QUALITY ASSESSMENT OF MESENCHYMAL STEM CELLS USING DEEP LEARNING BASED IMAGE ANALYSIS

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ABSTRACT

Quality Assessment of Mesenchymal Stem Cells using Deep Learning based Image Analysis

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Cell-based therapeutics is a current effective strategy for the potential curing of several human diseases. Mesenchymal stem cells (MSCs) are a heterogeneous group of cells that have been the subject of recent attention because of their clinically relevant therapeutic effects and transformative morphology. The success of MSCs to provide new remedies is dependent on their quality. Their quality can be assessed by examining their physical nature. Morphological evaluation has been a robust method for monitoring culture quality, but standard techniques are either subjective, destructive, or time consuming making real-time monitoring difficult. The goal is to develop an automated image analysis algorithm using deep learning to assess the viability of MSCs.

An algorithm using Keras and TensorFlow libraries in Python will be our main method for phase contrast microscope images of MSCs. The cell images are first preprocessed and then given to the U-Net architecture model for the segmentation of cells in the images. Results were validated using the manual outlining of cells by MSC culture experts as the ground truth. The segmentation algorithm demonstrated a Dice-Sorensen score of 0.89 ± 0.03 across 1755 train images, 0.85 ±

0.04 across 325 validation images, and 0.83 ± 0.06 across 15 test images. In summary, the proposed technique shows the potential to be incorporated into automated MSC quality control processes.

DEDICATION

I would like to dedicate my work to Mom and Dad who have led and shaped me into the person I am today. I would also like to thank Sarah Swift, who has been by my side in this process since the beginning. Thank you for your love and support.

ACKNOWLEDGMENTS

I would like to thank my graduate student mentor, Sakina Mohammed Mota, for her guidance, support, and help throughout my two years working in the Biomedical Optics Laboratory. I would especially like to thank her for the extensive help and expertise she has given me with this specific research study. I would not be able to do this research study without her.

Thanks also go to Dr. Kristen Maitland for leading the Biomedical Optics Laboratory and supporting and encouraging students such as myself to work on independent projects that interest them in the lab. Additionally, thank you to all of the people who have assisted along the way, regardless of the impact.

Finally, I would like to thank my parents, sister, my girlfriend, grandparents, and friends for always believing in me and offering their support and encouragement.

CHAPTER I

INTRODUCTION

Chronic diseases such as heart disease, cancer, diabetes, stroke, chronic lung disease, and arthritis are just a few of the leading causes of disability and death in both the United States and in the world. According to the National Center for Chronic Disease Prevention and Health Promotion, 6 in 10 adults in the U.S. have some form of a chronic disease. Since these diseases are incredibly common, there is a massive need for a therapeutic responses. Studies show that the approach of cell therapies has valuable potential to address this problem. Cell therapy is the transplantation of laboratory-expanded cells into patients to replace or repair damaged tissue and/or cells. Cytotherapies have the potential to treat heart disease, cancer, diabetes, musculoskeletal disease/trauma, and many forms of autoimmune disorders.

Mesenchymal stem cells, otherwise known as MSCs, are a diverse group of stem cells that can be differentiated into a variety of different types of cells. MSCs are multipotent, fast proliferating, and self-renewing which makes them an ideal candidate for testing. When these stem cells need to be injected into a patient, they need to be in a large quantity of cells. Unfortunately, With the growth of demand for these cells, it is impossible for current methods to keep up with demand. The main purpose of this work is to address current limitations in large-scale cell growth strategies by combining automated technology with cell expertise to monitor and evaluate cells during the culture process. A great method for evaluating the stem cell culture has been physical morphological evaluation. However, the current methods in today's research field are time inefficient and/or destructive. The deep learning algorithm in this study will not only be able to

quantitatively identity the number of cells in any given culture but will invasively be able to qualitatively characterize the MSCs .

