recreation is of utmost importance. The more recent adaptation - Micro-Net model - incorporates additional input image downsampling layer that circumvents max-

pooling process, thus maintaining the input features ignored by max-pooling layer. This way, more detailed contextual information is passed into the output layer, thus enabling better segmentation of adjacent cell nuclei [7]. This paper describes convolutional neural network-based algorithm for nuclei segmentation, which uses active contour layer for robust nuclei separation, and subsequent cell nucleus classifier for lymphocyte identification in H&E stained 20x magnification whole slide breast and colorectal cancer images.

2 Methods

2.1 Dataset

4 diagnostic H&E stained slides (2 breast, 2 colorectal cancer) were selected for the study. Slides were produced in National Center of Pathology, Lithuania and digitised with the Aperio ScanScope XT Slide Scanner (Aperio Technologies, Vista, CA, USA) using a x20 objective. Random 344 tiles of 256x256 pixels were generated and cell nuclei were annotated. 2 pixel-wide active contour borders surrounding each nucleus were added as a second layer to the nuclei segmentation masks. Dataset was split into 274 and 70 tiles for training and validation sets respectively. We applied robust image augmentation (rotation, flip, transpose, RGB augmentation, brightness adjustment, CLAHE) to obtain the final training set of 5206 images. Segmentation model was tested on 72 patches at 20x magnification extracted from breast cancer diagnostic slide obtained form open-access TCGA database (tile ID: TCGA_AN_A0AM) [8]. Cell classification model was trained on 11.032 lymphocytes and 10.922 other cell nuclei extracted from training set. Testing set of 1003 lymphocytes and 1185 other cell nuclei was prepared from the testing set.

2.2 Deep convolutional neural network model

The autoencoder architecture for nuclei segmentation is shown in Figure 1A. The model consists of 3 encoder and 3 decoder blocks consisting of 2 convolution layers, dropout (dropout rate 0.2) and max-pooling layers. Additionally, input image is downsampled, followed by 2 parallel blocks of 3 convolution layers, and added to the model after each max-pooling operation. We used *elu* activation after each convolution layer and *sigmoid* activation for the output layer. *Adam* optimizer was used with initial learning rate $lr = 0.001$, which was reduced by factor 0.1 if validation loss did not improve for 4 consecutive epochs (min $lr = 10^{-6}$). Dice coefficient was used to quantify model metrics with binary crossentropy dice loss as custom loss function.

$$Dice = \frac{2\,x\,TP}{(TP + FP) + (TP + FP)}$$

$$Crossentropy\ dice\ loss = 0.1\,x\ binary\ crossentropy + 0.9\,x\,(1 - Dice)$$

Nuclei classification model architecture is shown in Figure 1B. Model consists of 4 convolution layers, 2 max-pooling and 2 dense layers, where *relu* activation and batch normalization was used after each layer. *Softmax* activation was used for the output layer. We used *Adam* optimizer with initial learning rate $lr = 0.0001$, which was reduced by factor 0.1 if validation loss did not improve for 6 consecutive epochs (min $lr = 10^{-6}$). Accuracy was used as metrics with *binary crossentropy* as loss function. Models were trained on GeForce GTX 1050 GPU, 16 Gb RAM using Tensorflow machine learning library [9].

3 Results

We evaluated the performance of nuclei segmentation autoencoder and nuclei classifier CNN, metrics are provided in Table 1. Cell nuclei segmentation autoencoder achieved Dice coefficient of

0.85 and 0.82 for training and testing datasets respectively. Cell nuclei classifier reached 0.976 testing accuracy with precision, recall, F1 score of 0.968, 0.977, 0.972 respectively.

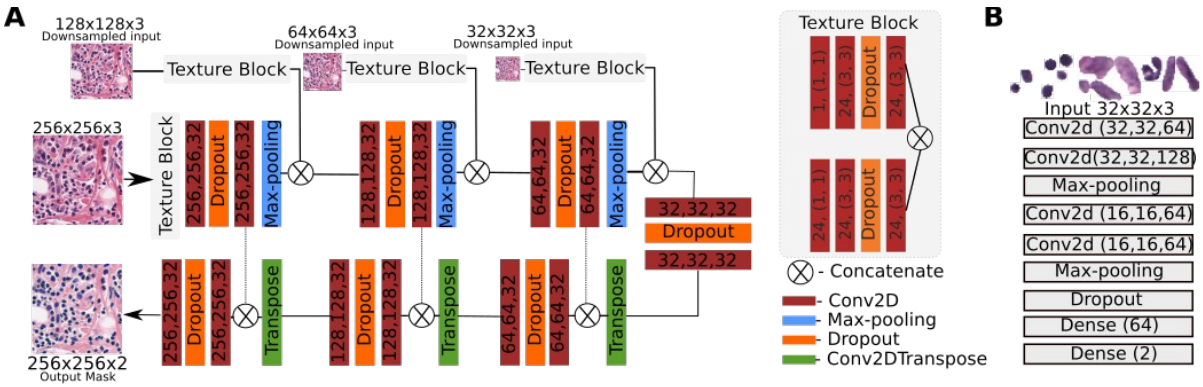

Figure 1: Architectures of convolutional neural networks. A - cell nuclei segmentation model. The model outputs 2-layered mask (nuclei - blue, active contour borders - green). Model output is overlaid on original image. B – cell nuclei classification model. Model input is cell nucleus cropped from original image along the segmentation mask contours. Nucleus is scaled to fit 32x32 pixel dimensions. Classifier is trained to detect lymphocytes from all segmented cell nuclei.

