Nuclei Segmentation with Recurrent Residual Convolutional Neural Networks based U-Net (R2U-Net)

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***Abstract*—Bio-medical image segmentation is one of the promising sectors where nuclei segmentation from high-resolution histopathological images enables extraction of very high-quality features for nuclear morphometrics and other analysis metrics in the field of digital pathology. The traditional methods including Otsu thresholding and watershed methods do not work properly in different challenging cases. However, Deep Learning (DL) based approaches are showing tremendous success in different modalities of bio-medical imaging including computation pathology. Recently, the Recurrent Residual U-Net (R2U-Net) has been proposed, which has shown state-of-the-art (SOTA) performance in different modalities (retinal blood vessel, skin cancer, and lung segmentation) in medical image segmentation. However, in this implementation, the R2U-Net is applied to nuclei segmentation for the first time on a publicly available dataset that was collected from the Data Science Bowl Grand Challenge in 2018. The R2U-Net shows around 92.15% segmentation accuracy in terms of the Dice Coefficient (DC) during the testing phase. In addition, the qualitative results show accurate segmentation, which clearly demonstrates the robustness of the R2U-Net model for the nuclei segmentation task.**

Keywords— Deep Learning, Convolutional Neural Networks; U-Net, R2U-Net, Nuclei Segmentation.

I. INTRODUCTION

People around the world are suffering from a multitude of different diseases including cancer, heart disease, chronic illness, brain related diseases such as tumors and Alzheimer's, and more common ailments such as diabetes. A recent study states that the well-known company "Pfizer" is going to stop working on new drugs to fight Alzheimer's disease and Parkinson's disease due to the prohibitive time and monetary expense required. Also according to this study, there exists a 10-year delay between the development of a new drug and the time it takes to bring it to market [1]. Medical imaging speeds up the assessment process of almost every disease from lung cancer to heart disease. The automatic nuclei segmentation and detection algorithms can help to unlock a cure faster for critical diseases like cancer, as well as the common cold. Identification of the cell's nuclei is the starting point for analysis, and there exists about 30 trillion cells each containing a nucleus full of DNA within the human body. Identifying a cell accurately can help researchers to observe how to react to a cell with respect to different treatments. As a result, researchers can understand the underlying biological process at work. This solution can help to ensure better treatment of patients, and it can accelerate both the treatment and drug discovery processes. Therefore, the computational pathology [2] and microscopy images play a big role in decision making for disease diagnosis, since these images able to provide a large amount of information for computer-aided diagnosis (CAD). This enables quantitative and qualitative analysis of these images with a very high throughput rate. Nowadays, computational pathology is becoming very popular in the field of medical imaging, and it has the potential to greatly benefit pathologists and patients. Therefore, this field has received significant attention from both the research community and clinical practice [3,4].

These computation approaches are able to provide faster and more efficient image analysis compared to alternative manual systems used by researchers and clinical scientists. These new methods can release pathologists from difficult and repeated routine efforts [5]. Since computational pathology and microscopic imaging is very challenging when performed manually, it may lead to large inter-observer variations [6]. CAD reduces experimenter bias significantly and provides accurate characterization of diseases [7]. Additionally, computational pathology provides reproducible and rigorous measurement of pathological image features that can be used for clinical follow up. These methods also help the study of personalized medicine and treatment, which significantly benefits patients. A prerequisite for clinical practice of CAD is nuclei classification, segmentation, and detection. These methods are considered to fall within the realm of basic annotated image analysis. These techniques provide different quantitative analyses including cellular morphology for features such as size, shape, color, texture. However, it is very difficult to achieve robust and accurate performance for these different tasks with pathological images for several reasons. First, the pathological and microscopy images contain background clutter including noise, artifacts (images are blurred sometimes), low signal to noise ratio (SNR), and poor depth resolution. This image degradation is usually due to the devices used for image acquisition. In addition, there is low contrast between the foreground and the background in the images. Second, there exists significant variation in the size, shape, and intercellular intensity between different nuclei and