As the number of cells in any given culture continue to rise, the distinct boundaries between cells begins to diminish and a gray area of accurate testing begins to develop. Any program given the task of determining the exact boundaries in each cell cluster will be an extremely difficult task. This can be attributed to multiple different difficulties. Arguably the biggest predicament when the cell culture has an unusually high amount of cells is the clarity of the specific image. When the pixels are not very clearly differentiated from cell and space, current segmentation algorithms fail to work as predicted. Current algorithms base their cell evaluation on the edge boundaries of the cell. When the cell boundaries become incredibly thin and the pixels become less distinct, standard techniques fail to work. Improper segmentation is the beginning of disastrous sequence of events. Failure to properly quantify the cell count and assess the quality of these cells can lead to improper use of these cells in the clinical field. Deep learning has the potential and ability to overcome these faults and open the door into a new style of cell segmentation. Deep learning contains multiple processing layers to learn and use various layers of abstraction [2, 3]. When it comes to the future of cell segmentation, it is becoming more and more transparent technology has the ability to overcome human error and reduce the time, effort, and energy into accurately assessing both the quantity and quality of these MSC cells cultures

Deep learning has recently been making a large splash in the technology field but has yet to be paired with cell segmentation. This represents the novelty of this research. This allows the process to be less dependent on cell experts and create a more objective discernment process. Another deep learning advantage is its ability to be able to asses with cell culture with no damage done to the physical cell culture. This new algorithm has the potential to bridge the gap between

the growing need for cells to be accurately controlled and the best available technology being used in the cell segmentation field

CHAPTER II

METHODS

There are multiple steps in the method used to obtain a valid deep learning algorithm. First, by visually inspecting the cells, experts in MSCs manually prepare the ground for the training and performance validation of the deep learning model. The input images are preprocessed and then are amplified to ensure the data received is sufficient. The images are then transferred to the U-Net architecture based deep learning model to obtain the segmentation output. The output's performance is compared using the ground truth that was manually obtained. **Figure 1** shown below is a step-by-step flow chart of the proposed image analysis method. These input images are acquired using a phase contrast microscope by an outside party.

