Scratch Assay Image Analysis Automation

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Abstract. In this brief proof-of-concept paper, we present an algorithm developed in Python to automate the analysis of images obtained in scratch assays. Our algorithm uses random forest, a classic machine learning technique, to train and segment scratch assay images. This enables an average time reduction of 84% on the analysis of the images, together with a procedure with replicable results.

Keywords: machine learning, medical images & software solution

1 Introduction

Scratch assays, also known as migration assays or wound healing assays, are experiments conducted to study the migration capacity of collective groups of cells [2,6]. These types of experiments are widely used in cancer research, as they enable the understanding of how cells mobilise and occupy space, especially in processes like metastasis and invasion of healthy tissue [3].

A two-dimensional migration assay usually involves four steps (See Figure 1.c). The first step consists of cultivating tumor cells on a dish until the point where cells have formed a confluent monolayer. Then a cell-free region is generated under determined conditions. A common practise is to scratch the surface of the dish with the tip of a pipette, making a wound by carefully removing cells. Then, after the scratch is done, the documentation process begins. This process consists of obtaining photos of the dish at pre-established periods. Finally, the images are analysed by measuring the cell-free gap, which provides insight into the cells' migratory potential [2,6].

There are several methods to analyse the images and acquire quantitative measures of the change in time of the cell-free region's area. However, most of these methods are conducted manually[1]. They involve measuring wound areas in every image of each experiment by hand, usually by drawing the contour of the cell-free region[2,6]. As a result, the whole process takes a considerable amount of time. Besides, the obtained measurements are highly operator dependant; hence, they are not easily replicable.
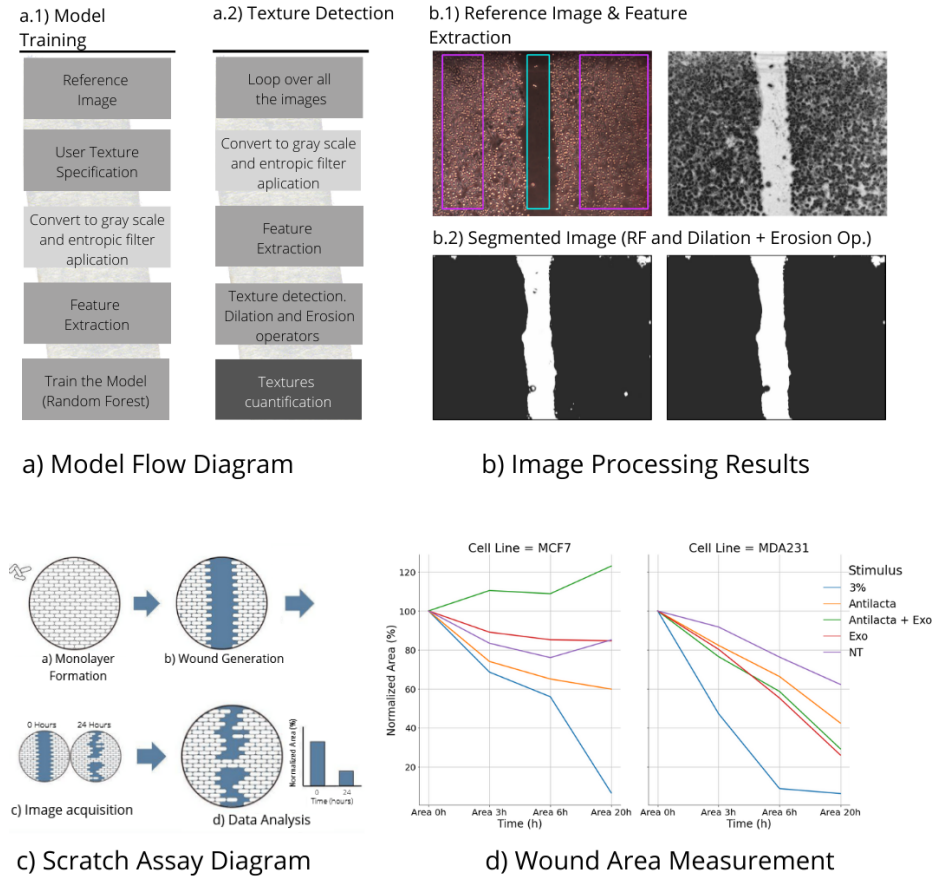
Fig. 1. a) Algorithm Flow Diagram, a.1) Training process. a.2) Texture Detection process. b) Image Processing Results b.1) Reference/training Image with user specified textures (left), Gray scale and entropy filter applied to the reference image (right). b.2) Segmented Image (left). Dilation and Erosion operators applied on the segmented image (right). c) Scratch assay diagram, edited from G.Regnard[5]. d) Wound area measurement.

To address these time-consuming and replicability issues, we implemented a supervised machine-learning algorithm to train and classify regions of scratch assay images into two classes (wound vs. cell) and measure the corresponding wound area. The algorithm was tested on scratch assays using two breast cancer cell lines (MCF7 and MDA-MB-231).

2 Methodology

The proposed method consists of using Scikit-learn's random forest algorithm [4] to train a model with a reference image chosen by the user. The model is fed with a features vector for each pixel in the image to classify it into one of two categories (wounded texture/cell texture). The features vectors are obtained by applying Gaussian blurring filters and also calculating the eigenvalues of the hessian matrix after Gaussian blurring. The pixels used to train the image are obtained by manual user selection. Then, the resulting model is used to segment the rest of the scratch assay images. Afterwards, dilatation and erosion operators are applied to reduce the wound regions inside cellular areas. Finally, the algorithm counts the pixels classified per each category and obtains a measurement of the segmentation. A summary of the main steps of the algorithm is displayed in Figure 1.a.

Our model was tested on scratch assay images executed in a study looking for a new immune therapy for breast cancer. Research that used MCF7 and MDA-MB-231 tumor cell lines, with MDA-MB-231 being the more aggressive one. Assessing their migratory potential is essential to understanding how tumorigenicity is mitigated/minimised when applying immune blockade (see anti-lactadherin stimuli in Figure 1.d).

3 Results

Our laboratory colleagues spent an average of 150 seconds processing one image, ranging from 50 to 300 seconds. While our model took an average of 24 seconds, ranging from 8 to 40 seconds, depending on the computational power of the machine where the code ran. As a result, processing an image with our model is approximately six times faster than processing it manually.

After applying the proposed algorithm to 80 images, we successfully segmented 60 images with just one training process. Afterwards, we noticed that our model segmented 20 images incorrectly. These images were brighter than the image we used to train the model. In light of this, we trained our model with one of the 20 images, and then we re-segmented these images, obtaining better results. From this experience, we can say that the main limitation of our model is that it is not invariant to changes in luminosity and colour contrasts in the images, which can be controlled by training the model in each scratch assay. As more tests are developed, and more images are obtained, a better model that addresses these problems could be achieved.

4 Conclusion

In this work, we implemented an algorithm to segment scratch assay images automatically. The proposed algorithm was tested with two cell lines, each one with five different stimuli and with forty images per cell line, showing promising results. The proposed model can produce consistent results and measurements six times faster than an average operator.

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