Microscopic Nuclei Classification, Segmentation, and Detection with improved Deep Convolutional Neural Networks (DCNN)

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Abstract

Background: Nuclei classification, segmentation, and detection from pathological images are challenging tasks due to cellular heterogeneity in the Whole Slide Images (WSI).

Methods: In this work, we propose advanced DCNN models for nuclei classification, segmentation, and detection tasks. The Densely Connected Neural Network (DCNN) and Densely Connected Recurrent Convolutional Network (DCRN) models are applied for the nuclei classification tasks. The Recurrent Residual U-Net (R2U-Net) and the R2UNet-based regression model named as the University of Dayton Net (UD-Net) are applied for nuclei segmentation and detection tasks respectively. The experiments are conducted on publicly available datasets including Routine Colon Cancer (RCC) classification and detection and the Nuclei Segmentation Challenge 2018 datasets for segmentation task. The performance of the proposed methods is compared against the existing approaches in terms of precision, recall, Dice Coefficient (DC), Mean Squared Error (MSE), F1-score, and overall testing accuracy by performing pixels and cell-level analysis.

Results: The experimental results demonstrate around 2.2% and 4.5% higher performance in terms of F1-score for nuclei classification and detection tasks when compared to the recently published DCNN based method. Also, for nuclei segmentation, the R2U-Net shows around 91.63% testing accuracy in terms of DC which is around 0.76% higher compared to the U-Net model.

Conclusion: The proposed methods demonstrate robustness with better quantitative and qualitative results in three different tasks for analyzing the WSI.

Keywords: Digital pathology; Nuclei detection; Nuclei segmentation; DRCN; R2U-Net; and UD-Net

Introduction

People all around the world are suffering from different diseases including cancer, heart disease, chronic illness, diabetes, and brain-related diseases including Alzheimer’s. On average it takes approximately 12 years to discover a new drug and bring it to market for complex diseases like cancer. Medical imaging speeds up the assessment process of almost every disease from lung cancer to heart disease, thus alleviating some of the time expense associated with these illnesses. The
automatic nucleus classification, segmentation, and detection algorithms can help to unlock a cure faster for more many diseases like cancer. Identification of the cell’s nuclei is the starting point to an analysis of about 30 trillion cells, each of which contains a nucleus full of DNA within the human body. Accurate detection of cells can help the researchers to determine how to react to a cell for different treatments. As a result, the researchers can understand the underlying biological process of cell-level analysis in a clinical workflow. This solution can help ensure better treatment of the patients, and it can accelerate the treatment and the drug discovery processes. Therefore, the computational pathology and microscopy images play an important role in decision making for disease diagnosis. These image analysis methods provide a wide range of information for computer-aided diagnosis (CAD) and enable a quantitative and qualitative analysis of images with a very high throughput rate. Nowadays, computational pathology has become a very popular research area, therefore this research field gains significant attention from both the research community and those working in clinical practice [1, 2, 3].

![Proposed system](image)

**Figure 1** Proposed system: the patches are extracted from the multi-scale slide as required. Three different DL models are applied for nuclei classification, segmentation, and detection tasks. Finally, the performance is evaluated with different performance metrics.

The proposed DL approaches can provide faster and more efficient image analysis compared to the manual system that the researchers and clinician-scientists are using currently, which is alleviated difficulty and required repeated routine efforts [4]. The pathological images are very challenging to analyze manually, as a result, it can lead to large inter-observer variations [5]. On the other hand, CAD reduces the bias significantly and provides a characterization of diseases accurately [6]. Additionally, computational pathology gives a reproducible and rigorous measurement of pathological image features which can be used for clinical follow-up. It may also help to study personalized medicine and treatment, which would significantly benefit patients. As a prerequisite of clinical practice of CAD, the nuclei classification, segmentation, and detection methods are considered for annotated image analysis.
with different DCNN based methods. These techniques provide different quantitative analyses including cellular morphology, such as size, shape, color, texture, and other imagenomics. However, it is a difficult task to achieve robust and accurate performance in pathological imaging for several reasons. First, the pathology and microscopy images contain background clutter with noise, artifacts (image are blur sometimes), low signal to noise ratio (SNR), and poor depth resolution. These degradations usually occurred during image acquisition. Second, these images contain low contrast between the foreground and the background. Third, variations occur in terms of size, shape, and intercellular intensity of the nuclei or cell. Fourth, it can be observed very often that the nuclei of cells are partially overlapped with one another.

Meanwhile, several methods have been proposed to tackle these issues with automatic nuclei classification, segmentation, and detection for pathological imaging. In the last few years, several surveys have been conducted, and CAD technologies in the field of biomedical imaging including computational pathology have been summarized [7, 8]. These reviews briefly discuss different techniques related to pre-processing, nuclei classification, segmentation, detection, and post-processing methods. One of the recently published papers discusses several techniques related to data acquisition and ground truth generation, image analysis, recognition, detection, segmentation, and survival analysis [9]. Another review was conducted on different approaches related to feature extraction, predictive modeling, and visualization in digital pathology applications [10]. A survey was conducted on nuclei detection, segmentation, and classification on hematoxylin and eosin (H&E) and immunohistochemistry (IHC) stained histopathology images. Due to availability of annotated data and huge computing power, the Convolutional Neural Network (CNN) shown state-of-the-art-accuracy on different classification, segmentation, and detection problems [11, 12]. For the classification task, the goal is to define the class probability from the input samples. For example, in the binary breast cancer recognition problem the system defines whether the input samples are either a benign or malignant class. Second, in most cases, deep CNN-based semantic segmentation techniques are used for nuclei segmentation, which describes the process of associating each pixel of an image with a class label and defining the proper contour of the target region from an input image. Third, in DCNN based cell detection task, the objectives are to identify the central or rectangular coordinate of certain cells and defining of the contour of a nucleus. However, due to the complex nature of pathological images, there are still several developments ongoing in this field to develop better deep learning-based methods with better accuracy. In this work, we have applied three different improved DCNN models for nuclei classification, segmentation, and detection problems and have treated each as an individual task. The overall project implementation diagram is shown in Figure 1. The contributions of this paper are summarized as follows:

- We have proposed an improved model named the Densely Connected Recurrent Convolutional Network (DCRN) and applied it to the nuclei classification task.
- An improved deep learning model called R2U-Net is applied to nuclei segmentation in this implementation.
The R2U-Net based regression model named “UD-Net” is proposed and used for end-to-end nuclei detection.

The experiments have been conducted on three different publicly available datasets and the results demonstrate superior performance compared to existing machine learning and recently published DL based methods. The rest of the paper has been organized in the following way: Section 2 explains related works and Section 3 describes three different DL methods with mathematical details. The database, results, and discussions are provided in Section 4. Conclusions and future directions are presented in Section 5.

Related works
Automatic nuclei classification, segmentation, and detection is a prerequisite for various quantitative and qualitative analysis in computational pathology. The morphological features are computed for different diseases including routine colon cancer, breast cancer, drug development, and others. In the last few years, different DCNN based approaches have been proposed and successfully applied to pathological image analysis problems and show superior performance on different benchmark datasets for classification, segmentation, and detection [12]. In 2009, the image features including shape, texture, and size of nuclei are considered to develop a classical method for nuclear pleomorphism grading for breast cancer detection tasks [13]. Malon et al. used a CNN for classifying mitotic and non-mitotic cells using features that include the color, shape, and texture information [14]. The cancerous nuclei are classified from lymphocyte or stromal based on morphological features in H&E stained for breast cancer image analysis problem, and a machine learning method was used to accurately segment tissue from the input samples [15]. A nuclei segmentation classification method was proposed using an AdaBoost classifier where the intensity, morphological, and texture features were used in [16]. However, recent studies have shown that the deep learning-based approaches demonstrate better classification accuracy for large-scale pathological image classification tasks [12]. In 2014, Wang et al. used hand-crafted features, and a cascaded ensemble CNN was applied for detecting nuclei and mitosis cells and achieved superior nuclei classification compared to classical machine learning methods [17]. Another deep learning-based approach was proposed for cell classification and was compared against a bag of features and canonical representations methods and achieved better performance [8]. In 2017, a histopathological image classification approach was proposed using a support vector machine (SVM), AdaBoost, and DCNN methods. The experiment was conducted on four different H&E stained image datasets, namely the prostate, breast, renal clear cell, and renal papillary cancer cell detection tasks. The results demonstrate that the color-encoder deep network achieves the best performance out of nine individual classical methods and showed around 91.2 percent testing accuracy in terms of F1-score that is the highest testing accuracy to date [18]. For the very first time, we here introduce the Densely Connected Network (DCN) [19] and proposed a Densely Connected Recurrent Networks (DCRN) model for nuclei classification tasks.

For the nuclei segmentation task, a novel contour-based “minimum-model” cell detection and segmentation approaches were proposed in 2012 where a priori information was used to detected contours independent of their shape and achieved
promising segmentation results [20]. The Nuclei membrane segmentation method was proposed using a CNN model from microscopic images in 2012 [21]. In 2015, Ronneberger et al. proposed a U-Net and applied this model for medical image segmentation tasks and achieved state-of-the-art performance [22]. A learning-based framework for robust and automatic nucleus segmentation was proposed that shows proper shape properties of nuclei in pathological images where a CNN base iterative region merging technique is applied. In 2016, a novel segmentation approach was exploited to separate individual nuclei by combining a robust selection-based shape sharing and a local repulsive deformable model which were tested in several scenarios for pathological image segmentation and showed state-of-the-art performance against existing approaches [23]. A simple CNN model-based nuclei segmentation approach was proposed in 2017 named the CNN2, and CNN3 models for the different number of output classes. For the two-class model, the network was applied to classify pixels as inside or outside of the nuclei regions. On the other hand, for the three-class problem, the model was used for classifying pixels as belonging to the inside, outside, or boundary of nuclei regions [24]. In the same year, D. J. Ho et al. proposed a fully 3D-CNN method for nuclei segmentation method from 3D microscopy images [25]. A promising deep learning-based one-step contour aware nucleus segmentation approach was proposed with a fully convolutional neural network to segment the nuclei from corresponding boundaries simultaneously in 2018 [26]. A 3D Convolutional Network was used to perform cell nuclei detection and segmentation simultaneously in microscopic images and the model was tested with two different datasets and achieved state-of-the-art accuracy in detection and segmentation tasks [27]. For medical image segmentation problems, an improved version of the U-Net deep learning model was proposed in 2018 where recurrent residual modules were incorporated into the U-Net instead of forwarding convolutional layers. The model was evaluated on different modalities of medical imaging including retinal blood vessel segmentation, skin cancer segmentation, and lung segmentation tasks, and shown superior performance against U-Net, and SegNet [28]. To generalize the R2U-Net model, this model was used for end-to-end nuclei segmentation tasks in 2018 [29]. However, this study demonstrated better results of a large-scale R2U-Net model for nuclei segmentation tasks.