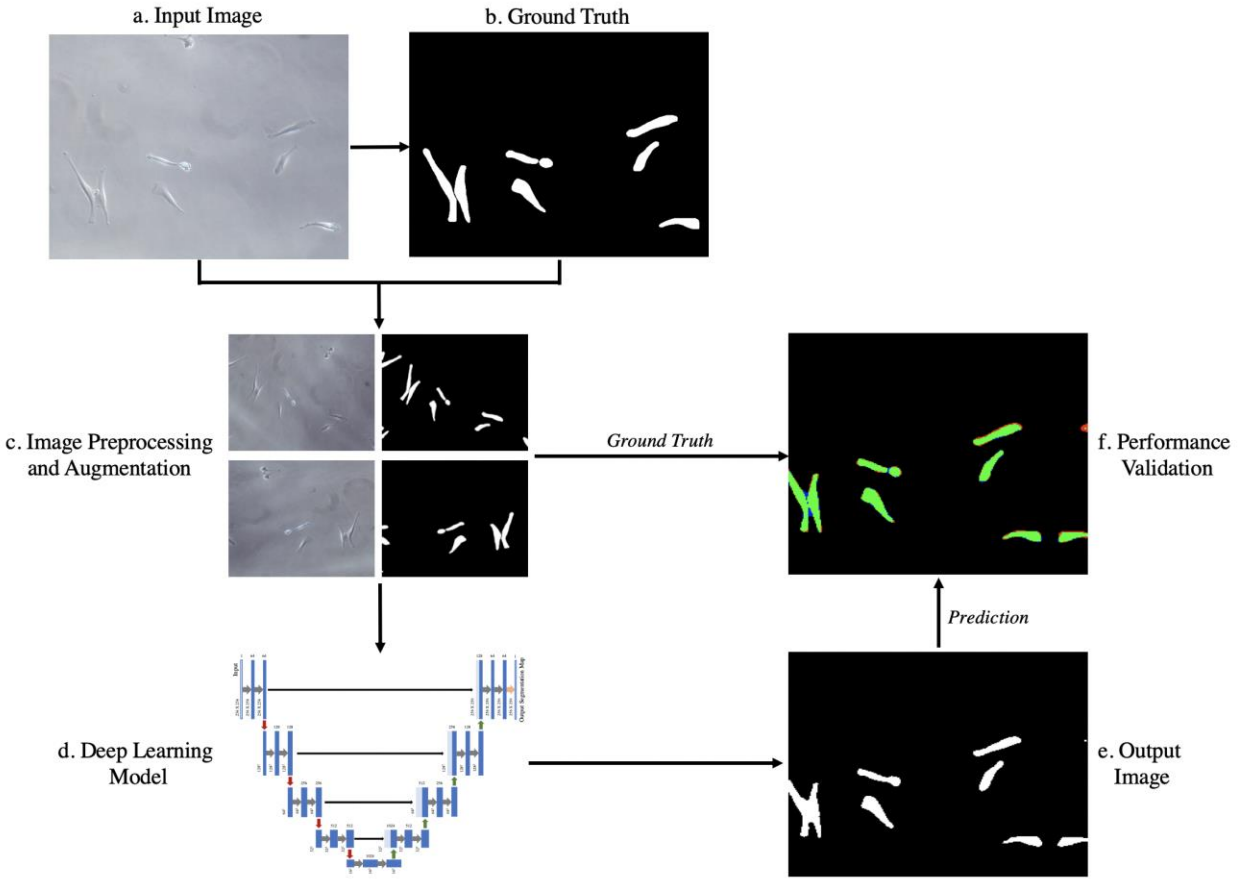

Figure 1: Pipeline of the proposed image analysis method

Dataset

Beginning with the samples, the human mesenchymal stem cells used in this were seeded at 100 cells/cm^2 in complete culture medium and cultured for 2 days before imaging. The cells were imaged under phase contrast on a Motic AE31 microscope using a Moticam 1SP 1.0 MP camera acquiring images with 1.56 pixels/µm, according to my PhD mentor, Sakina. A total of 47 images were acquired from three different cultures. In total these added up to 236 hMSCs. The acquired cell images were visually inspected by myself to manually outline cells within the images to generate the ground truth for the algorithm. These ground truth images were originally validated using ImageJ, but this process became extremely tedious and much less accurate than intended.

The next software used was Adobe Photoshop but these problems persisted. A promising software was LabelMe. LabelMe was unique because its primary use is to locate boundaries of objects in any industry. In this case, it was used to locate cells in the cell culture. However, after preparing over 50 images through LabelMe, it was impossible and an incredible waste of time to transfer the .tar files into .pdf or .jpeg files. Finally, Microsoft Paint was the software that was chosen to draw the ground truth. This process was extremely tedious and was much of the second half of 2019.

Image Preprocessing and Augmentation

All the images in the training, validation, and testing dataset are preprocessed. When only a few training samples are available, data augmentation is needed to teach the variability of cell properties.