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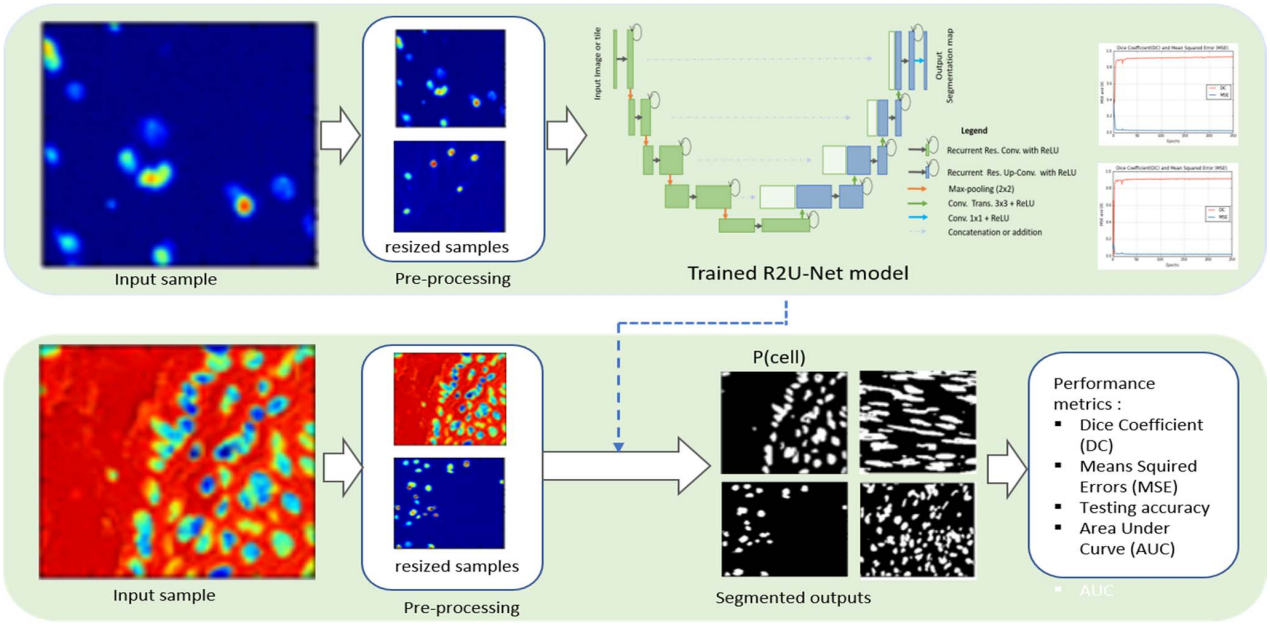

Fig. 1. Overall implementation diagram for nuclei segmentation. The upper part of the figure shows the training phase along with the R2U-Net model and lower part shows the steps related to the testing phase.

cells. Third, it can be observed very often that the nuclei or cells are partially overlapped with one another. There are several methods that have been proposed to tackle these issues with automatic nuclei/cell classification, segmentation, and detection for pathological images [8,11].

However, along with traditional image processing and computer vision-based approaches, there have been several surveys on deep learning-based approaches for pathological image analysis. Due to the available annotated data and large computing power, the DL approaches based on the Convolutional Neural Network (CNN) are providing state of the art accuracy on different computer vision problems [1]. A recent study shows that the DL based methods show great success in different modalities of the medical imaging domain including lung segmentation, diabetic identification, retina blood vessel segmentation, brain tumor detection, and many more [11].

To generalize the proposed R2U-Net model, it will be applied to nuclei segmentation in this work. The entire process diagram for nuclei segmentation with R2U-Net is shown in Fig. 1, and the contributions of this paper can be summarized as follows:

- This is the first time the R2U-Net has been applied to nuclei segmentation on a publicly available dataset, which was acquired from the Data Science Bowl Grand Challenge in 2018.
- The experimental results show promising quantitative and qualitative results in both the training and testing phases.
- The R2U-Net model shows approximately 92.15% segmentation accuracy in terms of the DC.

The rest of the paper has been organized in the following way: Section II explains related works and Section III presents the architecture of the R2U-Net model. The database, results, and discussions are provided in Section IV. Conclusions and future directions are presented in Section V.