For the nuclei detection task, two different approaches were primarily applied for nuclei detection: the first is detection-based counting, which requires a prior detection or segmentation in [30]. Another is a density estimation-based method that was used for nuclei detection without using segmentation methods in [31]. A framework with a supervised max-pooling CNN was trained to detect cell pixel regions using a Support Vector Machine (SVM) and outperformed against the hand-crafted feature-based approaches [32]. For nuclei detection, a stacked sparse autoencoder was used for non-nuclei and nuclei region detection with unsupervised fusion where a Softmax classifier was employed [33]. A CNN based regression model was used for nuclei detection and counting, where a fully convolutional neural regression network model was used to identify the density map of nuclei from an input image with arbitrary size [34].

However, in this study, we propose a new R2UNet-based regression model for end-to-end nuclei detection from pathological images. The recurrent convolutional
operations help the model to learn and represent features better compared to the staid forward convolutional operations. The robustness of the R2U-Net model has been discovered in several studies in the prior [28, 28].

**Proposed DCNN models**

**Densely Connected Recurrent Convolutional Network (DCRN)**

According to the basic structure of Densely Connected Networks (DCN) in [19], the outputs from the previous layers are used as the input for the subsequent layers. This architecture ensures the reuse of the features inside the model and shows better performance for classification tasks [19]. In this implementation, we have proposed an improved version of the DCN which is named as the Densely Connected Recurrent Network (DCRN) model for nuclei classification. The DCRN is the building block of several densely connected recurrent blocks and transition blocks are shown in Figure 2.

![Figure 2 The Densely Connected Recurrent Network model with recurrent, convolutional, and transition blocks.](image)

According to the basic mathematical details of DenseNet explained in [19], the $l^{th}$ layer receives all feature maps $(x_0, x_1, x_2, ...x_{l-1})$ from the previous layers as input:

$$x_l = H_l ([x_0, x_1, x_2, ...x_{l-1}])$$  \hspace{1cm} (1)

where $[x_0, x_1, x_2, ...x_{l-1}]$ is a concatenation of features from the 0, 1, ...., $l - 1$ layers, and $H_l(\cdot)$ is a single tensor. Let’s consider the $H_l(\cdot)$ input sample from the $l^{th}$ DCRN block containing 0, 1, ..., $F - 1$ feature maps as inputs to the recurrent convolutional layers. The convolutional layer performs three consecutive operations including Batch Normalization (BN), followed by a ReLU and a $3 \times 3$ convolution. The $H_{(i,j)}$ is a center pixel of a patch located in the input sample of the $k^{th}$ feature named $H_{(i,k)}(\cdot)$. Additionally, the output of the network represents with $H_{(i,k)}(t)$ for the $l^{th}$ layer and the $k^{th}$ feature map at time step t. The output can be expressed as in equation (2).

$$H_{(l,k)}(t) = \left( w_f^{(l,k)} \right)^T * H_{(i,j)}^{(l)} (t) + \left( w_r^{(l,k)} \right)^T * H_{r(i,j)}^{(l)} (t - 1) + b_{(l,k)}$$  \hspace{1cm} (2)

Here, $H_{(i,j)}^{(l)} (t)$ and $H_{r(i,j)}^{(l)} (t - 1)$ are the inputs to the standard convolution layers and the $l^{th}$ recurrent convolution layers respectively. The $w_f^{(l,k)}$ and $w_r^{(l,k)}$
values are the weights of the standard convolutional layers and the recurrent convolutional layers of the $l^{th}$ layer and $k^{th}$ feature map respectively. The term $b_{(l,k)}$ is the bias. The recurrent convolution operations are performed for the time steps $t$ [35, 36, 37]. The pictorial representation of the recurrent convolution operations for $t=2$ is shown in Figure 3. In the transition block, $1 \times 1$ convolutional operations are performed with BN followed by $2 \times 2$ average pooling layers. The DenseNet model consists of several dense blocks with feedforward convolutional layers and transition blocks, whereas the DCRN model uses the densely connected recurrent convolutional layers and transition blocks. For both DenseNet and the proposed DCRN models, we used 4 blocks with 3 layers per block, and a growth rate of 5 in this study. The model details for DenseNet and DCRN are shown in Table 1.

![Figure 3 Unfolded recurrent convolutional layer for time step t = 2.](image)

**R2U-Net**

We applied the R2U-Net model for nuclei segmentation for microscopic images in our prior study in 2018 [29]. However, in this study, we extended the nuclei segmentation tasks by applying a large-scale R2U-Net model and achieved better performance. The R2U-Net model is an improved segmentation model developed based on U-Net [22], Recurrent Convolutional Neural Networks (RCNNs) [36], and the Residual Network (ResNet) [38]. The conceptual diagram of the nuclei segmentation with the R2U-Net model is provided in Figure 4. The R2U-Net model consists of two main units that are encoding unit (shown in green) and the decoding unit (shown in blue). In both units, the recurrent residual convolutional operations are performed in the convolution blocks. A pictorial representation of the Recurrent Residual Convolutional Unit (RRCU) is shown in Figure 5.