Deep Learning Architecture for Cell Segmentation

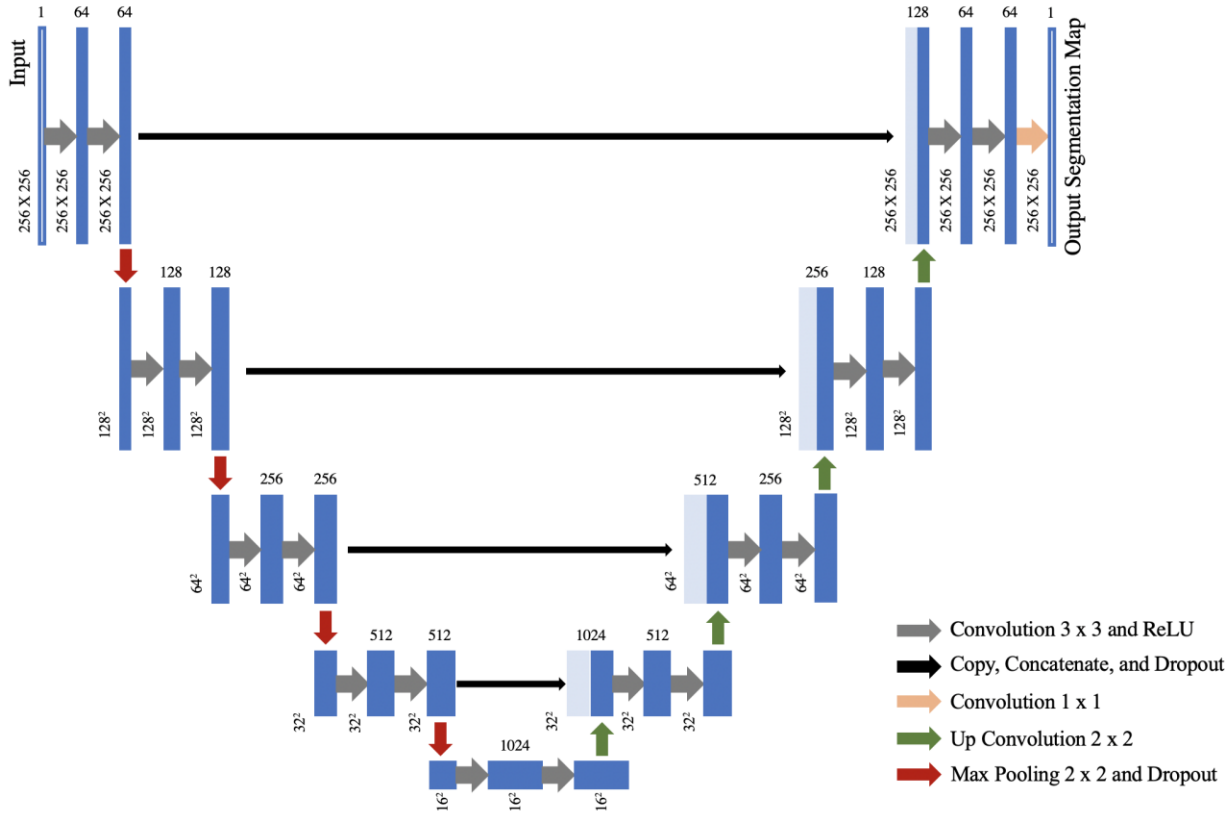

Figure 2: U-Net Architecture

There has been a lot of growth made in the deep learning field over the past years, especially in the field of segmentation. TensorFlow is an open-source software library for high-performance numerical computation. It has the ability to be paired with Python and create high-level computations. Its flexible architecture allows easy deployment of calculations across a variety of platforms. It also comes with strong support for both machine learning and deep learning. Keras is another high-level neural networks API, written in Python and capable of running on top of TensorFlow. Keras libraries were used for the deep learning application as is able to run much faster experimentations that most. It also allows easy prototyping, supports convolutional networks and runs seamlessly on both central processing units (CPU) and graphic

processing units (GPU). Deep learning networks generally require large amounts of processing power for training. The emergence of GPUs has made it possible for researchers to utilize powerful parallel technologies for training neural networks far more quickly, making it possible to learn on large datasets.

The U-Net architecture shown in **Figure 2** was implemented for our deep learning-based image segmentation [4]. The architecture consists of three different sections: the contraction, the bottleneck, and the expansion section. The contraction section is made of several blocks. Each block takes an input and applies two 3×3 convolution layers, followed by a rectified linear unit (ReLU) and a 2×2 max pooling. The number of feature maps doubles after each block so that architecture can learn the complex structures effectively. The bottleneck layer at the bottom mediates between the contraction layer and the expansion layer. It uses two 3×3 convolutional layers followed by ReLU and a 2×2 up convolution layer.

The reason deep-learning has been of such big interest as of late is because of its expansion section. Similar to the contraction layer, it also consists of multiple blocks. Each block passes the input to two 3×3 convolutional layers followed by ReLU and a 2×2 up sampling layer[5].. The input also gets appended by feature maps of the corresponding contraction layer every time. This action would ensure that the features that are learned while contracting the image will be used to reconstruct it. The number of expansion blocks is as same as the number of contraction blocks. After expansion, the resultant mapping passes through another 1×1 convolutional layer to map feature vectors with the desired number of classes [6].

The architecture uses a rather novel loss weighting scheme for each pixel such that there is a higher weight at the border of segmented objects. This loss weighting scheme helps the model to segment cells in a discontinuous fashion such that individual cells may be easily identified

within the binary segmentation map. Firstly, a pixel-wise softmax is applied to the resultant image which is followed by a cross-entropy loss function [7]. The idea is that even in segmentation every pixel has to lie in one of the classes. Hence, this method converts the segmentation problem into a multiclass classification one and it performs very well compared to the traditional loss functions.

