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Viability Analysis Of Drug-Treated Tumor Spheroids Using Machine Learning

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***Abstract*—3D spheroids that are able to mimic the microenvironment of tumors effectively have emerged as significant structures in cancer biology and drug development. This study aims to help cancer researchers monitor the changes in human liver cancer spheroids in response to drug treatment by offering a software tool for evaluating cell viability within 3D spheroids. A dataset of spheroid images are collected, processed, and classified using alternative machine learning models constructed with Random Forest, Logistic Regression, Support Vector Machine and Extreme Gradient Boosting methods. The classification performances of the models are evaluated in terms of the prediction accuracy, precision, recall, and F1-score values. Based on the test experiments conducted, Extreme Gradient Boosting model achieved the highest ratios for all of the performance metrics. Furthermore, a standalone desktop application is implemented to perform analyses of the images with the help of its user-friendly interface.**

***Keywords*—3D spheroids, spheroid behavior, viability analysis, image processing, image classification, machine learning.**

I. Introduction

There is an increasing interest in using three-dimensional (3D) spheroids for modeling cancer and tissue biology to advance clinical research. 3D spheroids are proven to mimic tumor behavior and microenvironment more effectively than conventional two-dimensional (2D) cell cultures [1], [2]. Cancer spheroids contain proliferating and nonproliferating cells, and well-oxygenated and hypoxic regions similar to in vivo tumors. Spheroids have been used to model various cancers including liver, breast, colon, and prostate, to identify novel anticancer therapeutics [3], [4].

The hanging drop method, which is frequently used in 3D spheroid studies, can be easily applied without expensive equipment, produces spheroids of similar size and gives reproducible results in various cancer cell lines [1], [2], [5]. The change in growth rate and the viability of the spheroids are two of the most important features utilized to assess how effective a cancer drug is in a hanging drop assay. Normally, visual inspection of the spheroid images need to be performed by an expert which slows down the process and, if there is no expert, results in data loss or misclassification. Recognizing the significance of this issue, the utilization of a machine learning (ML) method which operates on the spheroid images would improve the processing times of the experiments, mitigate the effects of data loss and misclassification. The primary aim of this study is to develop method to process the spheroid images for machine learning algorithms to characterize phenotypic changes in human liver cancer spheroids in response to drug treatment.

The rest of the paper is structured as follows: Section II gives a few number of related works on the software supported image analysis of spheroids, Section III explains the methodology used in the proposed model, Section IV shows and discusses the results of the experiments, finally Section V concludes the paper.

II. Related Work

Benning et al. [6] investigated the feasibility of efficacy of drug combinatorics or new compounds in 3D cell cultures. The authors analysed the viability of the spheroids through classifying their images using a Convolutional Neural Network (CNN) architecture based on AlexNet learning model. Regarding the dataset used for training and testing, totally 1200 spheroid images were annotated as the following three classes: unaffected (with a viability over 80%), mildly affected (with a viability between 40%–60%), and affected (with a viability less than 40%). The experiment results were evaluated in terms of the following criteria: accuracy, precision, recall, and F1-score. The model performed around 0.9 for each of these metrics.

Cosme et al. [7] examined the human melanoma C8161 spheroid morphology and drug responsiveness. The authors analyzed morphological parameters of the spheroids after photodynamic therapy (PDT) mainly using the following two software tools written in Matlab: AnaSP (ANAlyse SPheroids) [8] and ReViSP (Reconstruction and Visualization from a Single Projection) [9]. The paper emphasized that the morphometric parameters, particularly area and volume, could be used to assess the damage in spheroids.

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Triwibowo et al. [10] investigated on the classification of the mammogram images obtained from Kaggle to identify breast tumors. The authors processed the images to recognize the edges using the Canny edge detection method [11] and classified the images into whether having malignant breast tumor or not using Support Vector Machine (SVM) algorithm. Based on the test results mentioned, the proposed model achieved an accuracy of 95%.

As a related work on analysing the responses of 3D in vitro tumor models to drugs, Chen et al. [12] proposed SMART, a spheroid monitoring and AI-based recognition technique for assessing tumor spheroid behavior. For the learning model, the authors used a CNN-based architecture as the backbone, particularly PSP-U-Net for tumor spheroid boundary detection and evaluation of the invasiveness of tumor spheroids. The excess perimeter index and the multiscale entropy index were considered to evaluate the 3D tumor invasiveness analysis.

As an open source tool on spheroid image segmentation, Lacalle et al. [13] proposed SpheroidJ that would be used as a standalone application to allow the users to configure the segmentation parameters for handling a custom segmentation of the spheroid images with different sizes and shapes.

III. System Design and Implementation

Section III explains the protocol on the experimental design and gives the details on the implementation of the proposed system.