The recurrent operations are performed to different time steps, as shown in Figure 3 for $t = 2$, which means one forward convolution layer followed by two recurrent layers are used in a convolutional unit. The feature maps from the encoding unit are concatenated with the feature maps from decoding units. The Softmax layer is used at the end of the model to calculate the pixel label class probability. The network architecture and model parameters for R2U-Net are given in Table 1.
R2U-Net based regression model

In general, for cell detection and counting problems, the ground truth masks are created with a single-pixel annotation method where the individually annotated single-pixel represents an entire cell. The dataset that we have used in this study contains at least five to five hundred nuclei which are annotated manually with the center pixel annotation method. The annotations are then dilated with a $5 \times 5$ kernel and a Gaussian distribution is generated for the dilated region. In this regression model, we used the R2U-Net model to estimate the Gaussian density surface from the input samples instead of computing the classes directly or obtaining the pixel-level class probability. As the R2U-Net-based regression model is used for nuclei detection, we named this model the University of Dayton Network (UD-Net). For any input sample, a density surface $D(x)$ is generated based on a superposition of these Gaussians. The objective is for regressing this density surface for the corresponding input image $I(x)$. The target of the UD-Net model is minimized with the mean squared error between the predicted density and the target Gaussian density surface acts as the ultimate loss for the regression problem. In the testing phase, for a given input cell image $I(x)$, the UD-Net model computes the Gaussian density heat map $D(x)$. In prior work, a CNN-VGG architecture-based regression model was proposed in 2015 [39, 40, 41, 42]. However, in this work, we applied the UD-Net regression...
Table 1 The model architecture and the number of network parameters utilized for each model.

| Model/Tasks   | t | Architectures                                      | Params.(M) |
|--------------|---|---------------------------------------------------|------------|
| DenseNet/Class. | - | Blocks 4, layers 3, and growth rate 5             | 0.582      |
| DCRN/Class.   | 2 | Blocks 4, layers 3, and growth rate 5             | 0.582      |
| R2U-Net/Seg.  | 2 | 1->32(3)->64(3)->128(3)->256(3)->512(3)          | 0.983      |
| UD-Net/Det.   | 3 | 1->32(3)->64(3)->128(3)->256(3)->512(3)          | 1.038      |

model for nuclei detection tasks which is more powerful and robust compared to the existing methods.

Model Architectures

In this implementation, we used both DenseNet and the DCRN models with similar architectures for nuclei classification tasks as shown in Table 1. The main difference between these two models is that two feedforward convolutional layers are used for DenseNet whereas, for the DCRN model, we used two recurrent convolutional layers. For segmentation, we used the R2U-Net model with 0.98M network parameters implemented with t=2. We used the UD-Net model with time step t=3 that increases the number of network parameters to 1.038M, which shows better testing performance. The model architecture of the R2U-Net and UD-Net regression and the number of network parameters are shown in Table 1.

Experiments and results

To demonstrate the performance of the DCRN, R2U-Net, and R2U-Net based regression (UD-Net) models for nuclei classification, segmentation, and detection tasks. The datasets for the classification and detection tasks were taken from the recent study in [39] and the nuclei segmentation dataset was taken from the 2018 Data Science Bowl Grand Challenge dataset [43]. For this implementation, the Keras [44] and TensorFlow [45] frameworks were used on a single GPU machine with 56G of RAM and an NVIDIA GEFORCE GTX-980 Ti.

Figure 6 Example images from the dataset for nuclei classification.
Dataset for nuclei classification

This dataset contains 200 annotated samples for classification and detection tasks, where 100 samples are used for classification and the remaining 100 samples are used for detection. The actual sample size is $500 \times 500$ pixels. For both classification and detection tasks, randomly selected 80 percent of samples are used for training, and the remaining 20 percent of samples are used for the testing phase. Some of the randomly selected samples for the nuclei classification task are shown in Figure 6. For the classification task, the dataset has four different classes of routine colon cancer including Epithelial, Fibroblast, Inflammatory, and miscellaneous. The samples are annotated with respect to the center pixel of the cell and provided as a MAT file. Each of the large patch ($500 \times 500$ pixels) contains four different types of nuclei, however, we have observed that in some cases, the large patches do not contain all four types of nuclei cells. We have extracted patches with the size of $32 \times 32$ pixels to the center point of the cells from the large image patches. We have extracted 5,295 patches for epithelial, 5,424 patches for inflammatory, 4,220 patches for fibroblast, and 1,390 patches for miscellaneous. We have a total of 16,329 patches where 80 percent samples are used for training and the remaining 20 percent samples are used for validation as mentioned in [39]. The example patches are shown in Figure 7.

![Figure 7](image)

**Figure 7** Randomly selected patches for four different types of nuclei of routine colon cancer.

Dataset for nuclei segmentation

In 2018, the Data Science Bowl launched a competition with a mission to create an effective algorithm for automatic nucleus detection and segmentation. The nuclei segmentation database contains 735 images in total. The size of the samples is $256 \times 256$ pixels, where 650 images and their corresponding pixel-level annotation masks are released for training, and the remaining 65 samples for testing respectively. However, in this study, from the training set, 80 percent of the samples are used for training and the remaining 20 percent are used for validation and testing. The number of training and testing samples are 536 and 134 respectively. This database contains both single and multichannel images, hence, we have converted all samples to gray-scale representation. Figure 8 shows the input samples in the first rows and corresponding ground truth masks in the second row respectively.
Database for Nuclei Detection
The nuclei detection database contains 100 samples and 100 masks with single-pixel annotation [39, 46, 47]. The original size of the database samples is 500x500. Some of the randomly selected samples and corresponding dilated masks are shown in Figure 9. For nuclei detection, we extracted the non-overlapping patches with a size of 96x96 pixels from the input samples and corresponding masks. We used a total of 4,392 non-overlapping patches and masks. Of these patches, around 80 percent are used for training and the remaining 20 percent are used for testing.