II. Related Works

Automatic nuclei classification, segmentation, and detection is a prerequisite for various quantitative and qualitative analysis including morphological feature computation for different diseases including breast cancer and drug development. In the last few years, several different DCNN approaches have been proposed and successfully applied to medical image analysis problems. They show superior performance on different benchmark datasets for classification, segmentation, and detection tasks [1]. First, in classification tasks, the target is to identify the class probability of the input samples. For example, in the binary breast cancer recognition problem, the system defines a classification where the input sample is in the category of benign or malignant. Second, most segmentation techniques used for deep learning describe the process of associating each pixel of an image with a class label. Another objective of this task is to define the proper contour of an object. Third, in DCNN based detection tasks, the object is to identify the central coordinate of a certain object. Defining the bounding box of an object is also one of the goals of this task. For example, one goal could be to identify the center pixel coordinate of a nucleus. Several research groups are looking into the complex nature of pathological images. However, in this paper, the nuclei segmentation method is proposed using the R2U-Net.

In the last few years, several surveys have been conducted on different methods of CAD technologies in the field of digital biomedical imaging (including pathology) [8]. These reviews briefly discuss techniques related to pre-processing, nuclei classification, segmentation, detection, and post-processing.

One of these papers discusses several techniques related to data acquisition and ground truth generation, image analysis, recognition, detection, segmentation, and statistics relating to survival analysis [9]. Another review has been conducted on Whole Slide Imaging (WSI) including approaches related to feature extraction, predictive modeling, and visualization [10]. A survey conducted on nuclei detection, segmentation, and classification discusses performance on hematoxylin and eosin (H&E), and immunohistochemistry (IHC) stained histopathology images [11].

A novel contour based "minimum-model" cell detection and segmentation approach was proposed in 2012. This approach uses minimal a priori information and detects contours independent of their shape [12]. Nuclei membrane segmentation using CNNs was proposed for microscopic images [13]. Ronneberger et al. [14] proposed a CNN based approach called U-Net for general medical image segmentation. In addition, this model has been applied to a range of segmentation problems including nuclei segmentation. A learning-based framework for robust and automatic nuclei segmentation with shape preservation was also presented using pathological images as input data. The CNN model generates probability heat maps, on which an iterative region merging technique is applied for shape identification. In addition, a novel segmentation approach is applied to separated individual nuclei, combining a robust selection-based sparse shape model and a local repulsive deformable model, which have been tested in several scenarios for pathological image segmentation. They showed state-of-the-art performance against existing approaches in 2016 [15].

A very simple CNN based nuclei segmentation approach was proposed in 2017. In this work the CNN2 and CNN3 models are applied to two and three output classification structures respectively. For the two-class model, the network is used to classify a pixel as either inside or outside of a nucleus. On the other hand, the model with three classes is used to classify pixels as belonging to the inside, outside, or boundary of the nuclei [16]. In 2017, D.J. Ho et al. proposed a fully 3D nuclei segmentation method using a three-dimensional convolutional neural network [17]. A deep learning-based one-step contour aware nuclei segmentation approach was proposed for high resolution pathological images. In this implementation, the fully convolutional neural network (FCNN) is applied to segment the nuclei and the corresponding boundaries simultaneously [18]. A 3D Convolutional Network is used for joining cell nuclei detection and segmentation in microscopic images simultaneously. This method is tested on two different datasets and achieved the SOTA in detection and segmentation tasks [19].

For general medical image segmentation, an improved version of the U-Net deep learning model was proposed in 2018 where recurrent residual modules were used instead of feedforward convolutional layers, and this network was named R2U-Net. This new model was tested on different modalities of medical imaging tasks including retina blood vessel segmentation, skin cancer segmentation, and lung segmentation. The experimental results were compared against U-Net, and SegNet and show superior testing performance. This advanced and new hybrid deep learning model generalizes U-Net, residual, and recurrent convolutional neural networks [20]. However, we used the R2U-Net model for end-to-end nuclei segmentation in this implementation.

III. Recurrent Residual U-Net (R2U-Net)

The entire R2U-Net model is provided in the upper part of the Fig. 1. This model consists of two main units which are encoding units (shown in green) and decoding units (shown in blue). In both units, the recurrent residual convolutional operations are performed in each convolutional block in the encoding and decoding units. The conceptual diagram of the recurrent residual unit is shown in Fig. 2 (a). The recurrent operation is performed with respect to different time steps, which is shown in Fig. 2 (b). For the recurrent convolutional unit, $t = 2$, which means one general convolution layer and two recurrent layers are used in this convolutional unit. The feature maps from the encoding unit are concatenated with the feature maps from decoding units. For further details about R2U-Net, please see [20]. The softmax layer is used at the end of the model to calculate class probability. The model details and number of feature maps for this implementation are shown in Table I.

