## Nuclei Segmentation Using Deep Learning and Hyperspectral Imaging for High Accuracy

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Abstract: Computerized microscopy image analysis plays a crucial role in computer aided nuclei segmentation/diagnosis. Machine learning methods have emerged as a powerful tool in medical investigation and clinical practice. Utilization of RGB datasets to segment tissue fields has attracted considerable attention in the biomedical informatics industry as these large datasets are difficult to acquire and validate. This study provides an analysis of the differences in nuclei segmentation of human tissue using RGB images and Hyperspectral images (HSI) using the UNET Convolutional Neural network architecture. The paper introduces the popular neural network specifically designed for biomedical segmentation problems and it summarizes current deep learning achievements in tasks such as detection and segmentation. The results of this research qualify the potential usage of hyperspectral imaging to improve the quality of neural network segmentations given a smaller dataset of HSI volumes

**Area of Research:** In the histopathological field of biology, current computational methods of biological nuclei segmentation are done using large datasets of 3-color-channel images to train neural networks. The accuracy of these models are not comparable to experienced pathologists as they lack in precision. This study presents an alternative method to segment and analyze human tissue using Hyperspectral imaging. Hyperspectral imaging was proven to drastically increase the accuracy of neural networks with limited data compared to RGB images.

**Goal Of Research:** The goal of this project is to develop a program that can detect cancer from a prostate biopsy using Artificial Intelligence trained on a hyperspectral dataset.

**Methods:** Datasets of RGB, and HSI were used each containing 85 fields. To obtain ground truth masks, RGB fields were manually annotated and validated using the Label-Studio software. Each RGB tissue field had a replica in the Hyperspectral data format, so the same masks were implemented for both imaging types. Each tissue field was 966x606 pixels from the prostate but were cropped to smaller patches to improve training efficiency.

The spectral python package enabled specific HSI bands to be extracted from the entire data cube. Bands that contained pixels majorly representative of connective tissue were not selected to train on because chemical structure of nuclei were invisible. Without nuclear structure data, network quality will decrease.

Various dimensions of the UNET architecture were tested with the following patch sizes (length in pixels  $\times$  width in pixels):  $128\times128$ ,  $256\times256$ , and  $512\times512$ .  $64\times64$  patch sizes rendered the field of view too small for the network to detect individual instances of nuclei and  $1024\times1024$  yielded the issue of not enough training data as the fields were only  $966\times606$ .

The third dimension of an image represents the number of bands (color channels) in the image. The HSI model was trained using the HSI images with 1 to 12 color channels to test improvement compared to the RGB model with 3 color channel images. Each image was input as multi-dimensional Tensor of 64-bit floating point numbers.

The network was initially trained with 128×128, 256×256 and 512×512 patches of the tissue fields to test overfitting on different datasets. After training on RGB and 3-band HSI images for

25, 30, and 50 epochs, it was determined that 512×512 patch sizes were the most efficient, had less overfitting, and provided the highest average IoU (Intersection over Union). 128×128 and 256×256 patch sizes cropped out individual nuclei within the patch and caused the network to have a compromised training dataset.

Different dataset sizes were tested from 5 fields to 85 fields to test the effect of data size on model accuracy. In practice, large datasets are difficult to acquire and require vast amounts of time to annotate and validate. Therefore, if HSI provides comparable accuracy to the RGB model trained on a smaller dataset, it would prove to be a more efficient use of training resources.

To prevent testing the network on known images, the batches were split by 80% training and 20% testing. The model's optimizer was Adam, as it is an extended form of stochastic gradient descent and is commonly utilized in computer vision models. The loss function this research utilized was binary cross entropy.

**Results:** With a dataset of 5 tissue fields, the HSI model achieved 75% IoU and 85% F-score with 10 color channels. With a dataset of 5 fields, the RGB model achieved 66% IoU and 80% F-score. Both training methods caused saturation in training loss and validation loss as epochs increase. As the dataset size increased, both models achieved an accuracy of 81% when trained on 45 fields.

The maximum IoU achieved from the HSI dataset was 81% and an F-score of 91% when trained on 36 fields with 10 color channels. The RGB segmentation model produced a maximum IoU of 81% and a maximum F-score of 90% when given a dataset of 45 fields. IoU and F-score saturated as the number of training fields increased.

Different HSI datasets were trained on with 2 to 12 color channels. Each patch was 512×512 to maximize IoU as described. The training epochs ranged from 15-105 epochs according to the number of color channels per image (more color channels required more epochs).

The IoU threshold peaked at 80.9% and F-score peaked at 91% with 10 color bands with 105 epochs of training. As number of color channels increased after 10, IoU decreased, indicating training saturation.

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