Evaluation Metrics
The performance of the models for nuclei cell detection tasks is evaluated with different performance metrics including precision, recall, and F1-score which are
stated in equations (3) through (5). The True Positive (TP) refers to the number of nuclei cells correctly detected with respect to the ground truth, whereas False Positive (FP) represents the number of detected nuclei that are not in the ground truth. The number of ground truth nuclei cells that are un-detected are called False Negatives (FNs). The mathematical representation of precision, recall, and F1-score are shown in the following expression as follows:

\[
\text{precision} = \frac{TP}{TP + FP} \tag{3}
\]

\[
\text{recall} = \frac{TP}{TP + FP} \tag{4}
\]

\[
F1\text{-}score = \frac{2 \times \text{recall} \times \text{precision}}{\text{recall} + \text{precision}} \tag{5}
\]

**Training methods**

We used the DenseNet and DCRN with the same architecture and with the same number of network parameters for nuclei classification. In both models, we used a stochastic gradient descent (SGD) optimization method with a learning rate of 0.001, a weight decay of 1x10^-4, a momentum of 0.9, and cross-entropy loss. The entire model is trained for 100 epochs with batch size 32. For the segmentation task, we applied the Dice Coefficient (DC) and Mean Squared Error (MSE) loss. The DC is expressed in equation (6), where GT refers to the ground truth and SR refers to the segmentation result.

\[
DC = 2 \times \frac{|GT \cap SR|}{|GT| \cap |SR|} \tag{6}
\]

Another metric used to evaluate the performance of the segmentation algorithm is the MSE as defined in equation (7). In this case, \(Y\) represents ground truth and \(\bar{Y}\) represents the predicted outputs for an input sample with height \(h\) and width \(w\) and \(n = h \times w\).

\[
MSE = \frac{1}{n} \sum_{i=1}^{n} (Y_i - \bar{Y}_i)^2 \tag{7}
\]

We trained a segmentation model with 250 epochs and used an Adam optimizer with a learning rate of \(2 \times 10^{-4}\) and a batch size of 16. Finally, for detection with the UD-Net regression model, we used the Adam optimizer with a learning rate of \(2 \times 10^{-4}\) and measured mean squared error (MSE). The UD-Net model is trained for 500 epochs and with a batch size of 64.
Table 2: Nuclei classification accuracy and comparison against other machine learning and deep learning methods.

| Methods                        | Average F1-score | AUC    | Accuracy  |
|--------------------------------|------------------|--------|-----------|
| CRImage [40]                   | 0.488            | 0.684  | -         |
| Super-pixel descriptor [40]    | 0.6878           | 0.853  | -         |
| SoftMax CNN+SSPP [39]          | 0.7488           | 0.893  | -         |
| SoftMax CNN+NEP [39]           | 0.748            | 0.917  | -         |
| DenseNet [19]                  | 0.796            | 0.9493 | 0.9004    |
| Proposed (DRCN)                | 0.806            | 0.9536 | 0.9124    |

Results
Nuclei classification
We tested both DenseNet and DCRN models with the same setup and achieved 90.04% and 91.24% testing accuracy respectively. The experimental results and comparison against the existing nuclei classification approaches are shown in Table 2, the proposed DCRN shows superior performance for nuclei classification compared to DenseNet and other existing methods.

The area under the Receiver Operating Characteristic (ROC) curve for both DenseNet and DCRN is shown in Figure 10, which shows that DCRN provides the highest Area Under Curve (AUC) with similar network architecture and the same number of network parameters. This demonstrates the robustness of our proposed DCRN model over DenseNet for nuclei classification tasks.

![Figure 10](image)

Figure 10: Area under the ROC curve of the DenseNet and DCRN models for nuclei classification tasks.

Nuclei segmentation
In this implementation, we used a simple R2U-Net model where only 0.84 million network parameters were utilized. We considered the DC and MSE for observing training progress and performance in the training and testing phases. From the experiments, it has been observed that the model converged after 100 epochs, however, the training and evaluation continued until 250 epochs to ensure better convergence,
considering the lack of the number of samples available for training. In the testing phase, we achieved 90.86% and 91.62% testing accuracy in terms of DC score for nuclei segmentation with U-Net and R2U-Net models respectively.

![Figure 11](image_url) Qualitative results for both U-Net and R2U-Net models for nuclei segmentation, the first row shows the input samples, the second row shows the corresponding ground truth masks, the third and third row shows the outputs for U-Net and the fourth row show the outputs from R2U-Net model. The orange circles show the false detection of the U-Net model.

**Qualitative analysis**

Figure 11 shows some example outputs when using the U-Net and R2U-Net models for nuclei segmentation task where the first row shows the input images, the second row shows the ground truth mask for the corresponding input samples, the third row shows the output of U-Net model, and the fourth row represents the outputs for R2U-Net model. The proposed R2U-Net segmentation model shows better quantitative outputs compared to the U-Net model during the testing phase. Also, if we observe the input samples in the first column in the third row shows the false detection which is indicated with an orange circle whereas the R2U-Net shows very accurate segmentation results like ground truth in the second column. Likewise, we can observe the same false detection results in the second column. In column third, the black regions appear in the nuclei regions which false negative, however, the R2U-Net model shows accurate segmentation output in this case. The input shown in the fourth column contains a complex background where some regions are very likely the nuclei regions. The U-Net model fails to show the isolated nucleus that is marked with an orange circle whereas the R2U-Net successfully segmented.
Table 3 Nuclei detection accuracy of the proposed model and comparison against existing methods.

| Methods                      | Precision | Recall | mean F1-score |
|------------------------------|-----------|--------|---------------|
| CRImage[40]                  | 0.657     | 0.461  | 0.542         |
| CNN[40]                      | 0.783     | 0.804  | 0.793         |
| SSAE[46]                     | 0.617     | 0.644  | 0.630         |
| LIPSyM[46]                   | 0.725     | 0.517  | 0.604         |
| SC-CNN(M=1)[39]              | 0.758     | 0.827  | 0.791         |
| SC-CNN(M=2)[39]              | 0.781     | 0.823  | 0.802         |
| Proposed (UD-Net)            | **0.821** | **0.842** | **0.832**     |

and separated the individual nucleus successfully. Thus, the segmentation results demonstrate the robustness of the R2U-Net model for nuclei segmentation tasks from pathological images.