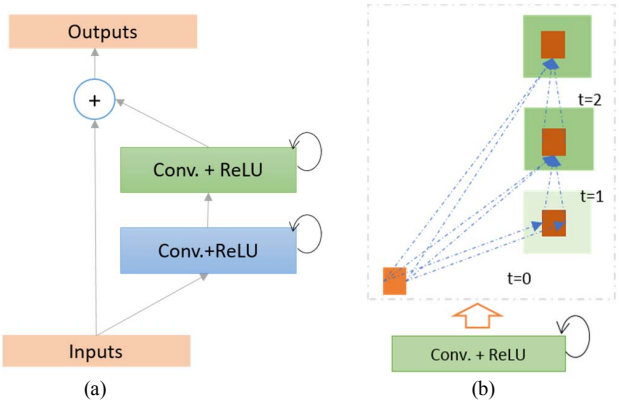

Fig. 2. Diagrams displaying (a) the recurrent residual convolutional unit and (b) the unfolded version of the recurrent convolutional unit.

IV. Results and Discussion

To demonstrate the performance of the Inception Recurrent Residual Convolutional Neural Network (IRRCNN) models [21], we have tested them on the nuclei segmentation and detection dataset from the 2018 Data Science Bowl Grand Challenge [23]. A discussion of this dataset is provided in this section. For this implementation, the Keras [24] and TensorFlow [25] frameworks were used on a single GPU machine with 56G of RAM and an NIVIDIA GEFORCE GTX-980 Ti.

A. Dataset

This work utilizes the dataset from the 2018 Data Science Bowl Grand Challenges [23], which contains 735 images in total. Of the total image set, 650 images contain pixel-level annotation for training and the remaining 65 samples are unlabeled to be used in testing. From the training set, 80% of the samples are used for training and remaining 20% are used for validation. The numbers of training and validation samples are 536 and 134 respectively. A selection of samples from the

training set are shown in Fig. 3, and Fig. 4 shows the normalized training samples. In the first row of images in Fig. 4, sample heat maps are shown, and in the second row of images red represents the nuclei region and blue represents the non-nuclei region.

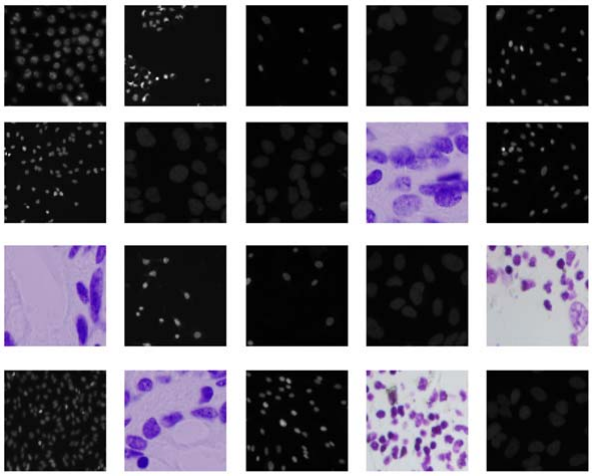

Fig. 3. Example samples from Nuclei Segmentation database.

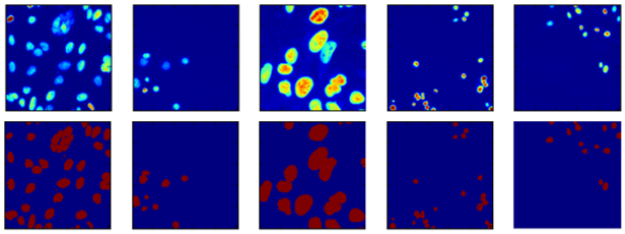

Fig. 4. Training image in the first row and second row shows the label images.

B. Evaluation Metrics

In the presented implementation, we considered the Dice Coefficient (DC) and Mean Squared Error (MSE) for observing training progress and performance in the training and testing phases. The DC is expressed in equation (1), where GT refers to the ground truth and SR refers the segmentation result.

$$DC = 2\,\frac{|GT \cap SR|}{|GT| + |SR|} \tag{1}$$

Another metric used to evaluate the performance of the segmentation algorithm is the MSE as defined in equation (2). In this case Y is represents desired outputs and $\hat{Y}$ represents predicted output. For an input sample with height h and width w and $n=h\times w$.