Performance Validation

After each test, the results were scored using a similarity coefficient known as Sorensen-Dice (DICE). It measures the agreement between the algorithm's output (A) and the interpretation of experts (B). The DICE score outputs a value between 0 and 1. A DICE score of 0 correlates to no overlap in the scores while a score of 1 represents complete overlap and in complete agreement.

$$DICE = \frac{2 \times |A \cap B|}{|A| + |B|}$$

CHAPTER III

RESULTS

Table 1 below shows the performance of the developed image analysis method for MSC segmentation. DICE scores are reported by each algorithm. The DICE score in the table gives the mean and standard deviation of the DICE score of all the images in the training, validation, and the testing dataset. The 95% confidence interval of the mean DICE score is achieved by allowing the deep learning algorithm 5 times over.

Table 1: Performance of the proposed segmentation algorithm

	Number of Images	DICE Score	95% Confidence Interval
Training (Culture 1 and 2)	1755	0.893 ± 0.024	(0.888, 0.898)
Validation (Culture 1 and 2)	325	0.851 ± 0.038	(0.850, 0.852)
Independent Testing (Culture 3)	15	0.819 ± 0.054	(0.812, 0.826)

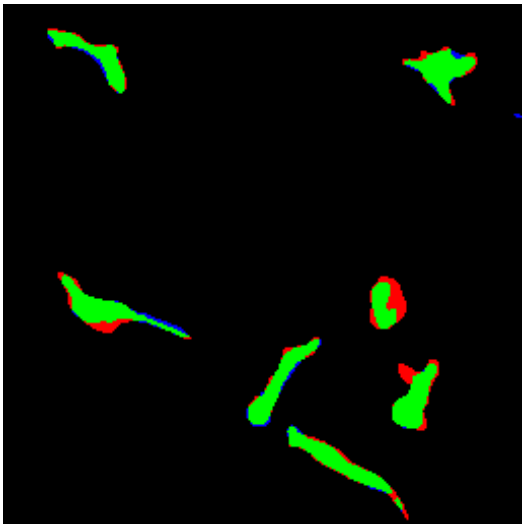

Figure 3: Segmentation Map

Figure 3 shows the segmentation map of one of the images from the independent test dataset. Each color signifies a different type of result with the independent testing and the ground truth. If a pixel is green, this correlates to true positive, which means the algorithm correctly identified cells that were indeed present. If a pixel is red, a cell is present there but the algorithm fails to properly annotate it, otherwise known as a false negative. A blue pixel represents the opposite, a background pixel that is segmented from the algorithm but no cells are present, known as a false positive). The majority of the pixels, which are black, represent the algorithm claiming these pixels are not cells and it is background, known as true negatives.

Every cell culture was analyzed using the method above. The DICE metric paired with the physical validation of the segmentation map show the true potential and results of the deep learning algorithm inspired by U-Net Architecture. Hence, it can be concluded that the deep learning-based image analysis method can segment MSCs non-invasively, rapidly, and with an accuracy of more than 80%.

CHAPTER IV

CONCLUSION

These results were obtained in March of 2020. However, with the emergence of COVID-19, the work going forward was slowed considerably. This work will be continued in Sakina's PhD proposal. As described above, the deep learning algorithm proved to be a useful tool and showed great promise to be a future direction to pursue in the cell image segmentation field. Currently, the developed method is able to accurately detect cell regions properly with an accuracy of more than 80%. Importantly, it does not damage the cells during the segmentation process. Image analysis is now going to replace a process that was once tedious and allow the process to be much more objective, rather than subjective to expert's approval. In conclusion, the proposed deep learning algorithm was a success, as it was able to detect cells in a cluster within a 80% accuracy. The system is able to be fully automated, quantitative, and non-invasive.

As stated above, the performance of this system is dependent on having high quality cell images, as the system will not work properly without this. As the system develops and becomes more complex, having the ability to also quantify images without the best clarity would be a robust improvement. The next step in this project is to work on the segmentation output to identify individual cells within a clump of cells. Once a cell is detected, the algorithm will be able to accurately distinguish between morphological features of different cells. Once an algorithm is able to detect these physical features, it can be used for a different algorithm to classify the cells based on viability. This will allow researchers to not only be able to accurately count the number of cells on any given culture, but it will also allow them to distinguish between which cells are usable and which are not able to be injected for clinical use.

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