![Training and validation accuracy for nuclei detection.](image)

**Figure 12** Training and validation accuracy of the UD-Net model for nuclei detection.

### Nuclei detection

The training and validation accuracy for the UD-Net model is shown in Figure 12. In this experiment, we developed a patch-based approach for nuclei detection, the quantitative results and comparison are shown in Table 3. A recently published paper reported a 0.802 F1-score as the highest testing accuracy for nuclei detection in 2016[39], whereas our proposed model shows a 0.8318 F1-score for nuclei detection which is approximately a 2.98% better performance compared to the SC-CNN model [39].

The qualitative results for the nuclei detection model for the patch (96 × 96 pixels) and original input image approaches are shown in Figure 13 and Figure 14 respectively. The first column shows the input patches, the second column shows the ground truth masks, and the third column represents the model outputs after thresholding with respect to a value of 0.5. Lastly, the fourth column shows the final outputs with blue and green solid circles, where the blue circles indicate the ground truths and the green circles represent the model outputs respectively. The quantitative results demonstrate that the UD-Net model can detect the nuclei very precisely.

After generating the patch-based outputs, we merged all the patches (96 × 96 pixels) to generate output for the entire input image. Figure 14 shows the outputs
of 250 × 250 pixels images which have cropped the merged images (480 × 480 pixels), where the first column shows the inputs, the second column is for a ground truth Gaussian density surface, the third column represents the model outputs and the fourth column shows the final outputs with blue and green dots to represent the ground truth and the center pixel of the network out for each nucleus respectively.

**Figure 13** Nuclei detection outputs with inputs, ground truth, model outputs after thresholding, and final outputs with a blue and green dot. The blue dot represents the ground truth and the green dot shows the center pixels of the network outputs.

**Analysis**

We conducted a set of experiments to evaluate three important tasks for nuclei classification, segmentation, and detection tasks. For classification, we applied DenseNet and an improved version of DenseNet called the DCRN. The DenseNet provides performance of 0.796 and 0.9493 in terms of F1-score and AUC whereas the proposed DCRN provides approximately 0.8060 and 0.9536 for F1-score and AUC respectively. The DCRN provides around 2.2% and 3.66% better performance in terms of F1-score and AUC against recently published results with a softmax Convolutional Neural Network (CNN) and a neighboring ensemble predictor (NEP) known as softmax CNN+NEP [39]. The proposed method shows 91.24% testing accuracy which is about 1.2% better when compared to DenseNet. Second, we used the R2Unet for segmentation and achieved 91.62% testing accuracy which is around 0.76%
better performance compared to the U-Net model. Third, the UD-Net regression model was applied for nuclei detection and achieved 82.17%, 84.23%, and 83.2% for precision, recall, and F1-score respectively. Overall, the proposed models provide superior performance for all three tasks. The testing time per sample for classification, segmentation, and detection are shown in Table 4.

Conclusion
In this study, we proposed three different models including the Densely Connected Recurrent Convolutional Network (DCRN), the Recurrent Residual U-Net (R2U-Net), and the R2U-Net-based regression named the University of Dayton Net (UD-Net) for nuclei classification, segmentation, and detection tasks respectively. These models were evaluated on three different publicly available datasets. Firstly, we achieved 91.14% testing accuracy for the nuclei classification task which is around 2.2% and 3.66% higher F1-score and AUC scores compared to recently published results. Secondly, the R2U-Net model shows 0.76% better testing accuracy against the U-Net model for nuclei segmentation tasks. Finally, for detection, we have achieved 83.2% testing accuracy in terms of F1-score, and the UD-Net model shows around 3% better F1-score compared to the existing methods. In the future, we would like to explore these models on more challenging datasets for computational pathology applications.

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Abbreviations
DCNN : Densely Connected Neural Network
DCRN : Densely Connected Recurrent Convolutional Network
RUU-Net : Recurrent Residual U-Net
UD-Net : University of Dayton Net
RCC : Routine Colon Cancer
SVM : Support Vector Machine
CAD : Computer Aided Diagnosis

Availability of data and materials
All of the databases used in this study are publicly available and willing to provide the codes the public on request.

Ethics approval and consent to participate
There is no animal or human study directly involved in this study.

Competing interests
To the best of our knowledge, no conflict of interest, financial or other, exists.

Consent for publication
All authors agreed for submitting this manuscript to this journal.

Authors’ contributions
All author listed in this paper contributed sufficiently in this work.