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2 \tag{2}$$

C. Results

We considered 250 epochs and used the Adam optimizer with a learning rate of 2×10^{-4}. Fig. 5 shows the DC and MSE with respect to the number of epochs during training. The validation DC and MSE are shown in Fig. 6.

From Figs. 5 and 6, it can be observed that the model converged after 100 epochs, the training and evaluation continued until 250 epochs were completed to ensure optimum convergence. From this experiment, we achieved approximately 92.15% testing accuracy for nuclei segmentation with the R2U-Net model.

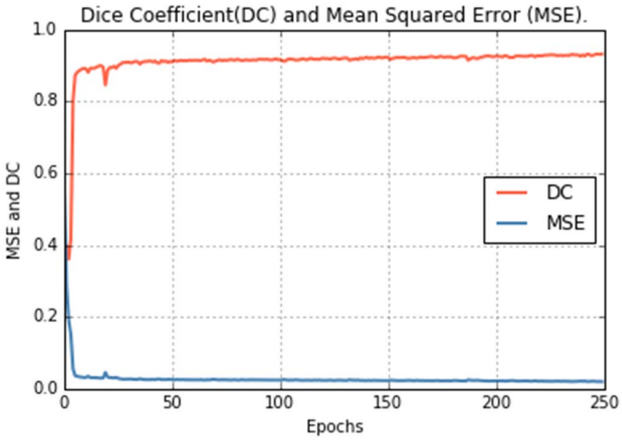

Fig. 5. Training accuracy in term of the Dice Coefficient (DC) and Mean Squared Error (MSE) for 250 epochs.

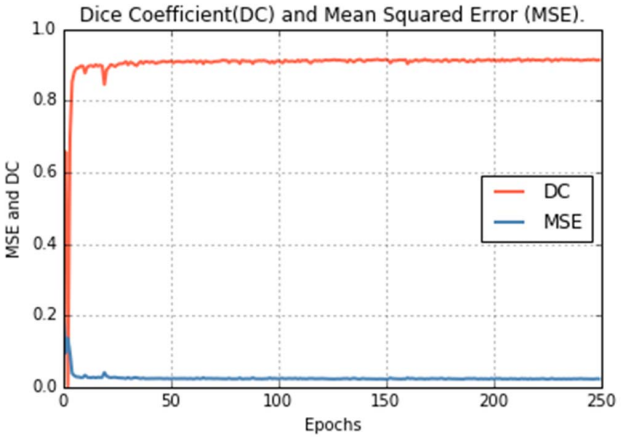

Fig. 6. Validation accuracy in terms of the Dice Coefficient (DC) and Mean Squared Error (MSE) for 250 epochs.

D. Analysis

Fig. 7 shows some classification samples when using the R2U-Net model for nuclei segmentation where the first, third, and fifth rows show the input samples. Likewise, the second, forth, and sixth rows represent the corresponding network outputs. Based on these results, our proposed segmentation model provides promising segmentation outputs during testing phase. In addition, if we observe the input samples in the second and fourth columns of the first row, there is strong separation between nuclei and similar objects of less interest. Similar behavior is displayed in the input sample in the fourth row of the fourth column.

The input shown in the fifth row of the third column contains a complex background. However, in all cases the results show similar segmentation outputs with respect to the desired outputs. This clearly demonstrates the robustness of R2U-Net for nuclei segmentation from pathological images. In this implementation, we used a simple R2U-Net model where