The confusion matrix for our cell classification model demonstrates that out of 1003 labeled lymphocytes only 20 were misclassified as false negative while 29 false positive observations were registered out of 1185 nuclei labeled as other cell types as shown in Figure 2A. Receiver-operating curve (ROC) shown in Figure 2B indicates low false positive rate of our lymphocyte classifier. Figure 2C represents testing output results of our model.

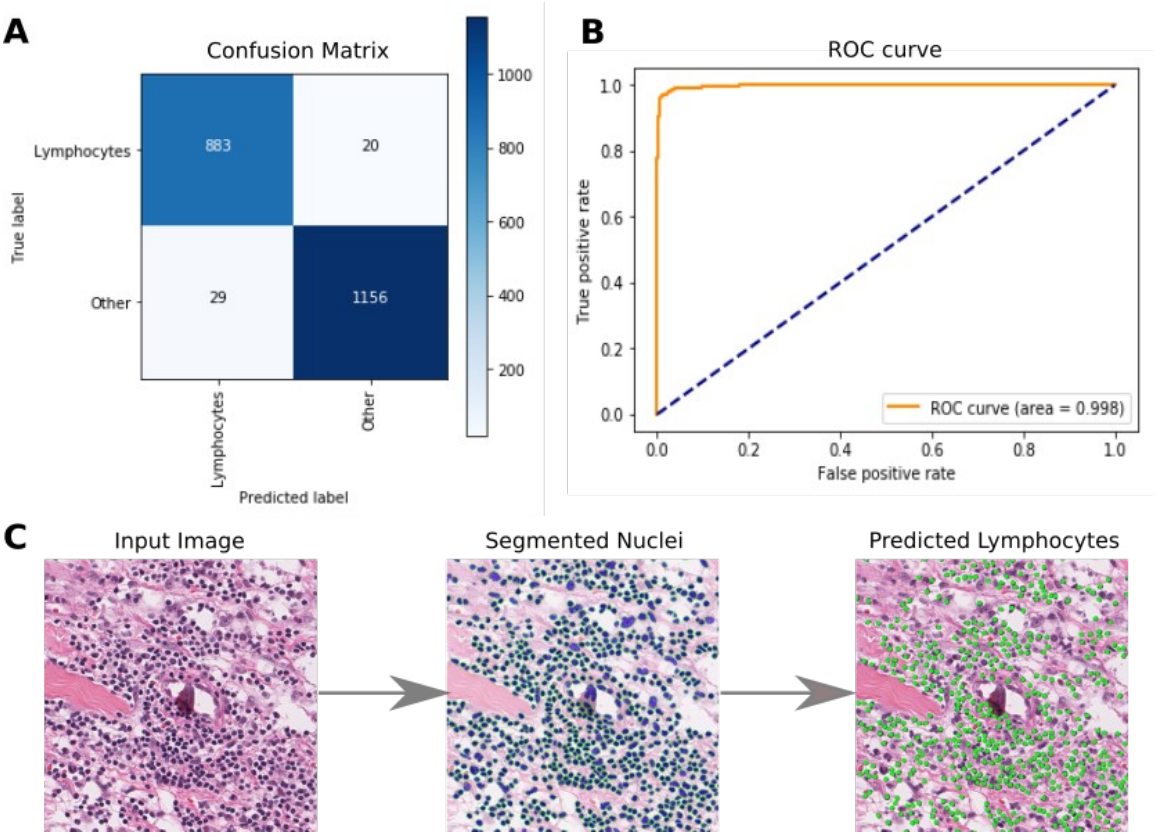

Figure 2:*Evaluation of cell nuclei classifier. A – confusion matrix of testing cell nuclei dataset, B – ROC curve*

depicting model performance metrics, C – predicted cell nuclei and lymphocytes overlaid on original input image.

Table 1: Performance metrics of deep neural network model. Segmentation performance statistics were obtained for nuclei segmentation autoencoder, classification performance metrics were acquired form cell nuclei classifier.

	Segmentation performance	Classification performance			
	Dice	Accuracy	F1 Score	Precision	Recall
Training	0.85	0.948	0.946	0.952	0.941
Testing	0.82	0.976	0.972	0.968	0.977

4 Conclusions

In this paper we propose an end-to-end deep learning-based algorithm for cell nuclei segmentation and consecutive lymphocyte identification in H&E stained 20x magnified breast and colorectal cancer whole slide images. Due to active contour layer, our cell nuclei segmentation model successfully separates adjacent nuclei, which is a common challenge in whole slide image analysis. Nuclei segmentation output mask is consecutively used to extract detected nuclei by predicted contours, which are fed into deep neural network for lymphocyte detection. Our proposed model detects lymphocytes with 0.976 accuracy and is a promising tool for future studies.

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