A. Dataset Construction

Spheroids were created using the liver cancer cell line Huh7 and the liver cancer stellate cell line LX2. The hanging drop method as shown in Fig. 1 was systematically implemented, following to the established standards specified by [14]. Spheroids have been separated in three different groups (i.e., DMSO without drug as control group, 2 microMolar Sorafenib as low-dose group, and 5 microMolar Sorafenib as high-dose group) each with 120 spheroids. The initial imaging of all groups has done at the end of the 7th day using a light microscope. Following the first imaging, all spheroids in each group captured by light microscopy have been recaptured at the end of the corresponding days shown in Fig. 2. This iterative procedure resulted in the compilation of a comprehensive dataset (i.e., 1512 spheroid images) that included the morphological changes demonstrated by the spheroids throughout time.

B. Image Processing

The images were taken in 10x and 4x magnified photos. Initially, all images were converted into grayscale. After then, two different image pre-processing steps were applied into corresponding different scaled microscopic images.

For the images obtained with 10x magnification, Contrast Limited Adaptive Histogram Equalization (CLAHE) [15] was used to improve contrast. CLAHE method splits an image into small regions known as tiles and separately applies histogram equalization to each tile. The method limits the

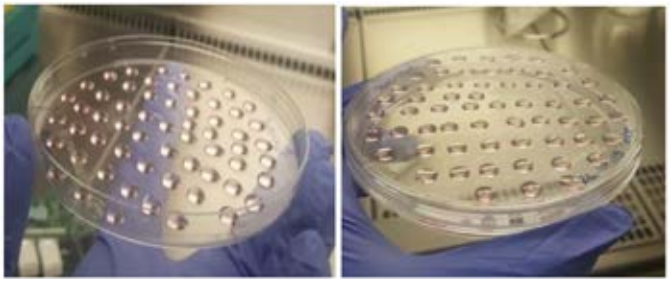


Fig. 1: Hanging-drop method.

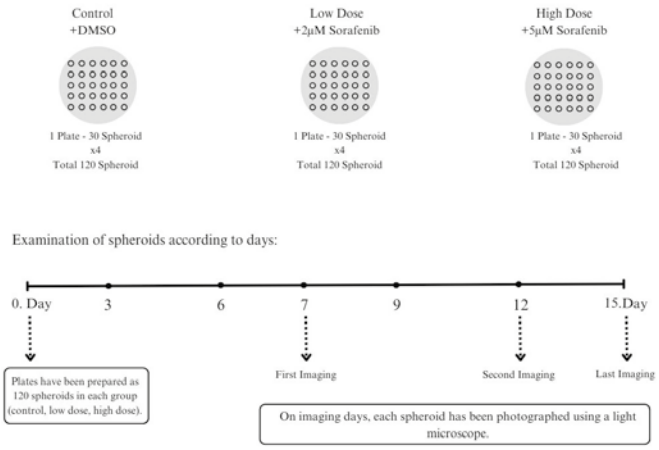


Fig. 2: Data collection.

contrast enhancement in each tile by clipping the histogram with a specified value, which was defined as 2 in our study. Gaussian blurring was the next operation to remove noise and smooth image surface. Then, binary threshold operation was applied into the images for the segmentation of foreground and background areas.

For the images obtained with 4x magnification, Canny edge detection [11] was used for removing noises, edge detection and thresholding. This operation served as the foundation for outlining spheroid boundaries.

At the end of each of these two pre-processing steps, morphological closing operations were applied to all images to bridge gaps within cell structures to improve their continuity and completeness for accurate feature calculation. Contour extraction is a critical step for identifying significant regions in the processed images to obtain the features that give useful insights about spheroid structure and activity.

Contours were detected from the binary image obtained by thresholding and canny edge detection. The largest of these many contours was chosen as the primary region of interest, which was used to calculate the important features for machine learning algorithm. Key features to extract were decided to be the geometric properties including area, perimeter, diameter, roundness, aspect ratio, solidity and convexity. The area, perimeter and diameter values were calculated by

default functions in OpenCV as pixels and were converted to the unit of millimeters with the scale factor. Roundness is a measurement of how closely the contour resembles a circle. It is calculated with the following formula that leads to 1 for an exact circle shape:

$$4\pi Area/Perimeter^2$$

The aspect ratio is the ratio of the longest dimension to the shortest dimension of the contour's smallest bounding rectangle. It shows us if the shape has become extended or compact. Solidity is the ratio of the contour area to the area of the convex hull. It represents the contour's concaveness, with higher values showing more convex forms. Convexity is the ratio of the contour perimeter to the perimeter of the convex hull. It indicates the degree of deformity or complexity of the contour form in relation to its convex hull. These features were considered to be used in the machine learning algorithm for the decision of cell viability, so that the extracted features from all the images were written into a file in comma separated values format.

C. Data Preparation

Before training the machine learning model, the entire data in the file produced at the end of the image processing pipeline was examined. One-hot encoding was applied to the categorical data. Then, numerical features were normalized using min-max scaling for ensuring consistency in scale between variables. For detecting and reducing the outliers within the data to ensure the robustness, the statistical Interquartile Range (IQR) method was employed on each numerical feature. By calculating the IQR (i.e., the difference between the 75th and 25th percentiles of the data) for each of the corresponding key feature, a numerical value observed outside the range starting from "25th percentile – 1.5 x IQR" to "75th Percentile + 1.5 x IQR" was considered as an outlier and was removed from the dataset. The finalised dataset constructed to be used in machine learning model was split into two subsets, particularly 70% for training and 30% for testing.