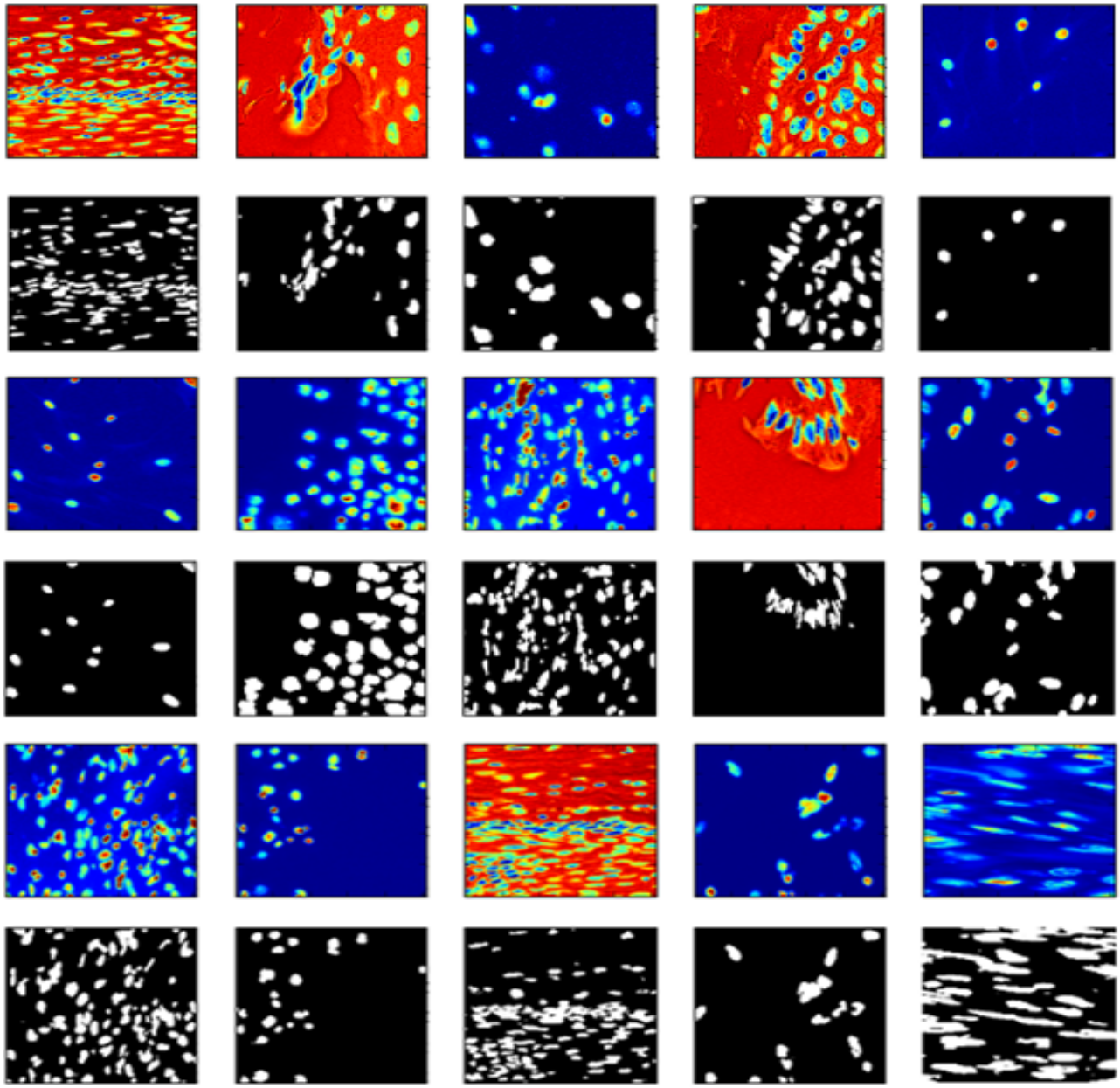

Fig. 7. Qualitative experimental outputs of the R2U-Net during the testing phase. The first, third, and fifth rows show the testing inputs, and the second, fourth, and sixth rows show the output images.

only 0.84 million network parameters were utilized. The network architecture along with the number of parameters is shown in Table I.

Table I. The R2U-Net architecture with different RCLs along with the number of parameters.

t	Network architectures	Number of Parameters (Millions)
2	1→16→32→64→128→64→32→16→1	0.845

V. Conclusion and Future Work

In this paper, we demonstrated the application to the R2U-Net model for nuclei segmentation. We evaluated the R2U-net model on the 2018 Data Science Bowl Grand Challenge dataset and achieved a promising accuracy of approximately 92.15% in terms of the Dice Coefficient (DC). In addition, the experimental results illustrate excellent qualitative performance. In future work, we plan to implement and investigate different deep learning models for nuclei classification and detection for digital pathological images.

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