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References
1. Van Norman, G.A.: Drugs, devices, and the fda: part i: an overview of approval processes for drugs. JACC: Basic to Translational Science 1(3), 170–179 (2016)
2. Rojo, M.G., Punys, V., Slodkowska, J., Schrader, T., Daniel, C., Blobel, B.: Digital pathology in europe: coordinating patient care and research efforts. Stud Health Technol Inform 150, 997–1001 (2009)
3. Rojo, M.G.: State of the art and trends for digital pathology. Stud. Health Technol. Inform 179, 15–28 (2012)
4. Pantanowitz, L., Sinard, J.H., Henricks, W.H., Fatheree, L.A., Carter, A.B., Contis, L., Beckwith, B.A., Evans, A.J., Lal, A., Parwani, A.V.: Validating whole slide imaging for diagnostic purposes in pathology: guideline from the college of american pathologists pathology and laboratory quality center. Archives of Pathology and Laboratory Medicine 137(12), 1710–1722 (2013)
5. López, C., Lejeune, M., Bosch, R., Korzynska, A., García-Rojo, M., Salvadó, M.-T., Alvaro, T., Callau, C., Roso, A., Jaén, J.: Digital image analysis in breast cancer: an example of an automated methodology and the effects of image compression. Studies in health technology and informatics 179, 997–1001 (2009)
6. Bueno, G., García-Rojo, M., Déniz, O., Fernández-Carrobles, M.M., Vállez, N., Salido, J., García-González, J.: Emerging trends: grid technology in pathology. Stud Health Technol Inform 179, 218–229 (2012)
7. Gurcan, M.N., Boucheron, L.E., Can, A., Madabhushi, A., Rajpoot, N.M., Yener, B.: Histopathological image analysis: A review. IEEE reviews in biomedical engineering 2, 147–171 (2009)
8. Cruz-Roa, A.A., Ovalle, J.E.A., Madabhushi, A., Osorio, F.A.G.: A deep learning architecture for image representation, visual interpretability and automated basal-cell carcinoma cancer detection. In: International Conference on Medical Image Computing and Computer-Assisted Intervention, pp. 403–410 (2013). Springer
9. Fuchs, T.J., Buhmann, J.M.: Computational pathology: challenges and promises for tissue analysis. Computerized Medical Imaging and Graphics 35(7-8), 515–530 (2011)
10. Kothari, S., Phan, J.H., Stokes, T.H., Wang, M.D.: Pathology imaging informatics for quantitative analysis of whole-slide images. Journal of the American Medical Informatics Association 20(6), 1099–1108 (2013)
11. Alom, M.Z., Taha, T.M., Yakopcic, C., Westberg, S., Siddike, P., Nasrin, M.S., Hasan, M., Van Essen, B.C., Awval, A.A., Asari, V.K.: A state-of-the-art survey on deep learning theory and architectures. Electronics 8(3), 292 (2019)
12. Litjens, G., Kooi, T., Bejnordi, B.E., Setio, A.A.A., Ciompi, F., Ghafoorian, M., Van Der Laak, J.A., Van Ginneken, B., Sánchez, C.I.: A survey on deep learning in medical image analysis. Medical image analysis 42, 60–88 (2017)
13. Dalle, J.-R., Li, H., Huang, C.-H., Leow, W.K., Roccoeau, D., Putti, T.C.: Nuclear pleomorphism scoring by selective cell nuclei detection. In: WACV (2009)
14. Malon, C.D., Cosatto, E.: Classification of mitotic figures with convolutional neural networks and seeded blob features. Journal of pathology informatics 4 (2013)
15. Yuan, Y., Failmezger, H., Rueda, O.M., Ali, H.R., Gräf, S., Chin, S.-F., Schwarz, R.F., Curtis, C., Dunning, M.J., Bardwell, H., et al.: Quantitative image analysis of cellular heterogeneity in breast tumors complements genomic profiling. Science translational medicine 4(157), 157–143157143 (2012)
16. Sharma, H., Zerbe, N., Heim, D., Wienert, S., Behrens, H.-M., Hellwich, O., Hufnagl, P.: A multi-resolution approach for combining visual information using nuclei segmentation and classification in histopathological images. In: VISAPP (3), pp. 37–46 (2015)

17. Wang, H., Cruz-Roa, A., Basavanahally, A., Gilmore, H., Shih, N., Feldman, M., Tomaszewski, J., Gonzalez, F., Madabhushi, A.: Cascaded ensemble of convolutional neural networks and handcrafted features for mitosis detection. In: Medical Imaging 2014: Digital Pathology, vol. 9041, p. 904104 (2014). International Society for Optics and Photonics

18. Singh, M., Zeng, Z., Kalaw, E.M., Giron, D.M., Chong, K.-T., Lee, H.K.: A study of nuclei classification methods in histopathological images. In: International Conference on Innovation in Medicine and Healthcare, pp. 78–88 (2017). Springer

19. Huang, G., Liu, Z., Van Der Maaten, L., Weinberger, K.Q.: Densely connected convolutional networks. In: Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition, pp. 4700–4708 (2017)

20. Wiener, S., Heim, D., Senger, K., Stenzinger, A., Beil, M., Hufnagl, P., Dietel, M., Denkert, C., Klauschen, F.: Detection and segmentation of cell nuclei in virtual microscopy images: a minimum-model approach. Scientific reports 2(1), 1–7 (2012)

21. Ciresan, D., Giusti, A., Gambardella, L., Schmidhuber, J.: Deep neural networks segment neuronal membranes in electron microscopy images. Advances in neural information processing systems 25, 2843–2851 (2012)

22. Ronneberger, O., Fischer, P., Brox, T.: U-net: Convolutional networks for biomedical image segmentation. In: International Conference on Medical Image Computing and Computer-assisted Intervention, pp. 234–241 (2015). Springer

23. Xing, F., Xie, Y., Yang, L.: An automatic learning-based framework for robust nucleus segmentation. IEEE transactions on medical imaging 35(2), 550–566 (2015)

24. Kumar, N., Verma, R., Sharma, S., Bhargava, S., Vahadane, A., Sethi, A.: A dataset and a technique for generalized nuclear segmentation for computational pathology. IEEE transactions on medical imaging 36(7), 1550–1560 (2017)