D. Experiments Using Machine Learning

Using the annotated spheroid images, the dataset produced was labeled in three different categories of cell viability (i.e., healthy, slightly dead, and extremely dead). Via training a machine learning model, the viability of the cells would be predicted based on the classification of the images by the model.

As the main backbone on the machine learning model, firstly, a supervised learning algorithm, Random Forest was chosen to be the classifier component of the system. Grid Search method was used for determining the best combination of hyper-parameters. The model was monitored using five-fold cross-validation technique. The performance of the model was evaluated in terms of prediction accuracy, precision, recall, and F1-score metrics. With the aim of both comparing the model with alternative models using some other machine learning algorithms and investigating for a possible improvement on the prediction accuracy, Logistic Regression, SVM and Extreme Gradient Boosting (XGBoost) techniques were also examined.

E. Experiment Results

The experiments were conducted using different machine learning models (i.e., Random Forest, Logistic Regression, SVM and XGBoost) with the same image dataset constructed.

Table I lists the performance results on the prediction of the categories of the images using the machine learning via alternative models.

TABLE I: PERFORMANCES OF THE MODELS

Model	Accuracy	Precision	Recall	F1-score
Random Forest	0.839	0.845	0.839	0.841
Logistic Regression	0.780	0.773	0.780	0.775
SVM	0.795	0.794	0.795	0.794
XGBoost	**0.844**	**0.851**	**0.844**	**0.845**

As emphasized in Table I, XGBoost performed the best amongst the models in terms of all the evaluation metrics.

Fig. 3 illustrates the confusion matrix results of the tests with each of the four models mentioned in Table I.

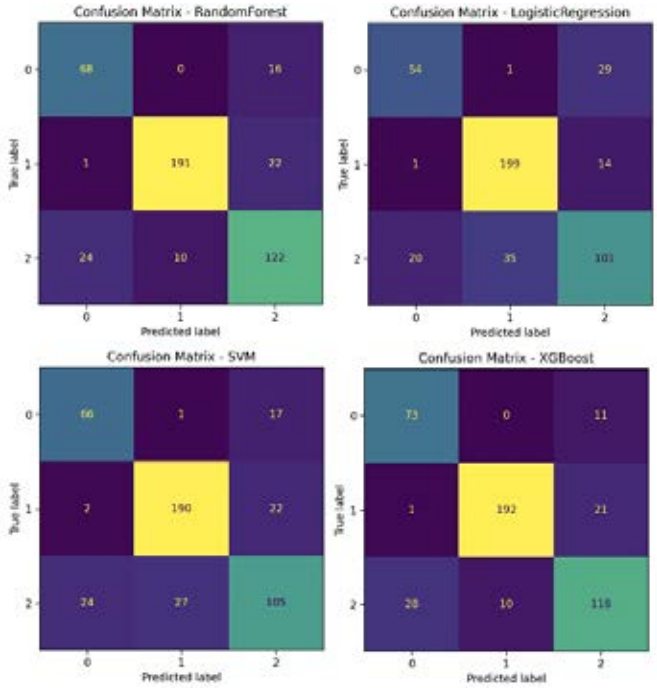


Fig. 3: Confusion matrix results of the models.

F. Standalone Application

To conduct the experiments in a systematic approach and in a fast way, a standalone desktop application was also implemented. A screenshot from the application showing the home page listing the main services is given in Fig. 4.

In deed, the objective of constructing such a ready-to-use standalone application incorporating the trained learning



Fig. 4: Home page of the application.

model is to make the users see the automatic prediction results easily.

Another useful service in our standalone application is that the users would be able to increment the dataset. Whenever the user takes a set of many new photos, the system processes the new images loaded and adds their extracted numerical features into the end of the data file. After retraining, the updated model would be ready-to-use.

IV. Conclusion

To be used in the classification of the 3D spheroids, a dataset of 1512 spheroid images have been collected from the real-time experiments conducted in the Genetics and Bioengineering Laboratory at Izmir University of Economics.

The images have been processed to obtain the following key features to represent the genetic properties of the spheroids: area, perimeter, diameter, roundness, aspect ratio, solidity and convexity.

The data has been trained for the construction of a machine learning model to classify the images in three different categories (i.e., healthy, slightly dead, and extremely dead). Alternative machine learning models have been employed using Random Forest, Logistic Regression, SVM and XGBoost methods. The classification performance of each different model has been evaluated in terms of the prediction accuracy, precision, recall, and F1-score values. Based on the test experiments conducted, XGBoost model achieved the highest performance ratios in terms of all the metrics; particularly 84.4% for accuracy, 85.1% for precision, 84.4% for recall, and 84.5% for F1-score.

Furthermore, a standalone desktop application has been implemented to perform analyses of the images. The application is ready-to-use on the fast prediction for the classification of the spheroid images. The dataset can also be incremented by the users via loading new images into the application.

As the future work, after employing the image processing techniques applied in our proposed system, deep learning algorithms, particularly CNN architectures would be examined to improve the performance of the classification of the spheroid images.

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