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NON-DESTRUCTIVE ANALYSIS OF SPHEROIDS USING ARTIFICIAL INTELLIGENCE: PRELIMINARY STUDY OF CONVOLUTIONAL NEURAL NETWORKS PERFORMANCE

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SUMMARY

The cultivation of cells in 3D has gained more interest in research once 3D architecture can be closer to full cell physiological functionality. The cell culture in a spheroid format has shown very promising results, further for bioprinting developing so fast during the last decade [1]. The interaction of spheroids and the matrix, or bioink, have proportionate new structures to be analyzed, especially if one would like to follow the whole system (spheroid and bioink) without fluorescent dyes [2]. In this paper, we present a non-destructive image analysis of the spheroid viability considering three different image datasets of fibroblast NIH-3T3 spheroids acquired in different culture conditions, each consisting of approximately 300 cell samples. The first two sets possess four possible cellular structures: living cells inside spheroids (the largest cell aggregate is the spheroid; it comprises cells with lower brightness values and a very well defined membrane with a dot placed at its center, characterizing its nucleus); living cells outside spheroids (cells characterized by their lower brightness values, very

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well-defined boundaries, the cell membrane, and easy identification of the nucleus, which looks like a dot in the center of the cell; those cells are found in the aggregates); dead cells (single-cell, characterized by high brightness and very light central formation, identifies as the cell nucleus); and background (well plate area, no cell structure is identified), while the third set consists only of living cells inside the spheroid and background. Each dataset was sampled with a proportion of 70%-20%-10% for training-testing-validation datasets. In order to improve the classification task and avoid overfitting, i.e., the lack of generalization of the network, several strategies were applied, such adding blurred images to the training dataset. The CNN architectures chosen were Wide ResNet [3], VGG16 with Batch Normalization [4], SqueezeNet [5], ResNext [6], MobileNetV3 Large [7], GoogLeNet [8] and AlexNet [9], each presenting a different technology for the convolutional layer (responsible for extracting the relevant features from the images) and the classification layer (responsible for actually classifying each image according to the features extracted from the convolutional layer) as well. VGG16 presented the highest F1-score: the harmonic average of the precision, the relation between True Positives and False Positives, and recall, the relation between True Positives and False Negatives, with mean value of 0.97. With the results achieved, the authors believe that using CNNs for cell classification is a promising tool for automating this task, thus, the next step of the presented work is to use VGG16 as a backbone for implementing a Neural Network that can automate the identification and cell counting in a spheroid image.

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