25. Fu, C., Ho, D.J., Han, S., Salama, P., Dunn, K.W., Delp, E.J.: Nuclei segmentation of fluorescence microscopy images using convolutional neural networks. In: 2017 IEEE 14th International Symposium on Biomedical Imaging (ISBI 2017), pp. 704–708 (2017). IEEE

26. Cui, Y., Zhang, G., Liu, Z., Xiong, Z., Hu, J.: A deep learning algorithm for one-step contour aware nuclei segmentation of histopathology images. Medical & biological engineering & computing 57(9), 2027–2043 (2019)

27. Ram, S., Nguyen, V.T., Limesand, K.H., Sabuncu, M.R.: Joint cell nuclei detection and segmentation in microscopy images using 3d convolutional networks. arXiv preprint arXiv:1805.02850 (2018)

28. Alom, M.Z., Yakopcic, C., Hasan, M., Taha, T.M., Asari, V.K.: Recurrent residual u-net for medical image segmentation. Journal of Medical Imaging 6(1), 014006 (2019)

29. Alom, M.Z., Yakopcic, C., Taha, T.M., Asari, V.K.: Nuclei segmentation with recurrent residual convolutional neural networks based u-net (r2u-net). In: NAECON 2018-IEEE National Aerospace and Electronics Conference, pp. 228–233 (2018). IEEE

30. Arteta, C., Lemptisky, V., Noble, J.A., Zisserman, A.: Learning to detect cells using non-overlapping extremal regions. In: International Conference on Medical Image Computing and Computer-assisted Intervention, pp. 348–356 (2012). Springer

31. Fiaschi, L., Köthe, U., Nair, R., Hamprecht, F.A.: Learning to count with regression forest and structured labels. In: Proceedings of the 21st International Conference on Pattern Recognition (ICPR2012), pp. 2685–2688 (2012). IEEE

32. Dong, B., Shao, L., Da Costa, M., Bandmann, O., Frangi, A.F.: Deep learning for automatic cell detection in wide-field microscopy zebrafish images. In: 2015 IEEE 12th International Symposium on Biomedical Imaging (ISBI), pp. 772–776 (2015). IEEE

33. Xu, J., Xiang, L., Liu, Q., Gilmore, H., Wu, J., Tang, J., Madabhushi, A.: Stacked sparse autoencoder (ssae) for nuclei detection on breast cancer histopathology images. IEEE transactions on medical imaging 35(1), 119–130 (2015)

34. Xie, W., Noble, J.A., Zisserman, A.: Microscopy cell counting and detection with fully convolutional regression networks. Computer methods in biomechanics and biomedical engineering: Imaging & Visualization 6(3), 283–292 (2018)

35. Alom, M.Z., Hasan, M., Yakopcic, C., Taha, T.M., Asari, V.K.: Inception recurrent convolutional neural network for object recognition. Machine Vision and Applications 32(1), 1–14 (2021)

36. Liang, M., Hu, X.: Recurrent convolutional neural network for object recognition. In: Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition, pp. 3367–3375 (2015)

37. Alom, M.Z., Hasan, M., Yakopcic, C., Taha, T.M., Asari, V.K.: Improved inception-residual convolutional neural network for object recognition. Neural Computing and Applications 32(1), 279–293 (2020)

38. He, K., Zhang, X., Ren, S., Sun, J.: Deep residual learning for image recognition. In: Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition, pp. 770–778 (2016)

39. Srirunkunwatana, K., Raza, S.E.A., Tsang, Y.-W., Sneed, D.R., Cree, I.A., Rajpoot, N.M.: Locality sensitive deep learning for detection and classification of nuclei in routine colon cancer histology images. IEEE transactions on medical imaging 35(5), 1196–1206 (2016)

40. Yuan, Y., Fallmeier, H., Rueda, O.M., Ali, H.R., Gräf, S., Chin, S.-F., Schwarz, R.F., Curtis, C., Dunning, M.J., Bardwell, H., et al.: Quantitative image analysis of cellular heterogeneity in breast tumors complements genomic profiling. Science translational medicine 4(157), 157–143157143 (2012)

41. Srirunkunwatana, K., Raza, S.E.A., Tsang, Y.-W., Sneed, D.R., Rajpoot, N.M.: A novel texture descriptor for detection of glandular structures in colon histology images. In: Medical Imaging 2015: Digital Pathology, vol. 9420, p. 942004 (2015). International Society for Optics and Photonics

42. Xie, Y., Xing, F., Kong, X., Su, H., Yang, L.: Beyond classification: structured regression for robust cell detection using convolutional neural network. In: International Conference on Medical Image Computing and
43. Data-science-bowl-2018 (2018). https://www.kaggle.com/c/data-science-bowl-2018
44. Keras (2019). https://github.com/keras-team/keras.git
45. Abadi, M., Barham, P., Chen, J., Chen, Z., Davis, A., Dean, J., Devin, M., Ghemawat, S., Irving, G., Isard, M., et al.: Tensorflow: A system for large-scale machine learning. In: 12th (USENIX) Symposium on Operating Systems Design and Implementation ((OSDI) 16), pp. 265–283 (2016)
46. Xu, J., Xiang, L., Liu, Q., Gilmore, H., Wu, J., Tang, J., Madabhushi, A.: Stacked sparse autoencoder (ssae) for nuclei detection on breast cancer histopathology images. IEEE transactions on medical imaging 35(1), 119–130 (2015)
47. Kuse, M., Wang, Y.-F., Kalasannavar, V., Khan, M., Rajpoot, N.: Local isotropic phase symmetry measure for detection of beta cells and lymphocytes. Journal of pathology informatics 2 (2011)